Sulphatide-specific antibody-mediated effects on the transcriptional profile of myelinating cultures

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) characterised by the formation of chronically demyelinated plaques of gliotic scar tissue associated with varying degrees of axonal injury and loss. The aetiology of MS remains inexplicit but it is now generally thought of as a 'complex trait' in which environmental factors disrupt immunological self-tolerance to myelin antigens in genetically susceptible individuals. The most obvious immunological abnormality associated with MS is a sustained intrathecal synthesis of immunoglobulins within the CNS, manifested by presence of oligoclonal bands of immunoglobulins when cerebrospinal fluid is analysed by isoelectric focusing. These immunoglobulins are derived from clonally expanded B cell populations sequestered in the CNS, but their pathophysiological significance remains obscure.

The specificity profile of this intrathecal antibody repertoire is complex; however, an increasing body of evidence indicates a significant component of this repertoire to be specific for lipids, in particular sulphatide. We therefore used a sulphatide-specific mouse monoclonal antibody (mAb) that mimics the specificity profile of components of the intrathecal response in patients to investigate its effects in myelinating cultures derived from embryonic rat spinal cord *in vitro*. These experiments focused on exploring the effects of mAb O4 in the absence of serum, a model situation which reproduces that seen in the CNS of patients with progressive forms of MS in which blood brain barrier damage is minimal.

We observed mAb O4 had no immediate effect on myelin integrity, but after 10 days inhibited myelination completely, an effect associated with a 50 % increase in the number of Iba-1^{+} microglia. To explore the underlying mechanism we performed a gene microarray on cultures treated with mAb O4 in the presence and absence of serum as a source of complement. In the absence of serum mAb O4 induced an unexpected pattern of transcriptional responses characterised by induction of many chemokines (including *Cxcl13, Cxcl11, Cxcl10, Cxcl9 and Ccl2*) and a large number of interferon sensitive genes (ISGs) more commonly associated in the development of innate and adaptive immunity to pathogens in

the CNS. These responses were not seen when cultures were treated with mAbs with no known CNS-specificity, and were abolished when serum was added as a source of complement.

These observations identify a novel antibody-dependent mechanism by which components of the intrathecal antibody repertoire may maintain a pro-inflammatory signalling environment in the CNS in the absence of input from the peripheral immune system. If validated in patients, these findings identify the intrathecal B cell repertoire as an important therapeutic target in MS.

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Author's Declaration

I, Tiia Semenoff, do here by declare that the work described in this thesis is original and was generated entirely as a result of my own effort. None of the data, submitted as part of this thesis, has been submitted for any other degree, either at the University of Glasgow, or any other institution.

Signature:……

Printed name: Tiia Semenoff

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Abbreviations

AAN	American academy of neurology
ADCC	antibody-dependent cellular cytotoxicity
ADEM	acute disseminated encephalomyelitis
ANOVA	analysis of variation statistical test
APC	antigen presenting cells
AQP4	aquaporin-4
ASC	antibody-secreting cells
ATP	adenosine triphosphate
BB	blocking buffer
BBB	blood-brain barrier
BLAST	basic local alignment search tool
BSA	bovine serum albumin
C-	control in the absence of serum
C+	control in the presence of serum
Ca^{2+}	calcium channel
CCL	chemokine (c-c motif) ligand
CCR	chemokine (c-c motif) receptor
cDNA	complementary DNA
CGT	ceramide galactosyl transferase
CNP	2',3'-Cyclic-nucleotide 3'- phosphodiesterase
CNS	central nervous system
CSF	cerebral spinal fluid
CST	ceramide sulfotransferase
CXCL	chemokine (c-x-c motif) ligand
CXCR	chemokine (c-x-c motif) receptor
DAPI	4', 6-diamidino-2-phenylindole
DM	differentiation medium
DMT	disease modifying treatment
DIV	days in vitro
dH_2O	distilled water
DMEM	Dulbecco's modified eagle medium
DNA	deoxyribonucleic acid
E11.5	embryonic day 11.5

EAAT1	excitatory amino acid transporter 1
EAE	experimental autoimmune encephalomyelitis
EGF	epidermal growth factor -
ELISA	enzyme-linked immunosorbent assay
FBS	foetal bovine serum
Fcμ	Fc receptor
FITC	fluorescein isothiocyanate
GalC	galactosylceramidase
GCRMA	robust multi-array average adjusted for GC content
GLAST	glutamate aspartate transporter
GFAP	glial fibrillary acidic protein
GO	gene ontology
GSB	gene-specific binding
GWAS	genome-wide association study
HBSS	Hanks balanced salt solution
IFN	interferon
IgG/M	immunoglobulin, isotype G/M
IgM-	isotype control in the absence of serum
IL	interleukin
IRF	interferon regulatory factor
ISG	interferon sensitive gene
K^+	potassium channel
L-15	Leibovitz's-15 medium
LPS	lipopolysaccharide
mAb	monoclonal antibody
MAG	myelin oligodendrocyte glycoprotein
MOG	myelin associated glycoprotein
MBP	myelin basic protein
MHC	major histocompatibility complex
MOBP	myelin-associated oligodendrocyte basic protein
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MS	multiple sclerosis
$Na_v1.2/1.6$	sodium channel
$NaHCO_3$	sodium bicarbonate

Na+ /K+ -ATPase	sodium-potassium adenosine triphosphatase
Nfasc155	neurofascin-155
NG2	neuron glial antigen 2
NMO	neuromyelitis optica
NO	nitric oxide
NSB	non-specific binding
O4-	mAb O4 in the absence of serum
O4+	mAb O4 in the presence of serum
OCP	oligoclonal band
OLIG2	oligodendrocyte transcription factor
OPC	oligodendrocyte precursor cell
qPCR	quantitative polymerase chain reaction
P1	postnatal day 1
Panther	protein analysis through evolutionary relationships
PBS	phosphate buffered saline
PCA	principle component analysis
PDGFα-R	platelet-derived growth factor subunit A receptor
PFA	paraformaldehyde
PLL	poly-L-lysine
PLP	proteolipid protein
PM	plating medium
PMS	progressive multiple sclerosis
PNS	peripheral nervous system
PPMS	primary progressive multiple sclerosis
PSA-NCAM	polysialylated neural cell adhesion molecule
RRMS	relapsing-remitting multiple sclerosis
RT-PCR	real-time PCR
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SPMS	secondary progressive multiple sclerosis
Th1/17	T helper cell types 1/17
TLR5	toll-like receptor 5
TMEV	Theiler’s murine encephalomyelitis virus
TNF	tumour necrosis factor
WHO	world health organization

1 Introduction

1.1 Mammalian central nervous system

The mammalian nervous system is a complex highly vascularized organ that is generally discussed in terms of two anatomically distinct parts, the peripheral nervous system (PNS) and the central nervous system (CNS). The CNS consists of the brain and spinal cord, which are enclosed by the meninges and protected from physical trauma by the skull and vertebrae of the spinal column, respectively. The PNS comprises the remainder of the nervous system *i.e.* that located outside of the brain and spinal cord. The primary function of the CNS is to integrate, coordinate and respond to information received from the PNS via a diverse array of sensory inputs. This is dependent on a complex network of cellular interactions involving neurons, macroglia (astrocytes and oligodendrocytes) and microglia. These interactions can be disrupted by toxins, infection, hypoxia and trauma, effects that are responsible for the functional deficits seen in neurological and psychiatric disease.

1.1.1 Neurons

Neurons are specialised cells that use chemical and electrical signals to transmit information. The human brain contains approximately 10^{11} neurons each of which comprises a cell body (the perikaryon) and a network of processes (neurites) that relay information to and from other cells. Neurons receive information via electrical impulses from dendrites, while information is transmitted onto other targets via the axon. The speed at which information is transmitted down an axon is dependent on three factors, its diameter, whether or not it is myelinated (*see Section 1.1.2.1*) and length of the internodes (Brill et al., 1977; Young et al., 2013); nerve conduction velocities in small un-myelinated fibres may be in the range of 0.5-2.0 m/s, whereas it may exceed 120 m/s in large myelinated fibres (Siegel and Sapru, 2011).

Metabolic and structural homeostasis along the length of the axon is maintained by axonal transport, a bidirectional mechanism that transports organelles, vesicles and proteins from the neuronal cell body. These cargos are transported via microtubules and include neurofilament proteins that polymerize to form

neurofilaments the major component of the axonal cytoskeleton (Griffin et al. 1995; Silber et al., 2002).

1.1.2 Glial cells of central nervous system

1.1.2.1 Oligodendrocytes

Oligodendrocytes derive from a variety of sites in the CNS during development. In the spinal cord 80 to 90 percent of oligodendrocytes derive from the ventral ventricular zone as oligodendrocyte precursor cells (OPC) which migrate and differentiate into myelin-forming oligodendrocytes (Bradl and Lassmann, 2010; Lu et al., 2002; Takebayashi et al., 2002; Zhou and Anderson, 2002). In addition, a small fraction of spinal cord oligodendrocytes are derived from OPC that migrate from the dorsal spinal cord (Bradl and Lassmann, 2010; Cai et al., 2005; Fogarty, Richardson and Kessaris, 2005; Vallstedt, Klos and Ericson, 2005). Migration patterns are thought to be influenced ventrally via sonic hedgehog and dorsally via bone morphogenetic protein gradients (Tekki-Kessaris et al., 2001; McTigue and Tripathi, 2008). In the forebrain, OPC populating the embryonic telencephalon derive from the medial ganglionic eminence and anterior entopeduncular area of the ventral forebrain (Bradl and Lassmann, 2010). These are joined by OPC originating in the lateral and/or caudal ganglionic eminences and postnatal cortex (Kessaris et al., 2006; Bradl and Lassmann, 2010). The different waves of cells compete against each other to populate the brain before differentiating into myelinating oligodendrocytes (Bradl and Lassmann, 2010). This is regulated by multiple positive and negative signalling mechanisms that include contributions from the extracellular matrix (Colognato and Tzvetanova, 2011), growth factors (Redwine et al., 1997; Spassky et al., 2001), chemotropic molecules (Jarjour et al., 2003; Tsai et al., 2002), and chemokines (de Castro and Bribian, 2005; Dziembowska et al., 2005).

Oligodendrocyte development involves multiple stages that can be followed using stage specific combinations of molecular markers which define the lineage development (*Fig. 1.1.2.1a*). This starts with a neural progenitor that differentiates into an OPC after which these cells lose their migratory and proliferative potential and differentiate into myelin protein expressing oligodendrocytes (Pfeiffer, Warrington and Bansal, 1993; He and Lu, 2013;

Stangel and Hartlung, 2002). This involves spatial-temporal regulation and integration of multiple, in some case redundant signalling pathways that include among others, Notch1 interactions with Jagged1 ligand on the axonal surface (Wang et al., 1998; Genoud et al., 2002; Radtke and Raj, 2003; Watkins et al., 2008; Bradl and Lassmann, 2010). This transition from OPC to a differentiated oligodendrocyte is a critical step in the development of myelinating lineage (Miller, 2002); however, survival of these pre-myelinating oligodendrocytes is ultimately dependent on their ability to make productive contacts with axons (Simons and Trajkovic, 2006). This interaction occurs within 5 to 12 hours as demonstrated by experiments on zebra fish *in vivo* and rodent cultures *in vitro*, respectively (Watkins et al., 2008; Czopka et al., 2013).

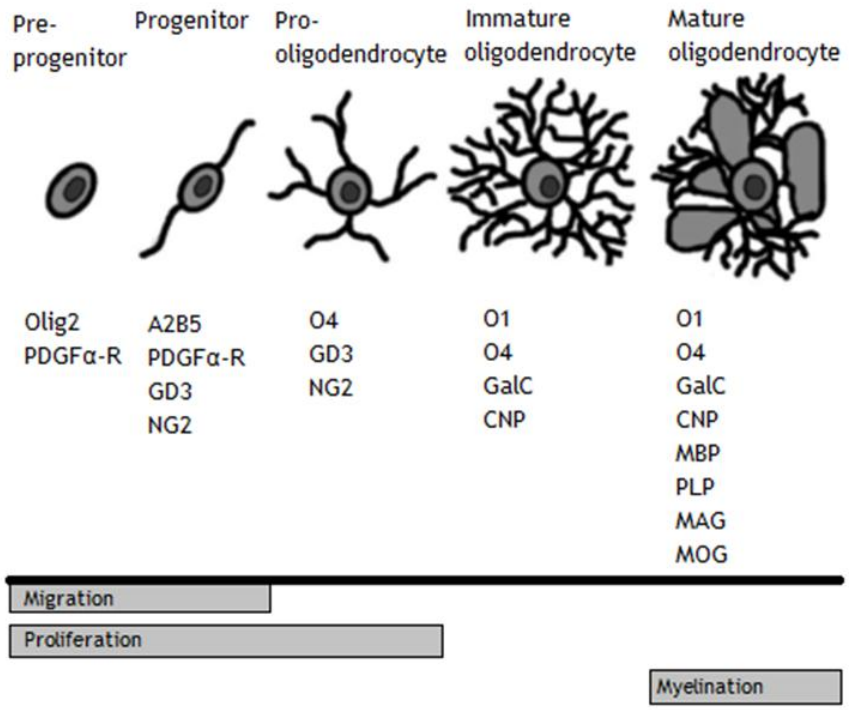


Figure 1.1.2.1a: Oligodendrocyte lineage indicated by their stage-specific markers. *OPC development into mature myelin forming oligodendrocytes can be visualised by the use of different markers. (Adapted from Stangel and Hartung, 2002).*

Information on human oligodendrocyte development and myelination is relatively sparse due to ethical issues, but studies on aborted embryos indicate oligodendrocyte development begins during the second trimester (Back et al., 2001; Jakovcevski et al., 2009). Similar to the situation in rodents, oligodendrogenesis in man exhibits ventral to dorsal progression (Pringle and

Richardson, 1993; Timsit et al., 1995; Jakovcevski et al., 2009) and there is a clear dissociation between the timing of oligodendrocyte differentiation and myelination in the forebrain (Back et al., 2001; Jakovcevski and Zecevic et al., 2005). Myelination is first observed in basal ganglia during the third trimester and somewhat later in the corpus callosum (Back et al., 2001; Jakovcevski, Mo, Zecevic, 2007). Myelination in human peaks during the first year of life but continues into adulthood (Stangel and Hartung, 2002). However, the adult CNS retains a large pool of mitotic neural/glial antigen 2 (NG2) positive OPCs (Miller, 2002), thus allowing myelination to continue at a low levels throughout life (Young et al., 2013). Under normal conditions turnover of adult OPC is slow (Young et al., 2013), but experimental studies demonstrate they can rapidly proliferate in response to tissue damage and differentiate to oligodendrocytes that will remyelinate axons (Levine, Reynolds and Fawcett, 2001; Reynolds et al., 2001; Miller, 2002; Wilson, Scolding and Raine, 2006; Nishiyama et al., 2009; Tripathi et al., 2010).

Mechanisms in myelination

The mechanisms that determine the timing of myelination are regulated at many levels. One factor is axonal diameter, as only axons with a diameter of > 0.2 μm are myelinated in the CNS (Simons and Trajkovic, 2006; Piaton et al., 2010; Bradl and Lassmann, 2010; Tripathi et al., 2010). Axonal calibre also determines the thickness of the myelin sheath (Friede, 1972; Piaton et al., 2010). Moreover, the axo-glial interaction has also been shown to increase axon diameter (Colello and Schwab, 1994; Mason et al., 2001; McTigue and Tripathi, 2008). These observations demonstrate axonal signals play a major role in regulating functional myelination *in vivo*, but *in vitro* synthesis of myelin-like structures is actually a default response of mature oligodendrocytes that can occur in the absence of axons (Lee et al., 2012). However, this uncoordinated myelin production may lead to detrimental consequences (Simons and Trajkovic, 2006), thus further indicating the importance of the control mechanisms *in vivo*. The molecular and spatial constraints that control myelination *in vivo* are still poorly understood but these clearly involve multiple, often redundant, mechanisms. Successful myelination is dependent on the interplay of an array of permissive and inhibitory axonal factors that include neural electrical activity which provides a positive signal for myelination (Gyllensten and Malmfors, 1963;

Demerens et al., 1996; Pianton et al., 2010) and down regulation of polysialylated neural cell adhesion molecule (PSA-NCAM), an inhibitor of myelination expressed at the axonal surface (Charles et al., 2000; Miller, 2002; Coleman et al. 2005). Other factors shown to modulate myelination include signal transduction involving components of the extracellular matrix, growth factors and metabolites such as adenosine and adenosine triphosphate (ATP) (Kuperman, Volpert and Okamoto, 1964; Maire, Medilanski and Straub, 1984; Stevens et al., 2002; Ishibashi et al., 2006; Colognato et al., 2007; Furusho et al., 2012; Bradl and Lassmann, 2010).

Oligodendrocyte function

For many years the function of the oligodendrocyte was considered to promote rapid saltatory conduction by ensheathing axons with myelin (Nave, 2010). This view was refined as studies demonstrated that formation of specific axo-glial domains was required for saltatory conduction (Vabnick and Shager, 1998; Tait et al., 2000). Differential clustering of sodium channels ($Na_v1.2$ channels that prior to myelination are replaced by $Na_v1.6$ channels) and potassium channels (K^+) are arranged in nodes of Ranvier and juxtaparanodal domains of myelinated axons, respectively (***Fig. 1.1.2.1b***; Boiko et al., 2001; Bhat et al., 2001). However it is now recognised these interactions also maintain a structural relationship between the axon and myelin sheath that facilitates the ability of oligodendrocytes to provide metabolic support to axon segments and somas through several growth and trophic factors (McTigue and Tripathi, 2008; Edgar and Nave, 2009; Nave, 2010; Fünfschilling et al., 2011). Demyelination not only disrupts this trophic support, but also renders axons more susceptible to damage by inflammatory mediators (McTigue and Tripathi, 2008); a combination of effects predicted to compromise axonal survival in chronic inflammatory demyelinating diseases such as multiple sclerosis (Franklin et al., 2012). Unlike the situation in the PNS where myelinating Schwann cells maintain a one to one relationship with axons, oligodendrocytes in the CNS interact with multiple axons and can assemble and maintain > 40 individual myelin sheaths (Pfeiffer, Warrington and Bansal, 1993; Podblieska et al., 2013). As a consequence, the loss of a single oligodendrocyte has the potential to compromise the functional and structural integrity of multiple axons.

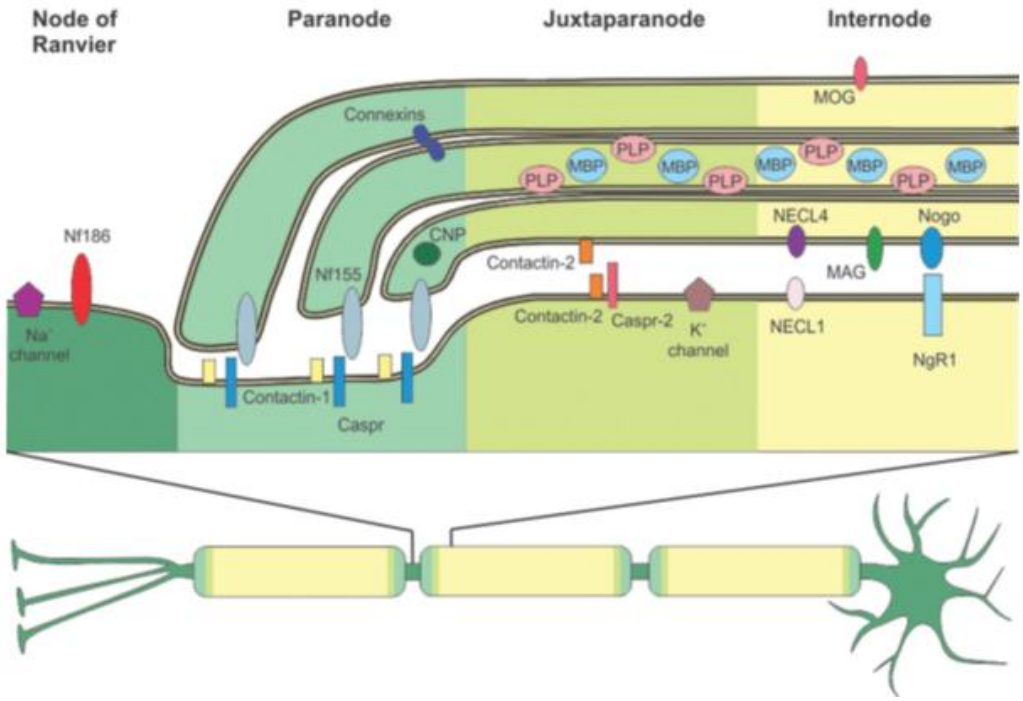


Figure 1.1.2.1b: Myelin protein composition in the CNS*. Myelin wraps around the axons at internodes, leaving small gaps, nodes of Ranvier, between each segment. Adjacent to them are the paranode and the juxtaparanode. Each of the intervals has a distinctive myelin protein composition. PLP and MBP are found embedded within the internodal myelin, when MOG is expressed on the surface of the sheath. MAG is expressed in the periaxonal space. Saltatory conduction by the nerve is enabled by separation of the sodium and potassium channels into nodes of Ranvier and juxtaparanode, respectively (Mayer and Mein, 2012).*

Structure and composition of CNS myelin

The molecular architecture of the myelin sheath requires the co-ordinated synthesis and assembly of myelin proteins and lipids into specific membrane domains (*Fig. 1.1.2.1b*). Initial biochemical analysis of compact internodal myelin revealed it was enriched in compacted lipids that constitute > 70 % of the dry weight (Dupree et al., 1998) and had a relatively simple protein composition; myelin basic protein (MBP) and proteolipid protein (PLP) accounting for > 80 % of its protein content (*Table 1.1.2.1;* Baumann and Pham-Dinh, 2001). However, it is now recognised CNS myelin also contains a several hundred quantitatively minor proteins (Ishii et al., 2009). Their functional significance is in general poorly understood but an increasing number are implicated as candidate autoantigens in MS including 2',3'-Cyclic-nucleotide 3'-phosphodiesterase, myelin-associated glycoprotein (MAG), myelin oligodendrocyte protein (MOG), neurofascin-155 (Nfasc155) and many others (Brunner et al., 1989; Burgoon,

Gilden and Owens, 2004; Nave and Trapp, 2008; Nave, 2010; Dutta and Trapp, 2011).

The function of MBP and PLP is to maintain the structural integrity of compact internodal myelin (Simons and Trajkovic, 2006). MBP associates with the cytoplasmic face of the membrane where it acts as 'glue' holding opposing membrane surfaces in close apposition (Readhead et al., 1987; Fitzner et al., 2006). To avoid this occurring within the oligodendrocyte cell body or its processes, mRNA transcripts encoding MBP are only translated after they are transported to sites of myelin assembly (Colman et al., 1982; Ainger et al., 1993; de Vries et al., 1997; Müller et al., 2013). In contrast, PLP is translated in the oligodendrocyte cell body and then transported to the myelin sheath via vesicular transport (de Vries et al., 1997; Bradl and Lassmann, 2010). mRNA transcripts of minor protein MOG (Quarles, 1997; Kuhle et al., 2007; Lee and Linker, 2012) are restricted to oligodendrocytes (Pham-Dinh et al., 1993; Iglesias et al., 2001). MOG N-terminal domain, the immunoglobulin-like domain, is expressed on the outermost lamellae of myelin sheath, therefore making the antigen accessible for antibodies (Brunner et al., 1989; Brehm et al., 1999). Although compact internodal membranes make up the bulk of the myelin sheath, its primary functions (supporting saltatory conduction and providing metabolic support to the axon) are dependent on specialised axo-glial junctional complexes that anchor the ends of the myelin sheath to the axonal surface (*Fig. 1.1.2.1b*). Formation of these junctional complexes is dependent on expression of Nfasc155 by oligodendrocytes which then interacts with Caspr/contactin complexes on the axonal surface (Tait et al., 2000; Bhat et al., 2001; Boyle et al., 2001; Charles et al., 2002; Sherman et al., 2005; Bonnon et al., 2007). In the absence of Nfasc155 protein mice fail to form electron dense paranodal junctions and develop a paranodal pathology characterised by frequent paranodal loop eversion (Sherman et al., 2005). Intriguingly a similar pathology is developed in mice lacking either ceramide galactosyl transferase (CGT) or ceramide sulfotransferase (CST) (Jarjour et al., 2008), enzymes that are required for the synthesis of galactosylceramide and sulphatide, respectively. These glycosphingolipids are highly enriched in CNS myelin and together account for approximately 25% of the membranes lipid content (*Table 1.1.2.1*; Markus and Popko, 2002; Quarles et al., 2006). They clearly play a major role in

stabilizing the paranodal domain of myelin sheaths, but how this is achieved is unclear. This may involve lipid protein interactions that facilitate segregation of proteins such as Nfasc155 into discreet membrane rafts or domains, an effect that may also impact on signal transduction in oligodendrocytes (Jackman et al., 2009). This is suggested by studies demonstrating antibodies that cross link myelin glycolipids in the plane of the membrane can trigger an influx of calcium leading to microtubule depolymerisation (Dyer and Benjamins, 1991; Ilyas et al., 2003); an effect that may contribute to the ability of sulphatide –specific antibodies to inhibit oligodendrocyte differentiation *in vitro* (Bansal et al., 1989; Kirschning et al., 1995; Ilyas et al., 2003).

Table 1.1.2.1: Composition of central nervous system myelin. *(*percent total lipid adapted from Quarles et al., 2006; ** percent of total protein by weight adapted from Baumann and Pham-Dinh, 2001).*

	Human	Rat
****Lipid***	***70***	***70.5***
Cholesterol	27.7	27.3
Galactolipids	27.5	31.5
Minor galactolipids		
Cerebrosides	22.7	23.7
Sulphatides	3.8	7.1
Phospholipids	43.1	44.0
Ethanolamine phosphatides	15.6	16.7
Serine phosphatides	4.8	7.0
Phosphatidylinositol	0.6	1.2
Lecithin	11.2	11.3
Sphingomyelin	7.9	3.2
Plasmalogens	12.3	14.1

*****Proteins***	
Myelin basic protein (MBP)	30
Proteolipid protein (PLP)	50
Myelin associated glycoprotein (MAG)	1
Myelin oligodendrocyte protein (MOG)	> 0.1
2'-3'-cyclic nucleotide 3'- phosphohydrolase (CNP)	4

1.1.2.2 Astrocytes

The astrocyte is the most numerous of mammalian glial cells and may outnumber neurons ten to one (Benvenieste, 1992). They exhibit a range of morphologies but are typically stellate and are identified using antibodies specific for glial fibrillary acidic protein (GFAP) or excitatory amino acid transporter 1 (EAAT1 or

GLAST) (Barnett and Linington, 2012). Astrocytes are functionally heterogeneous (Oberheim, Goldman and Nedergaard, 2012) and in the normal CNS contribute to the maintenance of CNS homeostasis by providing metabolic support to oligodendrocytes and neurons, removing glutamate from the extracellular milieu and maintaining blood brain barrier (BBB) function (Barnett and Linington, 2012; Barros, 2013; Lundgaard et al., 2013). Astrocytes respond rapidly to CNS injury or infection, undergoing astrocytosis and shifting their phenotype to become more 'reactive' (Sofroniew, 2005; Zamanian et al., 2012). Astrocyte reactivity is a broad definition and recent studies emphasise this represents a spectrum of phenotypes rather than a single entity; the astrocyte response to injury is dependent on the nature of the insult and in addition, it will change over time (Zamanian et al., 2012). Nonetheless, this is generally equated with proliferation, secretion of pro-inflammatory cytokines and ultimately formation of glial scar tissue. The latter plays an important role in wound healing as it forms a barrier that prevents inflammatory cells invading the CNS from the periphery. However, this scar formation also provides an obstacle for axonal regeneration and myelination (Fawcett and Asher, 1999; Sofroniew, 2005). Another damaging effect by reactive astrocytes on neural cells can be caused by their cytotoxic potential to generate nitric oxide radicals (Sofroniew, 2005). It has also been suggested the spectrum of astrocyte reactivity includes an intermediate phenotype that secrete factors which support remyelination and promote neurite survival (Liberto et al., 2004; Nash et al., 2011; 2013).

1.1.2.3 Microglia

Ten to twenty percent of cells in the brain and spinal cord are microglia (Lawson et al., 1990; Langmann, 2007), a resident population of mononuclear phagocytes derived from primitive myeloid progenitors that populate the CNS before embryonic day 8 (Ginhoux et al., 2010). During CNS development microglia are involved in clearance of apoptotic cells and prune inappropriate neural connections (David and Kroner, 2011). However, in the adult they not only support CNS homeostasis, but respond rapidly to molecular signals associated with infections, hypoxia, and neurodegeneration (Kulkarni et al., 2004; Nimmerjahn et al., 2005; Mirshafiey and Kianiaslani, 2013; David and Kroner, 2011). Microglia sense the micro-environmental changes via various surface

receptors, thus leading to severity-dependent increase in microglial numbers (Nimmerjahn, Kirchhoff and Helmchen, 2005).

These responses are similar to those seen in tissue specific macrophages, but it is now apparent microglia are a specialised population of cells with a distinct transcriptional profile (Hickman et al., 2013). This recent transcriptional study also revealed that even 'quiescent' microglia express high levels of anti-microbial peptides indicating they are primed to initiate innate host defence programs (Hickman et al., 2013). In advanced stages of neuronal diseases, microglia have been shown to function in a neurotoxic manner (Muzio et al., 2007), thus causing induced functional and structural damage to the neurons.

1.2 Demyelinating diseases

Demyelinating diseases of the CNS compromise a broad spectrum of disorders in which major functional deficits are caused by loss of myelin, an effect that compromises the function and structural integrity of myelinated axons. The most prevalent are acquired inflammatory demyelinating diseases such as multiple sclerosis (MS), acute disseminated encephalomyelitis (ADEM) and neuromyelitis optica (NMO) (Reindl et al., 2013). Myelin is also a prominent target in the leukodystrophies, a group of genetic disorders that result in varying degrees of de- and dysmyelination (Poser, 1961; Chu et al., 1983; Patay, 2005), and is implicated in several important psychiatric disorders including schizophrenia (Davies et al., 2003; Takahashi et al., 2011).

The aetiology of inflammatory demyelinating diseases remains poorly understood but in general they are considered complex traits in which an environmental factor triggers disease in genetically susceptible individuals, subsequent disease activity being driven by the development of autoimmune responses to CNS antigens. This is believed to be the case in ADEM, a monophasic disorder that is seen primarily in children (Van Haren et al., 2013). However although ADEM is often preceded by some prior, often apparently trivial infection, no specific pathogen has been unambiguously associated with disease induction (Lee and Linker, 2012). Nonetheless ADEM is associated with autoimmune response as demonstrated by the presence of antibodies to CNS antigens (Van Haren et al.,

2013), in particular autoantibodies to MOG that can recognise epitopes exposed at the membrane surface and may be present in up to 40 % of cases (McLaughlin et al., 2009; Di Pauli et al., 2011; Probstel et al., 2011). Similar autoimmune responses have been recognised in the other acquired CNS inflammatory demyelinating diseases of the spectrum, however, differences appear in age of the onset, clinical course, disease severity, treatment, and radiological, pathological and cerebrospinal features (Reindl et al., 2013).

1.2.1 Neuromyelitis optica

Neuromyelitis optica (NMO), previously known as Devic's disease, is a severe recurrent or progressive CNS inflammatory disease characterised by the preferential involvement of the optic tract and spinal cord (Lucchinetti et al., 2002; Lalive, 2008; Jarius et al, 2014). NMO was previously considered a variant of MS, but it is now known to be a distinct disease entity characterised by the presence of autoantibodies that target aquaporin-4 (AQP4) expressed preferentially at astrocyte foot processes at the blood brain barrier (Lennon et al., 2005; Reindl et al., 2013). However, the reason why patients develop a pathogenic autoanibody response to AQP4, the precise role of the antigen in lesion formation, and the mechanisms leading to demyelination and loss of function remain unknown (Howe et al., 2014). The frequency of anti-AQP4 antibodies is comparable between children and adults with NMO (Rostasy and Reindl, 2013); however adults with NMO develop severe disabilities more rapidly (Rostasy et al., 2013). The prognosis for adult patients with NMO is abominable as over 30 % die within 5 years (Huppke et al., 2010). Race and ethnicity have also indicated predisposal for the disease phenotype; NMO being most frequent in black, Asian and Indian population, whereas white population is rarely affected (Huppke et al., 2010). Clinical disease is associated with a range of pathologies but the primary insult is astrocyte injury and loss mediated by AQP4-specific autoantibodies. However, a significant number of patients with NMO as defined by clinical and magnetic resonance imaging (MRI) criteria are seronegative for AQP4 specific autoantibodies (Lennon et al., 2004; Lalive et al., 2006). The numbers reported are dependent on the assay used to detect the AQP4-specific response and estimates of the frequency of seronegative patients range from 5 to 40 % (Mader et al., 2011). These observations have led to

speculation of additional autoantigenic targets being involved in NMO, possibly as a consequence of epitope/antigen spreading (Lalive et al., 2006). MOG is one possible target as potentially demyelinating MOG-specific IgG_1 autoantibodies are present in some AQP4-IgG negative patients (Mader et al., 2011; Rostasy et al., 2013). However, while MOG-specific antibodies may well exacerbate demyelination, they cannot account for the astrocyte pathologies that are characteristic of NMO. Indeed it remains unclear whether NMO is a purely antibody-mediated disease, as several studies demonstrate circulating AQP4-specific antibodies have to cross the BBB in order to induce a pathological effect within the CNS (Bradl and Lassmann, 2013). This has raised speculation that T cell responses to AQP4 play a central role in the pathogenesis of NMO by providing T cell help for the AQP4-specific B cell response, while at the same time inducing an inflammatory response in the CNS; the latter leading to BBB disruption, penetration of AQP4-specific antibodies into the CNS, tissue damage and ultimately clinical disease (Saadoun et al., 2011). Evidence in support of this 'two hit' model for NMO is provided by reports that AQP4-specific $CD4^+$ T cells are encephalitogenic in mice (Nelson et al., 2010) and a similar, potentially encephalitogenic T cell response is present in patients (Varrin-Doyer et al., 2012).

1.2.2 Multiple sclerosis

Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of the CNS. It affects more than two million people worldwide (National MS Society, 2014; WHO, 2014) and is the major cause of acquired, non-traumatic chronic neurological disability among young adults in Western Europe and North America (WHO, 2014). MS is commonly diagnosed between the ages of 20 and 40, but may occur in all age groups. Some 5 % of cases develop their first clinical signs of disease before the age of 16 (paediatric MS; McLaughlin et al., 2009), whilst approximately 10 % of cases are diagnosed at ages > 50 (late onset MS; Martinelli et al., 2004).

MS is clinically heterogeneous (Lucchinetti et al., 2000; Barnett and Prineas, 2004) and at present no prognostic markers are available to either the subsequent course of disease, or to stratify patients for treatment. The most

common presentation is relapsing-remitting MS (RRMS) which is seen in approximately 80 % of cases (WHO, 2014) and is characterized by repeated, unpredictable episodes of disability followed by periods of complete or partial recovery. However, within five to fifteen years a majority of these patients will develop secondary progressive MS (SPMS; Steinman, 2001; Trapp et al., 1998; Lalive, 2008; Hafler, 2004) in which accumulation of disability continues in the absence of obvious clinical relapses or remissions; a disease course seen from onset in the 10 – 20 % of MS patients who present with primary progressive MS (PPMS; WHO, 2014).

This distinction between RRMS and progressive forms of the disease is important as it is associated with marked differences in the clinical efficacy of current treatments for MS. Disease activity in RRMS is due to repeated episodes of inflammatory demyelination associated with varying amounts of axonal injury and loss (Hafler, 2004; Pender et al., 2004). Symptoms often present sub-acutely, developing over a period of several days and then persist for varying lengths of time after which they dissipate resulting in partial or complete clinical recovery (Hafler, 2004). Historically RRMS was considered a white matter disease, but improvements in immunopathological and MRI techniques show that grey matter lesions do frequently occur in RRMS (Trapp et al., 1998; Kuhlmann et al., 2002; Frischer et al., 2009; Lassmann, 2013). Moreover in both cases demyelination and axonal injury are associated with perivascular infiltrates of macrophages, T cells and B cells (Lee and Linker, 2012; Bar-Or, 2005; Mirshafiey and Kianiaslani et al., 2013) that disperse and migrate into the surrounding parenchyma (Lucchineti et al., 2011; Lassmann, 2013). These focal inflammatory lesions are visualized as discrete areas of Gadolinium enhancement in magnetic resonance imaging (MRI) scans, a technique now used extensively to monitor disease activity in the CNS during clinical trials. The clinical importance of these gadolinium enhancing lesions in RRMS is apparent from the outcome of clinical trials of ‘anti-inflammatory’ disease modifying treatments (DMT) such as the beta interferons, Glatiramer acetate (Copaxone®), Natalizumab (Tysabri), Fingolimod (FTY720/Gilenya®) and Alemtuzumab (Lemtrada®) (Jacobs et al., 1996; Johnson et al., 2000; Polman et al., 2006; Hauser et al., 2008; Kappos et al., 2010; Cohen et al., 2010; Derwenskus, 2011). These treatments delay disability formation and suppress the development of gadolinium enhancing

lesions in the CNS of patients with RRMS, an effect associated with significant reductions in relapse frequency. However, they fail to halt accumulation of disability in patients with progressive MS (PMS).

In hindsight this is perhaps not unexpected as the inflammatory foci seen in the CNS of patients with RRMS are generally absent in PPMS and SPMS (Confareux et al., 2000; Gold, Linington and Lassmann, 2006; Zwemmer et al., 2008). This observation provides a rational explanation why patients with PMS fail to benefit from current disease modifying therapies (SPECTRIMS Study Group, 2001; Cohen et al., 2002), and supports an emerging consensus that accumulation of disease in PMS is driven by effector mechanism(s) sequestered within the CNS that are largely independent of input from the immune system (Fischer et al., 2009). This concept is supported by data derived from clinical trials, pathological investigations and clinical imaging studies that demonstrate accumulation of disability in PMS is due to axonal loss (Confareux et al., 2000; Bjartmar and Trapp, 2001; Trapp et al., 1998) associated with widespread microglial activation and an inflammatory response sequestered within the meninges. These meningeal infiltrates are often enriched in B cells and are associated with demyelination, axonal loss and microglial activation in the underlying parenchyma (Magliozzi et al., 2007; Magliozzi et al., 2010; Lassmann, 2013). Meningeal inflammation is clearly clinically significant as it correlates with a more rapid accumulation of disability (Serafini et al., 2004; Magliozzi et al., 2007; Choi et al., 2012), but the causal relationship between this sequestered inflammatory response and tissue damage in the parenchyma remains unclear. In addition to meningeal inflammation small numbers of infiltrating T and B cells are also found in the parenchyma where they are often associated with the active rim of slowly expanding white matter lesions in patients with progressive disease (Kutzelnigg et al., 2005; Lassmann, 2013). Remyelination is also less frequent in PMS (Kuhlmann et al., 2008; Podbielska et al., 2013) partly due to a deficit in oligodendrocyte recruitment, an effect predicted to exacerbate the development of chronically demyelinated plaques of glial scar tissue, the end stage of MS lesion development (Prineas et al., 2001; Lassmann, 2013).

The ultimate cause of chronic disability in MS is due to axonal injury and loss (Trapp et al., 1998; Mirshafiey and Kianiaslani, 2013). Axonal injury is most

prominent in acute inflammatory demyelinating lesions in patients with RRMS, but this continues after the acute inflammatory response resolves and may ultimately result in the loss > 80 % of axons in individual lesions (Kuhlmann et al., 2002). This axonal pathology is attributed to an axonal energy deficit caused by the effects of oxidative damage on mitochondrial metabolism (Lu et al., 2000; Dutta et al., 2006; van Horssen et al., 2008; Handel et al., 2011; Lee and Linker, 2012; Davies et al., 2013) in a hypoxic environment. Energy deficiency impairs the functional activity of axonal Na^+/K^+-ATPase resulting in increased intra-cellular sodium concentrations that reverse the axonal Na^+/Ca^{2+} exchanger. This leads to increased axonal calcium concentrations, an effect that is amplified by the simultaneous failure of energy-dependent axonal Ca^{2+} efflux pumps, therefore resulting in membrane depolarisation and conduction block of the axons (Smith, 2007). Ultimately this compromises the functional and structural integrity of the axon (Stys, 2004; Kurnellas et al., 2005; Nave and Trapp, 2008). This axonal pathology, however, does not occur in isolation, but is associated intimately with the demyelinating component of the disease. Not only are myelinating oligodendrocytes themselves highly susceptible to oxidative damage (Benarroch, 2009; McTigue and Tripathi, 2008), but demyelination itself also increases axonal susceptibility to oxidative stress due to their increased energetic demand. In response, conduction properties are partially repaired by increasing expression of $Na_v1.2$ channels along demyelinated axonal segments (Craner et al., 2003; Smith, 2007). However, in addition to oxidative effects mediated by free radicals (Smith et al., 2001), axonal survival is compromised by a variety of other mechanisms including proteolytic enzymes, cytokines, autoantibodies and Wallerian degeneration (Hohlfeld, 1997; Trapp et al., 1998; Bjartmar and Trapp, 2001; Mathey et al., 2007; Huizinga et al., 2008; Bradl and Lassmann, 2010; Haider et al., 2011; Elliot et al., 2012; Davies et al., 2013). Convincing arguments can be made about each of these mechanisms in disease pathogenesis, but their relative importance in patients with progressive disease is unknown. This highlights the major challenge faced in the field of MS research at present. Namely, there is an urgent need to understand how these pathological responses are maintained in the CNS of patients with progressive forms of MS. Thus, allowing development of effective treatments for the devastating disease.

1.2.2.1 Mechanisms associated with tissue damage in multiple sclerosis

MS is a human-specific disease that does not occur in any other species. Therefore, researchers have been forced to use pathological investigations that are largely based on autopsy tissue from patients with longstanding disease, and animal models that replicate some but not all aspects of the disease. These models include viral diseases; however, the majority of studies are based on experimental autoimmune encephalomyelitis (EAE). It is a T cell mediated autoimmune disease induced in susceptible species by sensitisation with CNS tissue homogenates, CNS myelin or purified myelin antigens such as MBP, PLP and MOG, or adoptive transfer of myelin antigen-specific T cells (Gold et al., 2006). EAE has had a major impact on our understanding of neuroinflammatory disease processes and was instrumental in the development of Glatiramer acetate (Copaxone) (Arnon et al., 1996) and Natalizumab (Tysabri) (Yednock et al., 1992) as a treatment for RRMS. Moreover, the model led to recognitions that formation of large 'MS-like' lesions in experimental animals requires contributions from both T- and B-cell dependent effector mechanisms. In this 'two-hit' model of lesion formation, BBB dysfunction and inflammation are initiated by reactivation of MHC class II restricted Th1 and Th17 T cells within the CNS (Minager and Alexander, 2003; Frohman et al., 2006; Kebir et al., 2007; Bettelli et al., 2007; Nelson et al., 2010). This inflammatory response is alone sufficient to induce severe neurological deficits, but in rodents and primates does not induce widespread demyelination (Lucchinetti et al., 1996; Raine et al., 1999; Lee and Linker, 2012). In these species extensive loss of myelin is only observed if autoantibodies that can bind to the myelin surface are also available to initiate antibody-dependent complement and/or cell mediated demyelination (Piddlesden et al., 1993; Elliot et al., 2012). These pathogenic autoimmune responses can target a variety of CNS autoantigens, but the demyelinating autoantibody response is restricted to a limited number of protein and lipid antigens (MOG, PLP, galactosyl ceramide and sulphatide) exposed at the myelin/oligodendrocyte surface (Linington et al., 1988; Fierz et al., 1988; Menon, Piddlesden, Bernard, 1997; Brehm et al., 1999; Rosenbluth et al., 1999; 2003; Morris-Downes et al, 2002; Kanter et al., 2006). However, this myelin damage may expose epitopes that provide further targets for antibody and/or T cell autoaggression (Matsuo et al., 1997) and ultimately extend to target myelin (e.g. MBP, PLP, MOG, MOBP) and non-myelin neural antigens (Fontana, Fierz and

Wekerle, 1984; Fierz et al., 1985; Sun and Wekerle, 1986), perpetuating disease activity in the CNS.

These observations lead to a consensus developing that views MS as an (auto)immune disease (Steinman, 1996; Lalive et al., 2006; Hundgeburth et al., 2013); a concept now supported by the clinical efficacy of anti-inflammatory disease modifying therapies (DMT) such as Natalizumab, Fingolimod (FTY720/Gilenya®) and Alemtuzumab (Lemtrada®), as well as the outcome of recent genome-wide association studies (GWAS) that implicate a primary role for T cell-mediated immunity in MS (Sawcer et al., 2011). However, there is still considerable debate as to the relative importance of different T and B cell subsets, their antigen-specificity and crucially whether or not autoimmunity plays a direct role in driving accumulation of disability in progressive forms of the disease (Bourquin et al., 2000). Until recently these questions focused solely on the role of the T cell division of the immune response, but it is now recognised that B cell dependent mechanisms also play an important if not crucial role in disease pathogenesis.

1.2.2.2 B-cell involvement in multiple sclerosis

A role for B-cells in MS was first discussed some 50 years ago following the demonstration MS was associated with intrathecal synthesis of immunoglobulin (Ig) (Kabat, Moore and Landow, 1942). The most obvious manifestation of this response is the presence of oligoclonal bands (OCB) of Ig in cerebrospinal fluid (CSF) which can be visualised by isoelectric focussing gels (Obermeier et al., 2008). This further enforced the implication of B cell dependent mechanism in MS, however, it was not until the last decade when clinical trials of B cell depletion in MS patients provided formal evidence of B cell activity in the disease development (Hauser et al., 2008; Kappos et al., 2011; *Table 1.2.2.2)*.

Table 1.2.2.2: Evidence for B cell dependent mechanisms in multiple sclerosis. *(Adapted from Mein et al., 2006)*

Evidence	Reference
Intrathecal immunoglobulin (Ig) production associated with a poly-specific antibody response and oligoclonal bands	Gilden, 2005; Walsh and Tourtellotte, 1986; Thompson, Kaufmann and Rudge, 1983; Sindic, Monteyne and Laterre, 1994; Derfuss et al., 2001; Derfuss et al., 2005; Reiber et al., 1998; Puccioni-Sohler et al., 1995; Bednarova, Stourac and Adam, 2005;
Clonal expansion and hypersomatic mutation of B cells sequestered within the CNS indicative of local antigen-driven affinity maturation	Qin et al., 1998; Owens et al., 1998; Baranzini et al., 1999; Smith-Jensen et al., 2000; Colombo et al., 2000; Colombo et al., 2003; Owens et al., 2003; Ritchie et al., 2004; Haubold et al., 2004; Corcione et al., 2004
Disease activity/progression correlate with B cell/plasmablast numbers in CSF and intrathecal IgM synthesis	Cepok et al., 2001; Cepok et al., 2005;
Decoration of myelin sheaths by antibodies and complement activation products	Warren, Catz and Steinman, 1995; O'Connor et al., 2005; Genain et al., 1999; Storch et al., 1998
A subset of patients benefit from plasma exchange	Keegan et al., 2005;
Clinical benefit of plasma exchange correlates with C9neo immune reactivity in lesions	Storch et al., 1998;
Demyelinating and axopathic autoantibodies identified in patient sera	Villar et al., 2003; Villar et al., 2005;
B-cell depletion by CD20-specific antibodies dramatically reduces disease activity	Stüve et al., 2005;

B cells develop from a common lymphoid progenitor in bone marrow after which immature B cells migrate to secondary lymphoid organs where they can differentiate into antibody secreting cells (*Fig. 1.2.2.2*). Members of the B-cell lineage normally comprise 10-25 % of circulating lymphoid cells (Corcione et al., 2005; Vyshika and Kalman, 2008) and can perform a wide variety of immune functions, any of which may contribute to their pathogenic activity in MS. This is not restricted to their ability to differentiate into antibody-secreting cells (ASC; plasma cells and/or plasmablasts) that may secrete demyelinating or axopathic autoantibodies (Elliott et al., 2012), but also includes their function as highly efficient antigen-presenting cells (APCs) (Lanzavecchia, 1985; Bar-Or et al., 2001;

Harp et al., 2010) and their immune regulatory properties (Mauri and Bosma, 2012; Yang et al., 2013). This diversity of B cell effector functions suggests involvement of different disease developmental mechanisms, which each may provide a novel therapeutic target.

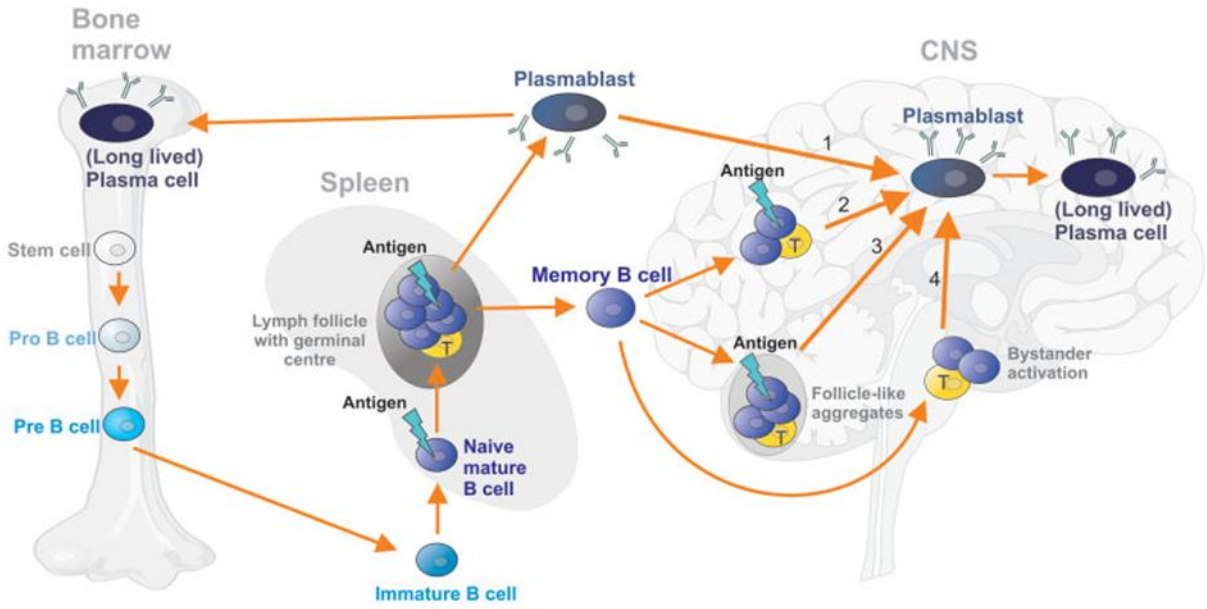


***Figure 1.2.2.2: Possible pathways in immunoglobulin production in the CNS**. B cell development starts in the bone marrow after which they travel to secondary lymphatic organs where antigen-driven germinal centre differentiation into memory B-cells and plasmablasts occurs. 1) Plasmablasts can either enter back into the bone marrow or the inflamed CNS. 2) Memory B cells after their entry to the CNS can differentiate into antibody-secreting cells in response to antigen outside of follicles, or 3) in response to follicle-like aggregates in the meninges. 4) Bystander reaction can cause the memory B cells to differentiate into plasmablasts (Meinl et al., 2006).*

1.2.2.3 Intrathecal antibody synthesis

Intrathecal Ig synthesis occurs in at least 90 % of patients with MS (Stangel et al., 2013), which is recognised with OCBs identification in CSF. This method is no longer mandatory for a diagnosis of MS if MRI criteria are fulfilled; however it is still widely used as it eliminates possibility of other inflammatory diseases. Synthesis of Ig within the CNS compartment in MS is substantial and may exceed 200 mg/day, although the average is nearer to 30 mg/day (Tourtellotte and Ma, 1978). The OCB patterns identified by isoelectric focusing patterns are patient-specific and persist for many years, although some variations will occur over time (Walsh and Tourtellotte, 1986; Colombo e al., 2003; Di Pauli et al., 2011;

Probstel et al., 2011; Kitley et al., 2012). As each OCB represents the product of a clonally expanded B cell population formed in the CNS (Obermeier et al., 2008) these observations indicate the CNS provides a long term survival niche for differentiating B cells and/or terminally differentiated plasma cells. These exhibit patterns of hypersomatic mutation indicative of antigen-specific maturation, presumably within the CNS compartment itself (Qin et al. 1998; Owens et al. 1998; Baranzini et al. 1999; Colombo et al. 2000; Owens et al. 2003; Monson et al., 2005). Mapping the specificity profile of this intrathecal antibody response with the help of antigen arrays has revealed it can recognise a large number of different lipid and protein autoantigens (Sellebjerg, Christiansen and Garred, 1998; Brennan et al., 2011; Quintana et al., 2012), as well as antigens derived from microbial pathogens (Yao et al., 2001; Derfuss et al., 2001). The latter includes a poly-specific immunoglobulin response that recognises measles, rubella and varicella-zoster viruses (MRZ reaction). This is generally found in patients diagnosed with MS but is absent in other neurological diseases (Luxton et al., 1995; Reiber et al. 1998; Jarius et al., 2006; Jarius et al., 2008). Altogether these studies demonstrate that a significant proportion of the intrathecal antibody responses associated with MS is directed against myelin-derived lipids, in particular sulphatide and its precursor galactosylceramide (Ryberg, 1978; 1980; Kasai et al., 1986; Ichioka et al. 1988; Kirschning et al., 1995; Kolodny et al., 1995; Ilyas et al., 2003; Kanter et al., 2006; Brennan et al., 2011). These glycosphingolipids are both exposed at the outermost surface of the myelin sheath and oligodendrocyte plasma membrane where they can provide targets for antibody-mediated demyelination in vivo (Fierz et al., 1988; Rosenbluth et al., 1999; 2003; Morris-Downes et al, 2002; Kanter et al., 2006).

Researchers may not have been able to the confirm antigen specificity of individual OCB, but clinical studies have established intrathecal antibody synthesis is associated with a poorer prognosis in MS. Specifically, OCB and/or elevated intrathecal Ig synthesis in patients with clinically isolated syndromes such as optic neuritis correlate with more rapid conversion to clinically definite MS, or in patients with relapsing remitting disease more rapid progression to SPMS (Villar et al., 2002; Nilsson et al., 2005; Tintore et al. 2008).

1.2.2.4 Evidence from MS lesions

Involvement of antibody-dependent mechanisms in the pathogenesis of demyelination has been suggested by reports identifying deposition of immunoglobulins and complement activation products in MS lesions (Compston et al., 1989; Genain etal., 1999; Lucchinetti et al. 2000; Storch et al., 1998). Lucchinetti et al., (2000) described four different patterns of demyelination in cortical MS lesion biopsies defined on the basis of immunopathological criteria. The most common (>50% of cases) were Type II lesions in which demyelination was associated a pattern of complement activation, immunoglobulin deposition and macrophage responses virtually identical to those induced by demyelinating antibodies in animals with EAE. Observations that lead the authors to speculate development of these lesions involved an autoantibody-response directed against the myelin surface. This concept is supported by demonstration of myelin sheaths being decorated at the leading edges of the lesion with immunoglobulin and c9neo depositions (Storch et al., 1998), myelin-specific autoantibodies associated with vesicular myelin debris (Raine et al., 1999; Genain et al., 1999), phagocytosis of these opsonized debris by activated macrophages (Prineas, 1985; Storch et al., 1998), and more recently the identification of demyelinating IgG autoantibodies in patient serum (Haase et al., 2001; Elliott et al., 2012). Nonetheless other groups have questioned the significance of these observations as similar patterns of complement deposition are seen in other, unrelated neurological disorders (Barnett and Prineas, 2004).

It is generally assumed that if autoantibodies are involved in the pathogenesis of MS these will recognise a small number of myelin-associated antigens, but the specificity of this pathogenic autoantibody response remains elusive. A large number of potential candidates have been identified over the years, but with the exception of MOG, none were validated as relevant targets for MS. The most recent was the Kir4.1 potassium channel, which despite exciting initial studies could not be proven to provide a relevant target from pathogenic autoantibodies in MS (Brickshawana et al., 2014). Interest in MOG developed from studies demonstrating it as an important target for demyelinating autoantibodies in EAE (Lebar et al., 1986, Lebar, Baudrimont and Vincent, 1989; Linington et al., 1986, 1988; Schluessner et al., 1987; Genain et al., 1995). Subsequently, detection of MOG-specific antibodies in patient sera (Kerlero et al., 1993; Diaz-Villoslada et

al., 1999; Lindert et al., 1999) and MS lesions (Raine et al., 1999; O'Connor et al., 2005) indicated their role in pathogenesis of MS. The claim that presence of MOG-specific autoantibodies correlated with a poor clinical prognosis (Berger et al., 2003) generated considerable controversy largely because at the time current immunoassay techniques were unable to distinguish between pathologically relevant and irrelevant antibodies (Kuhle et al., 2007). This, however, was resolved following development of cell based assays that specifically identify antibodies recognising the native extracellular domain of MOG expressed at the membrane surface; a pre-requisite if these antibodies are to mediate demyelination in vivo (Brehm et al., 1999; Lalive et al., 2006). Following the introduction of this methodology, potentially pathogenic MOG-specific antibodies were detected in less than 5 % of patients with classical adult onset disease, but were noted to occur at much higher frequencies in paediatric MS (approximately 20 %) and ADEM (approximately 40 %) (McLaughlin et al., 2009; Brilot et al., 2009; Selter et al., 2010; Probstel et al., 2011). These MOG-specific autoantibodies are predominantly of the complement fixing IgG_1 subclass and recognise surface epitopes of the native extracellular domain of MOG (O'Connor et al., 2010); characteristics that support the view they will exacerbate demyelination in seropositive patients. However, clinical significance of the antibody response remains unproven and its correlation with disease severity is still debated (Probstel et al., 2011).

1.2.2.5 Clinical evidence for antibody-dependent mechanisms in MS

Therapeutic plasma exchange is used to treat a variety of autoantibody mediated diseases including myasthenia gravis, systemic lupus erythematosus and Guillain-Barre syndrome, and is now recommended for patients with MS who develop acute relapses that fail to respond to steroids (Valbonesi et al., 1981; Weiner et al., 1989; Rodriquez et al., 1993; Bennetto et al., 2004; AAN Guideline, 2014). This was prompted by a review of previous studies suggesting plasma exchange may promote functional recovery in some patients (Weiner et al., 1989; Weinshenker et al., 1999; Keegan et al., 2002; Keegan et al., 2005; Schilling et al., 2006). These clinical observations are generally interpreted as an indication of autoantibody involvement in disease development. A retrospective analysis of patients who underwent a diagnostic brain biopsy revealed a

beneficial treatment to plasma exchange response correlated with lesional C9neo immunoreactivity (Keegan et al., 2005); a defining feature associated with antibody mediated tissue damage in the CNS (Lucchinetti et al., 2000). Subsequently, it was demonstrated that in patients with severe steroid non-responsive relapses, demyelinating IgG responses were significantly reduced following a plasma exchange treatment (Elliot et al., 2012). These observations provide persuasive evidence that autoantibody dependent mechanisms can be involved in MS; however, there is still considerable debate as to their clinical significance in the majority of patients. These concerns stem from two observations. First, plasma exchange is ineffective in most patients with MS; efficacy has only been noted in small number of patients with RRMS course (Keegan et al., 2002; 2005). Second, B cell depletion in RRMS reduces disease activity in the CNS, before any global effect on circulating Ig levels, which was first demonstrated in a clinical trial of Rituximab in RRMS (Hauser et al., 2008).

Rituximab is a monoclonal chimeric antibody that selectively depletes CD20+ B cells from the immune system and which was already licensed to treat lymphoma and rheumatoid arthritis (Coiffier et al., 1998; McLaughlin et al., 1998; Edwards et al., 2004). Hauser and his colleague's (2008) demonstrated a single course of Rituximab not only resulted in a rapid reduction in lesion activity in the brain, as demonstrated by MRI, but also reduced the relapse rate. However, this rapid response occurs before any significant reduction of serum Ig concentrations. In hindsight, this is not unexpected as CD20 is not expressed by antibody secreting plasma blasts or plasma cells (*Fig 1.2.2.2;* Cross et al., 2006). It also appears B cell depletion in the periphery has a minimal effect on intrathecal IgG synthesis or OCB patterns (Cross et al., 2006). These observations lead into an important dichotomy; immunopathological studies suggest involvement of antibody-mediated mechanisms in most MS cases when clinical studies provide significance only in a subset of MS patients.

1.3 Aim

To investigate this further we readdressed the prospect that complement independent antibodies targeting the myelin surface may mediate other

responses than demyelination. This is suggested by studies that indicate some naturally occurring IgM antibodies, one of them being sulphatide specific monoclonal O4, enhance oligodendrocyte survival and myelination in Theiler's murine encephalomyelitis virus (TMEV) mediated demyelination (Rodriguez and Lennon, 1990; Miller and Rodriquez, 1995; Asakura et al., 1996; Asakura et al., 1998; Asakura and Rodriguez, 1998; Warrington et al., 2000; Wright et al., 2009). Therefore, the aim of this thesis is to use the *in vitro* model of myelination to investigate the transcriptional changes occurring within the cultures in response to addition of mAb O4 in the cultures.

2 Methods

2.1 Animals

Sprague Dawley (Harlan Laboratories, UK) rats were used in the experiments. Animals were killed using a Schedule 1 method by a trained technician.

2.2 Myelinating culture system

Myelinating culture system was generated by producing a confluent monolayer of astrocytes and plating dissociated embryonic spinal cord directly on top (see below). The myelination was assessed after 18 days by use of immunocytochemistry and fluorescence microscopy. All culture preparation and maintenance was performed in an aseptic fashion, using laminar flow hoods. Only exception was the tissue dissection stage, which was performed on a bench using a microscope.

Myelinating cultures contain all cellular elements present in the CNS, including OPCs, astrocytes, neurons, and microglia. Myelination in these cultures results in large numbers of myelin sheaths associated with $Caspr^{+}$ paranodes and nodes of Ranvier (Elliot et al., 2012; Sorensen et al., 2008; Thomson et al., 2008). They therefore provide a simple model system in which effects on neuronal survival, neurite outgrowth, OPC proliferation and differentiation, and myelination can all be quantified by immunofluorescence microscopy. We quantify myelination determining the percentage of $SMI31^{+}$ axons ensheathed with proteolipid protein or myelin–oligodendrocyte glycoprotein positive myelin sheaths using pattern recognition based algorithms to differentiate myelin sheaths from immunoreactive oligodendrocyte cell bodies and processes.

2.2.1 Isolation and culturing of neurospheres

Neonatal P1 rats were decapitated, their brains were excised and transferred to Leibovitz's-15 medium (L-15; Invitrogen, Paisley, UK) on ice. Sterilised surgical instruments were used to cut the brains mid-sagitally, after which striatum was removed from each hemisphere and placed in 1 ml of L-15 on ice. Tissue was mechanically dissociated and titruated through a glass Pasteur pipette to produce single cell suspension.

The suspension was centrifuged at 800 rpm for 5 min at 4°C, the supernatant was disregarded and the cell pellet was resuspended into 20 ml of neurosphere medium [DMEM/F12 (1:1, DMEM containing 4,500 mg/L glucose), supplemented with 0.105 % $NaHCO_3$, 2 mM glutamine, 5,000 IU/ml penicillin, 5 µg/ml streptomycin, 5.0 mM HEPES, 0.0001 % bovine serum albumin, (all from Invitrogen), 25 µg/ml insulin, 100 µg/ml apotransferrin, 60 µM putrescine, 20 nM progesterone, and 30 nM sodium selenite (all from Sigma)]. The culture was supplemented with 20ng/ml (4µl) mouse submaxillary gland epidermal growth factor (EGF; R&D Systems). After which the cell suspension was placed into a non-coated 75 cm^3 tissue culture flask (Greiner, Gloucestershire, UK) and incubated at 37°C, in humidified atmosphere of 7 % CO_2/93 % air. The cultures were treated every two to three days with addition of 5 ml neurosphere medium and 4µl of 20 ng/ml EGF.

2.2.2 Astrocytes derived from neurospheres

After five to ten days *in vitro*, when the neurospheres had reached a critical size, they were titruated with a Pauster pipette to produce small spheres. The cell clusters were centrifuged at 800 rpm for 5 min at 4°C, and the supernatant was removed. The pellet was resuspended in low glucose (1.0 g/ml glucose) DMEM astrocyte media [supplemented with 10 % foetal bovine serum (FBS) and 2 mM L-glutamine]. Neurospheres were differentiated into astrocytes by plating them onto poly-lysine (PLL; 13 µg/ml, Sigma) -coated coverslips (13 mm diameter, VWR International, Leicestershire, UK) that were prepared by two hour incubation at 37°C, distilled sterile water wash, and overnight dry. 0.5 ml of neurosphere suspension was plated on the coverslips that was then topped up with 0.5 of astrocyte media. The cultures were incubated at 37°C and fed twice a week, until confluent astrocyte monolayer formed after 6-10 days.

2.2.3 Plating of dissociated embryonic spinal cord

Dissociated myelinating cultures were generated as previously described by Elliot et al., (2012). Myelinating cultures were produced from embryonic day (-E)15.5 rats. At E15.5, the pregnant female was euthanized in a carbon dioxide chamber. The abdomen was sterilised by 70 % ethanol. Sterilised scissors were used to

open the abdomen, and the gravid uterus was placed in sterile plastic Petri dish containing Hanks balanced salt solution (HBSS; Gibco, Invitrogen, Paisley) on ice. The foetuses were removed from the individual amniotic sacs and placed in fresh HBSS. They were decapitated just caudal to the attachment of cerebellum, rostral to the cervical flexure. Curved forceps were used to remove the skin and tissue covering the spinal cord, after which the meninges were stripped off to avoid peripheral nerve contamination, and the spinal cord was placed in 1ml of HBSS.

The spinal cords were broken down into smaller pieces using a Pasteur pipette. Enzymatic dissociation of tissue was produced by trypsin (100 µl of 2.5 % trypsin) and collagenase (100 µl of 1.33 % collagenase) in 1 ml HBSS, incubation of 15min. Enzymatic digestion was stopped by adding 2 ml of trypsin inhibitor (SD; 0.52 mg/ml soyabean trypsin inhibitor, 3.0 mg/ml bovine serum albumin and 0.04 mg/ml DNase). Cell mixture was spun down at 800 rpm for 5 min and the cell pellet was titruated with glass Pauster pipette into single cell solution and diluted with plating media (PM; 50 % Dulbecco's modified Eagle medium, 25 % heat inactivated horse serum, 25 % HBSS with Ca^{2+} and Mg^{2+}, and 2mM L-glutamine; Invitrogen) to make up 10 ml. Trypan Blue was used estimate cell number. Optimal density of 3 million cells/ml was generated.

The dissociated spinal cord cell mixture at cell density of 150,000cells/100µl was plated onto three coverslips containing an astrocyte monolayer. The coverslips were placed in a 35 mm Petri dish and the cells left to attach for two hours in the incubator. After which additional 350 µl of PM and 600 µl of insulin containing differentiation medium [DM+; DMEM containing 4,500 mg/L glucose, 10 ng/ml biotin and 0.5 % N1 hormone mixture [1 mg/ml apotransferrin, 20 mM putrescine, 4 mM progesterone, 6 µM selenium], 50 nM hydrocortisone and 0.5 mg/ml insulin (Sigma)] was added. Cultures were maintained for up to 28 days in a humidified atmosphere of 7 % CO_2/93 % air, at 37°C. Cultures were fed by removing 500 µl of the media and replacing it with 600 µl of fresh DM+ three times a week. At 12 days *in vitro* (DIV), insulin was excluded from the DM.

2.3 Hybridoma production

Frozen hybridomas (Z2 and O4) were thawed quickly under lukewarm water and washed in 20 ml RPMI-1640 media by centrifugation after which the media was removed. The cells were resuspended, placed in fresh media in capped 75 cm^3 tissue culture flask, and incubated at 37°C and 5 % CO_2/95 % air.

2.3.1 Antibody production

Antibodies were produced using Cell mAb Medium in a CELLine Device (BD Biosciences). The nutrient compartment was pre-wet with 25 ml of 100 % BD cell mAb medium. Cells were counted and resuspended in 15ml of the basal medium. The suspension was inoculated into the cell compartment. The nutrient compartment was filled with ~250 ml of mAb medium. Cells were incubated in 5 % CO2/95 % air incubator at 37°C. Every seven days, 13ml of cells in Ab medium were harvested from the cell compartment and centrifuged at 400g for 5min. The antibody-containing supernatant was collected and stored in -20°C. The remaining cell mixture was topped up with Ab medium, and the nutrient compartment was exchanged with fresh medium.

2.3.2 IgG_{2a} antibody purification

The IgG_{2a} supernatant was filtered with 0.45 µm filter to remove any particles. Hi-Trap Protein G Column HP (GE Healthcare Life Sciences) was washed with five bed volumes of 20 mM sodium phosphate buffer. The filtered antibody sample was applied to the column, after which the column was washed again. Then the antibody was eluted with one to three times of the column volume of 0.1 M Glycine into 100 µl of 1M Tris base measured in Eppendorf tubes. The sample concentrations were measured using NanoDrop 1000 Specrtophotometer and antibody containing samples were pooled and dialyzed against PBS. The column was regenerated with 20 % ethanol and stored at 4°C.

2.3.3 IgM antibody purification

The IgM supernatant was normalised to the binding buffer by addition of 0.8 M ammonium sulphate and filtered through 0.45 µm to remove any impurities. HiTrap IgM Purification HP (GE Healthcare Life Sciences) unit was equilibrated with 20 mM sodium phosphate, 0.8 M ammonium sulphate binding buffer. The sample was run through the column, after which the column was washed with binding buffer. The antibody was eluted with 20 mM sodium phosphate. The sample was nanodropped, and filtered through a filter unit (Merck Millipore). The purification column was regenerated by washing step involving regeneration buffer followed by binding buffer, then stored at 4°C.

In both cases (IgG and IgM), the proteins were appropriately concentrated. They were purity tested by SDS-PAGE non-reducing polyacrylamide gel, then stored at -20°C.

2.4 Antibody treatments of myelinating cultures

Treatments were completed in the presence or absence of rabbit serum as a source of complement. Cultures were treated with different antibody containing media at days 18 or 24. Antibodies were diluted into DM- medium at 20 µg/ml and filtered with 20 µm filter to remove any impurities. Cultures were treated by removing 500 µl and adding 600 µl antibody-containing medium. Controls were fed with DM-. Complement dependent experiments were additionally treated with 2 % rabbit serum (Sigma). Treatments were carried on either for 24 hours or ten days, in longer treatments feeding was done every two to three days.

2.5 Immunocytochemistry

To confirm these cultures reproduce the molecular organization and accessibility of auto-antigens present on myelinated axons *in vivo*, they were incubated with antibodies specific for myelin and axoglial antigens.

Staining was performed on various differently treated cultures after sterile incubations at 37°C for 24 hours or 10 days. Cultures were fixed in 50 µl 4 % PFA for 20 min, after which wash with 1xPBS was performed three times and excess was tapped off. The myelinating cultures were permeablised in 40 µl 0.5 % Triton X for 10 min. Coverslips were washed with PBS. Cultures were blocked for minimum of 30 minutes in 50 µl of blocking buffer [BB; 1 x phosphate buffered salin (PBS) containing 1 % bovine serum albumin and 10 % horse serum]. Next, myelinating cultures were blocked in primary antibodies diluted in 40 µl of BB. Wash with PBS was performed, after which blocking in secondary antibody diluted at 1:400 ratio into 40 µl of BB was executed. The myelinating cultures were washed in PBS and distilled water to remove salt traces, and mounted on labelled glass slides with 10 µl of Mowiol with DAPI. Cultures were dried out at 4°C overnight. In all cases where immunostaining was performed for surface exposed antigens live cells were incubated at 4°C with the primary antibody for 30 minutes. Cells were then washed extensively with PBS after which they were fixed and permeabilised as described above.

Images were captured using an Olympus BX51 fluorescent microscope and Image-Pro software. Quantitative analysis of neurite density and myelination was performed on 10 random images from each three coverslips that were taken at 10x magnification. Migroglia images were taken at 20x magnification. Neurite density, myelination and cell counts were quantified using analytical pipelines developed from CellProfiler cell image analysis software (Carpenter et al., 2006). These are available at https://qithub.com/muecs/cp.

Table 2.5: Immunocytochemistry primary and secondary antibodies

Primary antibody	Specificity	Host	Isotype	Dilution	Company	Secondary antibody
O4	Sulphatide	Mouse	IgM	1:500	From supernatant R & D Milteneyi	ms IgM 488
Z2	Myelin oligodendrocyte glycoprotein	Mouse	IgG2a	1:500	Our laboratory	ms IgG2a 488
AA3	Proteolipid Protein	Rat	IgG2a	1:100	Chemicon	rt IgG2a 488
SMI31	Phosphorylated neurofilament	Mouse	IgG1	1:1500	Abcam	ms IgG1 568
Iba1	Microglia	Rabbit	IgG	1:1000	Wako	rb IgG 568
Fab	Fab fragment	Mouse	IgG	1:150	Sigma	FITC

2.6 Generation of the Fab fragment of mAb Z2

The antibody was purified in the same manner as previously described (Andrew and Titus, 2000) and concentrated into 2 mg/ml stock. The protein was digested with papain (0.1mg/ml) dissolved in digestion buffer at equal volume to antibody solution at papain-to-antibody ratio of 1:20. Incubation was performed at 37°C and samples analysed after 4, 6, 8, 16, and 24 hours. The reaction was inactivated by addition of 0.3 M iodoacetamide to a final concentration of 0.03 M. To determine the optimum digestion time samples were analysed by SDS-PAGE under non-reducing conditions. Samples digested for 16 and 24 hours were pooled and dialysed against PBS overnight at 4°C. The Fab was then purified using Protein G column to remove undigested IgG and any Fc containing fragments (*Section 2.3.2),* unbound Fab was collected in the flow through. Fab was concentrated using a 10 K filter unit (Merck Millipore) and purity determined by SDS-PAGE under non-reducing conditions. Fab preparations were adjusted to 1 mg/ml in PBS and stored at -20^{o}C.

2.7 Gene expression analysis

2.7.1 RNA extraction and purification

RNA was extracted following the RNeasy Micro Kit protocol (Qiagen). One dish containing three cover slips of treated and control myelinating cultures were used to produce RNA samples. The culture supernatant was removed, centrifuged at 800 rpm for 5 min, after which the supernatant without the precipitate was stored at -20°C for further use. The cultures were gently washed with 1ml of filtered PBS. The cells were lysed with 350 µl of RLT buffer and sterile scraper was used to scrape the cells. The mixture was transferred into Qiashredder and centrifuged at 8000 g for 1 min. The samples were frozen at -80°C to aid the homogenisation.

Next the lysates were transferred into gDNA Eliminator spin columns (Qiagen) to remove contaminating gDNA and centrifuged at 8000 g for 30 sec. The flow through was mixed thoroughly with 350 µl 70 % ethanol, transferred into RNeasy MinElute spin column, and centrifuged at 8000g for 30 sec. The flow through was discarded and 700 µl RW1 was added, followed by 500 µl of RPE, and 500 µl 80 %

ethanol, each separated by centrifugation step at 8000 g for 30 sec and flow through removal. 14 µl of RNase free water was added directly on the filter centre and the extracted RNA was eluted to a new collection tube. The RNA concentration was Nanodropped and RNA was stored at -80°C.

2.7.2 Microarray

The RNA quality and integrity was assessed using Agilent Bioanalyser 6000 Nano LabChip platform system at Edinburgh Genomics at the University of Edinburgh Roslin Institute. The total RNAs were processed and labelled with biotin using Ambion WT Expression Kit following the Affymetrix GeneChip WT Terminal Labeling and Hybridization protocol. The processed RNAs were hybridized to Affymetrix GeneChip® Rat Gene 2.1 ST Arrays using manufacturer's protocols for using the Fluidics Station 450. The hybridized arrays were scanned on the Gene Array Scanner 3000-7G.

2.7.2.1 Partek Genomic Suite and Pathway analysis

Raw data from the microarray study were provided by the Roslin Intitute Polyomics Facility. The data analysis was carried out in Partek Genomics Suite and Pathway™ (version 6.6, Partek Inc., St. Louis, MO, USA) software. Probeset level data were normalized using GCRMA normalisation and summarised to transcript cluster level using One-Step Tukey's Biweight method. Details of the analyses are described in detail in the text (*Chapter 4*).

2.7.2.2 Panther gene list analysis

The PANTHER (protein analysis through evolutionary relationships) Classification System (http://www.pantherdb.org/) was used to cluster proteins and their genes according to their biological functions. Statistical enrichment was defined by Mann-Whitney test. Gene expression was considered significant if it satisfied a p-value cut-off of 0.05.

2.7.2.3 Interferon signature analysis

The interferome v2.0 (http://interferome.its.monash.edu.au/interferome/home.jspx), online database, allows experimental evidence on different interferon (IFN) subtypes to regulate IFN sensitive genes (ISGs).

2.8 Real time-polymerase chain reaction array

2.8.1 Total RNA extraction from myelinating cultures

RNA was extracted using RNEasy Micro Kit (Qiagen) as previously described.Cells were lysed in RLT buffer, homogenised using Qiashredder and stored at -80°C until RNA purification. Next the genomic DNA was removed and RNA was precipitated by addition of 70 % ethanol. This was followed by washing and elution of RNA from the RNeasy MinElute spin columns with 14 µl of RNeasy water. The resultant RNA was quantified using a NanoDrop and stored at -80°C to maintain RNA integrity until the assessment.

2.8.2 cDNA preparation

Sample cDNA was prepared from 500ng RNA using RT^2 First Strand Kits (Qiagen), as described by the manufactures. Genomic DNA elimination mix was prepared as follows:

Template RNA	≤ 8 µl
RNase free water	*
gDNA eliminator buffer	2 µl
Total volume	10 µl

* Reaction made up to total of 10 µl in H_2O

The mixture was incubated at 42°C for 5min, then immediately lifted on ice to stop the reaction.

To perform the reverse transcription, total volume of 10µl of master mixture per reaction sample was prepared as followed:

5xbuffer BC3	4 µl
Control P2	1 µl
RE3 reverse transcription mix	2 µl
RNase free water	3 µl
Total volume	*10 µl*

Each sample tube was vortexed and centrifuged. The thermocycler was set as the following for the reverse transcription reaction to be carried out:

1. 42°C 15 minutes
2. 95°C 5 minutes
3. 4°C Infinity

Volume of 91µl of RNase free water was added in each of the reaction samples, they were mixed, and placed in -20°C or continued to real-time PCR.

2.8.3 RT-PCR array

Master mix was prepared:

2xRT2 SYBR Green master mix	1350 µl
RNase free water	1248 µl
cDNA	102 µl
Total volume	*2700 µl*

Multichannel pipette was used to insert 25 µl of master mix in each of the 96-well RT^2 PCR Array plate (Qiagen) wells. The plate was centrifuged at 1000 g for 1 min.

The RT-PCR Array was performed on Applied Biosystems® 7500 Real-Time PCR Systems. The instrument was set as following:

1. 50°C 1 second
2. 95°C 10 minutes
3. 95°C 15 seconds
4. 60°C 1 minute

Steps three and four alternated for forty cycles after which data collection was performed.

2.8.4 Data analysis

Statistical analysis of the data was performed on free PCR array data analysis web portal (http://sabiosciences.com/pcrarraydataanalysis.php) using ΔΔCT method. This software allows automatic quantification of the C_T data provided by the array and calculation of the fold changes.

2.9 Quantitative real-time PCR

Change in the expression of selected genes of interest was verified using RNA obtained from additional cultures treated with mAb O4 or isotype control in the presence or absence of serum as a source of complement.

2.9.1 Total RNA extraction from myelinating cultures

RNA was prepared as previously described in *Section 2.6.1*.

2.9.2 Reverse transcription of RNA to cDNA

The RNA samples and QuantiTect Reverse Transcription kit (Qiagen) were thawed on ice. RNA samples were normalised to 500ng. cDNA synthesis reactions were accompanied by two control reactions:

1. –RT control, where reverse transcriptase was substituted for RNase free water as a control for genomic DNA contamination. Sample with the highest template RNA was used.
2. No template control, where template RNA was substitured for RNase free water for contamination of reagents.

Genomic DNA (gDNA) was eliminated from the RNA template by adding the following into a RNase free PCR tubes:

Template RNA	≤ 12 µl
RNase free water	*
gDNA wipeout buffer	2 µl
Total volume	*14 µl*

* Reaction made up to 14 µl in H_2O

The solution was incubated at 42°C for two minutes, then immediately set on ice to stop the reaction. Meanwhile, reverse transcriptase mixture was prepared as following and added into the RNA sample:

Reverse transcriptase mix	1 µl
Quantitative RT buffer	4 µl
RR primer mix	1 µl
Total volume	*6 µl*

The tubes were vortexed and centrifuged. The reverse transcription was carried out in thermocycler set for the following programme:

1. 42°C 20 minutes
2. 95°C 3 minutes
3. 4°C Infinity

RNase free water was used to dilute samples in 1:5 ratios, after which they were stored at -20°C.

2.9.3 Primer design

Specific primers were designed to amplify each target gene of interest identified on the microarray. Primer accuracy and efficiency was required to ensure accurate reaction. All primers were designed using Primer3: WWW primer tool (http://biotools.umassmed.edu/bioapps/primer3_www.cgi).

Specification for qPCR primers:

Primer size	18 to 23 base pairs (bp) (20bp optimal)
Melting temperature (T_m)	59.0°C to 61.0°C (60.0°C optimal)
GC content	40% to 65% (50% optimal)
Max self-complementarity	2.00
Max 3' complementarity	1.00

Basic local alignment search tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to ensure primer sequence specificity for the gene of interest. In cases where Primer3 software did not result any suitable results or BLAST found similarities between biological sequences, maximum self-complementarity was increased to 3 on Primer3: WWW primer tool.

Primer specificities were confirmed by qPCR reaction using any cDNA containing the transcript of interest. Specificity was considered accurate if appropriate size amplification occurred.

Table 2.8.3: Primer designs for the genes of interest.

Gene Symbol	Forward Primer (5' - 3')	Reverse Primer (5' - 3')
CCL2	TAGCATCCACGTGCTGTCTC	TGCTGCTGGTGATTCTCTTG
CCL5	CTT TGC CTA CCT CTC CCT CG	TCC CCA GCT GGT TAG GAC T
CCL7	CCA TCA GAA GTG GGT TGA GG	CAG AAA GGA CAA GGG TGA GG
CCL20	TTC ACA ACA CAG ATG GCC GA	TGC AGT GAT GTG CAG GTG AA
CXCL11	AAC GGT TCC AGG CTT CGT TA	TTG TCA CAG CCG TTA CTG GA
CXCL13	CCT GCT CGG AAT CTT AGT GTT	TGG GTT GAG TAT GGG AAG GA
IFN-α_4	CTC ATC TGC TGC TTG GAA TG	TTC TTG GGT TAG GGG AGG TT
IFN-γ	GCC CTC TCT GGC TGT TAC TG	CCA AGA GGA GGC TCT TTC CT
IL-1β	AAA AAT GCC TCG TGC TGT CT	TTG TCG TTG CTT GTC TCT CCT
IRF7	CAA GAG GAA ATG CTG GGT TG	AGA AGC CTG TGG TGG GAC T
SOCS1	AGC CAT CCT CGT CCT CGT	AAG GTG CGG AAG TGA GTG TC
STAT4	ACG CAC AGG AAA GCC TCA	CAC TGC TCA AGT CCA AAG TCA
CD14	TGG TCA ACA AAT CCT CTG CTT	ACC GAT GGA CAA CTT TCA GG
TNF	CCC CTT TAT CGT CTA CTC CTC A	TTC AGC GTC TCG TGT GTT TC
GAP-DH	ATG ACT CTA CCC ACG CAA G	TAC TCA GCA CCA GCA TCA CC

2.9.4 RT-PCR

The Affymetrix Array and chemokine array findings were validated by the use of primer specific qPCR. Front and reverse primers were mixed at 1:1 ratio. Quantitative PCR was performed on 7500 Fast machine set on the following programme:

1. 50°C 5 seconds
2. 95°C 10 minutes
3. 95°C 15 seconds
4. 60°C 1 minute

Steps 3 and 4 were altered for 40 cycles, after which data collection was performed.

2.10 Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was run for quantitative determination of chemokine concentrations within the sample supernatants as they were collected following myelinating culture treatments from three biological replicates. They were centrifuged at 800 rpm for 5 min to remove any precipitate and stored as aliquots at -20°C.

Commercially available ELISAs were performed as described in the manufacturer's instructions. All reactions were performed in duplicate. In all cases, reagents were prepared according to instructions. The standard curve was produced via two-fold dilution series. The samples of unknown protein concentrations were diluted based on values obtained from the RT-PCR Array and Affymetrix Array.

2.10.1 Rat CCL2 and CCL5 ELISA

Rat CCL2 and CCL5 were detected in the supernatants using ELISA construction kits from R&D Systems (MJE00 and MMR00, respectively). 50 µl of assay diluent was added into each well before they were topped up with 50ul of standard, control, or sample solution. The microplate was incubated for two hours in room temperature. The wells were aspirated and tapped on a paper towel, after which wash buffer was added. This was repeated five times. Then rat conjugate solution was added into the wells and left to incubate for two hours. Washing step was repeated five times and substrate solution was added into the wells for 30 minutes in room temperature and protection from light. Stop solution was pipetted into the wells and mixed gently by tapping the sides of the microplate. Finally, the plate was inserted in a reader set at 450 nm.

2.10.2 Rat CCL20 ELISA

Rat macrophage inflammatory protein 3α (MIP-3α) (CCL20) was detected in the supernatants using ELISA construction kits from Abnova (KA2000). 100 µl of standard or samples were pipetted into pre-coated 96-well plate wells after which it was covered and incubated at 37°C for 90 min. The wells were blotted on a paper towel and 100 µl of biotinylated anti-rat CCL20 antibody working solution was added into each well. The plate was incubated at 37°C for 60 min. The plate was washed three times with 1x PBS, each time allowing the plate to sit on a shaker for 1-2 min. Then, 100 µl of prepared avidin-biotin-peroxidase complex working solution was added into the wells for 30 min at 37°C. The washing step was repeated five times, after which 90 µl of prepared TMB colour developing agent into each well and incubated at 37°C for 20-25 min. 100 µl of

TMB stop solution was added into wells, and microplate reader was used to read the optical density absorbance at 450 nm.

2.10.3 Rat CXCL11 ELISA

Rat interferon inducible T cell alpha chemo-attractant (CXCL11) was detected in the supernatants using ELISA construction kits from Uscn Life Science Inc (E92071Ra). 100 µl of standard or samples were pipetted into pre-coated 96-well plate wells, after which it was covered and incubated at 37°C for 2 hours. The wells were aspirated, 100 µl of prepared detection reagent A was added and incubated for 60 mins at 37°C. The solutions were discarded and the wells were washed three times. 100 µl detection reagent B was added into the wells for 30 min incubation at 37°C. Washing step was repeated for five times and 90 µl of substrate solution was added. The well plate was incubated for 15-25 mins at 37°C, after which 50 µl of stop solution was added. The absorbance was measured by using the microplate reader at 450 nm.

2.10.4 Statistical Analysis

Standard curve was created by plotting the mean standard absorbance of each standard against their concentration, and best fit line was drawn. To determine the concentration of the target protein, the data was linearised by plotting the log of the mean sample concentrations (n=3) against the log of optical density; best fit line was determined by regression analysis. Diluted concentrations were multiplied by their dilution factors.

3 Antibody-mediated effects on myelin *in vitro*

3.1 Confirmation of antigen accessibility on myelin surface

Myelinating cultures were generated from dissociated embryonic (E15.5) rat spinal cord as previously described (Sorensen et al., 2008). Neurite outgrowth and myelination were assessed by immune fluorescence microscopy using antibodies specific for phosphorylated neurofilaments (clone SMI-31, red, *Fig. 3.1 A-ii & B-ii*) and PLP (clone AA3, green, *Fig. 3.1 B-i*). To confirm the epitope recognised by mAb O4 was exposed at the surface myelinating oligodendrocytes live cultures 24 DIV were incubated with mAb O4 at 4°C and then processed for immunofluorescence microscopy (green, *Fig. 3.1 A-i*). This confirmed that immunoreactivity for O4 was associated with the surfaces of linear structures identified as myelin sheaths and the cell bodies and processes of PLP^+ cells identified on the basis of their morphology as OPCs and oligodendrocytes.

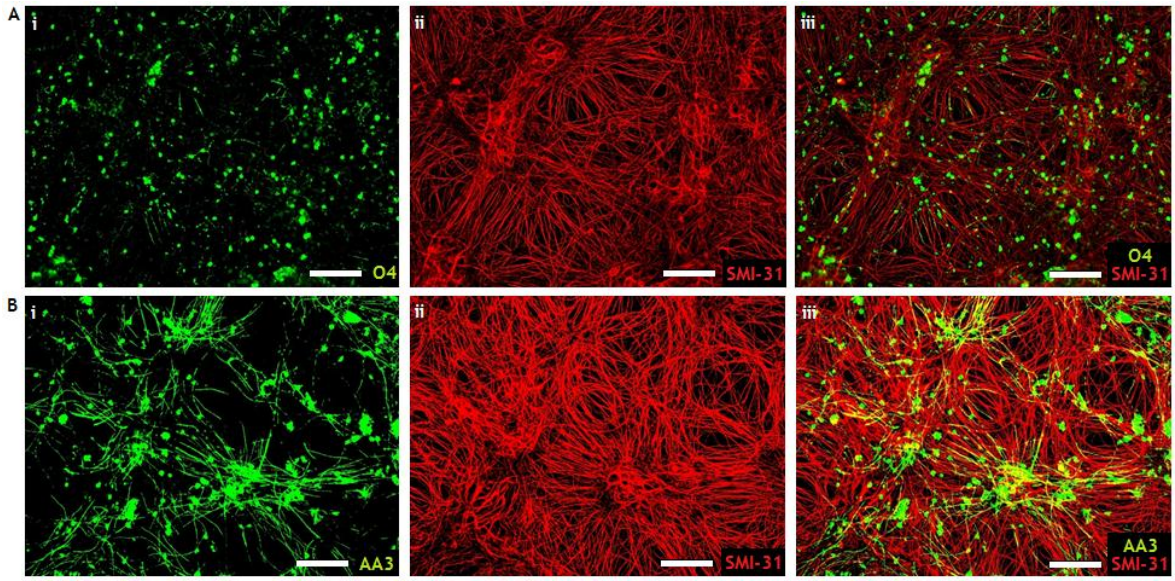


Figure 3.1: The O4 antigen is exposed on the surface of OPC, myelinating oligodendrocytes and myelin sheaths. *Live staining with mAb O4 (A-i, green) demonstrates its epitope(s) are accessible at the outer surface of OPC, myelinating oligodendrocytes and myelin sheaths. Two colour immunofluorescence microscopy for PLP (B-i, clone AA3, green) demonstrates the presence of terminally differentiated oligodendrocytes and myelin sheaths associated with axons (B-ii, clone SMI31, red); merged image (iii).All cultures analysed at 24 DIV. Bar represents 100 µm.*

3.2 IgM antibody mediated demyelination

Previous studies demonstrated myelinating cultures can be used to assess and quantify antibody-mediated demyelination *in vitro* (Elliott et al., 2012). To confirm mAb O4 was able to mediate this demyelinating effect in our cultures, they were treated with 20 µg/ml mAb O4 or its irrelevant mouse IgM (as an isotype control) in the presence of 2 % rabbit serum as a source of complement for 24 hours. Additional controls were provided by untreated cultures, and cultures treated with 2 % rabbit serum alone.

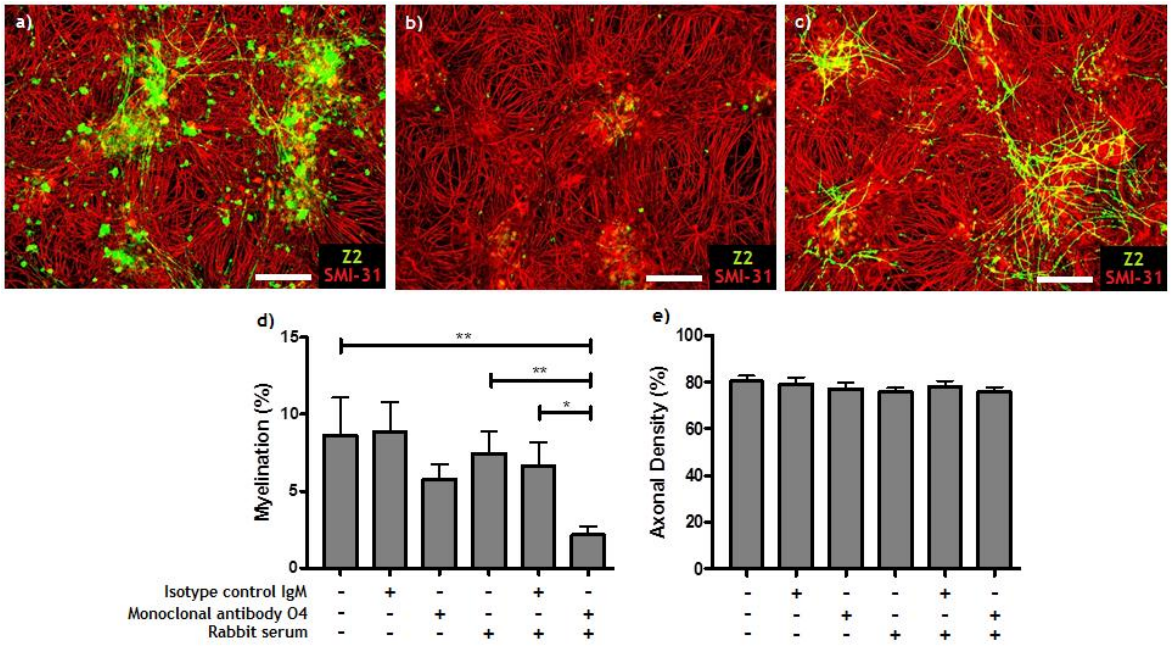


Figure 3.2: Acute demyelination by mAb O4 is dependent on an exogenous source of complement. *(b) Significant demyelination is only observed when cultures are incubated with mAb O4 in the presence of serum as an exogenous source of complement. mAb O4 has no corresponding effect on myelination (a & d, 5.80 ± 1.67 %) and neurite density (b, 77.90 ± 3.46 %) presented as (mean ± SD, n = 3, *p < 0.05, **p < 0.01, one-way ANOVA). Representative immune fluorescence images: (a) overnight incubation of cultures with mAb O4, (b) culture treated overnight with mAb O4 and 2 % rabbit serum and (c) culture incubated over night with control IgM and 2 % rabbit serum. Bar represents 100µm.*

Axonal density and myelination were quantified by computer aided analysis of immunofluorescent images (*Fig. 3.2a-c*). Incubating cultures for 24 hours with mAb O4, the IgM isotype control or serum alone had no statistically significant ($p > 0.05$) effect on myelination. However, a trend was observed suggesting treatment with mAb O4 or serum alone did mediate a slight decrease in myelin, as determined by quantifying myelination using mAb Z2 (specific for MOG) and

SMI-31 (specific for neurofilaments). In contrast, treatment with mAb O4 in the presence of serum as a source of complement resulted in a reduction of myelination by > 70 % relative to controls treated with serum alone (serum alone, 7.47 ± 2.47 %; serum plus mAb O4, 2.17 ± 0.98 %; P<0.01) (*Fig. 3.2*). Data presented in *Figure 3.2* were derived using three different preparations of mAb O4; one purified from culture supernatants in the laboratory, one purchased from R&D systems and the third provided by Miltenyi. There were no differences noted in their demyelinating activity in this *in vitro* study, and none had any effect on neurite density as determined by SMI-31 immunoreactivity.

3.3 IgG antibody mediated demyelination is Fc dependent

Demyelination by autoantibodies recognising epitopes exposed at the myelin/oligodendroglial surface is thought to be dependent on activation of complement (Elliot et al., 2012), a response which is Fc dependent. To test this hypothesis we investigated the demyelinating potential of the MOG-specific IgG_{2a} mAb Fab fragment of Z2 and its Fab fragment *in vitro*.

This Fab fragment (*Fig. 3.3d*) retained the ability of the parent antibody (*Fig. 3.3a*) to recognise its epitope on myelin and oligodendrocytes but was unable to induce significant demyelination when added to myelinating cultures in the presence of serum as a source of complement (*Fig. 3.3f*). This is in striking contrast to mAb Z2 which reduced myelination by >50 % in the presence of 2 % rabbit serum (*Fig. 3.3c & a*). Neither the mAb Z2 nor its Fab fragment had any effect on neurite density under any of the conditions investigated in this study (*Fig. 3.3h*).

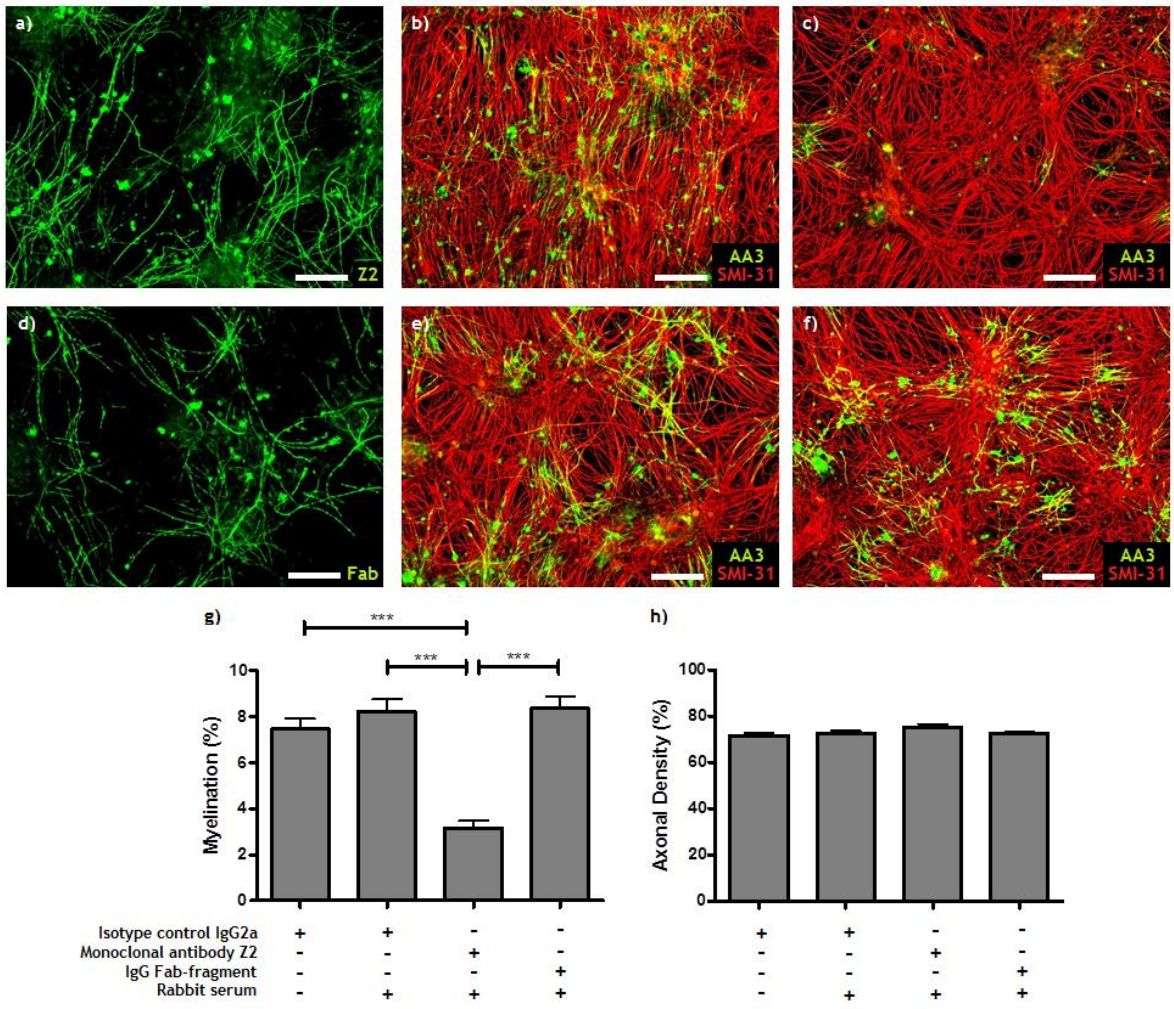


Figure 3.3: Acute antibody mediated demyelination is Fc- dependent*. MOG-specific mAb Z2 (a) and Fab fragment (d) epitopes are accessible at the outer myelinating oligodendrocytes and myelin sheaths. Overnight treatment of myelinating cultures with 20 µg/ml mAb Z2 or Z2 Fab in absence (b,e) or presence (c,f) of 2 % rabbit serum as a source of complement. As reported previously (Elliott et al., 2011) mAb Z2 mediates demyelination when an exogenous source of complement is available (c). In contrast, Z2 Fab is unable to mediate demyelination (f). Myelin as visualised by immunostaining for PLP (clone AA3; green) and neurites visualized using the neurofilament-specific antibody SMI-31 (red; d,e,g & h). Quantification of myelination (g) and neurite density (h) demonstrates myelin loss was only observed in the presence of serum when mAb Z2 was added to the cultures (3.16 ± 1.67 %), Z2 Fab had no effect (8.37 ± 2.80 %). Representative data from 30 images of one experiment (mean ± SD, ***p < 0.001, one-way ANOVA). Bar represents 100 µm.*

3.4 Complement-independent IgM mediated inhibition of myelination

Studies investigating the functional significance of autoantibody mediated effects in MS in general focus on their ability to induce demyelination by acute complement- and/or antibody-dependent cellular cytotoxicity (ADCC) dependent mechanism. Such experiments may replicate the situation seen in patients with

RRMS in which demyelination is associated with perivascular inflammation and BBB breakdown, but as outlined in the introduction this is not the case in patients with progressive disease in whom the BBB is apparently intact. In this situation availability of antibody and complement is effectively limited to that synthesised within the CNS compartment itself. In order to model this situation *in vitro* we decided to investigate the effects of longer treatments with mAb O4 in myelinating cultures in the absence of serum. This mAb was chosen as it reproduces the cellular specificity of a sulphatide-specific mAb isolated from a patient with MS (Kirschning et al., 1995; 1997). We therefore treated myelinating cultures from 18 to 19 DIV or from 18 to 28 DIV with 20µg/ml mAb O4 or the IgM isotype control.

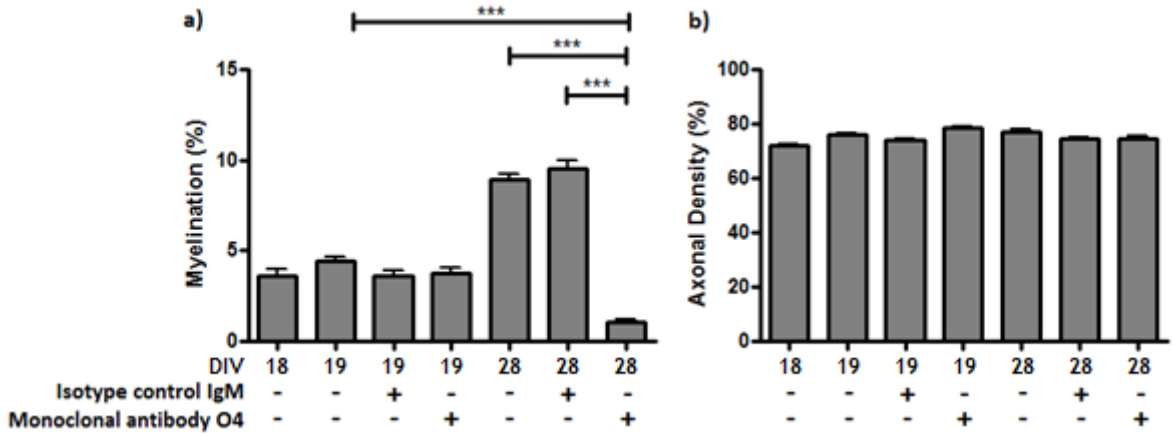


Figure 3.4: Prolonged antibody treatment in the absence of complement inhibits myelination*. Ten day treatment of myelinating cultures with 20 µg/ml O4 (sulphatide-specific monoclonal antibody) in the absence of complement resulted in demyelination (a). Prolonged isotype control treatment did not affect myelination. Full axonal density was established 18 DIV and remained intact after demyelination (b). Percentage values of myelination (a) and axonal density (b) were calculated in relation to cultures treated with equivalent concentrations of antibody or their isotype controls in the absence of complement for 24 hours or ten days (Mean ± SD; n=2; ***P < 0.001; one-way ANOVA).*

Analysis of immune fluorescent images revealed mAb O4 had no significant effect on axonal density (*Fig. 3.4b*) but following prolonged exposure significantly inhibited myelination (*Fig. 3.4a*). As demonstrated above, overnight incubation with mAb O4 (18 to 19 DIV) in the absence of an exogenous source of complement had no effect on myelination. In contrast, treatment for ten days not only completely blocked any further myelination, but also appeared to

induce some loss of pre-existing myelin sheaths. This effect was antigen-specific as it was not observed when cultures were treated with the IgM isotype control.

3.5 Antibody mediated inhibition of myelination is associated with increased microglial numbers

In addition to myelin and oligodendrocyte loss, previous studies demonstrate microglia are activated in demyelinating MS lesions (Barnett and Prineas, 2004) either in response to local injury or BBB disruption (Denk and Svoboda, 1997; Nimmerjahn, Kirchhoff and Helmchen, 2005). To investigate whether a similar effect can be induced *in vitro*, myelinating cultures (18 DIV) were treated with mAb O4 or isotype control and immunostained to identify Iba-1^{+} microglial cells after 24 hours and ten days.

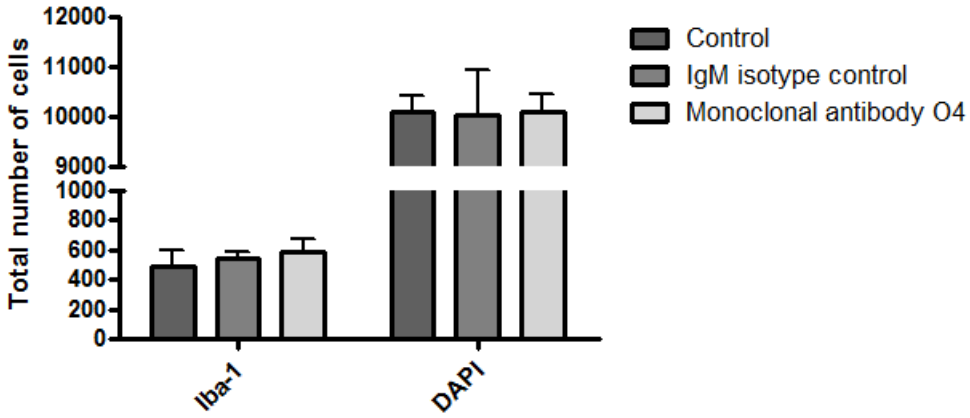


Figure 3.5a: Overnight antibody treatment has no effect on microglial numbers. *Antibody treatment in the absence of complement from 18 to 19 DIV did not affect microglial (a) or whole cell population (b) numbers. Representative data calculated as total of 30 images (mean ± SD, n=3, one-way ANOVA).*

Our results revealed no difference between Iba-1+ or total cell numbers per 30 fields in untreated (Iba-1 493 ± 183; DAPI 10091 ± 578), and isotype control (Iba-1 537 ± 79; DAPI 10023 ± 1556) or mAb O4 (Iba-1 580 ± 163; DAPI 10103 ± 601) treated myelinating cultures after 24 hours.

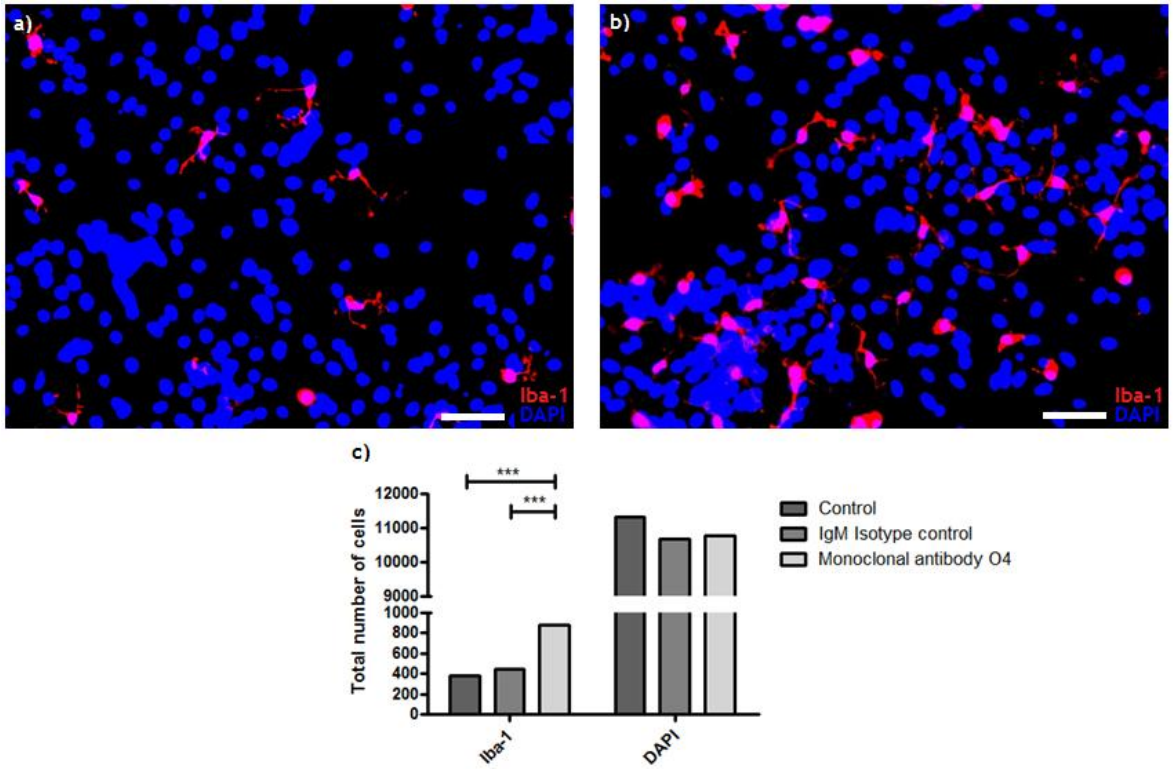


Figure 3.5b: Ten day antibody treatment increases number of microglia. *Myelinating cultures were treated from 18 to 28 DIV with an IgM isotype control (a) or mAb O4 (b). Prolonged treatment with isotype control IgM had no effect on microglial and total cell number (c) visualised by microglial-specific Iba-1 (red) and DAPI (blue), respectively. In contrast, ten day treatment with mAb O4 resulted in ~50 % increase in Iba-1^{+} cells when total DAPI number remained the same (a, b, c). Values shown represent total numbers of Iba1 and DAPI cells per 30 images. Representative data from one of three experiments (***p < 0.001, one-way ANOVA). Bar represents 50 µm.*

However, Iba-1^{+} microglial numbers in myelinating cultures treated for ten days with mAb O4 were found to more than double those in untreated or isotype control treated cultures (*Fig. 3.5b*; $p < 0.001$). This was not apparent in the total cell counts as microglial numbers account for < 5% of cells in these cultures. Comparison of untreated cultures at these two time points indicates there is no significant increase in microglial numbers over the course of this experiment in the absence of mAb O4.

3.6 Discussion

MS is a devastating disease that results in extensive demyelination associated with axonal loss and injury. Studies on patients indicate antibody-dependent mechanisms are involved in lesion formation (Cross et al., 2011). This data, however, is largely circumstantial (Cross et al., 2011), although numerous studies report MS is associated with increased levels of (auto-)antibodies in serum and CSF (Weber, Hemmer and Cepok, 2011). It is therefore important to develop *in vitro* techniques to determine how these antibodies may contribute to disease development and identify potential therapeutic leads to counter their effects in patients.

Myelinating cultures provide a valuable approach to study antibody mediated effects *in vitro* and in our study we decided to use the sulphatide-specific IgM mAb O4 as this antibody replicates multiple features associated with intrathecal autoantibody repertoire in MS patients. First, sulphatide is a major target for the intrathecal antibody response in patients with MS (Ilyas et al., 2003; Villar et al., 2005; Kanter et al., 2006; Brennan et al., 2011). Second, the presence of oligoclonal IgM anti-lipid antibodies in the CSF of MS patients is associated with a poor prognosis involving earlier second relapses, more relapses, and severed disability (Villar et al., 2005). Third, a human IgM mAb isolated from an MS patient is sulphatide-specific and exhibits a similar pattern of binding to the surface of oligodendrocytes in the rat brain cell cultures and tissue sections as mAb O4 (Kirschening et al., 1995; 1997). In addition, animal experiments demonstrate intrathecal synthesis of mAb O4 by hybridomal cells induces demyelination *in vivo* (Rosenbluth et al., 2003), whilst passive transfer of this antibody exacerbates disease severity in mice with EAE (Kanter et al., 2006). Lastly, *in vitro* studies from this laboratory demonstrated mAb O4 can mediate complement-dependent demyelination (see S*ection 3.2; 3.3*; Elliot et al., 2012).

Our data confirm these experiments in that binding of mAb O4 to the myelin/oligodendrocyte surface induces rapid complement-mediated demyelination (Elliot et al., 2012). In addition, we also found prolonged exposure to mAb O4 inhibits myelination even in the absence of serum as a source of complement. These results support the dominant view that anti-

glycolipid autoantibodies are pathogenic in patients with CNS demyelinating diseases. However, this interpretation has been questioned following reports stating mAb O4 and natural occurring human IgM autoantibodies enhance remyelination in TMEV model of inflammatory demyelination (Miller et al., 1994; Asakura et al., 1998; Warrington et al., 2000). This effect is attributed in part to surface binding of IgM antibodies inducing OPC proliferation and differentiation (Bansal, Gard and Pfeiffer, 1988). Why mAb O4 blocked rather than stimulated myelination in our current studies remains unclear, however, the outcome of antibody binding may be concentration dependent as we used antibody concentrations higher than those reported by Bansal and colleagues. Moreover, the myelinating cultures used in the present study contain large numbers of astrocytes and neurons that may provide an intrinsic source of complement components. Determining whether the myelination block and/or increase in microglial numbers seen following extended treatment with mAb O4 is complement-dependent is important and requires further investigation. However, it is apparent acute loss of myelin in this culture system is dependent on the availability of serum to provide an exogenous source of complement (Elliot et al., 2012); an effect we now confirm is Fc dependent. In this situation demyelination is presumed to be initiated by activation of the classical complement pathways following binding of C1q by antibody on the myelin/oligodendrocyte surface (Linington et al., 1989), although this has still to be formally demonstrated.

In our cultures, we observed rabbit serum alone induced a variable reduction in myelin. This was not statistically significant ($p > 0.05$) but may reflect rat oligodendrocyte sensitivity to damage mediated by antibody-independent activation of complement; an effect attributed to the failure of rat oligodendrocytes to the express membrane complement regulator CD59 (Zajicek et al., 1992; Wing et al., 1992; Piddlesden and Morgan, 1993). The significance of this effect *in vivo* is difficult to evaluate; however, it may be important in diseases associated with catastrophic damage to the BBB such as stroke. However, BBB damage is more subtle in MS and moreover, unlike the situation in experimental rodents, human oligodendrocytes do express CD59 (Scolding et al., 1998).

In the context of MS, the effect of prolonged treatment with mAb O4 in the absence of serum may in fact be more interesting than its ability to induce acute complement dependent demyelination. This experimental setting can be argued to mimic the situation within the CNS of patients with progressive forms of MS that exhibit little evidence of BBB dysfunction. In these patients there is very limited availability of complement and antibodies derived from the periphery, but there is substantial intrathecal synthesis of autoantibodies and possibly also, complement components. The former include antibodies recognising sulphatide, dominant target of mAb O4 in CNS myelin (Villar et al., 2005), whilst complement components in the CNS may be produced by microglia and astrocytes (Passinetti et al., 1992; Levi-Strauss and Mallat, 1987; Gasque et al., 1995; Jongen et al., 1997).

Our *in vitro* data demonstrate there is no obvious effect of mAb O4 on myelination after 24 hours, but after 10 days its presence not only completely blocks myelination but also mediates some loss of myelin already present at the start of the experiment. This observation supports the view intrathecal antibody responses to myelin-associated glycolipids are pathogenic rather than beneficial in MS. It will now be important to determine how mAb O4 disturbs myelination, and if this is specific for sulphatide or is a generalised effect mediated by any antibody that binds to the myelin/oligodendrocyte surface. Potential mechanisms include:

1) Steric hindrance due to the high density of Ig bound to the oligodendrocyte surface blocking molecular interactions with the extracellular matrix and/or axonal surface required to support OPC differentiation and myelination.
2) Dysmyelination due to destabilisation of immature myelin sheaths due to incorporation of Ig into the extracellular apposition (Rosenbluth and Moon, 2002; Rosenbluth and Schiff, 2009).
3) Cross-linking of surface microdomains leading to disruption of cytoskeleton and process retraction (Dyer and Benjamins, 1989; Dyer, 1993; Dyer and Matthieu, 1994), and/or a cellular stress response (Marta et al., 2005; Marta et al., 2008).

4) FcR mediated interactions between Ig on the myelin/oligodendrocyte and microglial cells (Marta et al., 2005).

The relative importance of these mechanisms can now be explored using Fab fragments derived from mAb O4 and other mAb recognising epitopes exposed at the myelin surface (eg. O1, an IgM mAb specific for galactocerebroside; O10, an IgM mAb specific for PLP; Z2, an IgG_{2a} mAb specific for MOG). These reagents would also be useful in investigating the relationship between inhibition of myelination by mAb O4 in the absence of serum and its effect on microglial cell numbers.

The demonstration continuous treatment with mAb O4 induced a significant increase in microglial cell numbers is potentially the most exciting result from these experiments, as it suggests a functional relationship may exist between the intrathecal antibody response (in the case represented by mAb O4) and microglial proliferation in patients with PMS (Prineas et al., 2001). This response could result, as previously mentioned, from antibody-mediated cross-linkage of microglial surface receptors, a concept supported by the observation treating mixed glial cell cultures with pentameric human Fcμ activates microglia (Howe et al., 2006). It was this observation that launched our interest in mapping transcriptional changes induced by mAb O4 in myelinating cultures.

4 Transcriptional profiling of antibody induced demyelination

In order to identify potential biomarkers associated with acute autoantibody-mediated demyelination a gene microarray study was performed on myelinating cultures. Five conditions were analysed (1) untreated cultures; (2) IgM isotype control alone (3) serum alone; (4) mAb O4 alone; (5) mAb O4 plus serum. All incubations were carried out for 24 hours at 37°C in 7 % CO_2/93 % air.

4.1 Processing of RNA samples

Total RNA was extracted from myelinating cultures (n = 3; in each case using 3 coverslips) treated as described above. In each case sample purity and RNA concentrations were determined by absorbance before samples were sent out for analysis at the Edinburgh Genomics laboratory. RNA integrity and quality was confirmed using the Agilent Bioanalyzer 6000 Nano LabChip platform system that utilises the RNA Integrity Number (RIN) algorithm to analyse RNA electropherograms (*Fig. 4.1a*). The RIN algorithm is calculated on the basis of the presence of degradation products (marked 'lower' in *Fig. 4.1b*) and the ratio of 28S to 18S ribosomal RNA. Samples were only deemed acceptable for the analysis if they had RIN scores of >8 (score of 10 corresponding to completely intact RNA, whilst a score of 1 indicating a completely degraded sample). All of our samples scored RIN $\geqslant$ 8.7, thus being eligible for further analysis.

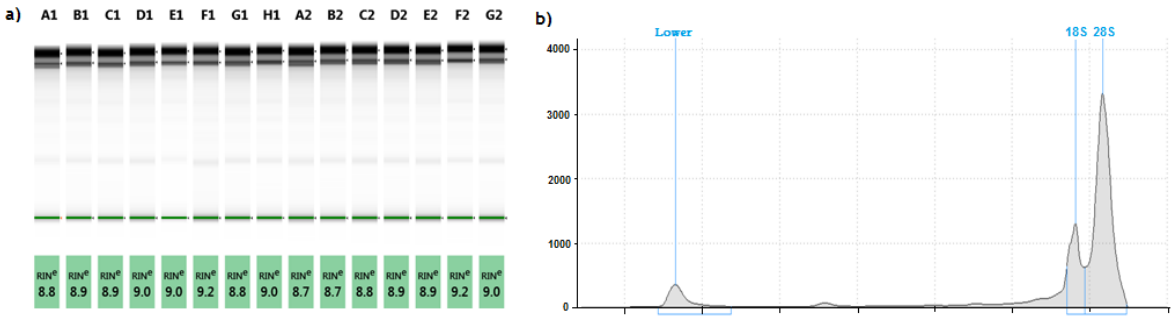


***Figure 4.1: Quality of RNA samples**. RNA was isolated from myelinating cultures treated overnight with mAb O4 or its isotype control in the presence or absence of serum as a source of complement. Additional controls were provided from untreated and serum alone treated cultures. a) Gel image assessment on Agilent Tapestation System expressing individual RIN scores. b) Representative electropherogram of high quality total RNA with and RNA integrity value of 9.*

4.2 Determining how antibodies influence gene expression in the myelinating culture

To establish how the different treatments influenced gene expression, the transcriptional profiles of myelinating cultures treated as described above were compared using Affymetrix GeneChip Rat Gene 2.1 ST array which comprise 28,407 distinct RefSeq probes that equate to 16,771 well-annotated gene transcripts. This was performed by Edinburgh Genomics at the University of Edinburgh Roslin Institute (https://genomics.ed.ac.uk/) using RNA samples labelled with biotin by means of the Ambion® WT Hybridisation protocol in combination with a Fluidics Station 450. After hybridisation the microarrays were scanned on the Gene Array Scanner 3000-7G and analysed using Partek Genomic Suite.

4.2.1 Pre-Processing of Affymetrix chip data using Partek Genomics Suite

Data processing was done using Partek Genomics Suite 6.6 Software after Edinburgh Genomics assessed the raw data qualitatively and pre-processed it using probe summarisation algorithms in series of stages to generate a set of expression measures for subsequent analysis. In brief this involved the following series of steps.

Background signal intensity was corrected on a chip-by-chip basis by a Robust Multi-array Average adjusted for GC content (GCRMA) algorithm which is designed to eliminate background noise due to non-specific hybridisation. Probe affinity occurs as a result of position-dependent base effect that can be calculated for each perfect match and mismatch value. Correction of the background noise occurs based on assumption that these values consist of optical noise, non-specific binding noise, and 'true' signal.
Therefore, three steps are required:

1. Optical background correction as optical noise is introduced by a scanner measuring hybridisation strength
2. Probe intensity adjustment via non-specific binding (NSB) that operates on the basis of affinity information and optical noise-adjusted mismatch intensities.

3 Probe intensity adjustment via gene-specific binding (GSB) that uses NSB-adjusted perfect match intensities for further correction of perfect match probe affinities.

Each of these values is adjusted by subtracting an affinity corrected mismatch value.

Normalisation is executed to unify the signal intensity distribution of each chip, therefore enabling the comparison and coordination of data analysis. This is done as all chips should have approximately the same distribution of perfect match values. In GCRMA, quantile normalisation is performed, where each perfect match value is plotted in number of dimensions and projected onto the diagonal, resulting in perfect match values on individual chips having the same distribution.

Probe summarisation. Signal intensities of individual probe sets are summarised to exons followed by summarisation to the corresponding genes, giving each entity a normalised expression measure. This is driven by the assumption that observed log-transformed perfect match values follow a linear additive model containing a probe affinity effect, a gene specific effect and an error term. Probe affinity effects are assumed to sum to zero, and the expression level is estimated by median polishing that protects against outlier probes. Summarisation to transcript cluster was performed by using One-step Tukey's Biweight method.

4.2.2 Signal plot of normalised data

Monitoring sample hybridisation is essential for quality control of the array data. Any variations in the data may result in irreproducible results. This can be avoided by using a set of control transcripts to detect differences in the hybridisation efficiency. Prior to hybridisation, fragmented cDNA is added in hybridisation cocktail containing controls that consist of standard concentrations (from the lowest to the highest) of four transcripts, bioB, bioC, bioD and creX. Signal intensities of the control transcripts on each chip are plotted, thus allowing differences in hybridisation efficacy to be limited. *Figure 4.2.2a* shows the signal intensities of the four hybridisation controls each of the 15 GeneChip

used in the array study. The plot suggests little variation between the hybridisation efficiency of each individual chip.

The frequency of the signal intensities of each chip was also plotted on a signal histogram (*Fig. 4.2.2b*). After labelled cDNA is hybridised to the chip, signal intensities are calculated for each set. Frequency of a range of signal intensities was plotted against probe sets on each chip. This allows control for hybridisation quality and sources for other non-biological variation, including efficiency of cDNA labelling or starting RNA quantity or quality. Each line represents an individual sample grouped into colour coded triplicates. Due to similar pattern, non-biological variables can be ruled out.

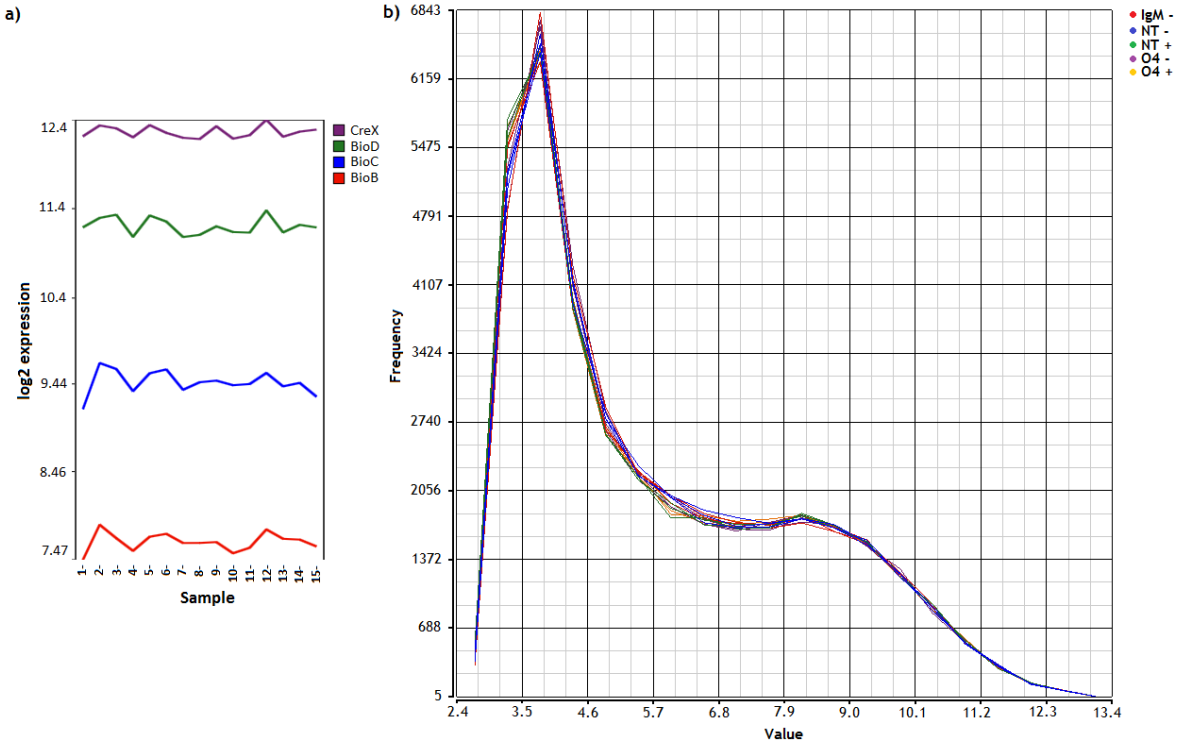


***Figure 4.2.2: Signal plot of normalised intensity values**. Biotin labelled sense-strand cDNA was generated from RNA samples and hybridised to each GeneChip and four control transcripts at control concentrations. The normalised signal intensities of colour coded hybridised controls (BioB, BioC, BioD, and CreX) indicated small variation within the control group (a). The occurring frequency of signal intensities for all probe sets on each chip. Each line represents represents individual GeneChip and thus an individual sample. Lines are coloured according to experimental grouping (n=3/group).*

4.2.3 Principle component analysis

Principle component analysis (PCA) is a statistical method that uses orthogonal transformation to allow easy visualisation of data sets by transforming a number

of correlated variables into a number of uncorrelated variables known as principle components. These are estimated from the eigenvectors of the covariance or correlation matrix of the original variables. Thus, PCA is a dimensionality reduction method, in which data is reduced into basic components deprived of any unnecessary parts allowing it to be easily visualised. The underlying assumption being that different conditions should result in different groups within a three dimensional plot.

The plot of PCA scores from our GeneChip data is illustrated in *Figure 4.2.3*. Samples from each condition were grouped in clusters, coloured accordingly to allow distinction between different groups of data and highlighting any outliers. Control untreated cultures (C-) and IgM isotype treated cultures in the absence of serum (IgM-) cluster together indicating treatment with an irrelevant IgM had little effect on gene transcription. Similarly data from cultures treated with serum alone (C+) and mAb O4 and serum (O4+) clustered together but at the other side of the plot. This was unexpected as this treatment results in demyelination and was therefore anticipated to be associated with a completely different profile. However, our results suggest the dominant effect is due to the presence of serum, rather than complement-dependent demyelination mediated by mAb O4. In contrast, cultures treated with mAb O4 alone (O4-) showed loose clustering that was well separated from all other conditions. This indicates this group to exhibit a markedly different transcriptional profile to the other four groups, but at the same time, more inter-group variance.

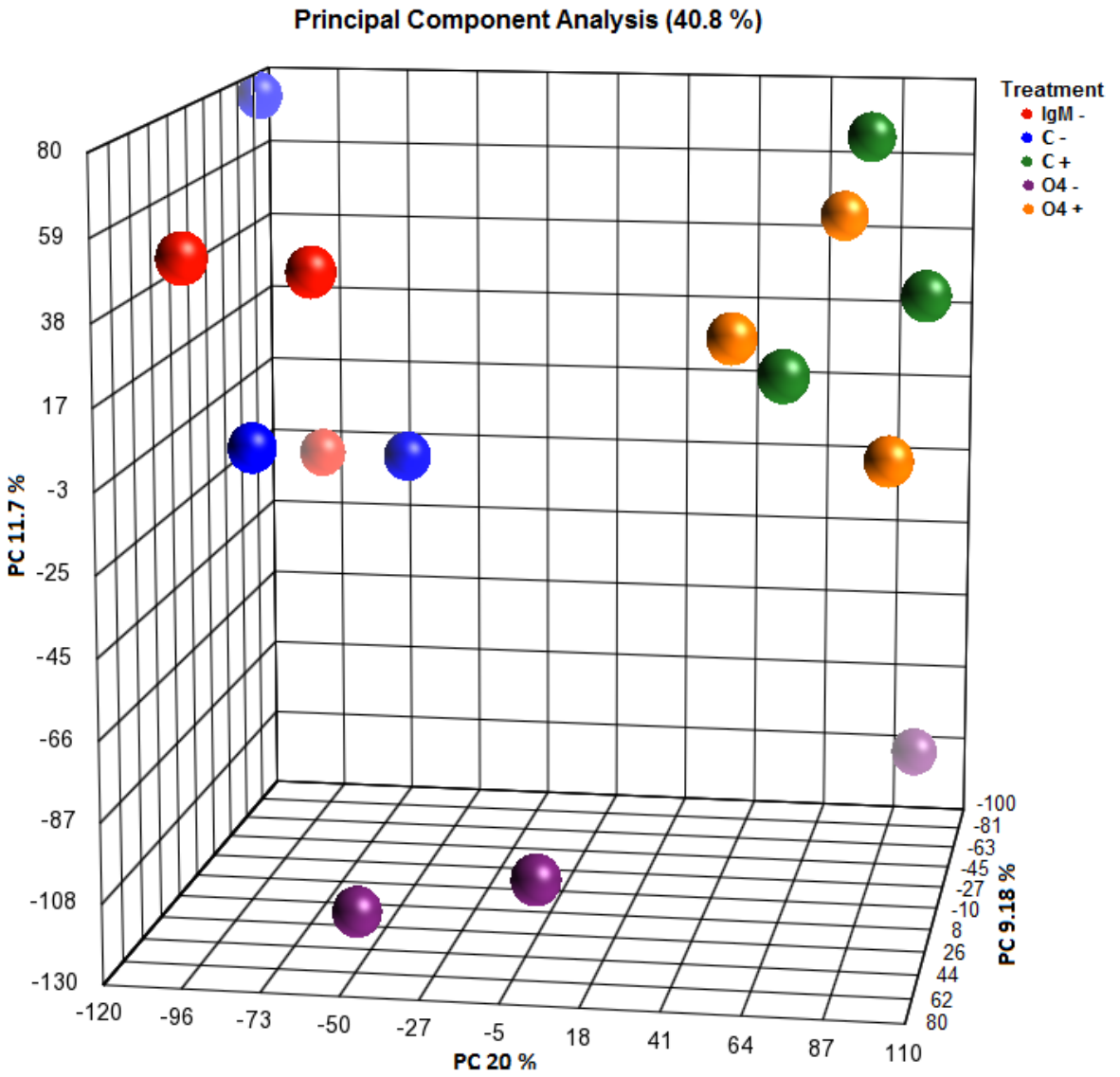


Figure 4.2.3: Principal component analysis of microarray samples*. Each sphere on the graph represents data from an individual GeneChip. Closer grouping indicates closer similarity of the transcriptional profiles. Samples are colour coded, thus allowing visualisation of the experimental groupings [untreated control (C-) blue; isotype control in the absence of serum (IgM-) red; mAb O4 in the absence of serum (O4-) purple; serum treated control (C+) green; mAb O4 in the presence of serum (O4+) yellow].*

4.2.4 Hierarchical cluster analysis

PCA indicates that adding mAb O4 to the cultures had a very different effect on gene expression compared to that seen in cultures treated with serum whether or not mAb O4 was present. To investigate this further hierarchical clustering was performed as this allows patterns of expression to be revealed within these large data sets. Hierarchy of the data sets is indicated by a dendrogram that shows how the microarrays are segmented together based on similarity of features. Groups merging at high values relative to their subgroup branching

values are candidates for natural clustering (Tibshirani et al., 2001). This method is considered unsupervised and unbiased as it does not take into account any experimental variables. The intergroup similarity can be measured via single-linkage, complete linkage or by group average. Therefore, hierarchical clustering should be treated with caution as using different methods generates in different dendrograms.

Our microarray data were clustered using Partek Genomic Suite and the results are shown in *Figure 4.2.4*. Hierarchy of the samples is indicated by the vertical dendrogram and is annotated by different colours for each set of triplicates. Gene clustering is specified by the horizontal dendrogram. Based on standardised intensities, the biological triplicates of each condition were similar and therefore cluster together. As already indicated by PCA untreated cultures (C-) and cultures treated with the IgM isotype control antibody in the absence of serum (IgM-) were very similar, indicating this control antibody had little effect of gene expression. However a completely different pattern was observed after cultures were incubated with mAb O4 alone (O4-) and this differed again from those derived from cultures exposed to serum whether or not mAb O4 was present. The latter observation indicates the effects of serum alone (C+) are more pronounced than those associated with antibody-mediated, complement-dependent demyelination.

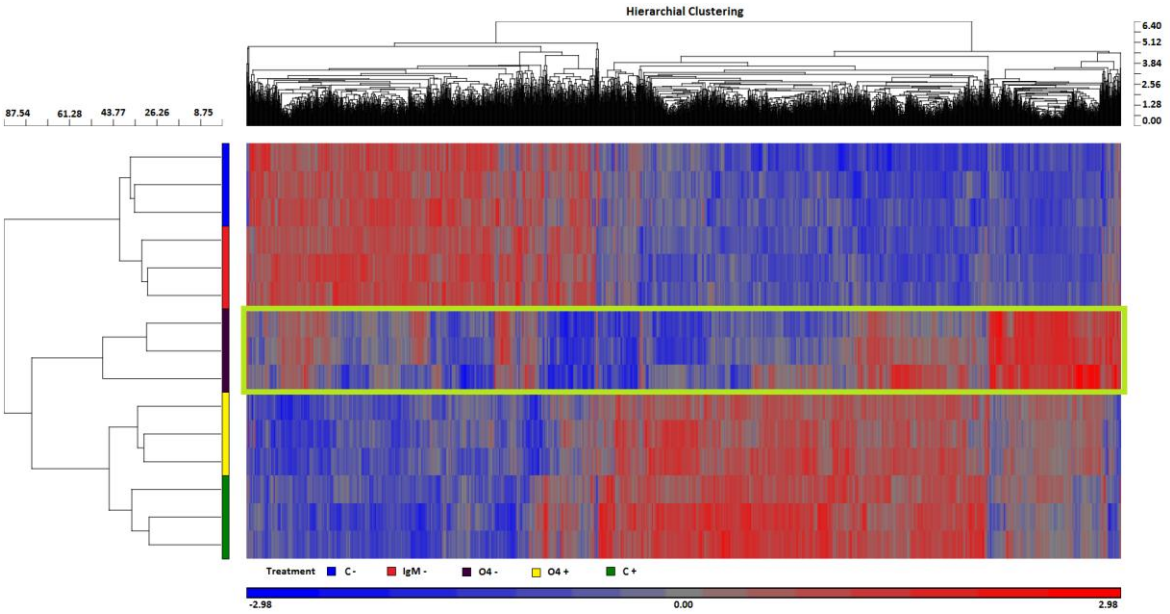


Figure 4.2.4: Hierarchical cluster analysis of antibody treated myelinating cultures. *This clustering displays signal intensities of 16,300 individual expressions relative to baseline. Only entities with a fold change of 1.4 or greater and -1.4 or lower were included. The signal intensity of each sample [untreated control (C-) blue; isotype control in the absence of serum (IgM-) red; mAb O4 in the absence of serum (O4-) purple; serum treated control (C+) green; mAb O4 in the presence of serum (O4+) yellow] is coloured according to their expression level relative to baseline. The algorithm scaled the normalised intensity to fit within the range -2.98 and 2.98. Green box marks the surprisingly different signal intensities of mAb O4 alone treatment.*

4.3 Gene expression analysis of Affymetrix chips using Partek Genomic Suite

Quality control processes should account for variation due to differences in RNA quality, sample labelling and hybridisation quality, so any differences observed between the sample groups should reflect genuine shifts in gene expression. To explore which of these changes may reflect biologically relevant effects normalised intensity values were analysed statistically using a GCRMA algorithm in Partek Genomic Suite. Differential expression analysis between groups was carried out by one-way ANOVA on the normalised expression values in combination with a Benjamini-Hochberg multiple testing corrections with a false discovery rate of 0.1. Initial gene lists were generated using the criteria ANOVA $\log_2$ fold change $\geq \pm 1.4$ with p-values ≤ 0.05.

4.3.1 The IgM isotype control antibody has no significant effects on gene expression in myelinating cultures

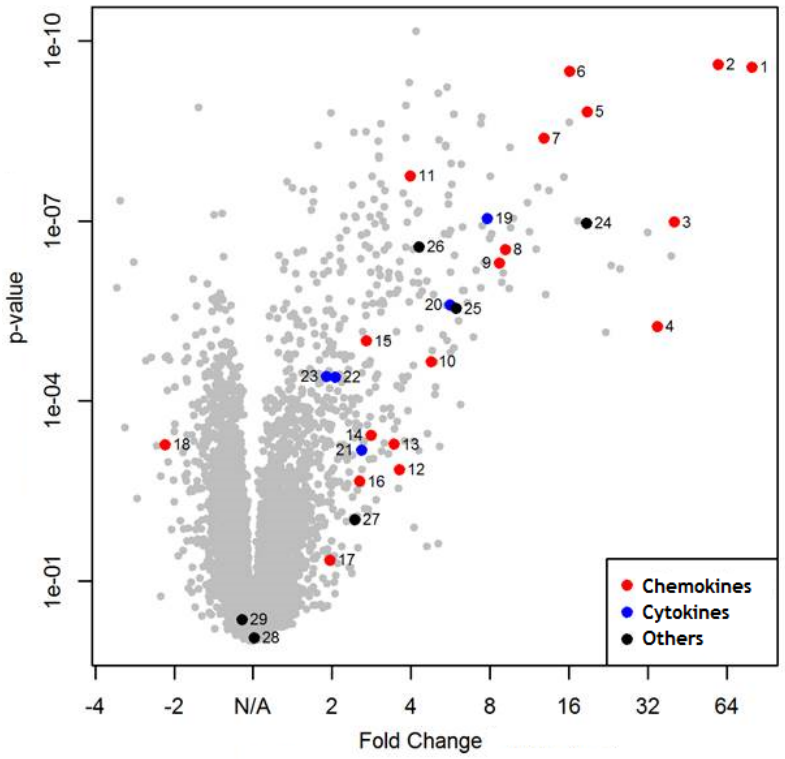
Comparison of untreated cultures and those exposed to the IgM isotype control antibody revealed the presence of this IgM had no significant effect on gene expression; threshold being set at ANOVA $\log_2$ fold change $\geqslant \pm 1.4$; p-value $\leqslant 0.05$). Thus, data from both untreated control and IgM isotype control treated cultures can be used as a baseline for comparative studies.

4.3.2 Genes identified as being differentially expressed in mAb O4 treated myelinating cultures

To investigate the effect of mAb O4 on gene expression in the absence of serum we compared the data sets obtained from cultures treated overnight with the IgM isotype control or mAb O4. The presence of mAb O4 was associated with the differential expression of 586 transcripts (ANOVA $\log_2$ fold change $\geq \pm 1.4$, p-values ≤ 0.05) of which 273 exhibited > 2.0-fold changes (*Appendix 8.1.1*). Presenting this data in the form of a Volcano plot (*Fig. 4.3.2*) demonstrates the effect of mAb O4 is skewed in favour of increased expression, 458 genes being up regulated against the 128 being down-regulated. The thirty-six most highly up regulated transcripts are listed in *Table 4.3.2*. Visual inspection of this data set reveals members of the chemokine family account for many of the most highly up regulated transcripts (9 of 36), in particular *Cxc11, Ccl9* and *Cxcl13,* the three genes most highly up regulated by mAb O4. In total, expression of 17 members of this family of 47 ligands was increased significantly, whereas only one family member was down regulated (*Ccl24*) (*Fig. 4.3.2*). In contrast, mAb O4 modulated the expression of only two chemokine receptors, *Ccr5* and *Cxcr5* which were up-regulated 1.9 and 1.5 fold respectively (*Appendix 8.1.1*). It should be noted mAb O4 also increased expression of a large number of other genes more commonly associated with innate immunity and infectious agents, including 2'-5'-oligoadenylate synthetase-like, myxovirus (influenza virus) resistance 1, *Il-1β, Il-1a* and *Cd14.*

Table 4.3.2: Differentially expressed genes in O4 antibody treated myelinating cultures in the absence of serum identified by Partek*. Microarray analysis was performed on Partek Genomic Suite. The data shown is sorted in manner of highest fold change to the cut-off of 1.4 in antibody treated cultures in comparison to isotype control treated cultures. Significance was calculated using one-way ANOVA. First 36 genes listed, rest can be found in the Appendix 8.1.1.*

Gene Symbol	Gene Name	Fold-Change	P-value
Cxcl11	chemokine (C-X-C motif) ligand 11	79.74	2.76E-10
Cxcl9	chemokine (C-X-C motif) ligand 9	59.29	2.49E-10
Cxcl13	chemokine (C-X-C motif) ligand 13	40.25	1.05E-07
Ifit3	interferon-induced protein with tetratricopeptide repeats 3	39.28	3.84E-07
Cxcl10	chemokine (C-X-C motif) ligand 10	34.87	5.75E-06
Oasl	2'-5'-oligoadenylate synthetase-like	32.03	1.55E-07
Ifit2	interferon-induced protein with tetratricopeptide repeats 2	25.12	6.36E-07
Mx1	myxovirus (influenza virus) resistance 1	23.25	5.58E-07
Rsad2	radical S-adenosyl methionine domain containing 2	22.19	7.29E-06
Cxcl17	chemokine (C-X-C motif) ligand 17	18.81	1.52E-09
Irf7	interferon regulatory factor 7	18.67	1.09E-07
Isg20	interferon stimulated exonuclease gene 20	17.34	1.01E-07
Gbp5	guanylate binding protein 5	16.10	2.29E-09
Ccl5	chemokine (C-C motif) ligand 5	16.05	3.26E-10
Apol9a	apolipoprotein L 9a	15.35	1.87E-08
F10	coagulation factor X	13.51	3.13E-08
Ddx60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60	13.06	1.70E-06
Zbp1	Z-DNA binding protein 1 NM_133564	12.93	1.10E-07
Ccl7	chemokine (C-C motif) ligand 7	12.86	4.22E-09
Lcn2	lipocalin 2	12.15	2.75E-08
Isg15	ISG15 ubiquitin-like modifier	12.04	2.96E-07
Usp18	ubiquitin specific peptidase 18	11.70	3.11E-06
LOC685067	similar to guanylate binding protein family, member	11.32	1.52E-07
LOC100366216	nuclear antigen Sp100-like	11.14	4.99E-08
Oas2	2'-5' oligoadenylate synthetase 2	9.82	9.20E-08
Lgals9	lectin, galactoside-binding, soluble, 9	9.60	1.31E-07
RT1-T24-3	RT1 class I, locus T24, gene 3	9.56	6.03E-09
Oas1b	2-5 oligoadenylate synthetase 1B	9.48	1.33E-06
Ccl19	chemokine (C-C motif) ligand 19	9.15	2.99E-07
Serpine1	serpin peptidase inhibitor, clade E (nexin, plasminog	8.99	7.34E-07
Cd72	Cd72 molecule	8.89	1.07E-06
Ccl12	chemokine (C-C motif) ligand 12	8.70	5.01E-07
Clec4a2	C-type lectin domain family 4, member A2	8.15	1.64E-07
Ifi27l2b	interferon, alpha-inducible protein 27 like 2B	8.05	1.85E-08
Mpa2l	macrophage activation 2 like	7.99	1.98E-07
Il1b	interleukin 1 beta	7.81	9.17E-08



#	Gene	Fold Change	p-value	Rank
Chemokines				
1	*Cxcl11	79.74	2.76E-10	1
2	Cxcl9	59.29	2.49E-10	2
3	*Cxcl13	40.25	1.05E-07	3
4	Cxcl10	34.87	5.75E-06	5
5	Cxcl17	18.81	1.52E-09	10
6	*Ccl5	16.05	3.26E-10	14
7	*Ccl7	12.86	4.22E-09	19
8	Ccl19	9.15	2.99E-07	29
9	Ccl12	8.70	5.01E-07	32
10	Ccl4	4.76	2.19E-05	82
11	Cxcl16	3.97	1.79E-08	102
12	Ccl3	3.62	5.36E-04	120
13	Cxcl1	3.45	5.24E-04	129
14	*Ccl20	2.82	3.74E-04	172
15	*Ccl2	2.71	9.99E-06	186
16	Cxcl2	2.55	1.39E-03	205
17	Ccl9	1.96	4.42E-02	304
18	Ccl24	-2.17	5.36E-04	16374
Interleukins				
19	*Il-1b	7.81	9.17E-08	36
20	Il-1a	5.65	2.50E-06	61
21	Il-18	2.60	6.49E-04	198
22	Il-7	2.06	4.00E-05	283
23	Il-10	1.91	3.90E-05	336
Others				
24	*Irf7	18.67	1.09E-07	11
25	*Cd14	5.94	2.88E-06	52
26	*Socs1	4.29	2.67E-07	90
27	*Tnf	2.44	9.47E-03	220
28	*Ifn-γ	1.01	8.81E-01	7497
29	*Ifn-α4	-1.10	4.36E-01	14292

Figure 4.3.2: Volcano plot demonstrating significance and expression values of chemokines, cytokines and other genes of interest in antibody treated myelinating cultures*. Chemokines (red), cytokines (blue) and others (black). The highest rank corresponds to the highest fold change indicated by ANOVA. Genes marked by * have been validated by qPCR.*

4.3.3 Genes identified in serum only treated myelinating cultures

Cluster analysis indicated that the presence of serum alone also induced significant changes in gene expression; however, these differed from the effect of mAb O4. Analysis of genes differently expressed in serum treated cultures compared to the untreated controls demonstrated this to be the case. The presence of serum was associated with differential expression of 959 transcripts (619 up-regulated, 340 down-regulated, ANOVA $\log_2$ fold change ≥ ± 1.4, p-values ≤ 0.05) of which 232 exhibited > 2.0-fold changes (*Fig. 4.3.3a*; *Appendix 8.1.2*). Again visual inspection of this data reveals many of the most differentially expressed genes belong to the chemokine family, although their ranking order differs from that seen in mAb O4 treated cultures (*Table 4.3.3b*) e.g. fold changes for *Cxcl13* (+40.25) and *Cxcl17* (+18.81) were far higher in mAb O4 treated cultures, than in cultures treated with serum (fold changes +3.45 and +2.30, respectively). Another interesting difference was found in the expression of *Ccl24*; in serum alone cultures it was up-regulated (fold change +2.10), whereas in mAb O4 treated cultures it was down-regulated (fold change -2.17).

Table 4.3.3a: Differentially expressed genes in serum alone treated myelinating cultures identified by Partek Genomic Suite. *These data show the fold change and significance of the entities that were up-regulated ≥ 1.4 serum treated cultures in comparison to control cultures. Significance was calculated using one-way ANOVA. First 36 genes listed, rest can be found in the Appendix 8.1.2.*

Gene Symbol	Gene Name	Fold Change	p-value
Lcn2	lipocalin 2	39.40	6.41E-10
Cxcl11	chemokine (C-X-C motif) ligand 11	34.86	2.15E-09
Ccl7	chemokine (C-C motif) ligand 7	28.72	2.89E-10
Cxcl5	chemokine (C-X-C motif) ligand 5	27.14	1.95E-05
Cxcl1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	23.12	1.69E-07
LOC363060	similar to RIKEN cDNA 1600029D21	14.93	9.30E-07
Cxcl9	chemokine (C-X-C motif) ligand 9	12.50	2.70E-08
Serpine1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	10.06	4.61E-07
Cxcl2	chemokine (C-X-C motif) ligand 2	9.78	1.64E-06
Cxcl10	chemokine (C-X-C motif) ligand 10	9.23	2.89E-04
Trem1	triggering receptor expressed on myeloid cells 1	9.20	1.72E-05
C3	complement component 3	8.92	5.49E-08
Mx1	myxovirus (influenza virus) resistance 1	7.95	2.38E-05
Ccl20	chemokine (C-C motif) ligand 20	7.86	1.07E-06
Ass1	argininosuccinate synthase 1	7.63	9.18E-07
Ifit3	interferon-induced protein with tetratricopeptide repeats 3	7.60	7.44E-05
Gbp5	guanylate binding protein 5	7.53	5.06E-08
Tgm1	transglutaminase 1	7.01	3.59E-07
Ptx3	pentraxin 3, long	6.96	9.55E-06
Il1rn	interleukin 1 receptor antagonist	6.74	1.11E-07
Slpi	secretory leukocyte peptidase inhibitor	6.49	2.13E-04
Bcl3	lymphoma 3	6.48	4.44E-06
Sell	selectin L	6.10	1.15E-03
Ccl6	chemokine (C-C motif) ligand 6	5.88	7.46E-04
Irf7	interferon regulatory factor 7	5.76	1.23E-05
Zc3h12a	zinc finger CCCH type containing 12A	5.60	1.35E-07
Birc3	baculoviral IAP repeat-containing 3	5.36	2.76E-09
Cd93	CD93 molecule	5.32	5.93E-07
Tnfaip2	tumor necrosis factor, alpha-induced protein 2	5.21	4.11E-09
Msr1	macrophage scavenger receptor 1	5.05	8.77E-06
Ccl4	chemokine (C-C motif) ligand 4	5.05	1.58E-05
Ifit2	interferon-induced protein with tetratricopeptide repeats 2	4.90	2.83E-04
Apol9a	apolipoprotein L 9a	4.80	3.40E-06
Rbp1	retinol binding protein 1, cellular	4.80	2.15E-06
Isg15	ISG15 ubiquitin-like modifier	4.71	2.15E-05
Mmp9	matrix metallopeptidase 9	4.69	7.11E-03

Table 4.3.3b: Differential expression of chemokines and their receptors by mAb O4 and serum.

mAb O4 alone					Serum alone			
Rank	**Gene**	**Fold Change**	**p-value**		**Rank**	**Gene**	**Fold Change**	**p-value**
1	*Cxcl11*	79.74	2.76E-10		2	*Cxcl11*	34.86	2.15E-09
2	*Cxcl9*	59.29	2.49E-10		3	*Ccl7*	28.72	2.89E-10
3	*Cxcl13*	40.25	1.05E-07		4	*Cxcl5*	27.14	1.95E-05
5	*Cxcl10*	34.87	5.75E-06		5	*Cxcl1*	23.12	1.69E-07
10	*Cxcl17*	18.81	1.52E-09		7	*Cxcl9*	12.50	2.7E-08
14	*Ccl5*	16.05	3.26E-10		9	*Cxcl2*	9.78	1.64E-06
19	*Ccl7*	12.86	4.22E-09		10	*Cxcl10*	9.23	0.000289
29	*Ccl19*	9.15	2.99E-07		14	*Ccl20*	7.86	1.07E-06
32	*Ccl12*	8.70	5.01E-07		24	*Ccl6*	5.88	0.000746
82	*Ccl4*	4.76	2.19E-05		30	*Ccl4*	5.05	1.58E-05
102	*Cxcl16*	3.97	1.79E-08		41	*Ccl2*	4.57	2.09E-07
120	*Ccl3*	3.62	0.000536		44	*Ccl5*	4.40	1.45E-07
129	*Cxcl1*	3.45	0.000524		59	*Cxcl16*	3.67	3.14E-08
172	*Ccl20*	2.82	0.000374		71	*Cxcl13*	3.45	0.001169
186	*Ccl2*	2.71	9.99E-06		78	*Ccl3*	3.22	0.002625
205	*Cxcl2*	2.55	0.001393		108	*Ccl12*	2.78	0.000325
304	*Ccl9*	1.96	0.044243		161	*Cxcl17*	2.30	0.000154
331	*Ccr5*	1.88	1.18E-04		213	*Ccl24*	2.10	0.000755
503	*Cxcr5*	1.54	2.08E-04		281	*Ccl19*	1.89	0.00625
16374	*Ccl24*	-2.17	0.000536					

4.3.4 Genes differentially expressed in response to antibody-mediated, complement-dependent demyelination (mAb O4 plus serum)

To identify genes differentially expressed in response to antibody-mediated demyelination we compared cultures treated with serum in the presence or absence of mAb O4. This analysis identified 21 differentially expressed genes (ANOVA log2 fold change ≥ ± 1.4, p-values ≤ 0.05) (*Table 4.3.4*) of which 4 were up-regulated and 17 down-regulated. In concordance with the observed loss of myelin induced by mAb O4 in the presence of serum, nine of these down regulated genes are expressed by oligodendrocytes and play important roles in myelination (Devaux and Gow, 2008; Soundarapadian et al., 2011; Van Strien, et al., 2011; Yao et al., 2012). Surprisingly no further enhancement of chemokine or immune gene expression was recognised.

Table 4.3.4: Differentially expressed genes in serum alone treated myelinating cultures identified by Partek*. These data show the fold change and significance of the entities that were up-regulated ⩾ 1.4 serum treated cultures in comparison to control cultures. Significance was calculated using one-way ANOVA. Genes marked by * are myelin/oligodendrocyte associated.*

Gene Symbol	Gene Name	Fold Change	p-value
Siglec5	sialic acid binding Ig-like lectin 5	5.46	5.96E-06
Clec4a2	C-type lectin domain family 4, member A2	2.92	6.68E-05
Tlr5	toll-like receptor 5	2.13	7.83E-05
Irg1	immunoresponsive gene 1	2.05	3.85E-05
Myo1d	myosin ID	-1.45	9.96E-06
**Sox10*	SRY (sex determining region Y)-box 10	-1.70	4.47E-05
**Plp1*	proteolipid protein 1	-1.75	1.47E-08
Gpr17	G protein-coupled receptor 17	-1.95	4.78E-05
Hhip	Hedgehog-interacting protein	-1.97	5.16E-06
Rdh5	retinol dehydrogenase 5 (11-cis/9-cis)	-1.99	6.95E-05
Map6d1	MAP6 domain containing 1	-2.02	1.38E-07
**Zfp488*	zinc finger protein 488	-2.12	4.28E-05
Fa2h	fatty acid 2-hydroxylase	-2.25	1.70E-08
**Myrf*	myelin regulatory factor	-2.26	7.16E-05
Ugt8	UDP glycosyltransferase 8	-2.33	1.91E-07
Plxnb3	plexin B3	-2.34	7.05E-08
**Cldn11*	claudin 11	-2.42	2.91E-05
**Mir2964*	microRNA mir-2964	-2.71	1.31E-05
**Mag*	myelin-associated glycoprotein	-3.00	3.07E-08
**Mog*	myelin oligodendrocyte glycoprotein	-3.17	3.58E-06
**Tgm1*	transglutaminase 1	-3.24	3.36E-05

4.4 Gene ontology clustering and pathway analysis

The initial gene ontology (GO) clustering was performed on Partek Genomic Suite to group genes on the basis of their functional hierarchy. The genes are functionally clustered according to three different factors, biological processes, molecular functions and cellular components. We performed GO clustering on differentially expressed genes with a fold change of 1.4 or higher with significance greater than 0.05. This was followed by pathway analysis on Partek Pathway™ that allows unbiased biological interpretation of the differentially expressed genes and groups them into influential pathways by utilising the KEGG database for rat.

Analysis of the Affymetrix dataset on Partek Genomic Suite generated long lists of differentially expressed genes for each treatment. To further analyse and validate this data we used two additional programmes, Panther and Interferome.

Panther (protein analysis through evolutionary relationships) Classification System 9.0 (http://www.pantherdb.org) is free online software that allows functional organisation of proteins and their genes. The system contains 7180 protein families that can be further divided into 52,768 functional subfamilies. The genes are mapped according to their biological functions by determining significant enrichment of functionally related genes. This then allows determination of the biological relevance of the data. Statistical enrichment is defined by the use of Mann-Whitney test that determines non-random distribution of numeric values in respect to the entire list of values. Expression of genes was considered significant whenever $P < 0.05$. Panther Gene Ontology™ allows organisation of proteins and their genes according to their molecular function, biological processes and cellular components. Panther Pathway Analysis consists of 176 pathways with over 21,000 components, therefore permitting mapping of the differently expressed genes into functional pathways.

Interferome v2.01 (http://interferome.its.monash.edu.au/interferome/home.jspx), is free online database that allows determination of type I, II and III interferon (IFN) regulated gene involvement. IFNs regulate cell proliferation, survival, migration and specialised functions in homeostatic and pathological processes, thus being heavily involved in infections, autoimmunity and inflammation. The programme has a default limit of 2.0 fold change for the gene expression, which we maintained in our search. Interferome database consists of human and mouse experiments, thus this can only be used as indicator as our experiments were performed on rat myelinating cultures.

4.4.1 Gene ontology clustering of cultures treated with O4 antibody in the absence of serum

When GO clustering was performed on the differentially expressed gene list obtained when mAb O4 treated cultures compared to those treated with IgM isotype control, 1358 biological processes were enriched with higher than $P<0.05$.

The ten most highly enriched processes identified using Partek Genomic Suite are listed in *Table 4.4.1a*. These processes are in general all associated with different aspects of immunity and/or responses to pathogens.

***Table 4.4.1a: Top 10 significantly enriched biological processes by Partek Genomic Suite**. Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the antibody in comparison to isotype treated cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	O4- vs IgM-score
immune system process	162.80	1.97E-71	19.71	5.02
response to external biotic stimulus	115.01	1.13E-50	20.19	4.98
response to external stimulus	113.32	6.10E-50	14.24	4.96
response to biotic stimulus	113.08	7.77E-50	19.60	4.98
immune response	113.05	8.00E-50	23.88	5.09
defense response	111.20	5.10E-49	20.12	5.11
regulation of immune system process	90.82	3.60E-40	16.45	4.90
positive regulation of immune system process	86.10	4.05E-38	20.91	5.01
response to cytokine	76.22	7.93E-34	20.00	5.33
response to other organism	75.21	2.18E-33	21.41	5.19

To complement this data we reanalysed this set of differentially expressed genes using Panther. This identified 20 biological processes that were significantly altered (*Fig. 4.4.1a*). Once again the most highly enriched processes were involved in various aspects of the immune response.

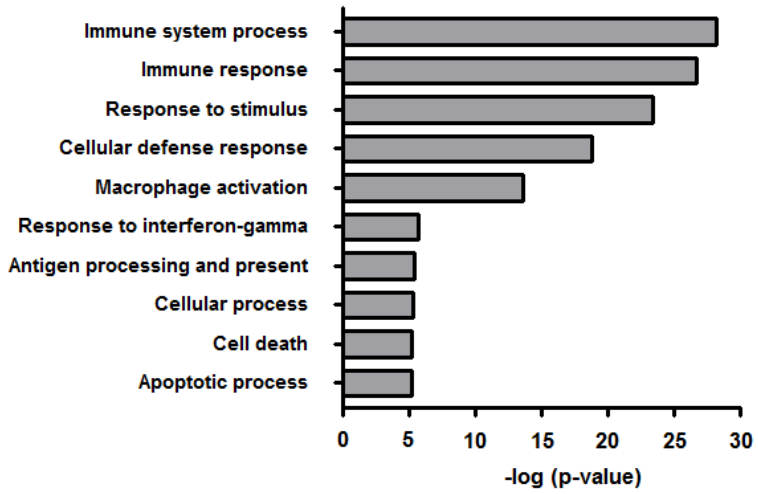


***Figure 4.4.1a: Top 10 enriched biological processes**. Using Panther software, gene ontology clustering was performed on the genes differentially expressed by ≥ 1.4-fold in the cultures treated with mAb O4compared to isotype cultures alone. The co-expression of functionally-related gene clusters was rated in order of significance.*

These analyses indicate that addition of mAb O4 to the myelinating cultures results in a transcriptional response that suggests binding of mAb O4 to the myelin/oligodendrocyte surface induces a state of 'heightened immune awareness'. This concept is supported by the observation that the most highly enriched molecular functions identified using Partek Genomic Suite (*Table 4.4.1b*) and Panther (*Fig. 4.4.1b*) are associated with over representation and enrichment of functions associated with cytokine/chemokine signalling.

Table 4.4.1b: Top 10 enriched molecular functions by Partek Genomic Suite. *Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the antibody in comparison to isotype treated cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	O4- vs IgM-score
cytokine receptor binding	37.07	7.96E-17	20.61	5.17
chemokine receptor binding	33.36	3.24E-15	44.74	5.82
cytokine activity	27.90	7.67E-13	20.00	5.42
binding	26.53	3.02E-12	4.58	4.75
receptor binding	25.06	1.30E-11	8.14	4.90
CCR chemokine receptor binding	20.85	8.79E-10	56.25	6.01
protein binding	20.44	1.33E-09	5.16	4.68
double-stranded RNA binding	18.52	9.09E-09	27.08	5.57
carbohydrate derivative binding	15.50	1.85E-07	6.18	4.99
G-protein coupled receptor binding	14.31	6.07E-07	12.15	5.45

Panther analysis found only eight molecular functions (*Fig. 4.4.1b*) to be significantly enriched. Again, the findings were similar to findings on Partek, as cytokine and chemokine activity, and receptor binding were found to be most highly overrepresented. Also peptidase and enzyme inhibitory activity were significantly overrepresented.

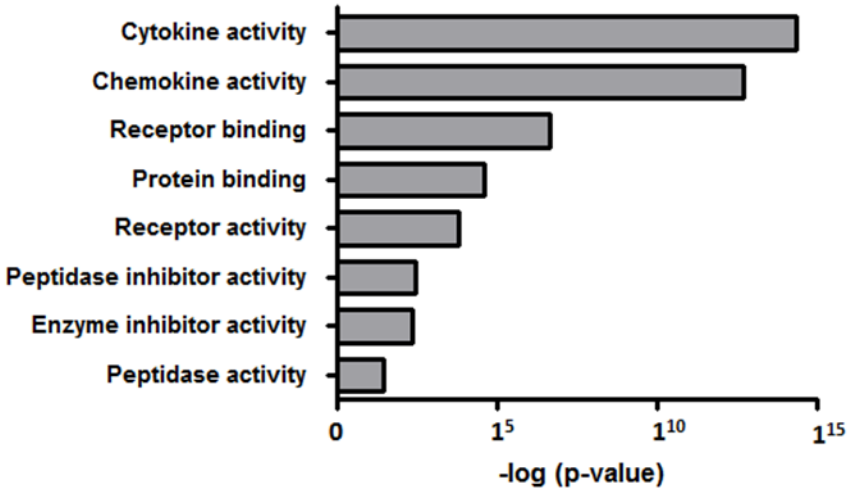


Figure 4.4.1b: Significant alteration of molecular function*. Reassessment of antibody treated cultures by Panther in similar manner as previously stated.*

Analysis using the Partek Genomic suite also identified significant enrichment for 90 cellular components. The ten most highly enriched are listed in *Table 4.4.1c* and include plasma membrane, cytoplasm and MHC protein complex. In contrast Panther analysis only identified enrichment for MHC protein complex.

Table 4.4.1c: Top 10 enriched cellular components by Partek Genomic Suite*. Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the antibody in comparison to isotype treated cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	O4- vs. IgM-score
plasma membrane part	35.19	5.20E-16	8.38	4.41
extracellular region part	23.22	8.25E-11	6.39	5.06
external side of plasma membrane	22.19	2.31E-10	15.17	4.76
extracellular space	21.60	4.14E-10	8.30	4.99
side of membrane	19.71	2.75E-09	13.21	4.70
cell surface	17.99	1.54E-08	10.66	4.58
MHC protein complex	16.94	4.40E-08	29.73	5.31
cytoplasm	15.49	1.87E-07	5.33	4.73
receptor complex	13.15	1.95E-06	11.73	4.44
vesicle	12.09	5.60E-06	5.67	5.10

The above analyses indicate that although mAb O4 is unable to induce acute demyelination in the absence of an exogenous source of complement, it triggers

a rapid transcriptional response involving genes commonly associated with innate and adaptive immunity. These include a number of interferon sensitive genes (ISG) which prompted us to use Interferome to analyse the 273 genes that were up regulated ≥ 2.0 –fold in this data set. Expression of the majority of these differentially expressed genes across all data bases was reported to be modulated by interferons. Limiting the search to data obtained using the nervous system revealed 53% (144 genes) were interferon sensitive. These genes were regulated by type I and II interferons, in most cases by both (*Fig. 4.4.1c*). Type I interferons are involved in immune and inflammatory regulations, when type II IFNs have been implicated in tumour growth inhibition.

Figure 4.4.1c: Interferon regulated genes identified in the differentially regulated gene list*. Interferon sensitive genes were found using Interferome software. Search limited to nervous system identified our genes to be type I and II specific.*

Pathway Analysis of serum independent antibody effect

Analysis of the differentially expressed gene list (mAb O4 vs IgM treated cultures) with Partek Pathway™ analysis software identified 29 enriched human disease pathways (*Table 4.4.1d*). These include a wide variety of viral, bacterial and parasitic disease associated pathways that share many common features in particular induction of chemokines (*Ccl2, Ccl5, Cxcl1, Cxcl2, Cxcl11*), interleukins (*Il-1a, Il-1β, Il-18*), and interferon regulatory factors (*Irf7, Irf9*).

Table 4.4.1d: Significantly altered human disease pathways. *Using Partek Pathway™ analysis software, differentially expressed genes with fold change of 1.4 or higher were organised in pathways in the cultures independently treated with monoclonal O4 antibody.*

Pathway Name	Function	% genes present in pathway	Enrichment Score	Enrichment p-value
Herpes simplex infection	Infectious diseases: Viral	24.68	42.14	5.00E-19
Influenza A	Infectious diseases: Viral	25.41	35.26	4.84E-16
Legionellosis	Infectious diseases: Bacterial	40.00	29.54	1.49E-13
Leishmaniasis	Infectious diseases: Parasitic	33.33	25.86	5.86E-12
Viral myocarditis	Cardiovascular diseases	28.99	25.67	7.10E-12
Tuberculosis	Infectious diseases: Bacterial	20.77	25.55	8.01E-12
Graft-versus-host disease	Immune diseases	34.69	25.23	1.11E-11
Staphylococcus aureus infection	Infectious diseases: Bacterial	40.00	23.20	8.37E-11
Type I diabetes mellitus	Endocrine and metabolic diseases	30.36	22.79	1.26E-10
Allograft rejection	Immune diseases	31.37	22.07	2.60E-10
Rheumatoid arthritis	Immune diseases	26.47	21.55	4.39E-10
Autoimmune thyroid disease	Immune diseases	28.07	20.22	1.65E-09
Measles	Infectious diseases: Viral	20.21	17.78	1.89E-08
Pertussis	Infectious diseases: Bacterial	25.00	17.29	3.09E-08
Malaria	Infectious diseases: Parasitic	30.00	16.28	8.54E-08
Viral carcinogenesis	Cancers: Overview	14.19	13.78	1.03E-06
Salmonella infection	Infectious diseases: Bacterial	20.59	13.64	1.19E-06
Epstein-Barr virus infection	Infectious diseases: Viral	13.02	12.30	4.53E-06
Chagas disease (American trypanosomiasis)	Infectious diseases: Parasitic	17.50	11.62	9.00E-06
HTLV-I infection	Infectious diseases: Viral	11.36	11.35	1.18E-05
Toxoplasmosis	Infectious diseases: Parasitic	16.47	10.89	1.86E-05
African trypanosomiasis	Infectious diseases: Parasitic	30.43	10.07	4.24E-05
Hepatitis C	Infectious diseases: Viral	15.48	9.53	7.29E-05
Hepatitis B	Infectious diseases: Viral	13.13	7.83	3.97E-04
Asthma	Immune diseases	21.74	5.83	2.93E-03
Systemic lupus erythematosus	Immune diseases	12.35	5.83	2.95E-03
Amoebiasis	Infectious diseases: Parasitic	10.00	4.01	1.81E-02
Amyotrophic lateral sclerosis (ALS)	Neurodegenerative diseases	12.20	3.38	3.41E-02
Prion diseases	Neurodegenerative diseases	13.79	3.27	3.80E-02

We also identified 13 enriched organismal system pathways (*Table 4.4.1e*) which with the exception of the osteoclast differentiation pathway are all involved/associated with immune functions. The immune system pathways involved factors including chemokines (*Ccl2, Ccl4, Ccl5*), interleukins (*Il-1a, Il-1B*) and *Cd80*.

Table 4.4.1e: Significantly altered organismal system pathways determined using Partek Pathway™. *The enriched pathways were determined as previously stated.*

Pathway Name	Function	% genes present in pathway	Enrichment Score	Enrichment p-value
NOD-like receptor signaling pathway	Immune system	37.78	26.84	2.21E-12
Chemokine signaling pathway	Immune system	19.72	25.14	1.21E-11
Antigen processing and presentation	Immune system	28.17	25.08	1.28E-11
Toll-like receptor signaling pathway	Immune system	26.67	23.97	3.88E-11
Cytosolic DNA-sensing pathway	Immune system	34.78	23.85	4.39E-11
Osteoclast differentiation	Development	18.29	12.94	2.40E-06
RIG-I-like receptor signaling pathway	Immune system	22.64	12.93	2.43E-06
Leukocyte transendothelial migration	Immune system	14.89	9.73	5.95E-05
Natural killer cell mediated cytotoxicity	Immune system	15.38	8.83	1.47E-04
Hematopoietic cell lineage	Immune system	15.15	7.43	5.95E-04
Intestinal immune network for IgA production	Immune system	14.29	4.00	1.83E-02
Complement and coagulation cascades	Immune system	12.50	3.98	1.88E-02
B cell receptor signaling pathway	Immune system	11.76	3.70	2.47E-02

Ten other enriched pathways (*Table 4.4.1f*) were also noted to be altered in the antibody treated cultures in comparison to their isotype control, these involved environmental and genetic information processing, cellular processes and metabolism.

Table 4.4.1f: Other significantly enriched pathways identified on Partek Pathway™. *The determination was done as previously stated.*

Pathway Name	Class	Function	% genes present in pathway	Enrichment Score	Enrichment p-value
Phagosome	Cellular Processes	Transport and catabolism	23.13	32.42	8.35E-15
Cytokine-cytokine receptor interaction	Environmental Information Processing	Signaling molecules and interaction	18.34	25.76	6.50E-12
Cell adhesion molecules (CAMs)	Environmental Information Processing	Signaling molecules and interaction	21.19	24.17	3.17E-11
NF-kappa B signaling pathway	Environmental Information Processing	Signal transduction	24.24	17.89	1.70E-08
Apoptosis	Cellular Processes	Cell growth and death	21.43	12.31	4.52E-06
Jak-STAT signaling pathway	Environmental Information Processing	Signal transduction	10.58	5.08	6.21E-03
Proteasome	Genetic Information Processing	Folding, sorting and degradation	14.63	4.72	8.90E-03
Primary bile acid biosynthesis	Metabolism	Lipid metabolism	23.08	4.02	1.79E-02
SNARE interactions in vesicular transport	Genetic Information Processing	Folding, sorting and degradation	15.38	3.63	2.65E-02
Endocytosis	Cellular Processes	Transport and catabolism	8.02	3.58	2.78E-02

Reanalysis of the significantly enriched gene set using Panther provided similar data in that five pathways were statistically overrepresented in particular inflammation pathway mediated by chemokine and cytokine signalling (*Fig. 4.4.1d*) that involved 25 out of the 241 differentially enriched genes including *Ccl2, Ccl5, Ccl7* and *Ccl20.*

Using statistical enrichment test on Panther recognised only immune response pathway to be to be significant. It involved 71 differentially enriched genes, including interleukins (*Il-1a, Il-1β*), interferon factors (*Irf7, Irf9*), tumor necrosis factor involved genes (*Tnf-aip2, Tnf-rsf1β, Tnf-sf10*).

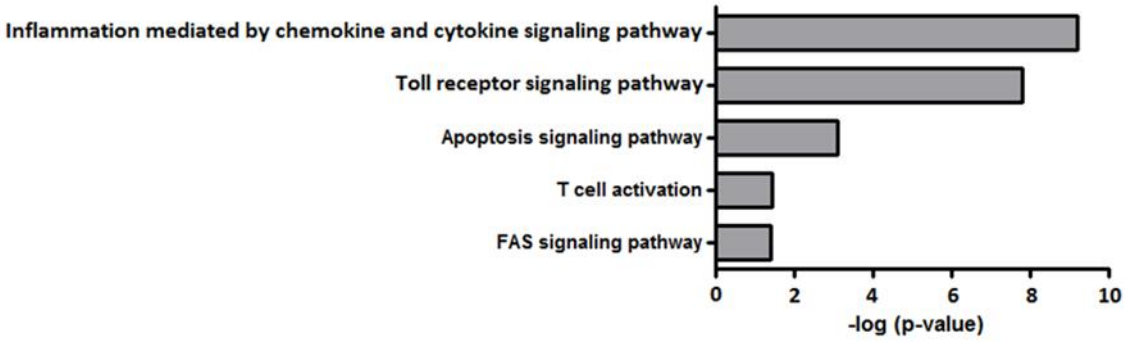


Figure 4.4.1d: Significantly altered biological pathways*. Panther Pathway analysis of genes upregulated by ≥ 1.4 fold change in complement independent antibody mediated effector mechanisms in myelinating cultures. Bonferroni correction for multiple testing was used to calculate the significance.*

4.4.2 Gene ontology clustering of cultures treated with serum alone

Presence of serum alone in the myelinating cultures indicated significantly regulated genes to be grouped into biological functions that respond to different stimuli, including stress, organic substance and biotic stimuli (*Table 4.4.2a*). Overall, Partek Genomic Suite analysis found 1449 biological processes to be enriched.

Table 4.4.2a: Top 10 enriched biological processes by Partek Genomic Suite*. Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the serum treated cultures in comparison to control cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	C+ vs C- score
response to external stimulus	75.67	1.38E-33	15.83	4.45
single-organism process	67.09	7.31E-30	8.17	4.17
response to external biotic stimulus	66.77	1.00E-29	19.81	4.36
response to biotic stimulus	63.36	3.05E-28	19.05	4.36
response to stress	62.87	4.95E-28	12.57	4.29
positive regulation of biological process	54.45	2.26E-24	10.09	4.25
response to organic substance	53.13	8.45E-24	12.02	4.31
single-organism cellular process	53.04	9.27E-24	8.40	4.22
defense response	52.92	1.04E-23	18.16	4.75
response to lipopolysaccharide	51.94	2.77E-23	24.42	4.53

When the microarray data was reanalysed on Panther, 26 significantly altered biological processes were recognised. Ten most highly altered processes presented in *Figure 4.4.2a*. These were involved in immune system processes and responses, and cellular death.

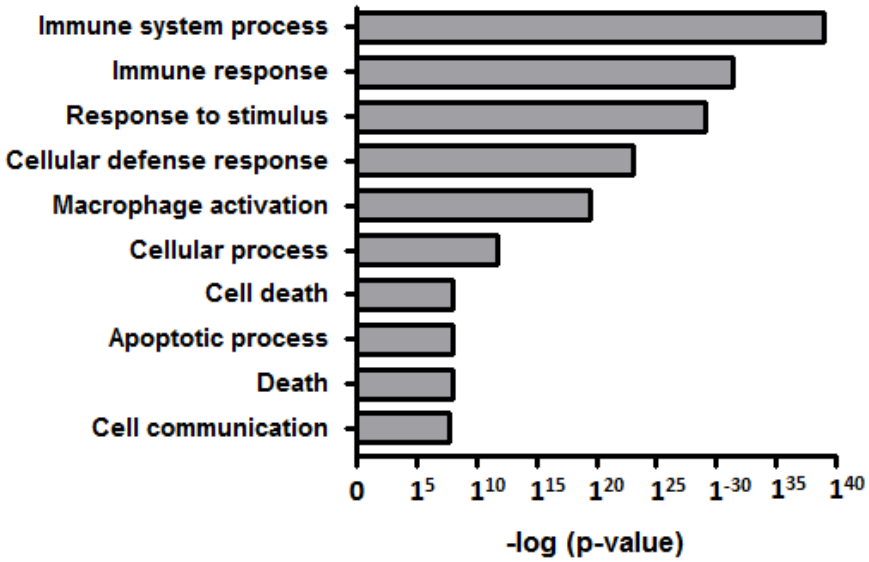


***Figure 4.4.2a: Top 10 altered biological processes**. Panther analysis of differentially expressed gene list in serum treated cultures compared to control. Analysis was performed similar to the method explained in section 4.4.1.*

In serum alone compared to untreated cultures, Partek Genomic Suite analysis reported 204 significant molecular functions. These, as in the mAb O4 treated cultures, were involved in chemokine activity and receptor binding (*Table 4.4.2b*). This was further recognised by using Panther analysis programme (*Fig. 4.4.2b*).

***Table 4.4.2b: Top 10 enriched molecular function by Partek Genomic Suite**. Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the serum treated cultures in comparison to control cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	C+ vs C- score
chemokine receptor binding	33.97	1.76E-15	52.63	5.38
chemokine activity	33.05	4.45E-15	58.06	5.32
cytokine receptor binding	24.98	1.42E-11	21.21	4.80
binding	24.53	2.23E-11	6.95	4.21
protein binding	24.06	3.54E-11	7.90	4.16
CCR chemokine receptor binding	19.88	2.32E-09	62.50	5.08
receptor binding	19.31	4.11E-09	10.49	4.14
identical protein binding	17.95	1.60E-08	10.85	4.01
G-protein coupled receptor binding	15.64	1.62E-07	16.57	4.78
cytokine activity	14.82	3.68E-07	18.46	4.95

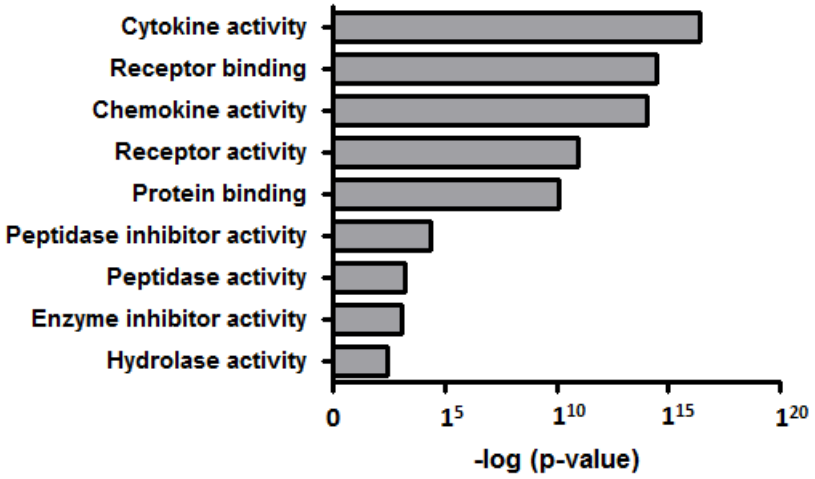


Figure 4.4.2b: Significantly altered molecular functions analysed on Panther.

Partek Genomic Suite analysis indicated genes differentially expressed by ≥ 1.4-fold to be grouped into 80 significantly enriched cellular components. Ten most significant components are shown in the *Table 4.4.2c*. Interestingly, these did not include the MHC protein complex function that was found to be the only enriched component by Panther analysis.

Table 4.4.2c: Top 10 enriched cellular components by Partek Genomic Suite. *Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the serum treated cultures in comparison to control cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	C+ vs C- score
extracellular region part	32.34	9.02E-15	9.93	4.55
extracellular space	30.40	6.29E-14	12.78	4.60
cytoplasm	22.74	1.33E-10	8.46	4.14
receptor complex	15.89	1.26E-07	16.76	4.66
vesicle	15.03	2.96E-07	8.69	4.69
plasma membrane part	13.99	8.43E-07	9.13	4.39
membrane-bounded vesicle	13.47	1.41E-06	8.58	4.72
extracellular vesicular exosome	11.86	7.07E-06	8.85	4.80
extracellular organelle	11.82	7.34E-06	8.84	4.80
extracellular membrane-bounded organelle	11.82	7.34E-06	8.84	4.80

Further analysis of the 232 genes regulated by ≥ 2.0-fold in serum only treatment on Interferome revealed 91% (212) to be ISGs. The number decreased to 100 genes (43%) after the search was limited to nervous system. These genes indicated specificity to type I or II, or them both (*Fig. 4.4.2c*), thus indicating a strong immunological signature.

Figure 4.4.2c: Serum alone compared to control treated differentially regulated genes are sensitive to type I and II interferons. *Interferome search limited to nervous system found 43% of the genes regulated by ≥ 2.0-fold to be interferon sensitive.*

Pathways in serum alone treated cultures

The genes differentially expressed in cultures treated with serum alone were grouped into human disease pathways by Partek Pathway™. These pathways function in different infectious diseases and cancer (*Table 4.4.2d*). The genes

present in these significantly enriched pathways include complement component 3 (*C3*), chemokines (*Ccl2, Ccl3, Ccl5*), interleukin (*Il-1a and Il-1B*), interferon factors (*Irf7, Irf9*), and *Rt1* genes.

Table 4.4.2d: Significantly altered human disease pathways. *Using Partek Pathway™ analysis software, differentially expressed genes with fold change of 1.4 or higher were organised in pathways in the cultures treated with serum alone.*

Pathway Name	Function	% genes present in pathway	Enrichment Score	Enrichment p-value
Influenza A	Infectious diseases: Viral	21.31	20.59	1.14E-09
Herpes simplex infection	Infectious diseases: Viral	18.83	19.77	2.60E-09
Legionellosis	Infectious diseases: Bacterial	33.33	18.84	6.57E-09
Rheumatoid arthritis	Immune diseases	22.06	12.83	2.67E-06
Pertussis	Infectious diseases: Bacterial	23.33	12.77	2.85E-06
HTLV-I infection	Infectious diseases: Viral	13.18	11.84	7.21E-06
Viral carcinogenesis	Cancers: Overview	14.84	11.57	9.49E-06
Salmonella infection	Infectious diseases: Bacterial	20.59	11.21	1.36E-05
Measles	Infectious diseases: Viral	17.02	10.10	4.12E-05
Graft-versus-host disease	Immune diseases	22.45	9.91	4.98E-05
Chagas disease (American trypanosomiasis)	Infectious diseases: Parasitic	17.50	9.30	9.15E-05
Type I diabetes mellitus	Endocrine and metabolic diseases	19.64	8.62	1.80E-04
Hepatitis B	Infectious diseases: Viral	15.15	8.19	2.77E-04
Hepatitis C	Infectious diseases: Viral	15.48	7.47	5.71E-04
Epstein-Barr virus infection	Infectious diseases: Viral	11.83	7.09	8.36E-04
Viral myocarditis	Cardiovascular diseases	15.94	6.75	1.17E-03
Malaria	Infectious diseases: Parasitic	20.00	6.71	1.22E-03
Allograft rejection	Immune diseases	17.65	6.46	1.56E-03
Tuberculosis	Infectious diseases: Bacterial	12.31	6.31	1.81E-03
Pathways in cancer	Cancers: Overview	10.53	6.11	2.21E-03
Leishmaniasis	Infectious diseases: Parasitic	16.67	6.05	2.36E-03
Autoimmune thyroid disease	Immune diseases	15.79	5.67	3.46E-03
Toxoplasmosis	Infectious diseases: Parasitic	12.94	5.07	6.27E-03
African trypanosomiasis	Infectious diseases: Parasitic	21.74	4.95	7.10E-03
Transcriptional misregulation in cancer	Cancers: Overview	10.08	3.54	2.89E-02
Renal cell carcinoma	Cancers: Specific types	12.24	3.00	4.96E-02

Altogether 10 organismal pathways were enriched in the Partek Pathway™ (*Table 4.4.2e*). The differentially expressed genes were mainly grouped in immune system pathways, apart from osteoclast differentiation, adipocytokine signalling and mineral absorption pathways that function in development, endocrine and digestive systems, respectively. The immune system pathways involved chemokines (*Ccl5, Cxcl10*), interleukins (*Il-1a, Il-1B*) and nuclear factor kappa-light-chain-enhancer of activated B cells (*NF-κB*).

Table 4.4.2e: Significantly enriched organismal system pathways identified by Partek Pathway™.

Pathway Name	Function	% genes present in pathway	Enrichment Score	Enrichment p-value
NOD-like receptor signaling pathway	Immune system	33.33	18.84	6.57E-09
Chemokine signaling pathway	Immune system	17.61	15.79	1.39E-07
Toll-like receptor signaling pathway	Immune system	22.67	14.80	3.75E-07
Cytosolic DNA-sensing pathway	Immune system	26.09	12.37	4.24E-06
RIG-I-like receptor signaling pathway	Immune system	22.64	10.79	2.06E-05
Antigen processing and presentation	Immune system	19.72	10.69	2.28E-05
Osteoclast differentiation	Development	18.29	10.42	2.99E-05
Adipocytokine signaling pathway	Endocrine system	13.21	3.71	2.44E-02
Hematopoietic cell lineage	Immune system	12.12	3.62	2.67E-02
Leukocyte transendothelial migration	Immune system	10.64	3.44	3.20E-02
Mineral absorption	Digestive system	13.51	3.01	4.94E-02

Other pathways (*Table 4.4.2f*) associated with the effect of addition of serum included pathways classified as indicative of environmental and genetic information processing, cellular processes, and metabolism.

Table 4.4.2f: Other significantly altered pathways in serum treated cultures identified using Partek Pathways™.

Pathway Name	Class	Group	% genes present in pathway	Enrichment Score	Enrichment p-value
Cytokine-cytokine receptor interaction	Environmental Information Processing	Signaling molecules and interaction	17.75	18.95	5.88E-09
NF-kappa B signaling pathway	Environmental Information Processing	Signal transduction	25.76	16.81	5.00E-08
Apoptosis	Cellular Processes	Cell growth and death	23.21	11.88	6.90E-06
Phagosome	Cellular Processes	Transport and catabolism	14.93	10.30	3.38E-05
HIF-1 signaling pathway	Environmental Information Processing	Signal transduction	16.47	8.62	1.80E-04
Jak-STAT signaling pathway	Environmental Information Processing	Signal transduction	14.42	7.65	4.78E-04
Cell adhesion molecules (CAMs)	Environmental Information Processing	Signaling molecules and interaction	13.56	7.37	6.31E-04
MAPK signaling pathway	Environmental Information Processing	Signal transduction	10.23	4.91	7.36E-03
Pyrimidine metabolism	Metabolism	Nucleotide metabolism	12.99	4.74	8.77E-03
Purine metabolism	Metabolism	Nucleotide metabolism	11.02	4.69	9.20E-03
One carbon pool by folate	Metabolism	Metabolism of cofactors and vitamins	25.00	4.65	9.52E-03
Cyanoamino acid metabolism	Metabolism	Metabolism of other amino acids	50.00	4.09	1.67E-02
DNA replication	Genetic Information Processing	Replication and repair	17.24	3.95	1.92E-02
Galactose metabolism	Metabolism	Carbohydrate metabolism	20.00	3.85	2.13E-02
Proteasome	Genetic Information Processing	Folding, sorting and degradation	14.63	3.78	2.29E-02
ErbB signaling pathway	Environmental Information Processing	Signal transduction	12.28	3.36	3.48E-02
Fanconi anemia pathway	Genetic Information Processing	Replication and repair	13.89	3.11	4.47E-02
Glycine, serine and threonine metabolism	Metabolism	Amino acid metabolism	16.00	3.10	4.50E-02
Cell cycle	Cellular Processes	Cell growth and death	10.34	3.05	4.75E-02

Reanalysis of the differentially expressed genes in the serum alone compared to control cultures on Panther (*Fig. 4.4.2d*) resulted in them being grouped into only four biological pathways that were significantly overrepresented.

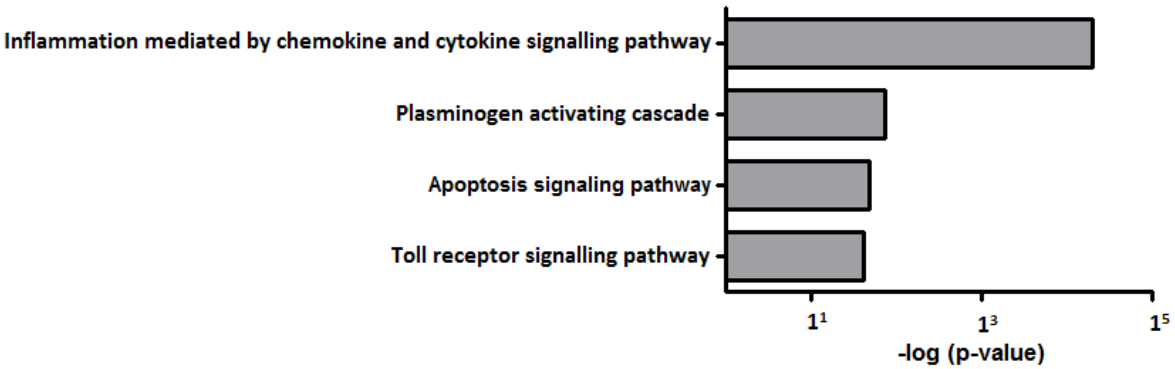


Figure 4.4.2d: Significantly overrepresented biological pathways determined using Panther software.

4.4.3 Gene ontology clustering of cultures treated with O4 antibody in the presence of serum

In previous chapter we observed complement dependent antibody mediated demyelination of myelinating cultures to occur after overnight incubation. Microarray data from this treatment in comparison to serum alone incubation interestingly resulted in only 4 genes (*Siglec5, Clec4a2, Tlr5, Irg1*) to be differentially expressed. When these were clustered together, 149 biological processes were found to be significantly enriched based on the Partek GO analysis. They were involved in central nervous system regulation by affecting its development and maintenance (*Table 4.4.3a*). When the differentially expressed genes by fold change of ≥1.4 were entered on Panther, no significantly altered pathways were found.

***Table 4.4.3a: Top 10 enriched biological processes by Partek Genomic Suite**. Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the mAb O4 and serum treated cultures in comparison to serum only cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	O4+ vs C+ score
regulation of toll-like receptor signaling pathway	13.09	2.07E-06	11.54	4.66
central nervous system myelin maintenance	12.78	2.81E-06	66.67	5.96
ensheathment of neurons	10.58	2.53E-05	5.08	5.13
axon ensheathment	10.58	2.53E-05	5.08	5.13
myelin maintenance	10.08	4.19E-05	20.00	5.96
positive regulation of toll-like receptor signaling pathway	9.70	6.14E-05	16.67	4.78
oligodendrocyte development	9.10	1.11E-04	12.50	4.26
glial cell development	7.51	5.46E-04	5.71	4.26
epidermal cell differentiation	6.84	1.07E-03	4.08	6.12
plasma membrane organization	6.61	1.35E-03	3.64	5.96

The Partek Genomic Suite GO analysis determined 11 molecular functions to be significantly enriched; these included different enzyme activities that allow chemical reaction catalysis (*Table 4.4.3b*). Again, none of them were found to be significant by the Panther analysis.

***Table 4.4.3b: All 11 enriched molecular functions by Partek Genomic Suite**. Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the mAb O4 and serum treated cultures in comparison to serum alone cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	O4+ vs C+ score
semaphorin receptor activity	5.11	6.01E-03	16.67	7.15
protein-glutamine gamma-glutamyltransferase activity	5.11	6.01E-03	16.67	4.47
transferase activity, transferring amino-acyl groups	4.61	1.00E-02	10.00	4.47
interleukin-1 receptor binding	4.42	1.20E-02	8.33	4.11
carbohydrate binding	4.02	1.79E-02	0.96	4.70
UDP-galactosyltransferase activity	4.02	1.79E-02	5.56	6.72
carboxy-lyase activity	3.87	2.09E-02	4.76	4.41
galactosyltransferase activity	3.52	2.97E-02	3.33	6.72
hydro-lyase activity	3.48	3.07E-02	3.23	4.41
carbon-carbon lyase activity	3.36	3.46E-02	2.86	4.41
carbon-oxygen lyase activity	3.14	4.33E-02	2.27	4.41

In addition, Partek Genomics Suite grouped the significantly expressed genes into nine significantly altered cellular components (*Table 4.4.3c*), the most highly enriched including neural modules such as paranode junction of axon, Schmidt-Lanterman incisures and myelin sheath, and cell associated junctions.

Table 4.4.3c: All 9 enriched cellular components by Partek Genomic Suite*. Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the mAb O4 and serum treated cultures in comparison to serum alone cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	O4+ vs C+ score
paranode region of axon	5.30	5.01E-03	20.00	7.51
basal part of cell	5.11	6.01E-03	16.67	4.54
Schmidt-Lanterman incisure	4.71	9.00E-03	11.11	7.51
myelin sheath	4.14	1.60E-02	6.25	7.51
Golgi-associated vesicle	3.92	1.99E-02	5.00	6.86
cis-Golgi network	3.82	2.19E-02	4.55	6.86
cell-cell junction	3.73	2.40E-02	0.82	4.51
intrinsic component of membrane	3.42	3.26E-02	3.03	4.47
cell-cell adherens junction	3.36	3.46E-02	2.86	4.47

Pathways associated with antibody mediated demyelinating cultures

The genes differentially expressed in cultures treated with antibody in the presence serum only identified three significantly enriched pathways (*Table 4.4.3d*), the cell adhesion molecules (CAMs), sphingolipid metabolism, and legionellosis. In all of them only approximately 2 % of genes were present in the pathway, these included *Cldn-11, Mag, Ugt-8* and *Tlr-5*. These genes encode for proteins that are involved in tight junctions, myelin protein and sphingolipids, and innate immunity. Reanalysis of the microarray data on Panther software found no pathways to be significantly altered.

Table 4.4.3d: Significantly altered biological pathways. *Using Partek Pathways™, the list of genes identified as being differentially expressed by ≥1.4, in the cultures treated with antibody in the presence of serum compared to serum treated cultures, was grouped into biological pathways.*

Pathway Name	Class	Function	% genes present in pathway	Enrichment Score	Enrichment p-value
Cell adhesion molecules (CAMs)	Environmental Information Processing	Signaling molecules and interaction	1.69	5.38	4.59E-03
Sphingolipid metabolism	Metabolism	Lipid metabolism	2.44	3.28	3.76E-02
Legionellosis	Human diseases	Infectious diseases: Bacterial	2.22	3.19	4.13E-02

Cultures treated with mAb O4 in the absence of serum and serum alone treated cultures share an immunological signature. The affecting pathways modulate similar groups of genes, chemokines being highly involved. In cultures treated with mAb O4 in the presence of serum, this signature disappears.

4.5 Validations

The Affymetrix array data allowed us to identify genes differentially expressed in myelinating cultures in response to a variety of conditions. Validation of this data with respect to the effects of mAb O4 in the presence and absence of serum was carried out by qPCR and by ELISA on a subset of genes of biological interest.

4.5.1 RT-PCR array

As the microarray data indicated there were pronounced differential effects of mAb and serum on expression of chemokines and cytokines, this was initially validated using a commercially available RT-PCR Array, which allowed us to profile changes in expression of 84 genes. This strategy confirmed mAb O4 had a pronounced effect on chemokine gene expression (Fig 4.5.1a).

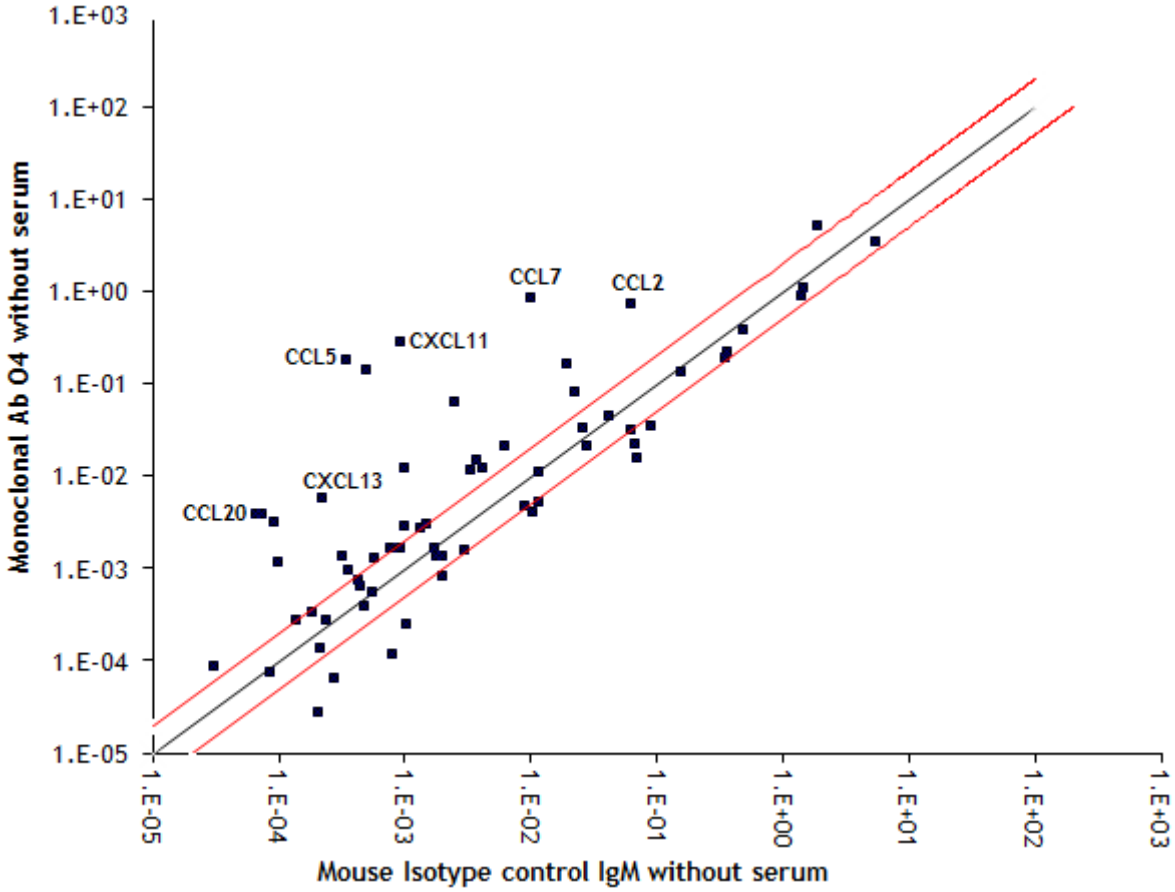


Figure 4.5.1a: Scatterplot of O4 treatment independent of complement indicating induced chemokine and cytokine response. *Myelinating cultures were treated as previously stated for 24 hours. mRNA was extracted from cultures at 25 DIV. cDNA was synthesised and analysed using RT^2 Profiler PCR Array as per manufactures instruction. Values shown are expression levels of each gene in the complement independent antibody samples versus their complment independent isotype control sample (n=1). Cut-off of 2.0, indicated by red lines, was used to indicate significantly regulated genes on the plot.*

When RNA extracted from the cultures treated with mAb O4 or the isotype control in the presence of serum were analysed using the same arrays it is apparent differential gene expression was far less. In this case (*Table 4.5.1b*), we observed *Cxcl13*, *Il-11* and *Bmp7* were differentially regulated to some extent, although this was not apparent from the microarray.

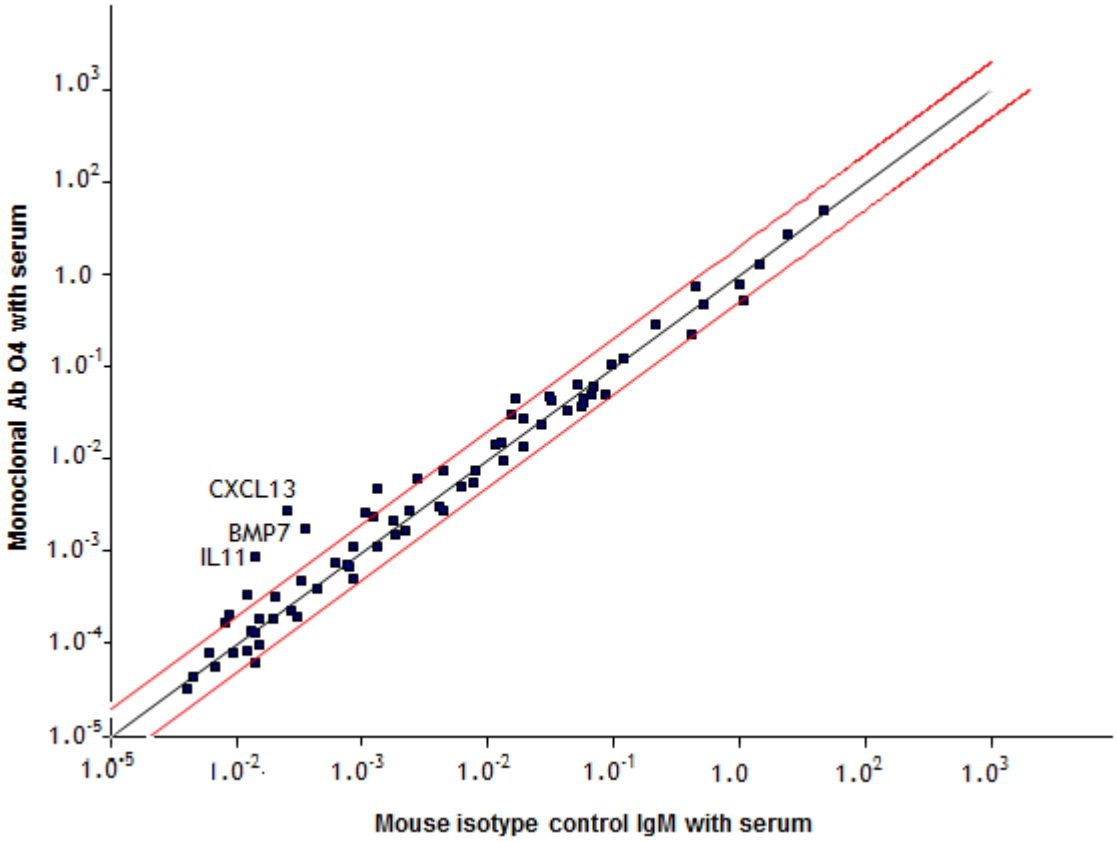


Figure 4.5.1b: O4 treatment with complement indicates no response in chemokine and cytokine expression. *Myelinating cultures were treated as previously stated for 24 hours. mRNA was extracted from cultures at 25 DIV. cDNA was synthesised and analysed using RT2 Profiler PCR Array as per manufactures instruction. Values shown are expression levels of each gene in the complement independent antibody samples versus their complment independent isotype control sample (n=1). Cut-off of 2.0, indicated by red lines, was used to indicate significantly regulated genes on the plot.*

4.5.2 Real-time PCR

In addition to RT-PCR array for chemokines and cytokines, quantitative RT-PCR was performed on genes (*Tables 4.5.2a*) with significant fold changes presented on the microarray. Mixture of differentially expressed chemokines was chosen not only based on their fold change values but by practical primer design and commercially available rat ELISA kits. These validations were performed using RNA isolated from four biological replicates. Significance was calculated based on their dCT values.

With the exception of *Soc1*, the genes of interest differentially expressed in mAb O4 treated cultures compared to the isotype control were all validated by qPCR (*Table 4.5.2a*). Two interferon genes, *Ifn-γ* and *Ifn-a_4*, were included in this

analysis to see if they might contribute to induction of ISG by mAb O4. There was no evidence for increased expression of *Ifn-γ* at this point; however, qPCR indicated mAb O4 treatment induced expression of *Ifn- a_4*.

***Table 4.5.2a: Antibody treatment without complement induces a chemokine and cytokine response**. Up-regulation of chemokines and cytokines in the presence of complement was investigated by incubating 24 DIV myelinating cultures with 20µg/ml O4 or their isotype control, plus 2% rabbit sera for 24 hours, after which mRNA was extracted. The fold changes from 4 biological repeats were calculated.*

	Array		qPCR					
Gene	**Fold Change**	**p-value**	**Fold Change**	**O4- dCT**	**SD**	**IgM- dCT**	**SD**	**Sig**
Cxcl11	79.74	2.76-10	936.55	0.86	1.43	10.06	1.56	**
Cxcl13	40.25	1.05-7	166.05	5.74	1.66	12.31	2.1	**
Irf7	18.67	1.09-7	247.53	-1.68	2.14	4.83	3.23	*
Ccl5	16.05	3.26-10	975.20	2.78	2.37	12.37	1.89	***
Ccl7	12.86	4.22-9	129.04	-0.32	0.93	6	1.92	**
Il-1β	7.81	9.17-8	53.35	6.54	1.75	11.78	1.4	**
Cd14	5.94	2.88-6	5.69	5.7	1	8.83	1.49	*
Socs1	4.29	2.67-7	2.79	11.94	1.24	12.8	2.64	ns
Ccl20	2.82	3.74-4	865.68	2.78	2.37	12.37	1.89	*
Ccl2	2.71	9.99-6	439.38	-0.04	0.85	7.4	2.6	*
Tnf	2.44	9.47-3	11.00	5.91	1.04	9.02	1.56	*
Ifn-γ	1.01	0.88	0.96	15.93	1.39	15.81	1.25	ns
Ifn-a4	-1.1	0.44	21.39	12.23	1.03	16.22	2.69	*

Repeating this analysis on samples obtained from cultures treated with mAb O4 in the presence of serum revealed this abolished all of the differential effects induced by mAb O4 alone, with the exception of *Il-1β*, which was elevated. The 4 differentially expressed genes (*Siglec5, Clec4a2, Tlr5, Irg1*) in the mAb O4 in the presence of serum compared to serum alone treated cultures were not validated in this study.

Table 4.5.2b: Antibody treatment with complement reduces chemokine and cytokine expression*. Up-regulation of chemokines and cytokines in the presence of complement was investigated by incubating 24 DIV myelinating cultures with 20 µg/ml O4 or their isotype control, plus 2% rabbit sera for 24 hours, after which mRNA was extracted. The fold changes from 4 biological repeats were calculated.*

	Array		qPCR					
Gene	**Fold Change**	**p-value**	**Fold Change**	**O4+ dCT**	**SD**	**NT+ dCT**	**SD**	**Sig**
Cxcl11	-1.21	0.32	2.72	1.65	2.03	2.38	1.3	ns
Cxcl13	2.24	0.02	12.31	7.82	1.13	9.93	1	ns
Irf7	1.28	0.29	2.88	-0.81	2.9	0.44	1.92	ns
Ccl5	-1.42	0.01	3.29	4.61	2.79	5.64	3.15	ns
Ccl7	-1.58	0.01	2.67	-1.17	1.53	-0.73	1.02	ns
Il-1β	1.57	0.01	12.52	6.17	1.28	9.11	0.83	*
Cd14	-1.01	0.85	6.77	4.31	0.94	6.06	0.95	ns
Socs1	1.25	0.09	1.38	12.75	0.76	12.78	0.68	ns
Ccl20	-1.3	0.21	5.89	4.61	2.79	5.64	3.15	ns
Ccl2	-1.08	0.52	4.28	-0.15	1.28	0.83	1.26	ns
Tnf	1.06	0.75	4.33	5.46	1.06	6.74	0.66	ns
Ifn-γ	1.02	0.75	4.48	15.09	1.84	16.33	2.09	ns
Ifn-α4	1.04	0.77	0.9	14.19	2.48	13.54	2.02	ns

4.5.3 ELISA

ELISA (enzyme-linked immunosorbent assay) allows measurement of total concentration of the cytokines in the culture supernatant. It should be noted that finding commercially available and effective rat ELISA kits was a challenge. Therefore, the analysis was only performed for five different cytokines, CCL2, CCL5, CCL20, CXCL11 and IL-1β. However, in all cases, the concentration of IL-1β was below the detection level of the commercially available ELISA kit. In the absence of serum, mAb O4 induced significant increases in CCL2, CCL5, CCL20 (*Fig. 4.5.3a*). A similar trend was observed for CXCL11, but this did not reach significance ($p < 0.05$), although antibody induced > 1,000 fold change in concentration compared to the controls.

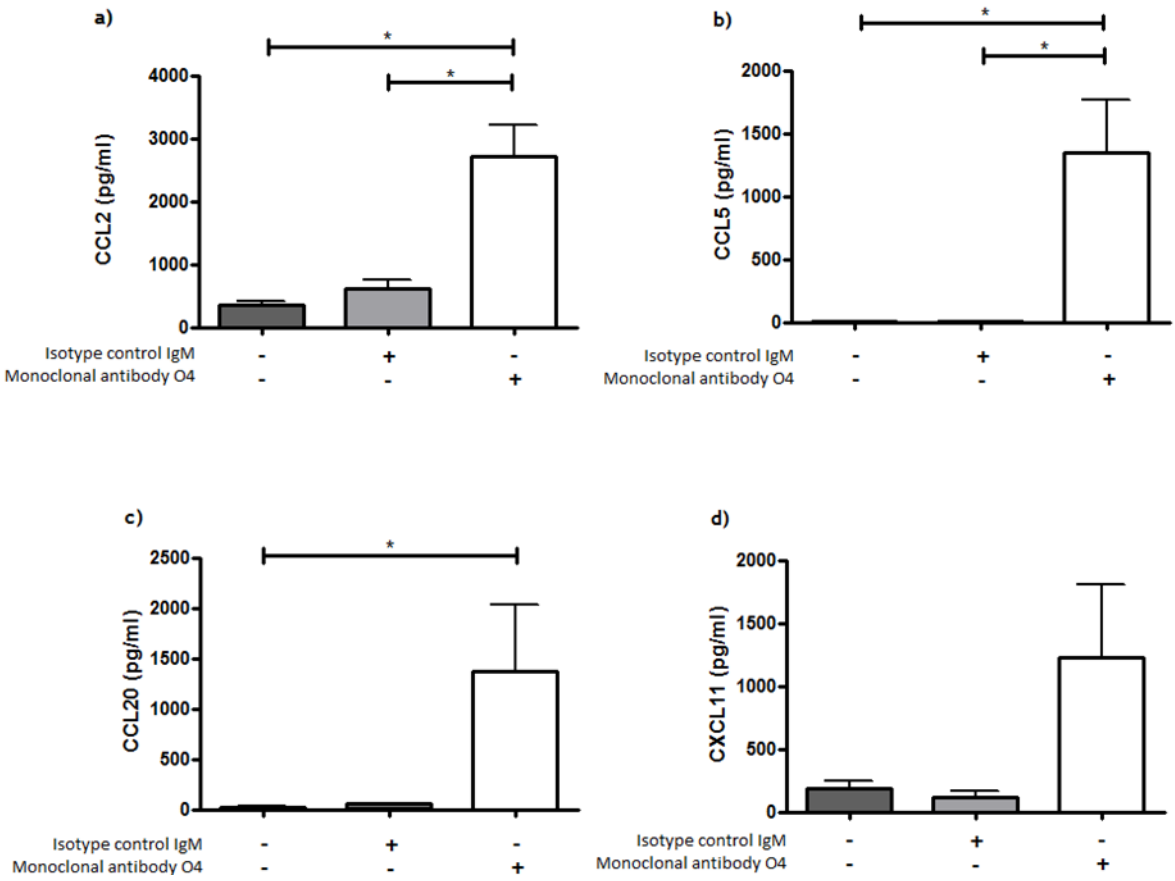


Figure 4.5.3a: Supernatant concentration of chemokines following complement independent antibody treatment*. Treatment was performed as previously mentioned. Chemokine concentration in the myelinating culture supernatant was measured by using ELISA. Significance was calculated using one-way ANOVA with Tukey's post-hoc test. n=3/group.*

Unfortunately, factors present in rabbit serum confounded attempts to use these assays to measure changes in chemokine concentrations induced in the presence of serum. Rabbit serum already gave high background values, and unexpectedly chemokine concentrations were generally lower in cultures treated with mAb O4 than in the presence of serum alone or the isotype control and serum (Fig. 4.5.3b).

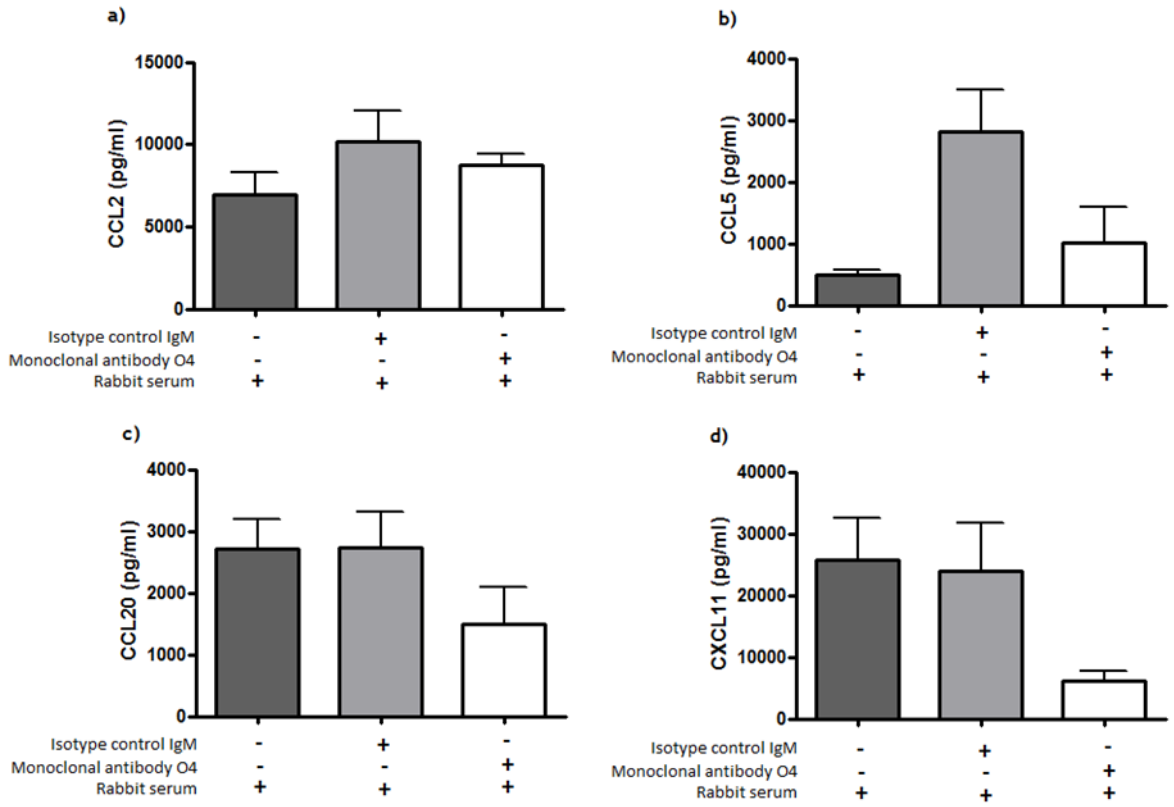


Figure 4.5.3b: Supernatant concentration of chemokines following antibody treatment in the presence of complement. *Treatment was performed as previously mentioned. Chemokine concentration in the myelinating culture supernatant was measured by using ELISA. Significance was calculated using one-way ANOVA with Tukey's post-hoc test. n=3/group.*

4.6 Discussion

This study reveals addition of a sulphatide-specific IgM mAb to myelinating cultures initiates a complex transcriptional response. Our analysis focused on two conditions:

1) The effect of mAb O4 in the absence of serum. We argue this model will allow us to explore the effects of intrathecal synthesis of sulphatide-reactive antibodies in the CNS of patients with progressive forms of MS; a disease state with minimal BBB dysfunction.

2) The effect of mAb O4 in the presence of serum. A model that may help in identification of specific transcriptional events associated with antibody-dependent, complement-mediated demyelination; a mechanism believed to contribute to lesion formation in a majority of patients with MS.

Our interest in the role of antibody-dependent effector mechanism(s) in MS derives from the fact there are no fully effective treatments for this devastating disease. Current treatments for MS are able to reduce relapse frequency in RRMS by targeting immune mechanisms that support the development of a T cell-mediated inflammatory response in the CNS. This strategy is however ineffective in patients with progressive forms of the disease. The failure of these 'anti-inflammatory' treatments in PMS led to speculation progressive MS was a purely neurodegenerative disease in which accumulation of disability was independent of any inflammatory disease process within the CNS. However, recent studies demonstrate this not the case (Frischer et al., 2009). Progressive (primary and secondary) MS is associated with extensive cortical pathology in which loss of myelin and neurites is associated with inflammation in the overlying meninges (Kutzelnigg et al., 2005; Magliozzi et al., 2007; Howell et al., 2011).These meningeal infiltrates are frequently enriched in B cells and may represent a ectopic lymphoid follicles (Magliozzi et al., 2007). In addition, widespread microglial activation is seen in throughout the CNS (Peterson et al., 2001; Prineas etal., 2001; Kutzelnigg et al., 2005; Magliozzi et al., 2007) and the active rim of slowly expanding lesions in white matter tracts is associated with recruitment of leukocytes, although this is far less pronounced than in early RRMS (Frischer et al., 2009). In response to these observations, a new hypothesis

was developed that attributed accumulation of disability in PMS to the effects of an inflammatory response sequestered in the CNS itself (Bradl and Lassmann, 2009; Fischer et al., 2013). However, the mechanisms that maintain this inflammatory response remain unknown. The gene expression data obtained in this study provides new insight into this problem as they demonstrate an antibody mimicking the specificity of the disease associated intrathecal antibody repertoire can induce a pro-inflammatory signalling environment *in vitro*.

In these experiments we used the sulphatide-specific mAb O4 to mimic the specificity of the intrathecal autoantibody repertoire in patients and explored its effects in myelinating cultures. In the absence of exogenous serum we believe this experimental model replicates the CNS environment in PMS in which BBB dysfunction is minimal. Unexpectedly this experiment revealed mAb O4 induced expression of a large number of chemokine genes [*Cxcl11* (80-fold), *Cxcl9* (59-fold), *Cxcl13* (40-fold), *Cxcl10* (35-fold), *Cxcl17* (19-fold); *Ccl5* (16-fold), *Ccl7* (13-fold), *Ccl20* (2.8-fold) and *Ccl2* (2.7-fold)], as well as many other transcripts associated with innate and adaptive immunity. Intriguingly, the vast majority of these are interferon sensitive genes (ISG) suggesting binding of mAb O4 to the myelin/oligodendrocyte surface triggers a cascade of responses mimicking those induced by infectious agents; a concept supported by gene ontology and pathway analysis of these genes. Extrapolating these observations to PMS suggests an intrathecal response directed against sulphatide, in addition to any direct detrimental effects it might have on myelin and oligodendrocytes (*Section 3*), will maintain inflammatory disease activity in the CNS. At present he mechanism(s) responsible for this state of 'heightened immune awareness' induced by mAb O4 are unknown. We speculate microglia and/or astrocytes are the major source of the chemokine response seen in these myelinating cultures, and that this is triggered by an activating signal derived from affected oligodendroglia. Potential candidates are damage associated molecular patterns (DAMPs) (Bianchi, 2007) generated in response to antibody-mediated cross-linking of sulphatide at the cell surface. These may include membrane vesicles shed from the oligodendroglial/myelin surface (Scolding et al., 1989), as well as molecular signals derived from the intracellular compartment such as high-mobility group box 1 (HMGB1), heat shock proteins, degraded DNA and RNA, S100 proteins and purine derivatives. Intriguingly, mAb O4 increases expression of

several receptors predicted to be involved in this response in particular triggering receptor expressed on myeloid cells 1 (*Trem-1*; fold change +4.6) and several C-type lectin receptors (*Clec4a2*, fold change +8.2; *Clec4a1*, fold change +2.9; *Clec4d*, fold change +2.8; *Clec2g*, fold change +2.5; *Clec4e*, fold change +2.4; *Clec4a3*, fold change +2.2). Determining the molecular mechanisms by which mAb O4 induces this chemokine signal, the identity of relevant ligand/receptor pairs and their biological/pathophysiological relevance in MS will require further studies.

However, with respect to chemokines they may not only provide signals to attract immune cells into the brain and spinal cord, but may also affect a wide range of resident cells that express appropriate receptors. CXCR1, CXCR2, and CXCR3 can be expressed by OPCs and oligodendrocytes (Nguyen and Stangel, 2001; Tsai et al., 2002; Omari et al., 2005), but apart from CXCR2 mediating the migratory arrest of OPC during their development (Tsai et al., 2002) their roles are unknown. Other studies demonstrate microglia and astrocytes also express CXCR3, as well as variable levels of CCR3, CCR4, CCR5, CCR6, CXCL1, CXCR2, CXCR4 and CXCR5 (Ransohoff, 2009). In the context of this study the role of CXCR3 may be particularly important as it is a receptor for CXCL11, CXCL10 and CXCL9, three of the chemokines most highly up regulated by mAb O4. However, irrespective of any resident cellular response triggered by these chemokines, together with CXCL13 they are also implicated in the recruitment of B cells into the CNS in a variety of disease models (Sellebjerg et al., 2009; Rainey-Berger et al., 2011; Kalinowska-Lyszczarz et al., 2011; Kowarik et al., 2012; Phares et al., 2013; Metcalf et al., 2013). This raises the possibility intrathecal synthesis of sulphatide-specific antibodies may support a positive feedback loop sustaining B cell recruitment into the CNS compartment. This hypothesis is supported by the observation mAb O4 also enhanced expression of *Cxcr5* (fold change +1.5), the receptor for CXCL13 which is implicated in the development of B cell enriched lymphoid follicle-like structures in the meninges of patients (Cyster et al., 2000; Magliozzi et al., 2004 2007). However, it should be noted the spectrum of chemokines induced by mAb O4 is very broad and in addition to any effect on B cells this is also predicted to enhance monocyte and T-cell recruitment into the CNS (*Table 6.0*). It should also be appreciated a large number of chemokines are expressed in MS tissues and at present it is unknown what contribution might be

made by the antibody-mediated effect described in this study (Mahad and Ransohoff, 2003; Biber et al., 2002; Furlan et al., 2005; Banisor et al., 2005).

Table 6.0: Chemokine receptor redundancy for the genes differentially expressed in our Affymetrix array *(Peprotech, 2011).*

Gene	Receptor	Responding cell type		Gene	Receptor	Responding cell type
CCL2	CCR2	Bs, Mo, actT, NK, iDC		CXCL1	CXCR1 > CXCR2	PMN
CCL3	CCR1, CCR5	Eo, Mo, actT, NK, iDC		CXCL2	CXCR2	PMN
CCL4	CCR5	Mo, act (Th1), NK, iDC		CXCL5	CXCR2	PMN
CCL5	CCR1, CCR3, CCR5	Eo, Bs, Mo, act, NK, iDC		CXCL9	CXCR3	act (TH1), NK
CCL7	CCR1, CCR2, CCR3	Eo, Bs, Mo, act, NK, iDC		CXCL10	CXCR3	act (TH1), NK
CCL9	CCR1	PMN, act		CXCL11	CXCR3, CXCR7	act (TH1), NK
CCL12	CCR2	Bs, Mo, actT, NK, iDC		CXCL13	CXCR3, CXCR5	B
CCL19	CCR7	T, act, mDC		CXCL16	CXCR6	actT
CCL20	CCR6	Tm, B, iDC		CXCL17	Unknown	Mo, iDC
CCL24	CCR3	Eo, Bs, actT (Th2), iDC				

** = qPCR validation, ** = qPCR and ELISA validation, Tm = memory T cell, B = B cell, actT = activated T cell, Bs = basophil, T = resting T cell, Eo = eosinophil, NK = natural killer cell, Mo = monocyte, PMN = neutrophil, mDC = mature dendritic cell, iDC = immature DC*

In summary, a significant body of evidence is now available demonstrating sulphatide-specific antibodies are a significant component of the intra-thecal repertoire and they are derived from clonally expanded B cell clones sequestered in the CNS (Baranzini et al., 1999; Qin et al., 1998; Owens et al., 2003; Rainey-Barger et al., 2011; Phares et al., 2011). These clonal expansions can be identified in CSF, meningeal infiltrates and parenchyma of patients with MS but recent studies demonstrate they are not permanently sequestered in the CNS but participate in bidirectional exchange across the BBB (von Budingen et al., 2012; Palanichamy et al. 2014; Stern et al., 2014). These observations support the therapeutic use of B cell depleting antibodies and mAb derived therapeutics that prevent lymphocytes from crossing the BBB in MS. However, our data suggests these treatments should be complemented by strategies designed to eliminate intrathecal populations of B cells and antibody secreting cells (plasmablasts and plasma cells); a treatment strategy we suggest will

disrupt an important feedback loop contributing to the maintenance of disease activity within the CNS.

In addition to effects on immune function within the CNS such treatments would also eliminate any direct effects of the intrathecal antibody response on myelin and/or axonal integrity. In *Section 3.4* we report prolonged exposure (ten days) to a high concentration of mAb O4 not only inhibited myelination, but also appeared induce demyelination. How these effects might be related to the transcriptional response induced by the antibody within 24 hours is unclear. However, the effect of mAb O4 on myelination was associated with a 50% increase in microglial numbers. Mitogenic colony-stimulating factors (CSFs) mediate microglial proliferation and/or activation (Sawada et al., 1990; Kreutzberg, 1996) and in this context it is interesting to note mAb O4 induced a significant increase in *Csf1* expression within 24 hours (*Appendix 8.1.2*; fold change + 1.8, $p = 9.7 \times 10^{-5}$). However, this finding was not yet validated at the protein level and it is premature to link these *in vitro* effects of mAb O4 on myelin and microglia to the widespread changes in microglial numbers and phenotype associated with lesion formation in MS.

Antibody-mediated demyelination is classically discussed as a complement-dependent phenomenon, but it appears endogenous synthesis of complement components is insufficient to support acute antibody-mediated demyelination (Elliott et al., 2011; this study). Nonetheless within 24 hours mAb O4 up-regulated expression of *C3* (fold change +7.5, $p = 1.2 \times 10^{-7}$) and *C3ar1* (fold change +2.6, $p = 1.4 \times 10^{-3}$) significantly. We therefore cannot rule antibody-mediated induction of these endogenous complement components does not contribute to the longer term effects of exposure to mAb O4 on myelination. It should also be considered this increase in component availability may also influence dendritic spine/synapse remodelling (Stephan, Barres and Stevens, 2012; Schafer and Stevens, 2010; Tremblay et al., 2011; Wang et al., 2010), and if so, it could play a significant role in the pathophysiology of MS, independently or in synergy with its effects on immune function and myelination.

The arguments above all stress the pathogenic potential of a sulphatide specific antibody, but other studies indicate may be beneficial in that it can stimulate

remyelination (Miller and Rodriquez, 1995; Warrington et al., 2000; Wright et al., 2009). We saw no evidence of this in the present study, but found binding mAb O4 to the oligodendrocyte/myelin surface was associated with a transcription response mimicking that induced by neurotrophic viruses. Why this occurs is unknown, however it suggests the ability of mAb O4 to enhance remyelination in TMEV infected mice is due to its ability to stimulate an immune response that enhances viral clearance rather than promoting OPC proliferation and differentiation.

These acute effects of mAb O4 on gene expression in the myelinating cultures were largely unexpected; the anticipation being the most informative transcription figures would be associated with antibody-dependent, complement-mediated demyelination. This was not the case as unexpectedly addition of serum alone induced a wide spectrum of changes in gene expression. These transcriptional changes have not been validated, but they suggest serum leakage across the BBB has multiple far reaching effects that might contribute to the pathogenesis of many CNS diseases. This requires further investigation, but possibly the most unexpected result of this study was that although mAb O4 induced demyelination in the presence of serum, this major pathogenic event was associated with only 21 significantly modulated transcripts (*Table 4.3.4*; 4 up-regulated, 17 down-regulated). The majority of genes that were down regulated are expressed by oligodendrocytes, and this is attributed to an antibody-mediated, complement-dependent loss of oligodendrocytes (Elliott et al., 2011). However, only four genes (*Siglec5, Clec4a2, Tlr5* and *Irg1*) were significantly up-regulated in this context. Validating these observations is important, but this should be performed using autologous serum to avoid potential artefacts due to xenogeneic serum effects.

At present we can only speculate on the functional significance of these changes. SIGLEC5 and CLEC4A (also known as dendritic cell immunoreceptor) contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) suggesting they may limit induction of a pro-inflammatory response following antibody/complement mediated demyelination (Crocker et al., 2007). However allocating functional significance to increased expression of *Tlr5* and *Irg1* is more difficult. Classical studies demonstrate bacterial flagellin is a major ligand for TLR5 and suggest it

may play an important role in regulating mucosal immunity (Hayashi et al., 2001; Takeda and Akira, 2005) whilst in the CNS it is up regulated on microglia in response to Borrelia burgdorferi, the cause of Lyme Disease (Neuroborreliosis) (Bernardino et al, 2008). Immunoresponsive gene 1 (IRG1) is now known to encode an enzyme that generates itaconic acid from cis-aconitate, a tricarboxylic acid cycle intermediate in microglia (Michelucci et al., 2012) and is highly up regulated in the CNS of mice with EAE (Carmody et al., 2002), but its functional relevance is unknown.

5 Future studies

This preliminary study of the effect of treating myelinating cultures with a sulphatide-specific mAb has many potential ramifications in particular with respect to the role a similar intrathecal response might play in the pathogenesis of PMS. In view that there are no treatments currently available to offer these patients it is a matter of some urgency this study is extended as soon as possible to:

a) Determine if patient CSF contains antibody and/or antigen-antibody complexes that can mediate a similar response to mAb O4 in myelinating cultures *in vitro*.
b) Define the antigen-specificity and mechanistic basis for this chemokine response *in vitro* and whether this can be reproduced using pre-formed antigen/antibody complexes.
c) Develop an animal model to investigate the consequences of this effect *in vivo*.

Our initial studies were performed using myelinating cultures derived from embryonic rat spinal cord, but to exploit the full range of genetic and immunobiological tools now available, future studies should be performed using cultures derived from embryonic mice. This methodology is well established and preliminary results suggest myelinating mouse and rat cultures respond in a similar manner to treatment with mAb O4 (Edgar, Linington, personal communication).

To address the questions outlined above one approach would be to repeat the transcriptional profiling described in this thesis, but with mouse cultures as the target and using RNAseq to obtain more complete information on how the cultures respond to different treatments over time. Once this is completed using a variety of myelin and non-myelin specific antibodies, pre-formed antigen-antibody complexes and all relevant controls, the most informative time point should be chosen to investigate the effects of selected CSF samples using the same methodology. In simple terms, size fractionation of CSF samples could be used to determine if effects on gene transcription are due to factors with a MW >100,000 but < 300,000 Daltons (potentially IgG) or immune complexes and/or monomeric IgM (MW > 300,000 Daltons). Ultimately this would provide a

series of differential gene expression profiles that can be compared directly to those induced using mAb O4 and other model antibodies/antibody-antigen complexes.

This strategy would also provide data defining the antigen specificity of the response i.e. whether it is sulphatide-specific or replicated using other model reagents. Combining this approach with immunofluorescence and/or *in situ* hybridisation studies would also allow us to determine which cell types are responsible for specific aspects of the response. These studies would also determine how to best measure effects of CSF samples on chemokine synthesis. One possibility would be to use a multi-flex ELISA or Luminex based assays to determine the concentrations of selected chemokines in supernatants harvested from treated and control cultures. This data could then be used to investigate potential correlations between these *in vitro* responses with clinical parameters such as accumulation of disability or treatment responses. Developing an animal model to explore these phenomena *in vivo* will be more challenging, although acute effects on gene expression, leucocyte recruitment, myelination and synaptic remodelling may be studied by direct intrathecal injection of antibody or antigen-antibody complexes. Longer term studies may, however, have to rely on delivery via osmotic pumps attached to intra-ventricular catheters.

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7 Appendix 1

Unless otherwise stated reagents were obtained from Sigma.

7.1 Buffers

Phosphate buffer saline (PBS)

8 g Sodium chloride

0.2 g Potassium chloride

0.2 g Potassium dihydrogen

1.18 g Disodium phosphate

Dissolved in approximately 800 ml of distilled water, adjusted to pH 7.4 with 1 M hydrogen chloride and topped up to 1 L with distilled water.

7.2 Myelinating culture media

7.2.1 Supplements

Soybean trypsin inhibitor

13 mg Soybean trypsin inhibitor

75 mg Bovine albumin factor V

1 mg DNase I from bovine pancreas

Made up in 25 ml Leibovitz medium 15 (Gibco, Invitrogen, Paisley)

1% Collagenase

20 ml Leibovitz medium 15

20 mg Collagenase type I

Insulin

100 mg Insulin from bovine pancreas

200 ml 10 mM Hydrogen chloride

Biotin

100 mg Biotin

100 ml 1 M Sodium hydroxide

Diluted 1:100 with distilled water

Hydrocortisone

100 mg Water soluble hydrocortisone

Dissolved in 27.6 ml distilled water

30% Glucose

30 g Glucose

Dissolved in 100 ml of sterile water

7.5% Sodium biocarbonate

7.5 g $NaHCO_3$

100 ml sterile water

1M HEPES

23.8 g HEPES

100 ml sterile water

10x Hormone mix

5 ml 30% glucose

3.75 ml 7.5% $NaHCO_3$

1 ml 1 M HEPES

250 mg Transferrin: apo-human

26 ml insulin solution

- 6.25 ml Human recombinant Insulin
- 19.75 ml Sterile water

25 ml 200 µM Putrescine

- 96.6 mg Putrescine
- 100 ml sterile water

25 µl Selenium

- 1 mg Sodium selenite
- 1.93 ml sterile water

25 µl Progesterone

- 1 mg Progesterone
- 1.59 ml 95% Ethanol

Top up to 250 ml with DMEM/F-12 medium

7.2.2 Tissue culture media

Differentiation medium

500 ml DMEM medium

5 ml Penicillin streptomycin

5 ml Sodium pyruvate

Insulin containing differentiation medium (DM+)

48.5 ml 1XDMEM with penicillin (100 U/ml)/streptomycin (100 µg/ml)/Glucose (4.500 mg/L)

250 µl N1 supplement

250 µl 10 µM Hydrocortisone

50 µl 1 µg/ml Biotin

1 ml 0.5 µg/ml Insulin

Differentiation medium (DM-)

49.5 ml 1XDMEM with penicillin (100 U/ml)/streptomycin (100 µg/ml)/Glucose (4.500 mg/L)

250 µl N1 supplement 100x

250 µl 10µM Hydrocortisone

50 µl 1 µg/ml Biotin

Neurosphere medium

210 ml DMEM-F12

25 ml 10x Hormone mix

5 ml 30 % Glucose

3.75 ml 7.5 % $NaHCO_3$

1.25 ml 1 M HEPES

2.5 ml L-glutamate

2.5 ml Penicillin Streptomycin

625 µl 4 % BSA in L15 media

Astrocyte medium

500 ml 1.0 g/L Glucose-containing DMEM

50 ml 10 % Fetal bovine serum

5 ml Penicillin streptomycin

2.5 ml Glutamine

Plating medium

50 % 1X DMEM (Gibco, Invitrogen, Paisley)

24 % Heat inactivated horse serum (Invitrogen, Paisley)

24 % 1X HBSS with Mg^{2+} and Ca^{2+} (Gibco, Invitrogen, Paisley)

2 % Glutamine (Sigma-Aldrich)

1.33µg/ml Poly-L-Lysine (PLL)

25 mg of poly-L-lysine hydrobromide

Dissolved in 6.25 ml of distilled water

7.3 Hybridoma media

Feeding medium (RPMI-1640)

50 ml fetal bovine serum

5 ml Penicillin streptomycin

5 ml L-glutamine

5 ml Sodium puryvate

5 ml Non-essential amino acids

0.5 ml 1000x Mercaptoethanol

Freezing medium

45 % Fetal Bovine Medium

45 % RPMI-1640 feeding medium

10 % Dimethyl sulphatide

7.4 IgG antibody purification buffers

20 mM Sodium Phosphate, pH 7.0 (Binding buffer)

0.542 g Sodium phosphate

1.637 g Disodium phosphate, Na_2HPO_4

1 L distilled water

0.1 M Glycine buffer (Elution buffer)

1.875 g Glycine

0.7 ml Concentrated hydrochloric acid

500 ml distilled water

1 M Tris base (Neutraliser)

70.55 Tris base

500 distilled water

7.5 IgM antibody purification buffers

20 mM Sodium phosphate, 8.0 M $(NH_4)_2SO_4$, pH 7.5 (Binding buffer)

1.084 g NaH_2PO_4, anhydrous

3.273 g Na_2HPO_4 x 7 H_2O

105.712 g $(NH_4)_2SO_4$

Quantum satis to 1 L with distilled water

20 mM Sodium phosphate, pH 7.5 (Elution buffer)

1.084 g NaH_2PO_4, anhydrous

3.273 g Na_2HPO_4 x 7 H_2O

Quantum satis to 1 L with distilled water

20 mM Sodium phosphate, pH 7.5 with 30 % isopropanol (Regeneration buffer)

1.084 g NaH_2PO_4, anhydrous

3.273 g Na_2HPO_4 x 7 H_2O

300 ml Isopropanol

Quantum satis to 1 L with distilled water

7.6 Materials for mAb Z2 fragmentation to Fab

Digestion buffer prepared fresh and stored on ice <10h

10 ml 1 x PBS

0.02 M EDTA

0.02 M Cysteine

Papain from papaya latex (equal volume to Ab, at 1:20 ratio)

0.3 M Iodoacetamide

10 ml PBS

0.0056 g/ml Iodoacetamide

8 Appendix 2

8.1 Partek Genomics Suite gene lists

8.1.1 Genes identified as being differentially expressed in mAb O4 treated myelinating cultures

Table 4.3.2: Differentially expressed genes in O4 antibody treated myelinating cultures in the absence of serum identified by Partek. *Microarray analysis was performed on Partek Genomic Suite. The data shown is sorted in manner of highest fold change to the cut-off of 1.4 in antibody treated cultures in comparison to isotype control treated cultures. Significance was calculated using one-way ANOVA.*

Gene Symbol	Gene Name	Fold-Change	P-value
Cxcl11	chemokine (C-X-C motif) ligand 11	79.74	2.76E-10
Cxcl9	chemokine (C-X-C motif) ligand 9	59.29	2.49E-10
Cxcl13	chemokine (C-X-C motif) ligand 13	40.25	1.05E-07
Ifit3	interferon-induced protein with tetratricopeptide repeats 3	39.28	3.84E-07
Cxcl10	chemokine (C-X-C motif) ligand 10	34.87	5.75E-06
Oasl	2'-5'-oligoadenylate synthetase-like	32.03	1.55E-07
Ifit2	interferon-induced protein with tetratricopeptide repeats 2	25.12	6.36E-07
Mx1	myxovirus (influenza virus) resistance 1	23.25	5.58E-07
Rsad2	radical S-adenosyl methionine domain containing 2	22.19	7.29E-06
Cxcl17	chemokine (C-X-C motif) ligand 17	18.81	1.52E-09
Irf7	interferon regulatory factor 7	18.67	1.09E-07
Isg20	interferon stimulated exonuclease gene 20	17.34	1.01E-07
Gbp5	guanylate binding protein 5	16.10	2.29E-09
Ccl5	chemokine (C-C motif) ligand 5	16.05	3.26E-10
Apol9a	apolipoprotein L 9a	15.35	1.87E-08
F10	coagulation factor X	13.51	3.13E-08
Ddx60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60	13.06	1.70E-06
Zbp1	Z-DNA binding protein 1 NM_133564	12.93	1.10E-07
Ccl7	chemokine (C-C motif) ligand 7	12.86	4.22E-09
Lcn2	lipocalin 2	12.15	2.75E-08
Isg15	ISG15 ubiquitin-like modifier	12.04	2.96E-07
Usp18	ubiquitin specific peptidase 18	11.70	3.11E-06
LOC685067	similar to guanylate binding protein family, member	11.32	1.52E-07
LOC100366216	nuclear antigen Sp100-like	11.14	4.99E-08
Oas2	2'-5' oligoadenylate synthetase 2	9.82	9.20E-08
Lgals9	lectin, galactoside-binding, soluble, 9	9.60	1.31E-07
RT1-T24-3	RT1 class I, locus T24, gene 3	9.56	6.03E-09
Oas1b	2-5 oligoadenylate synthetase 1B	9.48	1.33E-06
Ccl19	chemokine (C-C motif) ligand 19	9.15	2.99E-07
Serpine1	serpin peptidase inhibitor, clade E (nexin, plasminog	8.99	7.34E-07
Cd72	Cd72 molecule	8.89	1.07E-06
Ccl12	chemokine (C-C motif) ligand 12	8.70	5.01E-07
Clec4a2	C-type lectin domain family 4, member A2	8.15	1.64E-07
Ifi27l2b	interferon, alpha-inducible protein 27 like 2B	8.05	1.85E-08
Mpa2l	macrophage activation 2 like	7.99	1.98E-07

Il1b	interleukin 1 beta	7.81	9.17E-08
Dhx58	DEXH (Asp-Glu-X-His) box polypeptide 58	7.62	4.95E-07
C3	complement component 3	7.50	1.20E-07
Tap1	transporter 1, ATP-binding cassette, sub-family B (MDR/TA	7.43	1.89E-09
Siglec1	sialic acid binding Ig-like lectin 1, sialoadhesin	7.38	1.32E-06
Slamf9	SLAM family member 9	7.38	2.45E-09
Oas1i	2 ' -5 ' oligoadenylate synthetase 1l	7.11	6.62E-07
RGD1305184	similar to CDNA sequence BC023105	6.94	8.61E-06
Uba7	ubiquitin-like modifier activating enzyme 7	6.81	1.43E-06
Siglec5	sialic acid binding Ig-like lectin 5	6.58	2.33E-06
RT1-CE4	RT1 class I, locus CE4	6.38	1.46E-06
Cmpk2	cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial	6.31	1.46E-06
Il18bp	interleukin 18 binding protein	6.31	1.29E-07
Psmb9	proteasome (prosome, macropain) subunit, beta type, 9	6.23	1.15E-08
Slfn3	schlafen 3	6.18	1.16E-04
RGD1309362	similar to interferon-inducible GTPase	6.08	4.66E-06
Cd14	CD14 molecule	5.94	2.88E-06
Slfn1	schlafen 1	5.86	3.80E-07
Birc3	baculoviral IAP repeat-containing 3	5.85	1.68E-09
Emr1	EGF-like module containing, mucin-like, hormone receptor-like 1	5.82	1.30E-05
RT1-CE12	RT1 class I, locus CE12	5.73	2.50E-08
LOC684193	similar to sterile alpha motif domain containing 9-like	5.69	1.12E-08
Ebi3	Epstein-Barr virus induced 3	5.67	7.26E-07
Apol3	apolipoprotein L, 3	5.67	1.57E-05
RT1-S3	RT1 class Ib, locus S3	5.66	1.45E-07
Il1a	interleukin 1 alpha	5.65	2.50E-06
RT1-N3	RT1 class Ib, locus N3	5.58	1.69E-07
Psmb8	proteasome (prosome, macropain) subunit, beta type, 8	5.57	3.80E-08
Tmem106a	transmembrane protein 106A	5.56	5.30E-08
Sp100	SP100 nuclear antigen	5.50	6.07E-10
Ch25h	cholesterol 25-hydroxylase	5.49	9.81E-06
Itgal	integrin, alpha L	5.45	2.56E-06
Tapbp	TAP binding protein (tapasin)	5.43	5.82E-09
LOC688318	similar to leucine rich repeat containing 45	5.42	5.53E-09
Slc11a1	solute carrier family 11 (proton-coupled divalent metal ion tran	5.37	1.64E-07
Apol3	apolipoprotein L, 3	5.30	7.95E-06
Slpi	secretory leukocyte peptidase inhibitor	5.16	5.77E-04
Tnfaip2	tumor necrosis factor, alpha-induced protein 2	5.13	4.52E-09
Ifi47	interferon gamma inducible protein 47	5.11	7.53E-10
Rac2	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP b	4.99	5.93E-05
Plac8	placenta-specific 8	4.96	7.80E-05
LOC681182	similar to paired immunoglobin-like type 2 receptor beta	4.96	2.26E-05
Vcam1	vascular cell adhesion molecule 1	4.89	1.74E-08
Ifih1	interferon induced with helicase C domain 1	4.88	1.71E-06
Gpr31	G protein-coupled receptor 31	4.82	1.45E-05
Ccl4	chemokine (C-C motif) ligand 4	4.76	2.19E-05

Trem1	triggering receptor expressed on myeloid cells 1	4.64	3.49E-04
Scimp	SLP adaptor and CSK interacting membrane protein	4.61	1.01E-06
Tor3a	torsin family 3, member A	4.42	1.51E-06
Ms4a11	membrane-spanning 4-domains, subfamily A, member 11	4.34	2.99E-07
Pric285	peroxisomal proliferator-activated receptor A interact	4.33	1.73E-06
Oasl2	2'-5' oligoadenylate synthetase-like 2	4.30	6.23E-06
Socs1	suppressor of cytokine signaling 1	4.29	2.67E-07
Rtp4	receptor (chemosensory) transporter protein 4	4.29	1.09E-06
Itgam	integrin, alpha M	4.28	6.65E-04
Casp12	caspase 12	4.28	6.92E-08
Parp12	poly (ADP-ribose) polymerase family, member 12	4.27	7.06E-07
Ly6e	lymphocyte antigen 6 complex, locus E	4.24	7.49E-08
Slfn13	schlafen family member 13	4.20	7.02E-11
Irgm	immunity-related GTPase family, M	4.10	1.06E-06
MGC105567	similar to cDNA sequence BC023105	4.10	2.60E-05
Hck	hemopoietic cell kinase	4.09	4.71E-04
Psmb10	proteasome (prosome, macropain) subunit, beta type 10	4.08	2.98E-07
Cxcl16	chemokine (C-X-C motif) ligand 16	3.97	1.79E-08
Tap2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	3.96	2.24E-06
Ppm1n	protein phosphatase, Mg2+/Mn2+ dependent, 1N	3.94	5.01E-10
Cd274	CD274 molecule	3.91	5.09E-08
Msr1	macrophage scavenger receptor 1	3.91	3.88E-05
Abcb1b	ATP-binding cassette, subfamily B (MDR/TAP), member 1B	3.90	1.47E-04
Slfn5	schlafen family member 5	3.87	1.07E-07
Fgr	Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog	3.84	2.76E-07
Cpne2	copine II	3.83	4.15E-09
Tlr3	toll-like receptor 3	3.82	1.20E-09
Pram1	PML-RARA regulated adaptor molecule 1	3.79	4.69E-04
MGC108823	similar to interferon-inducible GTPase	3.77	4.71E-05
Sp140	SP140 nuclear body protein	3.75	3.46E-08
Ube2l6	ubiquitin-conjugating enzyme E2L 6	3.74	1.15E-07
Fcgr3a	Fc fragment of IgG, low affinity IIIa, receptor	3.72	4.02E-05
Slc4a1	solute carrier family 4 (anion exchanger), member 1	3.68	6.24E-05
Casp1	caspase 1	3.63	9.24E-07
Lgals5	lectin, galactose binding, soluble 5	3.62	4.72E-07
Ccl3	chemokine (C-C motif) ligand 3	3.62	1.39E-03
Naaa	N-acylethanolamine acid amidase	3.60	2.89E-03
Samd9l	sterile alpha motif domain containing 9-like	3.59	7.82E-07
Ifi44	interferon-induced protein 44	3.56	1.40E-06
Ly86	lymphocyte antigen 86	3.54	2.19E-04
Slc6a12	solute carrier family 6 (neurotransmitter transporter, betaine/GAB	3.54	5.61E-05
Cd74	Cd74 molecule, major histocompatibility complex, class II	3.52	7.13E-06
Gbp1	guanylate binding protein 1, interferon-inducible	3.51	1.60E-06
Pstpip1	proline-serine-threonine phosphatase-interacting protein 1	3.47	2.96E-04
Cxcl1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activi	3.45	5.24E-04
Bcl3	B-cell CLL/lymphoma 3	3.35	1.77E-04

Ncf1	neutrophil cytosolic factor 1	3.35	1.30E-04
Cd69	Cd69 molecule	3.32	1.65E-06
Stat2	signal transducer and activator of transcription 2	3.31	7.31E-07
LOC688318	similar to leucine rich repeat containing 45	3.29	9.24E-07
Icam1	intercellular adhesion molecule 1	3.28	2.55E-07
Lrrc25	leucine rich repeat containing 25	3.28	7.74E-04
Il1rn	interleukin 1 receptor antagonist	3.25	9.40E-06
RT1-CE1	RT1 class I, locus1	3.20	1.29E-07
Fam46a	family with sequence similarity 46, member A	3.18	3.19E-08
Has2	hyaluronan synthase 2	3.18	5.05E-04
Serping1	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1	3.18	5.80E-08
Rasa4	RAS p21 protein activator 4	3.17	3.09E-07
Gpr114	G protein-coupled receptor 114	3.15	2.43E-06
Plek	pleckstrin	3.14	1.35E-04
Trim21	tripartite motif-containing 21	3.14	1.02E-07
Ripk3	receptor-interacting serine-threonine kinase 3	3.12	1.98E-04
Car13	carbonic anhydrase 13	3.12	2.14E-06
Itgb2	integrin, beta 2	3.10	7.00E-04
Ido2	indoleamine 2,3-dioxygenase 2	3.08	3.35E-06
Nfkbie	nuclear factor of kappa light polypeptide gene enhancer in B-cell	3.08	2.41E-09
Irf9	interferon regulatory factor 9	3.07	2.45E-08
RT1-Da	RT1 class II, locus Da	3.06	8.32E-07
Nfkb2	nuclear factor of kappa light polypeptide gene enhancer in B-cells	3.04	7.97E-08
Gsap	gamma-secretase activating protein	3.04	2.36E-06
Upp1	uridine phosphorylase 1	3.03	1.47E-05
Lgals3bp	lectin, galactoside-binding, soluble, 3 binding protein	3.03	9.08E-09
Ifi35	interferon-induced protein 35	3.03	6.39E-07
Mov10	Moloney leukemia virus 10	3.02	8.49E-09
Trex1	three prime repair exonuclease 1	3.00	4.75E-09
RT1-N2	RT1 class Ib, locus N2	2.98	3.61E-05
Trim30	tripartite motif-containing 30	2.97	1.19E-06
LOC688318	similar to leucine rich repeat containing 45	2.94	3.68E-07
Rbp1	retinol binding protein 1, cellular	2.93	5.83E-05
Znfx1	zinc finger, NFX1-type containing 1	2.93	2.39E-05
Tuba1c	tubulin, alpha 1C	2.93	4.27E-05
Trim25	tripartite motif-containing 25	2.93	1.11E-07
Pycard	PYD and CARD domain containing	2.91	4.46E-08
Clec4a1	C-type lectin domain family 4, member A1	2.88	9.11E-04
Tnfsf10	tumor necrosis factor (ligand) superfamily, member 10	2.86	2.37E-06
Mvp	major vault protein	2.84	1.83E-08
Ccl20	chemokine (C-C motif) ligand 20	2.82	3.74E-04
Slfn2	schlafen 2	2.81	7.26E-07
Tlr7	toll-like receptor 7	2.80	2.17E-05
C1r	complement component 1, r subcomponent	2.79	8.64E-08
P2ry6	pyrimidinergic receptor P2Y, G-protein coupled, 6	2.79	1.84E-04
Irg1	immunoresponsive gene 1	2.79	1.66E-06

Lilrb4	leukocyte immunoglobulin-like receptor, subfamily B, member 4	2.79	7.22E-05
Samd9l	sterile alpha motif domain containing 9-like	2.79	2.47E-05
Clec4d	C-type lectin domain family 4, member D	2.78	9.39E-04
Nmi	N-myc (and STAT) interactor	2.78	2.88E-07
Hcls1	hematopoietic cell specific Lyn substrate 1	2.73	2.50E-04
Ripk2	receptor-interacting serine-threonine kinase 2	2.72	1.62E-07
Ccl2	chemokine (C-C motif) ligand 2	2.71	9.99E-06
Sod2	superoxide dismutase 2, mitochondrial	2.70	3.28E-09
Tapbpl	TAP binding protein-like	2.70	3.81E-06
RT1-Ba	RT1 class II, locus Ba	2.69	2.52E-05
Relb	v-rel reticuloendotheliosis viral oncogene homolog B	2.69	2.56E-07
Ddr2	discoidin domain receptor tyrosine kinase 2	2.69	2.55E-06
RGD1561730	similar to cell surface receptor FDFACT	2.65	4.85E-04
Lyn	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	2.62	4.59E-04
RT1-M3-1	RT1 class Ib, locus M3, gene 1	2.61	7.12E-06
RT1-CE5	RT1 class I, locus CE5	2.61	3.40E-05
C3ar1	complement component 3a receptor 1	2.60	1.41E-03
Il18	interleukin 18	2.60	6.49E-04
Ptx3	pentraxin 3, long	2.59	2.42E-03
Psme2	proteasome (prosome, macropain) activator subunit 2	2.59	5.41E-08
Muc15	mucin 15, cell surface associated	2.59	1.73E-07
Zc3h12a	zinc finger CCCH type containing 12A	2.58	3.03E-05
Sec14l4	SEC14-like 4 (S. cerevisiae)	2.57	4.31E-07
Fyb	FYN binding protein	2.55	2.60E-04
Cxcl2	chemokine (C-X-C motif) ligand 2	2.55	2.18E-03
Sla	src-like adaptor	2.55	1.98E-03
Itgax	integrin, alpha X	2.54	9.34E-05
Cyp4b1	cytochrome P450, family 4, subfamily b, polypeptide 1	2.49	3.09E-03
LOC688318	similar to leucine rich repeat containing 45	2.48	8.98E-05
Osmr	oncostatin M receptor	2.47	8.88E-08
Clec2g	C-type lectin domain family 2, member G	2.47	2.36E-04
LOC691141	hypothetical protein LOC691141	2.47	1.65E-04
LOC688318	similar to leucine rich repeat containing 45	2.47	2.97E-04
Piezo1	piezo-type mechanosensitive ion channel component 1	2.47	2.78E-04
Ifitm3	interferon induced transmembrane protein 3	2.45	8.34E-08
Clec4e	C-type lectin domain family 4, member E	2.44	4.10E-04
Casp7	caspase 7	2.42	3.40E-09
Rarres2	retinoic acid receptor responder (tazarotene induced) 2	2.41	9.78E-05
Epsti1	epithelial stromal interaction 1 (breast)	2.40	1.17E-04
Slamf8	SLAM family member 8	2.40	2.57E-08
Aif1	allograft inflammatory factor 1	2.39	8.01E-04
Cd80	Cd80 molecule	2.38	9.20E-05
Tf	transferrin	2.37	1.81E-05
Trim34	tripartite motif-containing 34	2.36	2.23E-06
Zc3hav1	zinc finger CCCH type, antiviral 1	2.36	3.99E-06
Trem3	triggering receptor expressed on myeloid cells 3	2.32	1.55E-03

Lsp1	lymphocyte-specific protein 1	2.32	1.45E-04
Rhoh	ras homolog family member H	2.32	7.71E-04
Ifitm2	interferon induced transmembrane protein 2	2.31	3.04E-05
Tlr2	toll-like receptor 2	2.31	2.35E-04
Rnf125	ring finger protein 125	2.31	2.40E-03
Aoah	acyloxyacyl hydrolase (neutrophil)	2.31	1.47E-04
Igsf6	immunoglobulin superfamily, member 6	2.29	9.78E-04
Stx11	syntaxin 11	2.28	2.83E-03
Cst7	cystatin F (leukocystatin)	2.28	1.47E-07
Cyba	cytochrome b-245, alpha polypeptide	2.26	4.35E-05
Capg	capping protein (actin filament), gelsolin-like	2.26	5.70E-04
Slc12a7	solute carrier family 12 (potassium/chloride transporters)	2.25	2.06E-05
Mrc2	mannose receptor, C type 2	2.25	1.69E-05
Selplg	selectin P ligand	2.25	7.85E-04
Tor1b	torsin family 1, member B	2.23	4.36E-06
Clec4a3	C-type lectin domain family 4, member A3	2.23	2.71E-03
Sh2d1b	SH2 domain containing 1B	2.23	1.82E-04
Pla2g16	phospholipase A2, group XVI	2.22	8.93E-05
Anxa4	annexin A4	2.22	8.34E-08
Exoc3l4	exocyst complex component 3-like 4	2.21	5.89E-07
Nfkbia	nuclear factor of kappa light polypeptide gene enhancer in B-	2.21	5.32E-04
Nlrp1a	NLR family, pyrin domain containing 1A	2.21	1.82E-04
Tnfrsf1b	tumor necrosis factor receptor superfamily, member 1b	2.20	1.25E-04
Mybphl	myosin binding protein H-like	2.18	8.20E-04
Batf2	basic leucine zipper transcription factor, ATF-like 2	2.18	4.91E-05
Ifi27	interferon, alpha-inducible protein 27	2.16	1.69E-05
LOC688318	similar to leucine rich repeat containing 45	2.16	7.18E-07
Il13ra1	interleukin 13 receptor, alpha 1	2.16	2.82E-07
Fbxw17	F-box and WD-40 domain protein 17	2.15	4.75E-07
Rnase9	ribonuclease, RNase A family, 9 (non-active)	2.15	2.23E-03
Parp10	poly (ADP-ribose) polymerase family, member 10	2.15	5.50E-06
Psme1	proteasome (prosome, macropain) activator subunit 1	2.14	1.92E-07
LOC498276	Fc gamma receptor II beta	2.14	5.41E-04
LOC681325	hypothetical protein LOC681325	2.14	3.59E-05
Xdh	xanthine dehydrogenase	2.13	4.29E-05
LOC691221	similar to CG1998-PA	2.13	6.36E-06
Cyp4v3	cytochrome P450, family 4, subfamily v, polypeptide 3	2.11	6.86E-05
Slc7a7	solute carrier family 7 (amino acid transporter light chain, y+L	2.11	9.41E-04
LOC100912449	protein FAM115C-like	2.08	3.79E-05
Trpm2	transient receptor potential cation channel, subfamily M	2.08	1.44E-04
Il7	interleukin 7	2.06	4.00E-05
Lif	leukemia inhibitory factor	2.05	2.32E-03
Sgms2	sphingomyelin synthase 2	2.05	2.15E-03
Lst1	leukocyte specific transcript 1	2.04	1.33E-03
LOC688318	similar to leucine rich repeat containing 45	2.03	9.62E-05
Tspo	translocator protein	2.03	1.71E-05

Il13ra1	interleukin 13 receptor, alpha 1	2.02	3.62E-06
Chi3l1	chitinase 3-like 1 (cartilage glycoprotein-39)	2.01	1.12E-05
Cdc42ep5	CDC42 effector protein (Rho GTPase binding) 5	2.01	1.30E-05
Vamp5	vesicle-associated membrane protein 5	2.01	7.06E-09
Bcl2a1	BCL2-related protein A1	2.01	1.34E-04
Tmem140	transmembrane protein 140	2.00	9.99E-05
Il4r	interleukin 4 receptor	2.00	2.51E-04
Slc15a3	solute carrier family 15, member 3	2.00	1.51E-03
Fcgr1a	Fc fragment of IgG, high affinity Ia, receptor (CD64)	1.99	2.67E-03
Hk3	hexokinase 3 (white cell)	1.99	3.52E-03
Dennd3	DENN/MADD domain containing 3	1.99	5.28E-04
Cp	ceruloplasmin (ferroxidase)	1.98	1.61E-09
Fmo5	flavin containing monooxygenase 5	1.98	1.24E-05
Rhbg	Rh family, B glycoprotein	1.97	3.05E-04
Stard5	StAR-related lipid transfer (START) domain containing 5	1.97	3.06E-06
Pnp	purine nucleoside phosphorylase	1.97	3.77E-07
Fcer1g	Fc fragment of IgE, high affinity I, receptor for; gamma polypept	1.96	2.43E-04
Timp1	TIMP metallopeptidase inhibitor 1	1.96	1.35E-05
Ubd	ubiquitin D	1.96	5.66E-04
Serpina3n	serine (or cysteine) peptidase inhibitor, clade A, member 3N	1.96	6.24E-05
Slco4a1	solute carrier organic anion transporter family, member 4a1	1.95	3.21E-05
Trib1	tribbles homolog 1 (Drosophila)	1.95	2.47E-03
Parm1	prostate androgen-regulated mucin-like protein 1	1.95	1.02E-05
Batf3	basic leucine zipper transcription factor, ATF-like 3	1.95	4.67E-04
Cd97	CD97 molecule	1.95	1.32E-04
Bicc1	bicaudal C homolog 1 (Drosophila)	1.94	1.99E-06
Pter	phosphotriesterase related	1.94	6.91E-07
Arhgap8	Rho GTPase activating protein 8	1.93	2.77E-04
Cd68	Cd68 molecule	1.93	1.70E-03
Pgf	placental growth factor	1.93	1.86E-03
Cmklr1	chemokine-like receptor 1	1.92	4.77E-04
Klrk1	killer cell lectin-like receptor subfamily K, member 1	1.92	4.64E-04
C1qtnf1	C1q and tumor necrosis factor related protein 1	1.92	8.74E-05
Il10	interleukin 10	1.91	3.90E-05
Flt3l	FMS-like tyrosine kinase 3 ligand	1.91	3.16E-06
Slc2a9	solute carrier family 2 (facilitated glucose transporter), me	1.89	2.40E-04
Ascc3	activating signal cointegrator 1 complex subunit 3	1.89	1.68E-05
LOC688318	similar to leucine rich repeat containing 45	1.89	5.90E-04
LOC689499	similar to Y97E10AL.1	1.88	5.90E-04
Ccr5	chemokine (C-C motif) receptor 5	1.88	1.18E-04
Parp3	poly (ADP-ribose) polymerase family, member 3	1.88	5.90E-06
Mocos	molybdenum cofactor sulfurase	1.88	4.52E-04
Aida	axin interactor, dorsalization associated	1.88	4.70E-07
Psmf1	proteasome (prosome, macropain) inhibitor subunit 1	1.86	7.90E-07
Ddit3	DNA-damage inducible transcript 3	1.86	8.13E-06
Lilrc2	leukocyte immunoglobulin-like receptor, subfamily C, member 2	1.85	2.24E-04

LOC686120	hypothetical protein LOC686120	1.85	1.02E-04
LOC686120	hypothetical protein LOC686120	1.85	1.02E-04
LOC688318	similar to leucine rich repeat containing 45	1.84	4.28E-04
Ifi204	interferon activated gene 204	1.83	1.38E-04
Ikbke	inhibitor of kappa light polypeptide gene enhancer in B-cells,	1.82	1.61E-04
Slc22a18	solute carrier family 22, member 18	1.82	1.28E-03
Cdk2ap2	cyclin-dependent kinase 2 associated protein 2	1.82	4.21E-05
Map3k8	mitogen-activated protein kinase kinase kinase 8	1.81	1.32E-03
Trim5	tripartite motif-containing 5	1.81	5.10E-05
Plin2	perilipin 2	1.80	7.92E-04
LOC688318	similar to leucine rich repeat containing 45	1.79	2.09E-05
RGD1304982	similar to RIKEN cDNA 2810025M15	1.79	5.25E-05
Lyz2	lysozyme 2	1.79	1.55E-04
Batf	basic leucine zipper transcription factor, ATF-like	1.78	9.77E-04
Vamp8	vesicle-associated membrane protein 8	1.78	3.48E-06
Dbx2	developing brain homeobox 2	1.78	3.53E-05
Tmco4	transmembrane and coiled-coil domains 4	1.78	2.81E-04
Csf1	colony stimulating factor 1 (macrophage)	1.78	9.70E-05
Rnf114	ring finger protein 114	1.78	5.52E-09
Tmem173	transmembrane protein 173	1.77	2.49E-03
Rgs1	regulator of G-protein signaling 1	1.77	1.07E-03
Pdlim1	PDZ and LIM domain 1	1.76	7.73E-04
Casp8	caspase 8	1.75	1.22E-05
Pnpt1	polyribonucleotide nucleotidyltransferase 1	1.75	1.07E-06
Rab13	RAB13, member RAS oncogene family	1.75	8.41E-05
RT1-CE15	RT1 class I, locus CE15	1.74	4.88E-04
Tubb6	tubulin, beta 6 class V	1.72	7.55E-06
Slc7a3	solute carrier family 7 (cationic amino acid transporte	1.72	5.34E-04
Plxnd1	plexin D1	1.72	3.59E-05
LOC688318	similar to leucine rich repeat containing 45	1.71	4.06E-04
Tagln2	transgelin 2	1.71	2.31E-04
Atp13a1	ATPase type 13A1	1.70	7.73E-07
Cyp27a1	cytochrome P450, family 27, subfamily a, polypeptide 1	1.70	5.02E-06
Gsdmd	gasdermin D	1.70	4.91E-08
Clic4	chloride intracellular channel 4	1.70	3.02E-08
LOC688318	similar to leucine rich repeat containing 45	1.69	4.66E-06
Hpx	hemopexin	1.68	1.08E-03
Atp10a	ATPase, class V, type 10A	1.68	3.26E-05
Trim69	tripartite motif-containing 69	1.68	1.04E-03
Hfe	hemochromatosis	1.67	6.64E-08
Frmpd1	FERM and PDZ domain containing 1	1.67	3.44E-03
Rtp3	receptor (chemosensory) transporter protein 3	1.67	6.85E-05
Rab19	RAB19, member RAS oncogene family	1.67	7.48E-05
Isoc2b	isochorismatase domain containing 2b	1.67	2.42E-04
Rnasel	ribonuclease L (2',5'-oligoisoadenylate synthetase-depe	1.66	2.51E-04
Nfkb1	nuclear factor of kappa light polypeptide gene enhancer in B-c	1.66	2.89E-04

RGD1561157	RGD1561157	1.66	8.32E-04
RT1-DMa	RT1 class II, locus DMa	1.65	6.72E-04
LOC688318	similar to leucine rich repeat containing 45	1.65	1.44E-04
Chadl	chondroadherin-like	1.65	2.97E-05
Nod1	nucleotide-binding oligomerization domain containing 1	1.65	1.90E-04
LOC685289	similar to paired immunoglobin-like type 2 receptor beta	1.64	1.81E-03
Usp25	ubiquitin specific peptidase 25	1.64	8.79E-06
LOC688318	similar to leucine rich repeat containing 45	1.63	3.12E-04
Fam49a	family with sequence similarity 49, member A	1.62	3.06E-06
Mst1r	macrophage stimulating 1 receptor (c-met-related tyrosine kina	1.62	1.27E-04
Clic1	chloride intracellular channel 1	1.61	3.78E-05
Irak3	interleukin-1 receptor-associated kinase 3	1.61	5.13E-04
Ms4a4a	membrane-spanning 4-domains, subfamily A, member 4A	1.61	2.91E-03
Nr1h3	nuclear receptor subfamily 1, group H, member 3	1.60	1.34E-03
AA926063	AA926063gene	1.60	1.24E-03
LOC680923	similar to paired immunoglobin-like type 2 receptor beta	1.60	3.05E-06
LOC688318	similar to leucine rich repeat containing 45	1.59	1.00E-03
Tnip1	TNFAIP3 interacting protein 1	1.59	1.82E-04
Fkbp11	FK506 binding protein 11	1.59	8.17E-04
Eci3	enoyl-Coenzyme A delta isomerase 3	1.59	1.67E-03
Bace2	beta-site APP-cleaving enzyme 2	1.58	2.41E-04
Irak2	interleukin-1 receptor-associated kinase 2	1.58	2.57E-03
LOC100910979	interferon-inducible GTPase 1-like	1.58	8.40E-04
Rsl1	regulator of sex limited protein 1	1.58	1.63E-04
Cnksr1	connector enhancer of kinase suppressor of Ras 1	1.58	1.92E-03
Aldh1l2	aldehyde dehydrogenase 1 family, member L2	1.57	2.36E-04
Pdpn	podoplanin	1.57	2.32E-04
Creb3l1	cAMP responsive element binding protein 3-like 1	1.56	7.17E-04
Iah1	isoamyl acetate-hydrolyzing esterase 1 homolog (S. cerevisiae)	1.56	5.78E-06
Erap1	endoplasmic reticulum aminopeptidase 1	1.56	3.12E-07
Ogfr	opioid growth factor receptor	1.56	3.21E-08
Lgals3	lectin, galactoside-binding, soluble, 3	1.55	3.61E-04
Pcsk1	proprotein convertase subtilisin/kexin type 1	1.55	1.25E-04
Adar	adenosine deaminase, RNA-specific	1.55	6.84E-05
Rgs16	regulator of G-protein signaling 16	1.54	3.46E-05
Trpv2	transient receptor potential cation channel, subfamily V, member 2	1.54	2.02E-03
Cxcr5	chemokine (C-X-C motif) receptor 5	1.54	2.08E-04
Tpm3	tropomyosin 3	1.54	6.41E-05
Agmo	alkylglycerol monooxygenase	1.53	3.35E-03
Lpcat3	lysophosphatidylcholine acyltransferase 3	1.53	7.56E-05
Stxbp2	syntaxin binding protein 2	1.53	1.23E-03
Tdrd7	tudor domain containing 7	1.53	4.66E-04
Rnaset2	ribonuclease T2	1.53	2.18E-04
Slc7a1	solute carrier family 7 (cationic amino acid transporter, y+ sys	1.52	9.67E-04
Mertk	c-mer proto-oncogene tyrosine kinase	1.52	1.00E-05
Rab20	RAB20, member RAS oncogene family	1.52	1.24E-03

Mylk	myosin light chain kinase	1.52	1.14E-05
P2rx4	purinergic receptor P2X, ligand-gated ion channel 4	1.51	1.07E-03
Sertad1	SERTA domain containing 1	1.51	1.70E-03
Vasp	vasodilator-stimulated phosphoprotein	1.51	1.34E-03
LOC688318	similar to leucine rich repeat containing 45	1.51	4.35E-04
Gch1	GTP cyclohydrolase 1	1.50	5.17E-04
Msn	moesin	1.49	5.56E-04
Nradd	neurotrophin receptor associated death domain	1.49	1.48E-03
LOC688318	similar to leucine rich repeat containing 45	1.49	4.64E-06
Ifngr2	interferon gamma receptor 2	1.49	1.38E-06
Dok1	docking protein 1	1.49	1.41E-04
Aldh3b1	aldehyde dehydrogenase 3 family, member B1	1.49	3.16E-03
Myd88	myeloid differentiation primary response 88	1.48	2.95E-04
Fkbp5	FK506 binding protein 5	1.48	1.17E-04
Grn	granulin	1.48	9.66E-05
Shc1	SHC (Src homology 2 domain containing) transforming protein 1	1.48	2.16E-04
Tmem243	transmembrane protein 243, mitochondrial	1.48	3.37E-07
Serpinb9	serpin peptidase inhibitor, clade B (ovalbumin), member 9	1.47	2.04E-03
LOC688318	similar to leucine rich repeat containing 45	1.47	5.97E-04
Hsh2d	hematopoietic SH2 domain containing	1.47	5.44E-06
Mfsd2a	major facilitator superfamily domain containing 2A	1.47	1.72E-03
Cd1d1	CD1d1 molecule	1.47	4.27E-04
Daxx	death-domain associated protein	1.46	4.25E-06
Slc35d2	solute carrier family 35, member D2	1.46	2.19E-03
Slc50a1	solute carrier family 50 (sugar transporter), member 1	1.46	7.53E-07
S1pr3	sphingosine-1-phosphate receptor 3	1.45	1.11E-04
Ptk2b	protein tyrosine kinase 2 beta	1.45	1.13E-04
Ctsh	cathepsin H	1.45	1.67E-05
Tmem63a	transmembrane protein 63a	1.45	2.67E-03
Bak1	BCL2-antagonist/killer 1	1.45	7.60E-04
Dnajc25	DnaJ (Hsp40) homolog, subfamily C, member 25	1.45	2.41E-04
Anxa1	annexin A1	1.45	3.73E-04
Mina	myc induced nuclear antigen	1.45	3.01E-03
Mad2l1bp	MAD2L1 binding protein	1.44	1.36E-04
B4galt1	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide	1.44	1.55E-03
Slc16a1	solute carrier family 16, member 1 (monocarboxylic acid transporte	1.43	3.26E-04
Enpp4	ectonucleotide pyrophosphatase/phosphodiesterase 4	1.43	3.50E-06
H6pd	hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)	1.43	3.13E-03
Gda	guanine deaminase	1.43	2.49E-03
Fgfrl1	fibroblast growth factor receptor-like 1	1.43	3.85E-04
Slc25a39	solute carrier family 25, member 39	1.43	6.82E-04
Plscr1	phospholipid scramblase 1	1.42	2.91E-03
Spats2l	spermatogenesis associated, serine-rich 2-like	1.42	3.87E-05
Prcp	prolylcarboxypeptidase (angiotensinase C)	1.42	4.33E-05
Vamp3	vesicle-associated membrane protein 3	1.42	1.16E-05
Ece1	endothelin converting enzyme 1	1.42	2.82E-08

Jag1	jagged 1	1.42	1.86E-04
Mgmt	O-6-methylguanine-DNA methyltransferase	1.42	1.49E-03
Ephx1	epoxide hydrolase 1, microsomal (xenobiotic)	1.41	1.53E-03
Il10rb	interleukin 10 receptor, beta	1.41	9.06E-05
RT1-CE10	RT1 class I, locus CE10	1.41	6.60E-04
Sectm1b	secreted and transmembrane 1B	1.41	1.54E-04
Coro1b	coronin, actin-binding protein, 1B	1.41	5.78E-05
Litaf	lipopolysaccharide-induced TNF factor	1.40	1.10E-04
Ethe1	ethylmalonic encephalopathy 1	1.40	2.09E-03
Eqtn	equatorin, sperm acrosome associated	-1.40	3.91E-04
LOC688318	similar to leucine rich repeat containing 45	-1.40	9.59E-04
Mdga2	MAM domain containing glycosylphosphatidylinositol anchor 2	-1.40	1.51E-04
PCDH8	PCDH8	-1.41	3.39E-04
Smoc1	FQ222878 // Smoc1 // SPARC related modular calcium binding 1 // 6q24 // 314280 ///	-1.41	1.26E-04
Notch2	notch 2	-1.41	2.65E-05
Cav2	caveolin 2	-1.41	8.37E-04
Hrsp12	heat-responsive protein 12	-1.41	8.06E-08
Dgcr8	DiGeorge syndrome critical region gene 8	-1.41	3.56E-03
Thsd7b	thrombospondin, type I, domain containing 7B	-1.41	3.39E-03
Tst	thiosulfate sulfurtransferase	-1.41	2.29E-04
Gabrg1	gamma-aminobutyric acid (GABA) A receptor, gamma 1	-1.41	1.15E-04
Fat3	FAT atypical cadherin 3	-1.41	6.86E-04
RGD1563159	RGD1563159	-1.41	3.12E-03
Ttpa	tocopherol (alpha) transfer protein	-1.41	8.21E-04
Tenm3	teneurin transmembrane protein 3	-1.41	1.69E-04
Clcn4	chloride channel, voltage-sensitive 4	-1.41	1.17E-03
Kndc1	kinase non-catalytic C-lobe domain (KIND) containing 1	-1.42	1.95E-04
Nme3	NME/NM23 nucleoside diphosphate kinase 3	-1.42	1.15E-04
LOC100363155	HAUS augmin-like complex, subunit 4-like	-1.42	4.03E-04
Htr3a	5-hydroxytryptamine (serotonin) receptor 3A, ionotropic	-1.42	2.78E-03
Cdh20	cadherin 20	-1.43	1.03E-04
Gatm	glycine amidinotransferase (L-arginine:glycine amidinotransferase)	-1.43	2.06E-06
Slc38a3	solute carrier family 38, member 3	-1.43	1.64E-03
Pdgfrb	platelet derived growth factor receptor, beta polypeptide	-1.43	1.89E-04
Gstt3	glutathione S-transferase, theta 3	-1.43	1.08E-04
Glud1	glutamate dehydrogenase 1	-1.43	3.23E-06
Fbxo2	F-box protein 2	-1.44	1.26E-05
Cav2	caveolin 2	-1.44	7.37E-05
Slc6a11	solute carrier family 6 (neurotransmitter transporter,	-1.44	1.03E-03
Parvb	parvin, beta	-1.44	3.82E-05
Enox1	ecto-NOX disulfide-thiol exchanger 1	-1.45	3.54E-04
Nckap5	NCK-associated protein 5	-1.45	1.11E-04
Rgs13	regulator of G-protein signaling 13	-1.45	3.48E-04
Plxnb3	plexin B3	-1.45	1.22E-04
Mmd2	monocyte to macrophage differentiation-associated 2	-1.45	8.01E-05

Ndp	Norrie disease (pseudoglioma)	-1.45	1.19E-05
Frem1	Fras1 related extracellular matrix 1	-1.45	2.07E-03
Pcdhb6	protocadherin beta 6	-1.45	1.18E-03
Nog	noggin	-1.46	4.26E-04
Dnai1	dynein, axonemal, intermediate chain 1	-1.46	2.70E-03
RGD1564308	similar to LOC495042 protein	-1.46	7.05E-05
Metrn	meteorin, glial cell differentiation regulator	-1.47	3.24E-05
Lrp4	low density lipoprotein receptor-related protein 4	-1.48	2.14E-04
Mfge8	milk fat globule-EGF factor 8 protein	-1.48	2.67E-04
Kcne1l	KCNE1-like	-1.48	2.75E-04
Dnah9	dynein, axonemal, heavy polypeptide 9	-1.49	4.81E-04
Zswim2	zinc finger, SWIM-type containing 2	-1.49	3.20E-05
Scrg1	stimulator of chondrogenesis 1	-1.49	1.11E-06
Sox5l1	SRY-box containing gene 5-like 1	-1.49	8.82E-04
Dnah9	dynein, axonemal, heavy polypeptide 9	-1.49	8.78E-04
Ago3	argonaute RISC catalytic component 3	-1.50	6.12E-05
Celsr2	cadherin, EGF LAG seven-pass G-type receptor 2	-1.50	3.11E-04
5031425E22Rik	RIKEN cDNA 5031425E22 gene	-1.51	1.27E-03
Ramp1	receptor (G protein-coupled) activity modifying protein 1	-1.51	2.14E-03
Folh1	folate hydrolase 1	-1.51	1.41E-04
Id4	inhibitor of DNA binding 4	-1.52	4.04E-05
Gpld1	glycosylphosphatidylinositol specific phospholipase D1	-1.52	3.62E-05
Lpar4	lysophosphatidic acid receptor 4	-1.52	2.08E-05
Mir568	microRNA mir-568	-1.52	5.93E-04
Lair1	leukocyte-associated immunoglobulin-like receptor 1	-1.52	1.70E-03
Ms4a14	membrane-spanning 4-domains, subfamily A, member 14	-1.53	3.55E-04
Mreg	melanoregulin	-1.53	1.37E-03
Cldn9	claudin 9	-1.54	9.51E-05
Kcnb2	M77482 // Kcnb2 // potassium voltage gated channel, Shab-related subfamily, member 2 //	-1.54	4.65E-04
Abhd3	abhydrolase domain containing 3	-1.54	2.76E-04
Zbtb20	zinc finger and BTB domain containing 20	-1.54	5.06E-04
Aamdc	adipogenesis associated, Mth938 domain containing	-1.54	1.67E-05
Btbd17	BTB (POZ) domain containing 17	-1.55	4.58E-04
Gabrb1	gamma-aminobutyric acid (GABA) A receptor, beta 1	-1.55	1.53E-04
Krtap3-3	keratin associated protein 3-3	-1.55	7.61E-04
Ntn4	netrin 4	-1.56	7.01E-06
Cd109	CD109 molecule	-1.56	6.39E-04
Smad9	SMAD family member 9	-1.56	2.87E-03
Hes5	hairy and enhancer of split 5 (Drosophila)	-1.59	1.14E-03
Slc15a2	solute carrier family 15 (H+/peptide transporter), member 2 // 1	-1.59	2.36E-05
Dnah9	dynein, axonemal, heavy polypeptide 9	-1.59	5.66E-04
Efemp1	EGF-containing fibulin-like extracellular matrix protein 1	-1.60	1.04E-03
Dnah9	dynein, axonemal, heavy polypeptide 9	-1.60	2.95E-03
Sorbs1	sorbin and SH3 domain containing 1	-1.61	1.30E-09
Igsf1	immunoglobulin superfamily, member 1	-1.61	1.07E-04
Veph1	ventricular zone expressed PH domain-containing 1	-1.61	6.10E-04

LOC688318	similar to leucine rich repeat containing 45	-1.62	2.70E-04
Tril	TLR4 interactor with leucine-rich repeats	-1.63	1.30E-04
LOC688318	similar to leucine rich repeat containing 45	-1.63	2.15E-03
LOC100363035	LOC100363035	-1.64	2.65E-06
C1ql1	complement component 1, q subcomponent-like 1	-1.64	2.72E-03
Pdzrn3	PDZ domain containing RING finger 3	-1.65	8.01E-06
LOC100909587	zinc finger protein 397-like	-1.66	1.02E-03
Lrrc55	leucine rich repeat containing 55	-1.68	4.64E-04
Ankfn1	ankyrin-repeat and fibronectin type III domain containing 1	-1.68	1.74E-04
LOC685158	similar to CG8138-PA	-1.69	3.21E-03
Cyp7b1	cytochrome P450, family 7, subfamily b, polypeptide 1	-1.69	9.34E-07
Notch3	notch 3	-1.70	1.17E-04
Capn6	calpain 6	-1.71	3.74E-04
Adamts9	ADAM metallopeptidase with thrombospondin type 1 motif, 9	-1.72	2.94E-03
Mir99a	microRNA mir-99a	-1.74	3.30E-03
Scn7a	sodium channel, voltage-gated, type VII, alpha	-1.77	1.95E-05
Slc26a3	solute carrier family 26, member 3	-1.78	1.63E-03
Gmnc	geminin coiled-coil domain containing	-1.78	3.70E-04
Rlbp1	retinaldehyde binding protein 1	-1.78	4.84E-05
Slco1c1	solute carrier organic anion transporter family, member 1c1	-1.79	1.10E-03
Timp4	tissue inhibitor of metalloproteinase 4	-1.79	2.15E-03
Rftn2	raftlin family member 2	-1.79	1.24E-05
Lix1	Lix1 homolog (chicken)	-1.79	4.38E-05
Gfral	GDNF family receptor alpha like	-1.81	2.22E-05
Pla2g5	phospholipase A2, group V	-1.81	1.82E-03
LOC100363484	DNA-damage-inducible transcript 4-like protein-like	-1.82	6.20E-04
Olr522	olfactory receptor 522	-1.84	7.35E-04
Mfap3l	microfibrillar-associated protein 3-like	-1.98	4.14E-06
Fzd8	frizzled family receptor 8	-1.99	5.88E-05
Aadat	aminoadipate aminotransferase	-1.99	2.43E-04
Aox1	aldehyde oxidase 1	-1.99	3.25E-04
Fezf2	Fez family zinc finger 2	-2.01	2.12E-03
Lrrc2	leucine rich repeat containing 2	-2.08	2.11E-05
Nkain4	K+ transporting ATPase interacting 4	-2.10	1.04E-03
Grin2c	glutamate receptor, ionotropic, N-methyl D-aspartate 2C	-2.11	1.78E-05
Galp	galanin-like peptide	-2.12	6.78E-05
Cldn19	claudin 19	-2.15	1.91E-05
Ccl24	chemokine (C-C motif) ligand 24	-2.17	5.36E-04
Fmo1	flavin containing monooxygenase 1	-2.24	1.80E-03
Doc2g	double C2-like domains, gamma	-2.32	5.55E-04
Tlr5	toll-like receptor 5	-2.44	1.89E-05
Ppp1r1b	protein phosphatase 1, regulatory (inhibitor) subunit 1B	-2.56	2.16E-05
Papss2	3'-phosphoadenosine 5'-phosphosulfate synthase 2	-2.84	4.93E-07
Stab1	stabilin 1	-3.08	2.76E-04
Slc40a1	solute carrier family 40 (iron-regulated transporter), member 1	-3.20	4.70E-08
Sucnr1	succinate receptor 1	-3.29	1.32E-06

8.1.2 Genes identified in serum only treated myelinating cultures

Table 4.3.3a: Differentially expressed genes in serum alone treated myelinating cultures identified by Partek*. These data show the fold change and significance of the entities that were upregulated ≥ 1.4 serum treated cultures in comparison to control cultures. Significance was calculated using one-way ANOVA.*

Gene Symbol	Gene Name	Fold Change	p-value
Lcn2	lipocalin 2	39.40	6.41E-10
Cxcl11	chemokine (C-X-C motif) ligand 11	34.86	2.15E-09
Ccl7	chemokine (C-C motif) ligand 7	28.72	2.89E-10
Cxcl5	chemokine (C-X-C motif) ligand 5	27.14	1.95E-05
Cxcl1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity	23.12	1.69E-07
LOC363060	similar to RIKEN cDNA 1600029D21	14.93	9.30E-07
Cxcl9	chemokine (C-X-C motif) ligand 9	12.50	2.70E-08
Serpine1	serpin peptidase inhibitor, clade E (nexin, plasminog	10.06	4.61E-07
Cxcl2	chemokine (C-X-C motif) ligand 2	9.78	1.64E-06
Cxcl10	chemokine (C-X-C motif) ligand 10	9.23	2.89E-04
Trem1	triggering receptor expressed on myeloid cells 1	9.20	1.72E-05
C3	complement component 3	8.92	5.49E-08
Mx1	myxovirus (influenza virus) resistance 1	7.95	2.38E-05
Ccl20	chemokine (C-C motif) ligand 20	7.86	1.07E-06
Ass1	argininosuccinate synthase 1	7.63	9.18E-07
Ifit3	interferon-induced protein with tetratricopeptide repeats 3	7.60	7.44E-05
Gbp5	guanylate binding protein 5	7.53	5.06E-08
Tgm1	transglutaminase 1	7.01	3.59E-07
Ptx3	pentraxin 3, long	6.96	9.55E-06
Il1rn	interleukin 1 receptor antagonist	6.74	1.11E-07
Slpi	secretory leukocyte peptidase inhibitor	6.49	2.13E-04
Bcl3	lymphoma 3	6.48	4.44E-06
Sell	selectin L	6.10	1.15E-03
Ccl6	chemokine (C-C motif) ligand 6	5.88	7.46E-04
Irf7	interferon regulatory factor 7	5.76	1.23E-05
Zc3h12a	zinc finger CCCH type containing 12A	5.60	1.35E-07
Birc3	baculoviral IAP repeat-containing 3	5.36	2.76E-09
Cd93	CD93 molecule	5.32	5.93E-07
Tnfaip2	tumor necrosis factor, alpha-induced protein 2	5.21	4.11E-09
Msr1	macrophage scavenger receptor 1	5.05	8.77E-06
Ccl4	chemokine (C-C motif) ligand 4	5.05	1.58E-05
Ifit2	interferon-induced protein with tetratricopeptide repeats 2	4.90	2.83E-04
Apol9a	apolipoprotein L 9a	4.80	3.40E-06
Rbp1	retinol binding protein 1, cellular	4.80	2.15E-06
Isg15	ISG15 ubiquitin-like modifier	4.71	2.15E-05
Mmp9	matrix metallopeptidase 9	4.69	7.11E-03
Serpina3n	serine (or cysteine) peptidase inhibitor, clade A, member 3N	4.69	3.18E-08
Lgals9	lectin, galactoside-binding, soluble, 9	4.66	4.61E-06
Ch25h	cholesterol 25-hydroxylase	4.64	2.42E-05

Lif	leukemia inhibitory factor	4.61	6.20E-06
Lyve1	lymphatic vessel endothelial hyaluronan receptor 1	4.60	6.88E-05
Usp18	ubiquitin specific peptidase 18	4.59	1.84E-04
Ccl2	chemokine (C-C motif) ligand 2	4.57	2.09E-07
Oasl	2'-5'-oligoadenylate synthetase-like	4.42	2.60E-04
Orm1	orosomucoid 1	4.41	1.31E-06
Ccl5	chemokine (C-C motif) ligand 5	4.40	1.45E-07
Chi3l1	chitinase 3-like 1 (cartilage glycoprotein-39)	4.18	1.45E-08
Cd14	CD14 molecule	4.15	2.11E-05
LOC688318	similar to leucine rich repeat containing 45	4.09	2.79E-03
Rab32	RAB32, member RAS oncogene family	4.07	3.92E-06
Upp1	uridine phosphorylase 1	3.98	2.09E-06
Tubb6	tubulin, beta 6 class V	3.97	1.17E-09
Itgam	integrin, alpha M	3.96	9.82E-04
Akr1b8	aldo-keto reductase family 1, member B8	3.93	2.07E-03
Cyp4b1	cytochrome P450, family 4, subfamily b, polypeptide 1	3.90	1.81E-04
Gldn	gliomedin	3.84	1.01E-04
Rsad2	radical S-adenosyl methionine domain containing 2	3.79	4.60E-03
Il1r2	interleukin 1 receptor, type II	3.79	2.71E-03
Isg20	interferon stimulated exonuclease gene 20	3.77	9.59E-05
Pgf	placental growth factor	3.74	7.41E-06
Ifi27l2b	interferon, alpha-inducible protein 27 like 2B	3.70	1.51E-06
Cxcl16	chemokine (C-X-C motif) ligand 16	3.67	3.14E-08
RT1-CE4	RT1 class I, locus CE4	3.65	3.46E-05
Cd36	CD36 molecule (thrombospondin receptor)	3.63	1.30E-03
Osmr	oncostatin M receptor	3.61	3.05E-09
Mpa2l	macrophage activation 2 like	3.58	1.70E-05
Psmb8	proteasome (prosome, macropain) subunit, beta type, 8	3.58	6.37E-07
Fmo5	flavin containing monooxygenase 5	3.56	4.07E-08
Gal	GMAP prepropeptide	3.56	2.48E-05
Tf	transferrin	3.48	6.55E-07
Sod2	superoxide dismutase 2, mitochondrial	3.48	3.58E-10
Cxcl13	chemokine (C-X-C motif) ligand 13	3.45	1.17E-03
Hrct1	histidine rich carboxyl terminus 1	3.43	8.26E-07
Fosl1	fos-like antigen 1	3.34	1.09E-04
Slfn3	schlafen 3	3.31	2.47E-03
Psmb9	proteasome (prosome, macropain) subunit, beta type, 9	3.30	6.68E-07
Casp12	caspase 12	3.29	4.51E-07
Lsp1	lymphocyte-specific protein 1	3.23	8.81E-06
Ccl3	chemokine (C-C motif) ligand 3	3.22	2.63E-03
Fgr	Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog	3.20	1.07E-06
Clec4d	C-type lectin domain family 4, member D	3.16	4.02E-04
LOC685067	similar to guanylate binding protein family, member	3.11	1.29E-04
Il17rb	interleukin 17 receptor B	3.07	1.42E-05
Ube2l6	ubiquitin-conjugating enzyme E2L 6	3.07	5.31E-07
Oas2	2'-5' oligoadenylate synthetase 2	3.06	5.80E-05

Serping1	serpin peptidase inhibitor, clade G (C1 inhibitor), member 1	3.05	8.20E-08
Vsig4	V-set and immunoglobulin domain containing 4	3.03	1.07E-04
Srgn	serglycin	3.03	1.33E-03
Clec4a2	C-type lectin domain family 4, member A2	3.03	5.13E-05
Gda	guanine deaminase	3.02	2.13E-07
Piezo1	piezo-type mechanosensitive ion channel component 1	3.00	5.77E-05
P4ha3	prolyl 4-hydroxylase, alpha polypeptide III	2.99	6.00E-06
Il1a	interleukin 1 alpha	2.99	1.30E-04
Fndc3c1	fibronectin type III domain containing 3C1	2.98	4.55E-07
RT1-CE12	RT1 class I, locus CE12	2.98	2.10E-06
LOC688318	similar to leucine rich repeat containing 45	2.97	8.15E-07
Vgf	VGF nerve growth factor inducible	2.97	4.77E-05
Spry4	sprouty homolog 4 (Drosophila)	2.96	1.97E-03
Timp1	TIMP metallopeptidase inhibitor 1	2.93	1.87E-07
Reg3b	regenerating islet-derived 3 beta	2.91	1.21E-04
Csf2rb	colony stimulating factor 2 receptor, beta, low-affinit	2.90	5.32E-03
Rgs1	regulator of G-protein signaling 1	2.88	7.45E-06
Alpl	alkaline phosphatase, liver/bone/kidney	2.86	3.31E-07
Trib1	tribbles homolog 1 (Drosophila)	2.86	8.90E-05
Ptgs1	prostaglandin-endoperoxide synthase 1	2.85	3.56E-04
Abcb1b	ATP-binding cassette, subfamily B (MDR/TAP), member 1B	2.84	1.07E-03
Lilrb4	leukocyte immunoglobulin-like receptor, subfamily B, member 4	2.84	6.24E-05
Vcam1	vascular cell adhesion molecule 1	2.83	9.71E-07
Dhx58	DEXH (Asp-Glu-X-His) box polypeptide 58	2.82	1.77E-04
Oas1b	2-5 oligoadenylate synthetase 1B	2.80	8.88E-04
Uba7	ubiquitin-like modifier activating enzyme 7	2.79	2.99E-04
Ccl12	chemokine (C-C motif) ligand 12	2.78	3.25E-04
Itgax	integrin, alpha X	2.76	4.68E-05
LOC681182	similar to paired immunoglobin-like type 2 receptor beta	2.75	8.56E-04
LOC688318	similar to leucine rich repeat containing 45	2.74	3.80E-05
Serpinb2	serpin peptidase inhibitor, clade B (ovalbumin), member 2	2.73	1.79E-04
Itga8	integrin, alpha 8	2.72	5.30E-07
Tuba1c	tubulin, alpha 1C	2.71	7.94E-05
Ier3	immediate early response 3	2.66	2.14E-04
Stat5a	signal transducer and activator of transcription 5A	2.63	1.69E-05
Ripk3	receptor-interacting serine-threonine kinase 3	2.62	7.04E-04
Oaf	OAF homolog (Drosophila)	2.61	6.10E-06
Cyp1a1	cytochrome P450, family 1, subfamily a, polypeptide 1	2.60	1.66E-04
Oas1i	2 ' -5 ' oligoadenylate synthetase 1I	2.58	3.43E-04
Rab20	RAB20, member RAS oncogene family	2.56	1.56E-06
Gda	guanine deaminase	2.55	2.34E-07
Tmem106a	transmembrane protein 106A	2.55	1.40E-05
Icam1	intercellular adhesion molecule 1	2.54	2.44E-06
Rtp4	receptor (chemosensory) transporter protein 4	2.53	5.65E-05
Dhrs9	dehydrogenase/reductase (SDR family) member 9	2.52	8.06E-05
Rab27b	RAB27B, member RAS oncogene family	2.52	4.36E-05

Rnf125	ring finger protein 125	2.52	1.23E-03
Ms4a11	membrane-spanning 4-domains, subfamily A, member 11	2.50	2.14E-05
Ido2	indoleamine 2,3-dioxygenase 2	2.50	2.08E-05
Sp140	SP140 nuclear body protein	2.49	1.13E-06
Pdlim1	PDZ and LIM domain 1	2.48	1.81E-05
Dbx2	developing brain homeobox 2	2.47	6.35E-07
Calcb	calcitonin-related polypeptide, beta	2.47	7.56E-06
Pram1	PML-RARA regulated adaptor molecule 1	2.45	6.52E-03
Rgs16	regulator of G-protein signaling 16	2.45	4.56E-08
Slc16a3	solute carrier family 16, member 3 (monocarboxylic acid transporter)	2.44	8.01E-05
Shc4	SHC (Src homology 2 domain containing) family, member 4	2.43	7.52E-05
Olr1	oxidized low density lipoprotein (lectin-like) receptor 1	2.43	9.91E-05
Mmp3	matrix metallopeptidase 3	2.43	1.85E-03
Ddx60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60	2.42	6.60E-03
Rdh5	retinol dehydrogenase 5 (11-cis/9-cis)	2.41	8.51E-06
Parm1	prostate androgen-regulated mucin-like protein 1	2.41	8.51E-07
Oasl2	2'-5' oligoadenylate synthetase-like 2	2.38	4.54E-04
Tap1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	2.37	5.51E-06
Sox7	SRY (sex determining region Y)-box 7	2.37	1.81E-04
Nfkbia	nuclear factor of kappa light polypeptide gene enhancer in B-cells	2.37	2.86E-04
Fstl3	follistatin-like 3 (secreted glycoprotein)	2.36	3.34E-04
Cdc42ep5	CDC42 effector protein (Rho GTPase binding) 5	2.36	2.01E-06
Psmb10	proteasome (prosome, macropain) subunit, beta type 10	2.36	2.61E-05
Phlda1	pleckstrin homology-like domain, family A, member 1	2.35	1.18E-03
Cmpk2	cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial	2.35	8.64E-04
Cp	ceruloplasmin (ferroxidase)	2.34	1.85E-10
Eya2	eyes absent homolog 2 (Drosophila)	2.33	1.09E-07
LOC689800	similar to osteoclast inhibitory lectin	2.33	9.26E-04
C1r	complement component 1, r subcomponent	2.31	5.91E-07
Emp3	epithelial membrane protein 3	2.31	3.46E-04
Cxcl17	chemokine (C-X-C motif) ligand 17	2.30	1.54E-04
Slc26a2	solute carrier family 26 (sulfate transporter), member	2.29	1.39E-04
Anxa1	annexin A1	2.29	3.50E-07
Aldh1l2	aldehyde dehydrogenase 1 family, member L2	2.29	1.39E-06
RT1-T24-3	RT1 class I, locus T24, gene 3	2.28	6.26E-05
Galnt3	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosam	2.28	1.93E-05
Ifih1	interferon induced with helicase C domain 1	2.27	4.50E-04
Lgals3bp	lectin, galactoside-binding, soluble, 3 binding protein	2.26	1.72E-07
Sema3a	sema domain, immunoglobulin domain (Ig), short basic domain, secreted	2.26	3.51E-06
Sp100	SP100 nuclear antigen	2.26	7.39E-07
Tap2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	2.25	2.03E-04
Micall2	MICAL-like 2	2.25	1.39E-07
Tapbp	TAP binding protein (tapasin)	2.24	6.11E-06
Nfkb2	nuclear factor of kappa light polypeptide gene enhancer in B-cells	2.24	1.62E-06
Il18bp	interleukin 18 binding protein	2.24	1.93E-04

Gsap	gamma-secretase activating protein	2.23	4.11E-05
Lgals3	lectin, galactoside-binding, soluble, 3	2.23	2.31E-06
Lrrc15	leucine rich repeat containing 15	2.23	2.99E-06
Slc2a9	solute carrier family 2 (facilitated glucose transporter), member 9	2.22	4.00E-05
Clcf1	cardiotrophin-like cytokine factor 1	2.21	1.84E-03
Ifi44	interferon-induced protein 44	2.21	8.68E-05
Batf3	basic leucine zipper transcription factor, ATF-like 3	2.20	1.29E-04
LOC688318	similar to leucine rich repeat containing 45	2.19	3.73E-05
Il1b	interleukin 1 beta	2.19	4.18E-04
Mmp13	matrix metallopeptidase 13	2.19	5.12E-03
Cd80	Cd80 molecule	2.19	2.07E-04
Ifitm3	interferon induced transmembrane protein 3	2.18	3.25E-07
Scimp	SLP adaptor and CSK interacting membrane protein	2.17	3.33E-04
Clic1	chloride intracellular channel 1	2.17	5.13E-07
Parp12	poly (ADP-ribose) polymerase family, member 12	2.16	1.74E-04
Hmga2	high mobility group AT-hook 2	2.15	6.09E-03
Slc35d3	solute carrier family 35, member D3	2.14	5.37E-05
Frmpd1	FERM and PDZ domain containing 1	2.14	2.11E-04
S100a6	S100 calcium binding protein A6	2.14	4.71E-05
Plp2	proteolipid protein 2 (colonic epithelium-enriched)	2.14	1.06E-04
RT1-CE1	RT1 class I, locus1	2.13	6.66E-06
Dennd3	DENN/MADD domain containing 3	2.13	2.61E-04
Il13ra1	interleukin 13 receptor, alpha 1	2.12	3.55E-07
Nyx	nyctalopin // Xq12 // 302516 ///	2.11	2.74E-06
RT1-S3	RT1 class Ib, locus S3	2.11	2.40E-04
Junb	jun B proto-oncogene	2.11	3.39E-04
Rasl11a	RAS-like family 11 member A	2.10	4.46E-05
Irgm	immunity-related GTPase family, M	2.10	2.59E-04
Tagln2	transgelin 2	2.10	1.57E-05
Tnfrsf12a	tumor necrosis factor receptor superfamily, member 1	2.10	2.27E-04
A3galt2	alpha 1,3-galactosyltransferase 2	2.10	4.76E-04
Ccl24	chemokine (C-C motif) ligand 24	2.10	7.55E-04
Il13ra1	interleukin 13 receptor, alpha 1	2.09	2.33E-06
RGD1561113	similar to Hypothetical UPF0184 protein C9orf16 homolog	2.08	2.83E-06
Hbegf	heparin-binding EGF-like growth factor	2.08	2.87E-03
Slc11a1	solute carrier family 11 (proton-coupled divalent metal ion transporter), member 1	2.07	2.54E-04
LOC688318	similar to leucine rich repeat containing 45	2.07	2.99E-03
LOC686151	similar to cell division cycle associated 5	2.07	2.13E-04
Cckbr	cholecystokinin B receptor	2.06	4.44E-04
LOC691141	hypothetical protein LOC691141	2.06	8.84E-04
Ripk2	receptor-interacting serine-threonine kinase 2	2.06	3.25E-06
Slc16a1	solute carrier family 16, member 1 (monocarboxylic acid transporter0, member 1	2.06	8.62E-07
Ckap2	cytoskeleton associated protein 2	2.05	1.56E-04
Tgfb3	transforming growth factor, beta 3	2.05	8.10E-05
Met	met proto-oncogene	2.05	7.60E-06

Glb1l2	galactosidase, beta 1-like 2	2.05	1.83E-05
Hectd2	ECT domain containing 2	2.04	7.24E-08
Irak2	interleukin-1 receptor-associated kinase 2	2.03	1.08E-04
Bicc1	bicaudal C homolog 1 (Drosophila)	2.03	1.15E-06
Prg4	proteoglycan 4	2.02	3.78E-04
Spry1	sprouty homolog 1, antagonist of FGF signaling (Drosophila)	2.02	1.29E-03
LOC688318	similar to leucine rich repeat containing 45	2.00	2.16E-05
Mybl2	myeloblastosis oncogene-like 2	2.00	2.57E-03
Ttk	Ttk protein kinase	2.00	8.84E-04
Galp	galanin-like peptide	2.00	1.28E-04
Slfn13	schlafen family member 13	2.00	8.45E-08
Trem3	triggering receptor expressed on myeloid cells 3	1.99	5.56E-03
Fgfrl1	fibroblast growth factor receptor-like 1	1.99	1.44E-06
Stat2	signal transducer and activator of transcription 2	1.99	9.56E-05
Relb	v-rel reticuloendotheliosis viral oncogene homolog B	1.99	7.35E-06
Rin1	Ras and Rab interactor 1	1.98	5.32E-07
Ifi27	interferon, alpha-inducible protein 27	1.98	4.67E-05
P2ry6	pyrimidinergic receptor P2Y, G-protein coupled, 6	1.98	3.37E-03
Nmi	N-myc (and STAT) interactor	1.97	1.20E-05
Il4r	interleukin 4 receptor	1.97	2.87E-04
Ppat	phosphoribosyl pyrophosphate amidotransferase	1.97	1.77E-05
Cdkn2b	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	1.97	6.78E-05
Eno3	enolase 3, beta, muscle	1.97	9.49E-04
Mlf1ip	MLF1 interacting protein	1.97	8.15E-04
Shmt1	serine hydroxymethyltransferase 1 (soluble)	1.96	3.41E-04
Mvp	major vault protein	1.96	1.15E-06
Mcm3	minichromosome maintenance complex component 3	1.95	1.69E-03
Cd97	CD97 molecule	1.95	1.27E-04
Dtl	denticleless E3 ubiquitin protein ligase homolog (Drosophila)	1.94	7.58E-04
Parp3	poly (ADP-ribose) polymerase family, member 3	1.94	3.89E-06
Gli2	GLI family zinc finger 2	1.93	9.93E-06
Uhrf1	ubiquitin-like with PHD and ring finger domains 1	1.91	4.49E-04
Ier5l	immediate early response 5-like	1.91	4.21E-05
Aurkb	aurora kinase B	1.91	4.82E-07
Rmi2	RMI2, RecQ mediated genome instability 2, homolog (S. cerevisiae)	1.90	1.53E-05
S100a4	S100 calcium-binding protein A4	1.90	8.84E-04
Ccl19	chemokine (C-C motif) ligand 19	1.89	6.25E-03
Slamf8	SLAM family member 8	1.89	5.16E-07
Tuba8	tubulin, alpha 8	1.89	1.44E-04
Etv5	ets variant 5	1.89	5.99E-05
Extl1	exostosin-like glycosyltransferase 1	1.88	1.86E-04
Gpr114	G protein-coupled receptor 114	1.88	3.86E-04
Mocos	molybdenum cofactor sulfurase	1.87	4.85E-04
Znfx1	zinc finger, NFX1-type containing 1	1.86	1.66E-03
Arsj	arylsulfatase family, member J	1.86	5.07E-04
Npy2r	neuropeptide Y receptor Y2	1.86	6.00E-05

Irf9	interferon regulatory factor 9	1.85	6.46E-06
Pnp	purine nucleoside phosphorylase	1.85	9.20E-07
Slc13a5	solute carrier family 13 (sodium-dependent citrate transporter), member	1.84	3.82E-05
Rarres2	retinoic acid receptor responder (tazarotene induced) 2	1.84	1.48E-03
Egln3	EGL nine homolog 3 (C. elegans)	1.84	6.26E-06
Ly6e	lymphocyte antigen 6 complex, locus E	1.84	1.61E-04
RT1-CE5	RT1 class I, locus CE5	1.84	1.18E-03
RT1-N3	RT1 class Ib, locus N3	1.84	1.15E-03
Adck4	aarF domain containing kinase 4	1.84	1.06E-04
Clec2d	C-type lectin domain family 2, member D	1.83	9.75E-05
Eci3	enoyl-Coenzyme A delta isomerase 3	1.83	2.31E-04
Pric285	peroxisomal proliferator-activated receptor A interacting complex 285	1.83	2.18E-03
Vav3	vav 3 guanine nucleotide exchange factor	1.83	3.11E-06
Cd274	CD274 molecule	1.82	8.27E-05
Acy1	aminoacylase 1	1.82	2.81E-06
Ppm1n	protein phosphatase, Mg2+/Mn2+ dependent, 1N	1.82	1.37E-06
Npm3	nucleophosmin/nucleoplasmin, 3	1.82	1.20E-05
Dusp6	dual specificity phosphatase 6	1.82	1.70E-03
Rgs8	regulator of G-protein signaling 8	1.81	8.87E-07
Gas7	growth arrest specific 7	1.81	2.61E-03
Mcm3	minichromosome maintenance complex component 3	1.81	7.60E-04
Cdt1	chromatin licensing and DNA replication factor 1	1.80	1.06E-04
Casp7	caspase 7	1.80	1.73E-07
Ccdc74a	coiled-coil domain containing 74A	1.80	1.69E-04
Mybl1	myeloblastosis oncogene-like 1	1.80	3.90E-03
Nab2	Ngfi-A binding protein 2	1.80	4.59E-06
Nptxr	neuronal pentraxin receptor	1.79	6.48E-06
Tpx2	TPX2, microtubule-associated, homolog (Xenopus laevis)	1.79	5.34E-04
Wdyhv1	WDYHV motif containing 1	1.79	1.18E-05
Psme2	proteasome (prosome, macropain) activator subunit 2	1.79	5.16E-06
Cdc6	cell division cycle 6	1.79	4.96E-04
LOC498276	Fc gamma receptor II beta	1.78	3.57E-03
Bub1	BUB1 mitotic checkpoint serine/threonine kinase	1.78	8.42E-04
Sec14l4	SEC14-like 4 (S. cerevisiae)	1.77	3.77E-05
LOC681325	hypothetical protein LOC681325	1.77	3.53E-04
Npy2r	neuropeptide Y receptor Y2	1.77	1.49E-04
Socs2	suppressor of cytokine signaling 2	1.77	1.15E-03
LOC688318	similar to leucine rich repeat containing 45	1.77	1.02E-04
Ebi3	Epstein-Barr virus induced 3	1.77	5.04E-03
Mir1949	microRNA mir-1949	1.77	5.15E-03
Hmga1	high mobility group AT-hook 1	1.76	5.44E-05
LOC100360619	ribosomal protein L28-like	1.76	1.11E-03
Tagln	transgelin	1.76	1.66E-03
Ifitm2	interferon induced transmembrane protein 2	1.76	6.87E-04
Tspo	translocator protein	1.76	1.13E-04

Trip10	thyroid hormone receptor interactor 10	1.76	1.31E-04
Chdh	choline dehydrogenase	1.76	3.90E-05
Mcm10	minichromosome maintenance complex component 10	1.76	7.88E-04
Nes	nestin	1.76	1.40E-05
Sdf2l1	stromal cell-derived factor 2-like 1	1.76	2.17E-04
Hk2	hexokinase 2	1.75	3.52E-03
Ntn1	netrin 1	1.75	8.17E-04
Cpne2	copine II	1.75	1.46E-05
Tmem26	transmembrane protein 26	1.75	4.68E-03
Dnph1	2'-deoxynucleoside 5'-phosphate N-hydrolase 1	1.74	8.03E-04
Pola2	polymerase (DNA directed), alpha 2, accessory subunit	1.74	2.48E-04
Galr2	galanin receptor 2	1.74	1.52E-05
Ifi35	interferon-induced protein 35	1.74	2.61E-04
Ier2	immediate early response 2	1.74	4.51E-03
Ucn2	NM_133385 // Ucn2 // urocortin 2 // 8q32 // 170896	1.73	1.37E-03
Fam26e	family with sequence similarity 26, member E	1.73	3.28E-03
H6pd	hexose-6-phosphate dehydrogenase (glucose 1-dehydrogenase)	1.73	1.47E-04
Samd9l	sterile alpha motif domain containing 9-like	1.73	9.51E-04
Spc25	SPC25, NDC80 kinetochore complex component, homolog (S. cerevisiae)	1.72	1.37E-03
Ehd2	EH-domain containing 2	1.72	2.51E-06
Phf19	PHD finger protein 19	1.72	1.03E-04
Slfn2	schlafen 2	1.72	1.98E-04
Slamf9	SLAM family member 9	1.71	3.44E-04
Myl12a	myosin, light chain 12A, regulatory, non-sarcomeric	1.71	4.45E-05
Hspa2	heat shock protein 2	1.71	7.16E-06
Nradd	neurotrophin receptor associated death domain	1.71	1.79E-04
Pkp2	plakophilin 2	1.71	4.15E-04
Slc5a3	solute carrier family 5 (sodium/myo-inositol cotransporter), member	1.71	1.27E-04
Csf1	colony stimulating factor 1 (macrophage)	1.71	1.75E-04
Tnip1	TNFAIP3 interacting protein 1	1.70	6.02E-05
Ptpn9	protein tyrosine phosphatase, non-receptor type 9	1.70	3.71E-06
Hjurp	Holliday junction recognition protein	1.70	1.55E-03
LOC100911280	vascular endothelial growth factor receptor 1-like	1.70	5.65E-03
Mov10	Moloney leukemia virus 10	1.70	8.48E-06
Slc39a8	solute carrier family 39 (zinc transporter), member 8	1.69	1.42E-05
Hist1h1b	histone cluster 1, H1b	1.69	2.44E-03
Galnt4	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 4	1.69	3.22E-04
LOC684193	similar to sterile alpha motif domain containing 9-like	1.69	4.74E-04
Fbxw17	F-box and WD-40 domain protein 17	1.69	1.49E-05
Kcnn3	potassium intermediate/small conductance calcium-activated channel	1.69	1.93E-04
LOC100359563	ribosomal protein S20-like	1.69	3.54E-05
Plcd3	phospholipase C, delta 3	1.69	3.93E-03
Gss	glutathione synthetase	1.68	5.05E-07
Msn	moesin	1.68	7.41E-05

Ece1	endothelin converting enzyme 1	1.68	5.93E-10
Slc35d2	solute carrier family 35, member D2	1.68	2.25E-04
Car13	carbonic anhydrase 13	1.68	1.33E-03
Asgr1	asialoglycoprotein receptor 1	1.67	6.00E-03
LOC688318	similar to leucine rich repeat containing 45	1.67	6.19E-05
Ifi47	interferon gamma inducible protein 47	1.67	3.73E-05
Casp1	caspase 1	1.67	1.80E-03
Gtse1	G-2 and S-phase expressed 1	1.67	5.40E-05
Kif22	kinesin family member 22	1.67	1.80E-03
Lingo3	leucine rich repeat and Ig domain containing 3	1.67	1.67E-03
Pdpn	podoplanin	1.67	8.81E-05
RGD1304982	similar to RIKEN cDNA 2810025M15	1.66	1.55E-04
Eif4ebp1	eukaryotic translation initiation factor 4E binding protein 1	1.66	1.48E-03
Agpat9	1-acylglycerol-3-phosphate O-acyltransferase 9	1.66	3.96E-04
LOC100359600	karyopherin alpha 2-like	1.65	6.05E-04
Trim25	tripartite motif-containing 25	1.65	9.70E-05
Mzt2b	mitotic spindle organizing protein 2B	1.65	3.16E-06
Pcsk1	proprotein convertase subtilisin/kexin type 1	1.65	4.47E-05
Prrg4	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)	1.65	1.27E-03
Tapbpl	TAP binding protein-like	1.64	1.06E-03
Tmem100	transmembrane protein 100	1.64	3.48E-04
Nfkb1	nuclear factor of kappa light polypeptide gene enhancer in B-cells	1.64	3.46E-04
Chtf18	CTF18, chromosome transmission fidelity factor 18 homolog (S. Cerevisiae)	1.64	1.56E-05
Shmt2	serine hydroxymethyltransferase 2 (mitochondrial)	1.64	2.25E-06
Slc25a39	solute carrier family 25, member 39	1.64	4.98E-05
Ebpl	emopamil binding protein-like	1.64	3.15E-04
Nts	neurotensin	1.64	5.36E-03
Rras	related RAS viral (r-ras) oncogene homolog	1.64	3.30E-06
RGD1563065	similar to 3110047P20Rik protein	1.64	6.38E-04
Birc2	baculoviral IAP repeat-containing 2	1.64	4.25E-05
Nr1h3	nuclear receptor subfamily 1, group H, member 3	1.63	1.02E-03
Gys1	glycogen synthase 1, muscle	1.63	4.81E-05
Casp8	caspase 8	1.63	4.02E-05
Slfn5	schlafen family member 5	1.63	7.17E-04
Mcm6	minichromosome maintenance complex component 6	1.63	5.39E-03
Kif18a	kinesin family member 18A	1.63	5.68E-03
Pmaip1	phorbol-12-myristate-13-acetate-induced protein 1	1.63	3.43E-03
Mms22l	MMS22-like, DNA repair protein	1.62	4.67E-03
Creld2	cysteine-rich with EGF-like domains 2	1.62	1.44E-04
Prdx5	peroxiredoxin 5	1.62	1.79E-04
Ccnf	cyclin F	1.62	4.73E-03
Dio3	deiodinase, iodothyronine, type III	1.62	9.13E-04
Bard1	BRCA1 associated RING domain 1	1.62	6.98E-03
LOC681766	hypothetical protein LOC681766	1.61	1.40E-04
Gadd45b	growth arrest and DNA-damage-inducible, beta	1.61	9.52E-04
Trip13	thyroid hormone receptor interactor 13	1.61	3.05E-05

Pfkfb3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	1.61	1.04E-05
Rad18	RAD18 homolog (S. cerevisiae)	1.61	1.31E-05
Emp2	epithelial membrane protein 2	1.61	3.97E-03
Myo1e	myosin IE	1.61	2.07E-05
Sertad1	SERTA domain containing 1	1.61	6.76E-04
Tradd	TNFRSF1A-associated via death domain	1.61	7.10E-04
Tmem154	transmembrane protein 154	1.61	2.86E-03
Slc44a5	solute carrier family 44, member 5	1.60	3.06E-05
B4galt1	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide	1.60	2.31E-04
Ethe1	ethylmalonic encephalopathy 1	1.60	1.80E-04
Gbp1	guanylate binding protein 1, interferon-inducible	1.60	3.82E-03
Trex1	three prime repair exonuclease 1	1.60	1.33E-05
Fsip1	fibrous sheath interacting protein 1	1.60	3.04E-05
Serinc2	serine incorporator 2	1.60	1.16E-03
Cmklr1	chemokine-like receptor 1	1.60	4.54E-03
RGD1565054	similar to 60S acidic ribosomal protein P1	1.60	5.34E-03
Scp2	sterol carrier protein 2	1.60	3.11E-04
F3	coagulation factor III (thromboplastin, tissue factor)	1.59	9.42E-05
Ikbke	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon	1.59	1.09E-03
Kcnf1	potassium voltage-gated channel, subfamily F, member 1	1.59	1.35E-03
LOC303566	E2F1-inducible gene	1.59	2.89E-03
Stard5	StAR-related lipid transfer (START) domain containing 5	1.59	8.53E-05
Plin2	perilipin 2	1.59	3.98E-03
Trmt61a	tRNA methyltransferase 61 homolog A (S. cerevisiae)	1.59	4.25E-05
Anxa4	annexin A4	1.59	1.29E-05
RT1-CE15	RT1 class I, locus CE15	1.58	1.77E-03
Nfkbie	nuclear factor of kappa light polypeptide gene enhancer in B-cell	1.58	1.08E-05
Tecta	tectorin alpha	1.58	1.36E-03
Hist1h2ao	histone cluster 1, H2ao	1.58	6.01E-03
LOC688318	similar to leucine rich repeat containing 45	1.58	7.10E-04
Chst11	carbohydrate (chondroitin 4) sulfotransferase 11	1.58	2.27E-05
Pak1ip1	PAK1 interacting protein 1	1.58	3.70E-03
Rangrf	RAN guanine nucleotide release factor	1.58	2.72E-04
LOC685108	similar to proteolipid protein 2	1.58	1.77E-03
Socs1	suppressor of cytokine signaling 1	1.57	3.62E-03
Cenpm	centromere protein M	1.57	8.42E-04
Creb3l1	cAMP responsive element binding protein 3-like 1	1.57	6.54E-04
Poc1b	POC1 centriolar protein homolog B (Chlamydomonas)	1.57	6.07E-04
Cdc45	cell division cycle 45	1.57	6.64E-04
Phlda3	pleckstrin homology-like domain, family A, member 3	1.57	2.89E-04
Fam49a	family with sequence similarity 49, member A	1.57	6.22E-06
Dyrk3	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3	1.57	1.58E-03
Slc7a8	solute carrier family 7 (amino acid transporter light chain, L system), member 8	1.56	8.57E-05
Axl	Axl receptor tyrosine kinase	1.56	1.47E-05
Trim34	tripartite motif-containing 34	1.56	5.55E-04

Trim21	tripartite motif-containing 21	1.56	4.12E-04
LOC688318	similar to leucine rich repeat containing 45	1.55	3.01E-04
Ap1s1	adaptor-related protein complex 1, sigma 1 subunit	1.55	9.16E-07
Layn	layilin	1.55	1.70E-03
Itpkb	inositol-trisphosphate 3-kinase B	1.55	3.95E-05
Sowahc	sosondowah ankyrin repeat domain family member C	1.55	1.04E-05
RGD1564552	similar to ribosomal protein L21	1.55	5.88E-03
Pno1	partner of NOB1 homolog (S. cerevisiae)	1.55	1.14E-05
Pycard	PYD and CARD domain containing	1.55	1.37E-04
S1pr3	sphingosine-1-phosphate receptor 3	1.54	3.18E-05
Arhgap18	Rho GTPase activating protein 18	1.54	3.92E-05
Ubash3b	ubiquitin associated and SH3 domain containing, B	1.54	2.13E-07
LOC688318	similar to leucine rich repeat containing 45	1.54	2.63E-04
Sema7a	semaphorin 7A, GPI membrane anchor	1.54	1.94E-04
Hopx	HOP homeobox	1.54	1.72E-05
Fau	Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV) ubiquitously expressed	1.54	1.02E-03
Fbl	fibrillarin	1.54	4.27E-04
Tpm4	tropomyosin 4	1.54	1.51E-04
Smpdl3b	sphingomyelin phosphodiesterase, acid-like 3B	1.54	8.83E-04
Pter	phosphotriesterase related	1.53	3.42E-05
Phldb3	pleckstrin homology-like domain, family B, member 3	1.53	1.17E-04
LOC688318	similar to leucine rich repeat containing 45	1.53	2.16E-07
Klf10	Kruppel-like factor 10	1.53	3.18E-03
Iffo2	intermediate filament family orphan 2	1.53	2.13E-06
Edf1	endothelial differentiation-related factor 1	1.53	1.08E-04
Lgals5	lectin, galactose binding, soluble 5	1.53	3.72E-03
Litaf	lipopolysaccharide-induced TNF factor	1.53	1.66E-05
Ereg	epiregulin	1.53	3.73E-04
Aida	axin interactor, dorsalization associated	1.53	1.69E-05
Flna	filamin A, alpha	1.53	2.66E-05
C1qtnf5	C1q and tumor necrosis factor related protein 5	1.53	1.04E-04
Bdnf	brain-derived neurotrophic factor	1.53	2.36E-03
RGD1562079	RGD1562079	1.53	3.14E-06
Nod1	nucleotide-binding oligomerization domain containing 1	1.53	6.58E-04
Pdxk	pyridoxal (pyridoxine, vitamin B6) kinase	1.53	9.00E-05
Mrps18b	mitochondrial ribosomal protein S18B	1.52	1.88E-04
Steap1	six transmembrane epithelial antigen of the prostate 1	1.52	3.50E-03
Pinx1	PIN2/TERF1 interacting, telomerase inhibitor 1	1.52	2.10E-06
Rbp2	retinol binding protein 2, cellular	1.52	1.76E-03
Pnpt1	polyribonucleotide nucleotidyltransferase 1	1.52	1.44E-05
Nme2	NME/NM23 nucleoside diphosphate kinase 2	1.52	2.92E-03
Xbp1	X-box binding protein 1	1.52	1.49E-07
Prr16	proline rich 16	1.52	1.91E-05
Lipa	lipase A, lysosomal acid, cholesterol esterase	1.52	9.71E-05
Fkbp11	FK506 binding protein 11	1.52	1.67E-03
Flt3l	FMS-like tyrosine kinase 3 ligand	1.52	1.34E-04

Rbpms2	RNA binding protein with multiple splicing 2	1.52	1.08E-04
LOC100362338	ribosomal protein L35a-like	1.52	3.68E-05
Kctd14	potassium channel tetramerisation domain containing 14	1.51	1.08E-03
Ginm1	glycoprotein integral membrane 1	1.51	4.68E-04
Srebf1	sterol regulatory element binding transcription factor 1	1.51	2.80E-03
Cyp4v3	cytochrome P450, family 4, subfamily v, polypeptide 3	1.51	4.76E-03
Elk3	ELK3, member of ETS oncogene family	1.51	6.54E-06
LOC688318	similar to leucine rich repeat containing 45	1.51	4.56E-03
Lbh	limb bud and heart development	1.51	1.01E-05
Lta4h	leukotriene A4 hydrolase	1.51	1.23E-04
LOC100363408	BolA-like protein 2-like	1.51	2.91E-04
Pde9a	phosphodiesterase 9A	1.51	4.66E-05
Abcg3l3	ATP-binding cassette, subfamily G (WHITE), member 3-like 3	1.51	1.98E-06
LOC100360439	ribosomal protein L36-like	1.51	3.44E-04
Tgif1	TGFB-induced factor homeobox 1	1.51	2.45E-03
LOC100362751	ribosomal protein P2-like	1.50	3.54E-04
LOC680700	similar to ribosomal protein L10a	1.50	7.66E-04
Pold1	polymerase (DNA directed), delta 1, catalytic subunit	1.50	1.28E-04
Pfdn1	Pfdn1 // prefoldin subunit 1	1.50	2.24E-04
Lrrc10b	leucine rich repeat containing 10B	1.50	5.35E-04
Pdzrn4	PDZ domain containing RING finger 4	1.50	5.52E-04
Zc3hav1	zinc finger CCCH type, antiviral 1	1.50	1.66E-03
Myl9	myosin, light chain 9, regulatory	1.50	2.10E-03
Mob3a	MOB kinase activator 3A	1.50	3.50E-06
RGD1561530	similar to Tle6 protein	1.50	8.11E-04
Wdr62	WD repeat domain 62	1.49	1.17E-05
Dnaaf3	dynein, axonemal, assembly factor 3	1.49	1.20E-03
Brca1	breast cancer 1, early onset	1.49	6.11E-04
RGD1559639	similar to ribosomal protein L10a	1.48	4.39E-04
Plekho2	pleckstrin homology domain containing, family O member 2	1.48	1.26E-04
Cth	cystathionase (cystathionine gamma-lyase)	1.48	2.88E-03
Slc12a7	solute carrier family 12 (potassium/chloride transporters), member 7	1.48	4.60E-03
Aprt	adenine phosphoribosyl transferase	1.48	4.11E-04
Tlr3	toll-like receptor 3	1.48	1.01E-04
Sema3e	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E	1.48	2.54E-06
Hspb1	heat shock protein 1	1.48	3.16E-04
P2rx4	purinergic receptor P2X, ligand-gated ion channel 4	1.48	1.62E-03
Snx18	sorting nexin 18	1.48	5.87E-03
Prkcdbp	protein kinase C, delta binding protein	1.48	2.79E-03
Lsm7	LSM7 homolog, U6 small nuclear RNA associated (S. cerevisiae)	1.48	9.30E-04
Slco4a1	solute carrier organic anion transporter family, member 4a1	1.47	2.07E-03
RGD1310335	similar to RIKEN cDNA C330027C09	1.47	6.56E-03
LOC686066	similar to 60S ribosomal protein L38	1.47	1.13E-04
Mt2A	metallothionein 2A	1.47	8.43E-05
Tnfrsf1a	tumor necrosis factor receptor superfamily, member 1a	1.47	1.92E-05

St3gal1	ST3 beta-galactoside alpha-2,3-sialyltransferase 1	1.47	6.08E-03
Ipo5	importin 5	1.47	1.33E-04
Gins2	GINS complex subunit 2 (Psf2 homolog)	1.47	3.67E-03
Erbb2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2	1.47	1.96E-03
Aspg	asparaginase homolog (S. cerevisiae)	1.46	1.06E-03
Iqgap3	IQ motif containing GTPase activating protein 3	1.46	8.13E-05
Tpm3	tropomyosin 3	1.46	1.75E-04
Dph5	DPH5 homolog (S. cerevisiae)	1.46	7.69E-04
LOC688318	similar to leucine rich repeat containing 45	1.46	6.17E-04
Ftsj3	FtsJ homolog 3 (E. coli)	1.46	5.75E-05
Tmem2	transmembrane protein 2	1.46	2.50E-03
Stat3	signal transducer and activator of transcription 3 (acute-phase response factor)	1.46	1.18E-07
Thop1	thimet oligopeptidase 1	1.46	1.06E-05
Chadl	chondroadherin-like	1.46	2.94E-04
Lyar	Ly1 antibody reactive	1.46	2.45E-04
LOC688318	similar to leucine rich repeat containing 45	1.46	2.37E-08
Spry2	sprouty homolog 2 (Drosophila)	1.46	9.77E-06
C1qtnf1	C1q and tumor necrosis factor related protein 1	1.45	4.61E-03
LOC688318	similar to leucine rich repeat containing 45	1.45	2.00E-03
Fanci	Fanconi anemia, complementation group I	1.45	3.99E-03
Ift43	intraflagellar transport 43 homolog (Chlamydomonas)	1.45	1.48E-03
Kpna2	karyopherin alpha 2	1.45	1.84E-05
Galr1	galanin receptor 1	1.45	7.62E-04
Psme1	proteasome (prosome, macropain) activator subunit 1	1.45	1.10E-04
Vhl	von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase	1.45	3.95E-05
Ube2t	ubiquitin-conjugating enzyme E2T (putative)	1.45	4.59E-03
Chaf1b	chromatin assembly factor 1, subunit B (p60)	1.45	5.34E-03
Slc20a1	solute carrier family 20 (phosphate transporter), member 1	1.44	5.51E-06
Aifm2	apoptosis-inducing factor, mitochondrion-associated 2	1.44	8.43E-05
Ercc1	excision repair cross-complementing rodent repair deficiency	1.44	1.41E-03
Aimp2	aminoacyl tRNA synthetase complex-interacting multifunctional protein 2	1.44	2.54E-06
Rps13	ribosomal protein S13	1.44	4.90E-05
RGD1309350	similar to transthyretin (4L369)	1.44	2.21E-03
LOC688318	similar to leucine rich repeat containing 45	1.44	9.11E-04
Polr1b	polymerase (RNA) I polypeptide B	1.44	1.53E-04
Dnajc25	DnaJ (Hsp40) homolog, subfamily C, member 25	1.44	2.68E-04
Tor1b	torsin family 1, member B	1.44	2.35E-03
Bhlhe40	basic helix-loop-helix family, member e40	1.44	2.55E-04
Gale	UDP-galactose-4-epimerase	1.44	1.94E-04
Mthfd1l	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like	1.44	2.46E-03
LOC100910714	60S ribosomal protein L36a-like	1.44	7.43E-04
Cebpb	enhancer binding protein (C/EBP), beta	1.44	2.44E-04
Polr1e	polymerase (RNA) I polypeptide E	1.43	3.43E-06
Psma6	proteasome (prosome, macropain) subunit, alpha type 6	1.43	4.42E-04
Pole2	polymerase (DNA directed), epsilon 2, accessory subunit	1.43	3.48E-03

Rgs4	regulator of G-protein signaling 4	1.43	2.62E-03
Timeless	timeless circadian clock	1.43	1.95E-03
Orai3	ORAI calcium release-activated calcium modulator 3	1.43	5.39E-04
Gins4	GINS complex subunit 4 (Sld5 homolog)	1.43	1.88E-03
Dnali1	dynein, axonemal, light intermediate chain 1	1.43	5.29E-04
Ifngr2	interferon gamma receptor 2	1.43	3.92E-06
Snrpa	small nuclear ribonucleoprotein polypeptide A	1.43	5.16E-06
Zmym6nb	ZMYM6 neighbor	1.43	1.02E-03
Tmco4	transmembrane and coiled-coil domains 4	1.43	7.01E-03
Dnai2	dynein, axonemal, intermediate chain 2	1.43	5.76E-03
LOC100361103	ribosomal protein L21-like	1.43	6.66E-03
Psrc1	proline/serine-rich coiled-coil 1	1.43	3.72E-04
Scg2	secretogranin II	1.43	1.16E-04
Ap5b1	adaptor-related protein complex 5, beta 1 subunit	1.42	2.11E-03
RGD1564259	similar to OTTHUMP00000040155	1.42	4.22E-03
Mphosph10	M-phase phosphoprotein 10 (U3 small nucleolar ribonucleoprotein)	1.42	3.27E-03
Ezr	ezrin	1.42	2.79E-05
B3galnt1	beta-1,3-N-acetylgalactosaminyltransferase 1 (globoside blood group)	1.42	6.93E-04
Tes	testis derived transcript	1.42	1.17E-04
Tonsl	tonsoku-like, DNA repair protein	1.42	1.75E-03
LOC688318	similar to leucine rich repeat containing 45	1.42	4.60E-03
LOC100360501	ribonuclease inhibitor-like	1.41	5.26E-06
Dsn1	DSN1, MIND kinetochore complex component, homolog (S. cerevisiae)	1.41	5.87E-03
Slc37a1	solute carrier family 37 (glycerol-3-phosphate transporter), member 1	1.41	5.43E-04
Tfcp2l1	transcription factor CP2-like 1	1.41	3.82E-03
Efemp2	EGF-containing fibulin-like extracellular matrix protein 2	1.41	7.66E-05
Eif5a	eukaryotic translation initiation factor 5A	1.41	1.29E-04
Dok5	docking protein 5	1.41	1.26E-04
Fpgs	folylpolyglutamate synthase	1.41	1.54E-03
Heatr2	HEAT repeat containing 2	1.41	1.26E-04
Ncapg2	non-SMC condensin II complex, subunit G2	1.40	1.85E-03
Mtmr11	myotubularin related protein 11	1.40	8.78E-05
RGD1311458	similar to cDNA sequence BC027231; hypothetical protein MGC27931	1.40	1.29E-03
Jun	jun proto-oncogene	1.40	3.51E-04
Rps5	ribosomal protein S5	1.40	2.01E-04
Mapkapk3	mitogen-activated protein kinase-activated protein kinase 3	1.40	1.28E-04
Arhgef37	Rho guanine nucleotide exchange factor (GEF) 37	-1.40	8.18E-04
Wwc2	WW and C2 domain containing 2	-1.40	2.65E-04
Adcy8	adenylate cyclase 8 (brain)	-1.40	8.79E-05
Slc2a13	solute carrier family 2 (facilitated glucose transporter), member 13	-1.41	4.15E-04
Klf11	Kruppel-like factor 11	-1.41	1.90E-05
Gpr155	G protein-coupled receptor 155	-1.41	1.18E-03
Itga9	integrin, alpha 9	-1.41	2.48E-03
Shisa9	shisa homolog 9 (Xenopus laevis)	-1.41	3.27E-05

Sez6l	seizure related 6 homolog (mouse)-like	-1.41	9.12E-05
Mdk	midkine	-1.41	1.79E-03
Ctnna2	catenin (cadherin associated protein), alpha 2	-1.41	5.25E-05
LOC688318	similar to leucine rich repeat containing 45	-1.41	3.74E-05
Acot2	acyl-CoA thioesterase 2	-1.41	2.86E-03
Bcas1	breast carcinoma amplified sequence 1	-1.41	1.83E-03
Zdhhc14	zinc finger, DHHC-type containing 14	-1.41	6.81E-06
Reps2	RALBP1 associated Eps domain containing 2	-1.41	5.63E-05
L1cam	L1 cell adhesion molecule	-1.41	3.62E-03
Hrsp12	heat-responsive protein 12	-1.41	7.30E-08
Map2k6	mitogen-activated protein kinase kinase 6	-1.42	9.95E-04
Hmgn5	high mobility group nucleosome binding domain 5	-1.42	3.84E-03
Csrp2	cysteine and glycine-rich protein 2	-1.42	4.30E-03
Abtb1	ankyrin repeat and BTB (POZ) domain containing 1	-1.42	1.05E-03
Kank1	KN motif and ankyrin repeat domains 1	-1.42	1.04E-04
Galnt6	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 6	-1.42	2.71E-03
Mob3b	MOB kinase activator 3B	-1.42	7.59E-06
Galnt13	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 13	-1.42	2.30E-06
Mei4	meiosis-specific, MEI4 homolog (S. cerevisiae)	-1.43	3.83E-03
Ctnna2	catenin (cadherin associated protein), alpha 2	-1.43	6.61E-06
Dscaml1	Down syndrome cell adhesion molecule-like 1	-1.43	7.15E-04
LOC688318	similar to leucine rich repeat containing 45	-1.43	4.80E-05
Tp53bp2	tumor protein p53 binding protein, 2	-1.43	2.28E-05
Mir342	microRNA mir-342	-1.43	2.11E-03
Tspan11	tetraspanin 11	-1.43	3.37E-04
LOC688318	similar to leucine rich repeat containing 45	-1.43	6.32E-03
LOC688318	similar to leucine rich repeat containing 45	-1.43	6.32E-03
Stap1	signal transducing adaptor family member 1	-1.44	8.24E-04
Omg	oligodendrocyte-myelin glycoprotein	-1.44	9.34E-06
Pde1b	phosphodiesterase 1B, calmodulin-dependent	-1.44	6.65E-03
Hps4	Hermansky-Pudlak syndrome 4	-1.44	3.19E-03
Rnf144a	ring finger protein 144A	-1.44	5.64E-07
Limch1	LIM and calponin homology domains 1	-1.44	1.58E-04
LOC688318	similar to leucine rich repeat containing 45	-1.45	2.42E-03
LOC689081	similar to cystatin E2	-1.45	2.34E-05
Slc38a3	solute carrier family 38, member 3	-1.45	1.27E-03
Adrbk2	adrenergic, beta, receptor kinase 2	-1.45	7.67E-04
Nanos1	nanos homolog 1 (Drosophila)	-1.45	3.96E-04
Dkk3	dickkopf WNT signaling pathway inhibitor 3	-1.45	2.09E-03
Snx30	sorting nexin family member 30	-1.46	2.94E-04
Gpld1	glycosylphosphatidylinositol specific phospholipase D1	-1.46	8.68E-05
LOC100912162	uncharacterized LOC100912162	-1.46	2.69E-06
Rnase2	ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin)	-1.46	9.13E-04
Als2cl	ALS2 C-terminal like	-1.46	3.97E-07

Tspan8	tetraspanin 8	-1.46	1.01E-03
LOC688318	similar to leucine rich repeat containing 45	-1.46	1.85E-04
Grm3	glutamate receptor, metabotropic 3	-1.46	1.24E-03
RGD1565616	RGD1565616	-1.46	3.02E-07
Igsf9b	immunoglobulin superfamily, member 9B	-1.46	4.46E-05
Pllp	plasmolipin	-1.46	3.70E-04
LOC688318	similar to leucine rich repeat containing 45	-1.47	1.75E-04
Gabbr1	gamma-aminobutyric acid (GABA) B receptor 1	-1.47	9.42E-08
Hs3st1	heparan sulfate (glucosamine) 3-O-sulfotransferase 1	-1.47	1.93E-03
Doc2a	double C2-like domains, alpha	-1.47	2.35E-03
Cdkn1c	cyclin-dependent kinase inhibitor 1C	-1.47	3.43E-04
Map6d1	MAP6 domain containing 1	-1.47	3.09E-05
Fbrsl1	fibrosin-like 1	-1.47	9.82E-06
Tmcc2	transmembrane and coiled-coil domain family 2	-1.47	1.13E-04
Cbln1	cerebellin 1 precursor	-1.47	1.57E-03
Sv2b	synaptic vesicle glycoprotein 2b	-1.47	4.16E-04
Vwa3a	von Willebrand factor A domain containing 3A	-1.47	2.60E-03
Pthlh	parathyroid hormone-like hormone	-1.47	4.46E-03
Ankfn1	ankyrin-repeat and fibronectin type III domain containing 1	-1.48	1.49E-03
Fam227a	family with sequence similarity 227, member A	-1.48	6.82E-03
Phactr3	phosphatase and actin regulator 3	-1.48	1.07E-03
LOC689725	similar to chromosome 9 open reading frame 79	-1.48	3.92E-04
Akr1c19	aldo-keto reductase family 1, member C19	-1.48	6.06E-03
Plp1	proteolipid protein 1	-1.48	4.29E-07
Cfd	complement factor D (adipsin)	-1.48	4.66E-03
Bmf	Bcl2 modifying factor	-1.48	6.11E-03
Gjc2	gap junction protein, gamma 2	-1.49	5.80E-04
Erbb3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	-1.49	1.68E-04
Cmtm5	CKLF-like MARVEL transmembrane domain containing 5	-1.49	1.58E-03
Cdc42ep2	CDC42 effector protein (Rho GTPase binding) 2	-1.49	9.51E-04
Astn2	astrotactin 2	-1.49	3.02E-04
Car8	carbonic anhydrase 8	-1.49	5.35E-05
Dnah9	dynein, axonemal, heavy polypeptide 9	-1.49	8.56E-04
Fam53b	family with sequence similarity 53, member B	-1.49	3.32E-05
Gstt3	glutathione S-transferase, theta 3	-1.49	4.26E-05
Adhfe1	alcohol dehydrogenase, iron containing, 1	-1.50	2.16E-03
Ms4a14	membrane-spanning 4-domains, subfamily A, member 14	-1.50	5.04E-04
LOC685513	similar to Guanine nucleotide-binding protein G(I)/G(S)/G(O) gamma-12 subunit precursor	-1.50	5.74E-04
Gpr146	G protein-coupled receptor 146	-1.50	7.34E-04
Cdh20	cadherin 20	-1.50	3.56E-05
Timp3	TIMP metallopeptidase inhibitor 3	-1.50	2.86E-03
Plag1	pleiomorphic adenoma gene 1	-1.50	2.60E-04
Tnfrsf11a	tumor necrosis factor receptor superfamily, member 11a, NFKB activator	-1.50	4.16E-05
LOC100359766	LOC100359766	-1.51	1.79E-03
Ppp1r1a	protein phosphatase 1, regulatory (inhibitor) subunit 1A	-1.51	1.05E-04

Zfhx4	zinc finger homeobox 4	-1.51	2.28E-03
Tmem229a	transmembrane protein 229A	-1.51	2.28E-03
Ly6h	lymphocyte antigen 6 complex, locus H	-1.51	9.31E-04
LOC688318	similar to leucine rich repeat containing 45	-1.51	3.42E-03
LOC688318	similar to leucine rich repeat containing 45	-1.51	6.95E-03
Slitrk6	SLIT and NTRK-like family, member 6	-1.51	1.58E-04
Nppc	natriuretic peptide C	-1.52	5.82E-03
Cnp	2',3'-cyclic nucleotide 3' phosphodiesterase	-1.52	1.79E-06
Tspan33	tetraspanin 33	-1.52	3.33E-05
Clstn2	calsyntenin 2	-1.52	9.79E-07
Scd1	stearoyl-Coenzyme A desaturase 1	-1.52	1.02E-06
Paqr8	progestin and adipoQ receptor family member VIII	-1.52	1.55E-06
Adcy5	adenylate cyclase 5	-1.53	8.08E-08
Utp14b	UTP14, U3 small nucleolar ribonucleoprotein, homolog B	-1.53	1.54E-03
Abca2	ATP-binding cassette, subfamily A (ABC1), member 2	-1.53	2.57E-08
Lhfpl1	lipoma HMGIC fusion partner-like 1	-1.53	9.31E-04
LOC688318	similar to leucine rich repeat containing 45	-1.53	1.75E-03
Gfral	GDNF family receptor alpha like	-1.53	3.40E-04
Gng7	guanine nucleotide binding protein (G protein), gamma 7	-1.53	4.15E-04
Pex5l	peroxisomal biogenesis factor 5-like	-1.53	6.61E-03
Npc1	Niemann-Pick disease, type C1	-1.54	1.01E-06
LOC688318	similar to leucine rich repeat containing 45	-1.54	5.96E-03
Mme	membrane metallo-endopeptidase	-1.54	1.64E-04
Pdk4	pyruvate dehydrogenase kinase, isozyme 4	-1.55	7.08E-03
Samt2	spermatogenesis associated multipass transmembrane protein 2	-1.55	8.98E-05
Pdgfb	platelet-derived growth factor beta polypeptide	-1.55	3.50E-03
Fbxo32	F-box protein 32	-1.55	1.48E-03
Pip4k2a	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	-1.55	8.67E-06
Sema5a	sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5A	-1.55	3.55E-07
Cux2	cut-like homeobox 2	-1.55	4.42E-05
Mmd2	monocyte to macrophage differentiation-associated 2	-1.55	1.90E-05
Mfge8	milk fat globule-EGF factor 8 protein	-1.56	1.05E-04
Atrn	attractin	-1.56	1.57E-08
Adcyap1r1	adenylate cyclase activating polypeptide 1 receptor 1	-1.56	1.26E-06
Gdpd2	glycerophosphodiester phosphodiesterase domain containing 2	-1.56	8.78E-05
Prkcg	protein kinase C, gamma	-1.56	2.92E-03
Mir2964	microRNA mir-2964	-1.57	6.88E-03
Lrrc1	leucine rich repeat containing 1	-1.57	3.14E-04
Trim36	tripartite motif-containing 36	-1.57	4.93E-05
LOC365085	similar to nidogen 2	-1.57	5.68E-04
C1ql1	complement component 1, q subcomponent-like 1	-1.58	4.61E-03
Dnah9	dynein, axonemal, heavy polypeptide 9	-1.58	6.77E-04
Zdhhc22	zinc finger, DHHC-type containing 22	-1.58	2.89E-03
Pip4k2a	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha	-1.58	3.05E-07
Cxxc4	CXXC finger protein 4	-1.58	1.87E-03

Hes1	hairy and enhancer of split 1 (Drosophila)	-1.58	1.58E-03
Caskin2	cask-interacting protein 2	-1.58	1.11E-06
Id4	inhibitor of DNA binding 4	-1.58	1.76E-05
LOC100360891	LOC100360891	-1.58	8.81E-06
Zfp804a	zinc finger protein 804A	-1.59	4.43E-05
RGD1309821	similar to KIAA1161 protein	-1.59	7.33E-07
RGD1559896	similar to RIKEN cDNA 2310022B05	-1.60	3.78E-05
Cyp2d4	cytochrome P450, family 2, subfamily d, polypeptide 4	-1.60	1.81E-03
Cbr1	carbonyl reductase 1	-1.60	7.62E-05
Dnah9	dynein, axonemal, heavy polypeptide 9	-1.60	1.34E-04
RGD1561963	similar to Dedicator of cytokinesis protein 10 (Protein zizimin 3)	-1.60	8.07E-08
Csmd1	CUB and Sushi multiple domains 1	-1.60	1.27E-03
St6galnac3	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 3	-1.60	1.51E-06
Notch2	notch 2	-1.60	1.52E-06
Ndrg1	N-myc downstream regulated 1	-1.60	7.72E-04
Elmo1	engulfment and cell motility 1	-1.60	2.96E-07
Ppp1r1b	protein phosphatase 1, regulatory (inhibitor) subunit 1B	-1.60	3.73E-03
Hrh3	histamine receptor H3	-1.61	1.94E-04
LOC688318	similar to leucine rich repeat containing 45	-1.61	5.76E-03
Plekha1	pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1	-1.61	1.53E-06
Rlbp1	retinaldehyde binding protein 1	-1.61	2.21E-04
Pdzrn3	PDZ domain containing RING finger 3	-1.62	1.10E-05
LOC688318	similar to leucine rich repeat containing 45	-1.62	3.30E-03
Myo1d	myosin ID	-1.63	8.68E-07
Scrg1	stimulator of chondrogenesis 1	-1.63	1.64E-07
Gabrb1	gamma-aminobutyric acid (GABA) A receptor, beta 1	-1.63	6.04E-05
Unc5b	unc-5 homolog B (C. elegans)	-1.63	2.02E-06
Scube2	signal peptide, CUB domain, EGF-like 2	-1.64	4.62E-03
Plcl1	phospholipase C-like 1	-1.64	1.20E-04
Col23a1	collagen, type XXIII, alpha 1	-1.64	6.21E-03
Nrbp2	nuclear receptor binding protein 2	-1.64	1.04E-06
Eya4	eyes absent homolog 4 (Drosophila)	-1.64	4.12E-03
Mir99a	microRNA mir-99a	-1.64	6.50E-03
Adam11	ADAM metallopeptidase domain 11	-1.65	4.08E-04
LOC100360891	LOC100360891	-1.65	1.47E-05
Fzd4	frizzled family receptor 4	-1.65	2.86E-03
Mycl1	v-myc myelocytomatosis viral oncogene homolog 1, lung carcinoma derived (avian)	-1.65	2.11E-04
Thsd7a	thrombospondin, type I, domain containing 7A	-1.66	8.34E-05
Grin3a	glutamate receptor, ionotropic, N-methyl-D-aspartate 3A	-1.66	9.88E-07
Bche	butyrylcholinesterase	-1.66	1.25E-04
Dnah9	dynein, axonemal, heavy polypeptide 9	-1.66	1.70E-03
Olfml1	olfactomedin-like 1	-1.66	8.86E-05
Tst	thiosulfate sulfurtransferase	-1.66	8.85E-06
Slc15a2	solute carrier family 15 (H+/peptide transporter), member 2	-1.66	1.12E-05

Gamt	guanidinoacetate N-methyltransferase	-1.66	3.63E-06
Mfsd4	major facilitator superfamily domain containing 4	-1.66	1.37E-03
Lrp4	low density lipoprotein receptor-related protein 4	-1.67	2.32E-05
LOC688318	similar to leucine rich repeat containing 45	-1.67	1.09E-04
Slc12a2	solute carrier family 12 (sodium/potassium/chloride transporter), member 2	-1.67	3.81E-08
Fat3	FAT atypical cadherin 3	-1.67	2.93E-05
Amotl2	angiomotin like 2	-1.68	1.99E-04
Kcne1l	KCNE1-like	-1.68	2.90E-05
Gmnc	geminin coiled-coil domain containing	-1.68	7.89E-04
Nkd1	naked cuticle homolog 1 (Drosophila)	-1.69	7.46E-04
St18	suppression of tumorigenicity 18	-1.69	2.23E-05
Csmd3	CUB and Sushi multiple domains 3	-1.69	5.28E-05
LOC688318	similar to leucine rich repeat containing 45	-1.69	3.23E-04
Adamtsl2	ADAMTS-like 2	-1.69	1.06E-03
Slc7a10	solute carrier family 7 (neutral amino acid transporter light chain, Asc system), member 10	-1.69	4.17E-03
Eci2	enoyl-CoA delta isomerase 2	-1.69	1.97E-04
Slco1c1	solute carrier organic anion transporter family, member 1c1	-1.69	2.12E-03
LOC688318	similar to leucine rich repeat containing 45	-1.69	1.55E-04
Abhd3	abhydrolase domain containing 3	-1.70	5.20E-05
Wisp2	WNT1 inducible signaling pathway protein 2	-1.70	5.36E-03
Glud1	glutamate dehydrogenase 1	-1.70	8.66E-08
Akr1b10	aldo-keto reductase family 1, member B10 (aldose reductase)	-1.71	1.08E-04
Mrc2	mannose receptor, C type 2	-1.71	4.72E-04
Rbpjl	ecombination signal binding protein for immunoglobulin kappa J region	-1.71	1.57E-04
Nuak1	NUAK family, SNF1-like kinase, 1	-1.71	1.30E-03
P2rx6	purinergic receptor P2X, ligand-gated ion channel, 6	-1.72	1.00E-03
LOC688318	similar to leucine rich repeat containing 45	-1.72	2.14E-03
LOC688318	similar to leucine rich repeat containing 45	-1.72	2.14E-03
Cyp27b1	cytochrome P450, family 27, subfamily b, polypeptide 1	-1.73	8.96E-05
Pex11a	peroxisomal biogenesis factor 11 alpha	-1.73	1.83E-04
Ldlrap1	low density lipoprotein receptor adaptor protein 1	-1.74	2.07E-04
Tmem63c	transmembrane protein 63c	-1.74	5.21E-05
Mfap3l	microfibrillar-associated protein 3-like	-1.75	2.44E-05
LOC691920	similar to kinesin-like motor protein C20orf23	-1.75	1.91E-03
Mat2a	methionine adenosyltransferase II, alpha	-1.76	1.08E-03
Klhl4	kelch-like family member 4	-1.77	9.03E-05
Dusp15	dual specificity phosphatase 15	-1.78	1.99E-04
Cyp27b1	cytochrome P450, family 27, subfamily b, polypeptide 1	-1.79	6.56E-04
Mir9-2	microRNA mir-9-2	-1.80	9.77E-05
Padi2	peptidyl arginine deiminase, type II	-1.80	2.37E-05
Cyp2c11	cytochrome P450, subfamily 2, polypeptide 11	-1.80	4.62E-03
Lrrc55	leucine rich repeat containing 55	-1.80	1.71E-04
Cpxm1	carboxypeptidase X (M14 family), member 1	-1.81	1.53E-03
Lmx1a	LIM homeobox transcription factor 1 alpha	-1.81	5.75E-04

Ano4	anoctamin 4	-1.81	5.71E-07
Cd74	Cd74 molecule, major histocompatibility complex, class II invariant chain	-1.81	2.52E-03
Ephb1	Eph receptor B1	-1.81	9.43E-07
Slc26a3	solute carrier family 26, member 3	-1.82	1.27E-03
Omd	osteomodulin	-1.82	8.14E-04
Dnah9	dynein, axonemal, heavy polypeptide 9	-1.82	5.66E-04
Nipal4	NIPA-like domain containing 4	-1.83	1.34E-03
Klk6	kallikrein related-peptidase 6	-1.83	3.05E-05
Slc4a4	solute carrier family 4, sodium bicarbonate cotransporter, member 4	-1.84	1.30E-06
LOC100359587	LOC100359587	-1.84	5.59E-05
Slc13a3	solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 3	-1.86	2.48E-05
Cldn11	claudin 11	-1.86	4.73E-04
Fam92b	family with sequence similarity 92, member B	-1.86	7.90E-04
Capn6	calpain 6	-1.87	1.10E-04
Bbox1	butyrobetaine (gamma), 2-oxoglutarate dioxygenase (gamma-butyrobetaine hydroxylase) 1	-1.87	1.41E-03
Draxin	dorsal inhibitory axon guidance protein	-1.87	6.77E-03
Fa2h	fatty acid 2-hydroxylase	-1.87	1.97E-07
Adrb2	adrenoceptor beta 2, surface	-1.87	1.81E-03
Cldn19	claudin 19	-1.88	9.96E-05
Sox10	SRY (sex determining region Y)-box 10	-1.88	1.06E-05
LOC688318	similar to leucine rich repeat containing 45	-1.89	1.34E-03
Lix1	Lix1 homolog (chicken)	-1.89	2.08E-05
Ppp1r16b	protein phosphatase 1, regulatory subunit 16B	-1.89	4.75E-07
Slc6a9	solute carrier family 6 (neurotransmitter transporter, glycine), member 9	-1.90	2.48E-07
Ugt8	UDP glycosyltransferase 8	-1.90	2.42E-06
Agmo	alkylglycerol monooxygenase	-1.91	1.75E-04
Pbld1	phenazine biosynthesis-like protein domain containing 1	-1.91	6.57E-05
Tmem200c	transmembrane protein 200C	-1.93	3.24E-05
LOC688318	similar to leucine rich repeat containing 45	-1.93	3.51E-03
Ca2	carbonic anhydrase 2	-1.95	5.50E-04
Pla2g5	phospholipase A2, group V	-1.95	8.10E-04
Aspa	aspartoacylase	-1.97	8.85E-04
Thsd7b	thrombospondin, type I, domain containing 7B	-1.98	1.92E-05
Gabrg1	gamma-aminobutyric acid (GABA) A receptor, gamma 1	-1.98	2.70E-07
LOC688318	similar to leucine rich repeat containing 45	-1.98	2.31E-03
LOC100362373	LOC100362373	-1.98	4.08E-06
Bcan	brevican	-1.98	1.42E-07
Plxnb3	plexin B3	-1.98	5.58E-07
LOC100359587	LOC100359587	-1.98	2.24E-05
Sult1a1	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	-1.98	1.72E-03
Mcam	melanoma cell adhesion molecule	-2.01	9.51E-04
Prodh	proline dehydrogenase (oxidase) 1	-2.01	4.81E-04
LOC100910790	spondin-2-like	-2.03	1.96E-03

Ms4a7	membrane-spanning 4-domains, subfamily A, member 7	-2.06	3.28E-05
Ppp1r14a	protein phosphatase 1, regulatory (inhibitor) subunit 14A	-2.06	5.68E-04
Gpr62	G protein-coupled receptor 62	-2.06	1.03E-04
Atp10b	ATPase, class V, type 10B	-2.06	1.44E-06
LOC100910367	uncharacterized LOC100910367	-2.07	1.96E-05
LOC100362373	LOC100362373	-2.10	1.83E-06
Igfbpl1	insulin-like growth factor binding protein-like 1	-2.11	6.11E-03
Cdh19	cadherin 19, type 2	-2.11	1.85E-05
Gpr17	G protein-coupled receptor 17	-2.13	1.71E-05
Rftn2	raftlin family member 2	-2.15	1.03E-06
Grin2c	glutamate receptor, ionotropic, N-methyl D-aspartate 2C	-2.16	1.38E-05
Tll2	tolloid-like 2	-2.17	6.76E-05
Pygm	phosphorylase, glycogen, muscle	-2.18	5.76E-05
Tspan2	tetraspanin 2	-2.18	3.57E-09
Hes5	hairy and enhancer of split 5 (Drosophila)	-2.19	1.83E-05
Notch3	notch 3	-2.20	4.11E-06
Mir3546	microRNA mir-3546	-2.20	1.24E-03
LOC688318	similar to leucine rich repeat containing 45	-2.22	1.26E-05
LOC360228	WDNM1 homolog	-2.22	5.76E-03
Kndc1	kinase non-catalytic C-lobe domain (KIND) containing 1	-2.25	1.07E-07
Mir2964	microRNA mir-2964	-2.26	7.21E-05
Fam69c	family with sequence similarity 69, member C	-2.27	2.94E-06
Evi2a	ecotropic viral integration site 2A	-2.29	4.27E-05
Veph1	ventricular zone expressed PH domain-containing 1	-2.30	6.62E-06
Kank4	KN motif and ankyrin repeat domains 4	-2.32	4.17E-05
Tmem88b	transmembrane protein 88B	-2.34	1.10E-04
Timp4	tissue inhibitor of metalloproteinase 4	-2.39	1.09E-04
Aox1	aldehyde oxidase 1	-2.39	4.89E-05
Hmgcs2	3-hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial)	-2.43	2.85E-05
Ccdc3	coiled-coil domain containing 3	-2.45	7.07E-03
Acot1	acyl-CoA thioesterase 1	-2.46	1.12E-03
Mag	myelin-associated glycoprotein	-2.48	1.89E-07
LOC363337	similar to RIKEN cDNA 1700081O22	-2.48	4.46E-04
Myrf	myelin regulatory factor	-2.50	2.69E-05
Nkain4	Na+/K+ transporting ATPase interacting 4	-2.51	2.14E-04
Slc6a11	solute carrier family 6 (neurotransmitter transporter), member 11	-2.52	4.24E-07
Fmo1	flavin containing monooxygenase 1	-2.54	6.73E-04
Ctnna3	catenin (cadherin associated protein), alpha 3	-2.54	6.53E-03
Tmem125	transmembrane protein 125	-2.55	1.39E-05
Gpd1	glycerol-3-phosphate dehydrogenase 1 (soluble)	-2.69	2.56E-05
Tril	TLR4 interactor with leucine-rich repeats	-2.71	2.28E-07
Gpr20	G protein-coupled receptor 20	-2.73	1.42E-06
Stab1	stabilin 1	-2.74	6.25E-04
Papss2	3'-phosphoadenosine 5'-phosphosulfate synthase 2	-2.76	6.44E-07
S1pr5	sphingosine-1-phosphate receptor 5	-2.77	1.19E-07
Gpr37	G protein-coupled receptor 37	-2.79	7.74E-07

Slc40a1	solute carrier family 40 (iron-regulated transporter), member 1	-2.80	1.48E-07
LOC100910839	leucine zipper protein 2-like	-2.82	4.45E-04
P2ry12	purinergic receptor P2Y, G-protein coupled, 12	-2.82	8.86E-04
Hapln2	hyaluronan and proteoglycan link protein 2	-2.83	5.79E-06
Clmn	calmin	-2.85	4.36E-08
Mog	myelin oligodendrocyte glycoprotein	-2.98	5.85E-06
Gpr34	G protein-coupled receptor 34	-3.01	2.56E-03
Slc4a5	solute carrier family 4, sodium bicarbonate cotransporter, member 5	-3.14	6.97E-03
Agt	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	-3.22	3.41E-03
LOC679711	similar to RIKEN cDNA 5031410I06	-3.39	7.12E-05
Tlr5	toll-like receptor 5	-3.47	9.56E-07
Car14	carbonic anhydrase 14	-3.70	1.12E-04
P2ry13	purinergic receptor P2Y, G-protein coupled, 13	-4.13	1.50E-06
Sucnr1	succinate receptor 1	-4.15	2.54E-07
Opalin	oligodendrocytic myelin paranodal and inner loop protein	-4.20	1.12E-06
Hhip	Hedgehog-interacting protein	-4.32	3.57E-09
Wnt2b	wingless-type MMTV integration site family, member 2B	-4.84	4.18E-03
Cckar	cholecystokinin A receptor	-4.93	6.44E-03
Mal	mal, T-cell differentiation protein	-5.15	4.78E-06
C1qtnf3	C1q and tumor necrosis factor related protein 3	-5.63	5.19E-03
Aplnr	apelin receptor	-7.35	5.22E-03

8.2 Gene ontology

8.2.1 Gene ontology clustering of cultures treated with O4 antibody in the absence of serum

8.2.1.1 *Table 4.4.1a: Significantly enriched biological processes by Partek Genomic Suite. Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the antibody in comparison to isotype treated cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	O4- vs IgM-score
immune system process	162.80	1.97E-71	19.71	5.02
response to external biotic stimulus	115.01	1.13E-50	20.19	4.98
response to external stimulus	113.32	6.10E-50	14.24	4.96
response to biotic stimulus	113.08	7.77E-50	19.60	4.98
immune response	113.05	8.00E-50	23.88	5.09
defense response	111.20	5.10E-49	20.12	5.11
regulation of immune system process	90.82	3.60E-40	16.45	4.90
positive regulation of immune system process	86.10	4.05E-38	20.91	5.01
response to cytokine	76.22	7.93E-34	20.00	5.33
response to other organism	75.21	2.18E-33	21.41	5.19
response to stress	71.11	1.31E-31	9.60	4.93
multi-organism process	68.64	1.55E-30	15.85	5.20
positive regulation of biological process	66.97	8.22E-30	7.53	4.79
positive regulation of response to stimulus	66.53	1.28E-29	11.57	5.02
defense response to other organism	64.12	1.42E-28	23.45	5.07
response to molecule of bacterial origin	63.30	3.24E-28	21.19	4.88
immune effector process	63.00	4.36E-28	24.06	5.06
response to lipopolysaccharide	61.39	2.19E-27	21.32	4.86
regulation of response to stimulus	58.64	3.42E-26	8.55	4.85
response to organic substance	58.02	6.31E-26	9.00	4.85
cellular response to chemical stimulus	57.26	1.35E-25	10.15	4.94
positive regulation of cellular process	57.02	1.72E-25	7.52	4.72
cellular response to organic substance	53.42	6.31E-24	10.87	4.91
cellular response to cytokine stimulus	52.31	1.91E-23	19.39	5.40
cellular response to molecule of bacterial origin	51.30	5.24E-23	28.80	4.77
cellular response to biotic stimulus	50.50	1.18E-22	27.21	4.78
cellular response to lipopolysaccharide	50.06	1.81E-22	28.93	4.80
regulation of immune response	48.63	7.60E-22	16.93	4.91
regulation of cytokine production	48.20	1.16E-21	17.10	4.82
response to stimulus	47.33	2.77E-21	5.59	4.76
inflammatory response	46.58	5.87E-21	20.57	4.64

response to wounding	44.06	7.35E-20	15.38	4.66
regulation of defense response	43.80	9.49E-20	17.90	5.07
response to virus	43.53	1.25E-19	25.00	5.75
regulation of response to stress	42.82	2.54E-19	12.45	5.00
response to lipid	42.53	3.39E-19	11.24	4.76
regulation of immune effector process	42.23	4.55E-19	20.53	4.94
regulation of multicellular organismal process	41.96	5.98E-19	8.17	4.52
response to interferon-gamma	41.45	9.98E-19	45.65	5.87
regulation of leukocyte migration	40.90	1.73E-18	31.03	5.00
positive regulation of immune response	40.48	2.64E-18	19.05	4.97
innate immune response	39.19	9.59E-18	22.76	5.05
taxis	38.90	1.27E-17	21.79	5.04
chemotaxis	38.90	1.27E-17	21.79	5.04
regulation of innate immune response	38.37	2.17E-17	28.42	5.23
cellular response to lipid	38.07	2.93E-17	16.60	4.62
regulation of response to external stimulus	37.19	7.03E-17	13.90	4.92
positive regulation of leukocyte migration	37.19	7.04E-17	33.82	5.15
response to oxygen-containing compound	37.05	8.09E-17	9.05	4.77
antigen processing and presentation	36.95	9.01E-17	31.58	5.80
leukocyte activation	36.47	1.45E-16	16.28	4.57
negative regulation of viral process	35.81	2.81E-16	42.22	5.70
negative regulation of multi-organism process	35.75	2.99E-16	31.94	5.49
regulation of localization	35.73	3.04E-16	8.40	4.70
positive regulation of cytokine production	35.65	3.29E-16	20.37	5.09
response to bacterium	35.46	3.99E-16	20.25	5.16
cell chemotaxis	35.41	4.19E-16	26.80	5.33
regulation of locomotion	34.97	6.51E-16	12.59	5.13
regulation of multi-organism process	34.89	7.05E-16	19.88	5.12
positive regulation of signal transduction	34.76	8.05E-16	10.07	5.10
chemokine activity	34.37	1.18E-15	51.61	5.94
positive regulation of signaling	34.27	1.31E-15	9.80	5.09
response to interferon-beta	34.20	1.40E-15	91.67	5.88
regulation of signal transduction	34.12	1.52E-15	7.91	4.87
cell activation	33.93	1.84E-15	14.57	4.64
positive regulation of response to external stimulus	33.59	2.57E-15	20.39	5.21
positive regulation of transport	33.17	3.94E-15	11.90	4.86
positive regulation of cell communication	33.09	4.25E-15	9.67	5.10
positive regulation of locomotion	33.00	4.67E-15	16.24	5.15
response to interleukin-1	32.87	5.32E-15	30.14	4.94
defense response to virus	31.98	1.29E-14	27.38	5.72

positive regulation of immune effector process	31.75	1.62E-14	22.31	5.03
myeloid leukocyte activation	31.47	2.16E-14	32.26	4.76
positive regulation of cell motility	31.41	2.28E-14	16.29	4.97
regulation of leukocyte chemotaxis	31.02	3.39E-14	33.93	5.17
regulation of intracellular signal transduction	31.01	3.40E-14	9.30	4.94
regulation of cell proliferation	30.96	3.57E-14	8.80	4.67
single-organism process	30.66	4.82E-14	4.87	4.58
regulation of cell migration	30.55	5.42E-14	12.60	4.95
positive regulation of cellular component movement	30.43	6.10E-14	15.79	4.97
positive regulation of intracellular signal transduction	30.40	6.27E-14	11.16	5.15
regulation of symbiosis, encompassing mutualism through parasitism	30.35	6.60E-14	25.56	5.39
regulation of cell motility	30.28	7.08E-14	12.31	4.95
defense response to bacterium	30.13	8.20E-14	20.14	4.89
positive regulation of cell migration	30.12	8.27E-14	16.06	4.98
regulation of signaling	30.09	8.54E-14	7.26	4.88
response to tumor necrosis factor	29.91	1.03E-13	26.51	5.35
negative regulation of viral genome replication	29.72	1.23E-13	56.52	5.65
positive regulation of adaptive immune response	28.87	2.90E-13	28.57	5.44
leukocyte migration	28.71	3.39E-13	22.64	5.10
positive regulation of leukocyte chemotaxis	28.71	3.42E-13	35.42	5.21
adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	28.71	3.42E-13	35.42	4.46
regulation of cellular component movement	28.65	3.60E-13	11.61	4.91
leukocyte chemotaxis	28.57	3.90E-13	28.17	5.29
regulation of mononuclear cell proliferation	28.55	4.01E-13	19.71	5.07
cellular response to oxygen-containing compound	28.50	4.21E-13	10.53	4.86
regulation of cell activation	28.47	4.34E-13	13.83	4.70
regulation of cell communication	28.20	5.64E-13	7.12	4.86
positive regulation of defense response	27.84	8.10E-13	20.83	4.80
regulation of leukocyte proliferation	27.82	8.28E-13	19.15	5.07
response to organic cyclic compound	27.76	8.81E-13	9.41	4.52
regulation of viral process	27.05	1.79E-12	27.94	5.70
regulation of adaptive immune response	26.83	2.23E-12	21.90	5.35
regulation of lymphocyte proliferation	26.80	2.30E-12	19.12	5.11
positive regulation of adaptive immune response based on somatic recombination of immune receptors built from	26.76	2.39E-12	27.54	5.34

immunoglobulin superfamily domains				
cellular response to interferon-beta	26.63	2.71E-12	100.00	6.31
regulation of leukocyte activation	26.51	3.07E-12	13.90	4.77
regulation of I-kappaB kinase/NF-kappaB signaling	26.45	3.27E-12	18.84	5.06
cellular response to interferon-gamma	26.40	3.42E-12	46.43	5.91
positive regulation of multicellular organismal process	26.09	4.65E-12	11.16	5.03
antigen processing and presentation of peptide antigen	26.02	5.03E-12	33.33	5.50
positive regulation of leukocyte mediated immunity	25.93	5.49E-12	26.39	5.10
regulation of transport	25.92	5.52E-12	8.40	4.79
regulation of leukocyte mediated immunity	25.66	7.15E-12	19.83	4.86
cellular response to interleukin-1	24.96	1.45E-11	31.37	5.10
regulation of leukocyte apoptotic process	24.89	1.55E-11	28.81	4.69
single-organism cellular process	24.88	1.57E-11	5.00	4.57
positive regulation of metabolic process	24.73	1.82E-11	6.88	4.71
regulation of lymphocyte migration	24.38	2.59E-11	46.15	5.42
regulation of viral genome replication	24.31	2.78E-11	40.63	5.65
lipopolysaccharide-mediated signaling pathway	24.21	3.07E-11	52.38	4.85
positive regulation of lymphocyte mediated immunity	24.16	3.22E-11	25.71	5.10
positive regulation of chemotaxis	24.15	3.26E-11	24.05	5.36
regulation of chemotaxis	23.97	3.91E-11	21.21	5.31
adaptive immune response	23.90	4.17E-11	25.35	4.59
regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	23.56	5.84E-11	20.79	5.18
regulation of nitric oxide biosynthetic process	23.30	7.59E-11	34.15	4.68
regulation of tumor necrosis factor production	23.19	8.51E-11	26.15	4.62
cellular response to tumor necrosis factor	22.92	1.11E-10	25.76	5.47
positive regulation of cell activation	22.69	1.40E-10	16.05	5.16
regulation of developmental process	22.65	1.46E-10	7.19	4.48
positive regulation of behavior	22.54	1.62E-10	20.83	5.40
single-organism transport	22.48	1.72E-10	6.79	4.59
response to endogenous stimulus	22.47	1.75E-10	7.76	4.50
negative regulation of biological process	22.46	1.75E-10	5.94	4.72
positive regulation of calcium ion transport	22.45	1.78E-10	29.41	5.30
leukocyte mediated immunity	22.45	1.78E-10	29.41	3.97
positive regulation of T cell activation	22.35	1.97E-10	20.62	5.31

regulation of protein metabolic process	21.97	2.88E-10	7.17	4.76
positive regulation of tumor necrosis factor production	21.86	3.21E-10	38.71	4.81
regulation of apoptotic process	21.85	3.25E-10	8.03	5.01
granulocyte chemotaxis	21.39	5.16E-10	33.33	4.52
regulation of lymphocyte activation	21.37	5.23E-10	13.39	4.99
positive regulation of cellular metabolic process	21.37	5.26E-10	6.74	4.63
response to nitrogen compound	21.32	5.52E-10	8.78	4.65
regulation of programmed cell death	21.30	5.64E-10	7.93	5.01
myeloid leukocyte migration	21.26	5.82E-10	27.27	4.78
positive regulation of cell proliferation	21.18	6.33E-10	9.33	4.54
positive regulation of innate immune response	21.17	6.39E-10	25.00	4.64
granulocyte migration	21.03	7.39E-10	32.50	4.52
regulation of molecular function	20.94	8.08E-10	6.89	4.78
regulation of cell killing	20.92	8.20E-10	24.62	5.21
positive regulation of molecular function	20.75	9.72E-10	8.33	4.82
positive regulation of developmental process	20.74	9.88E-10	8.91	4.37
biological regulation	20.72	1.00E-09	4.55	4.71
antigen processing and presentation of peptide antigen via MHC class I	20.60	1.13E-09	35.29	5.68
regulation of T cell proliferation	20.56	1.17E-09	19.79	5.39
regulation of cell death	20.48	1.27E-09	7.67	4.95
positive regulation of cell killing	20.45	1.32E-09	25.86	5.31
response to chemical	20.39	1.40E-09	5.44	4.83
positive regulation of leukocyte activation	20.30	1.53E-09	15.48	5.27
immune response-regulating signaling pathway	20.20	1.69E-09	19.39	4.22
regulation of lymphocyte mediated immunity	20.02	2.02E-09	19.19	5.03
regulation of behavior	19.99	2.08E-09	15.86	5.25
antigen processing and presentation of endogenous antigen	19.96	2.14E-09	100.00	6.33
activation of innate immune response	19.84	2.42E-09	33.33	4.67
antigen processing and presentation of exogenous antigen	19.78	2.56E-09	43.48	4.82
regulation of T cell activation	19.77	2.59E-09	15.09	5.15
regulation of phosphorylation	19.70	2.78E-09	8.11	4.80
cellular response to organic cyclic compound	19.69	2.80E-09	12.83	4.50
regulation of leukocyte mediated cytotoxicity	19.68	2.82E-09	24.59	5.29
regulation of phosphate metabolic process	19.54	3.27E-09	7.31	4.79
activation of immune response	19.51	3.35E-09	17.70	4.86
regulation of interferon-gamma production	19.44	3.60E-09	24.19	5.25

regulation of phosphorus metabolic process	19.37	3.88E-09	7.28	4.79
positive regulation of leukocyte proliferation	19.36	3.90E-09	19.57	5.27
negative regulation of cellular process	19.36	3.92E-09	5.89	4.69
regulation of biological process	19.24	4.39E-09	4.55	4.71
positive regulation of leukocyte mediated cytotoxicity	19.21	4.54E-09	25.93	5.40
regulation of biological quality	19.15	4.81E-09	6.39	4.82
positive regulation of lymphocyte activation	19.14	4.87E-09	15.83	5.29
regulation of inflammatory response	19.01	5.53E-09	14.55	5.05
positive regulation of leukocyte differentiation	18.90	6.17E-09	20.24	4.61
negative regulation of immune system process	18.89	6.25E-09	14.46	5.02
cytokine-mediated signaling pathway	18.85	6.51E-09	16.28	5.40
response to organonitrogen compound	18.51	9.12E-09	8.53	4.55
lymphocyte mediated immunity	18.48	9.46E-09	30.00	4.11
regulation of natural killer cell chemotaxis	18.05	1.45E-08	85.71	6.11
locomotion	17.99	1.54E-08	8.98	4.79
positive regulation of mononuclear cell proliferation	17.98	1.55E-08	19.10	5.38
regulation of lymphocyte apoptotic process	17.86	1.75E-08	28.57	4.88
regulation of T cell mediated cytotoxicity	17.86	1.75E-08	28.57	5.61
regulation of sequence-specific DNA binding transcription factor activity	17.79	1.88E-08	12.12	5.05
regulation of metabolic process	17.68	2.09E-08	5.24	4.72
regulation of protein transport	17.55	2.38E-08	11.41	4.82
defense response to protozoan	17.43	2.70E-08	50.00	5.10
regulation of lymphocyte chemotaxis	17.43	2.70E-08	50.00	5.95
positive regulation of interleukin-6 production	17.26	3.20E-08	30.56	5.34
positive regulation of cytokine secretion	17.21	3.36E-08	24.53	4.92
positive regulation of nitric oxide biosynthetic process	17.12	3.68E-08	34.48	4.78
positive regulation of I-kappaB kinase/NF-kappaB signaling	17.03	4.02E-08	16.98	5.49
regulation of ERK1 and ERK2 cascade	17.00	4.12E-08	16.10	5.16
regulation of phagocytosis	16.94	4.40E-08	29.73	4.85
transport	16.88	4.65E-08	5.92	4.58
regulation of establishment of protein localization	16.82	4.96E-08	10.80	4.95
positive regulation of phosphorus metabolic process	16.81	5.02E-08	8.44	4.92
positive regulation of phosphate metabolic process	16.81	5.02E-08	8.44	4.92
positive regulation of macromolecule metabolic process	16.79	5.12E-08	6.41	4.57

response to interferon-alpha	16.78	5.16E-08	58.33	5.31
regulation of calcium ion transport	16.72	5.45E-08	15.83	5.11
regulation of macrophage chemotaxis	16.69	5.63E-08	75.00	4.77
positive regulation of NF-kappaB transcription factor activity	16.67	5.73E-08	20.00	5.15
response to abiotic stimulus	16.66	5.83E-08	7.64	4.74
antigen processing and presentation of endogenous peptide antigen	16.63	5.99E-08	100.00	6.92
positive regulation of T cell mediated cytotoxicity	16.63	5.99E-08	28.95	5.78
positive regulation of ion transport	16.59	6.25E-08	15.70	5.21
positive regulation of sequence-specific DNA binding transcription factor activity	16.51	6.74E-08	14.93	5.42
regulation of T cell mediated immunity	16.50	6.84E-08	23.21	5.57
positive regulation of T cell mediated immunity	16.47	7.03E-08	25.53	5.73
positive regulation of lymphocyte proliferation	16.28	8.51E-08	18.18	5.46
positive regulation of interleukin-8 production	16.27	8.62E-08	44.44	5.42
regulation of multicellular organismal development	16.25	8.74E-08	7.00	4.37
T cell activation	16.14	9.83E-08	14.60	4.65
biological_process	16.11	1.01E-07	3.99	4.68
neutrophil migration	16.05	1.08E-07	31.25	3.77
neutrophil chemotaxis	16.05	1.08E-07	31.25	3.77
innate immune response-activating signal transduction	16.05	1.08E-07	31.25	4.61
lymphocyte chemotaxis	16.04	1.08E-07	53.85	6.70
cell migration	15.99	1.14E-07	9.34	4.92
defense response to Gram-positive bacterium	15.97	1.16E-07	24.49	4.82
positive regulation of phagocytosis	15.96	1.17E-07	36.00	4.83
cell activation involved in immune response	15.95	1.19E-07	18.99	4.89
leukocyte activation involved in immune response	15.95	1.19E-07	18.99	4.89
regulation of interleukin-6 production	15.84	1.31E-07	20.29	5.07
regulation of vasculature development	15.79	1.39E-07	13.73	5.04
regulation of angiogenesis	15.77	1.42E-07	14.29	5.07
lymphocyte activation	15.77	1.42E-07	11.96	4.48
positive regulation of lymphocyte migration	15.75	1.44E-07	42.11	5.84
antigen processing and presentation of exogenous peptide antigen	15.75	1.44E-07	42.11	4.77
regulation of leukocyte differentiation	15.68	1.56E-07	13.64	4.63
positive regulation of cell differentiation	15.67	1.56E-07	9.09	4.38
regulation of mononuclear cell migration	15.63	1.64E-07	66.67	4.69
regulation of protein secretion	15.60	1.68E-07	15.52	4.59

regulation of NF-kappaB import into nucleus	15.57	1.74E-07	34.62	4.99
positive regulation of chemokine production	15.57	1.74E-07	34.62	5.59
regulation of toll-like receptor signaling pathway	15.57	1.74E-07	34.62	4.96
positive regulation of phosphorylation	15.56	1.75E-07	8.57	4.68
cellular response to stimulus	15.48	1.90E-07	4.92	4.71
leukocyte cell-cell adhesion	15.40	2.05E-07	29.41	5.29
ion transport	15.39	2.07E-07	7.74	4.41
regulation of primary metabolic process	15.39	2.08E-07	5.26	4.74
positive regulation of protein transport	15.34	2.17E-07	13.38	4.97
establishment of localization	15.30	2.26E-07	5.75	4.58
response to protozoan	15.27	2.33E-07	40.00	5.10
toll-like receptor signaling pathway	15.27	2.33E-07	40.00	4.80
regulation of type I interferon production	15.19	2.52E-07	33.33	6.09
regulation of macromolecule metabolic process	15.11	2.75E-07	5.27	4.72
regulation of cellular localization	15.08	2.83E-07	7.88	4.83
regulation of protein localization	15.06	2.88E-07	9.62	4.84
positive regulation of ERK1 and ERK2 cascade	14.94	3.24E-07	17.65	5.38
response to ionizing radiation	14.89	3.42E-07	15.60	5.17
positive regulation of macrophage chemotaxis	14.87	3.49E-07	83.33	4.93
secretion by cell	14.86	3.51E-07	11.42	5.08
regulation of protein phosphorylation	14.85	3.56E-07	7.90	4.76
negative regulation of innate immune response	14.83	3.64E-07	38.10	5.95
eosinophil chemotaxis	14.74	3.97E-07	60.00	5.41
regulation of antigen processing and presentation	14.74	3.97E-07	60.00	5.38
regulation of cellular protein metabolic process	14.72	4.04E-07	6.83	4.82
positive regulation of catalytic activity	14.64	4.39E-07	8.01	4.67
peptide antigen binding	14.52	4.94E-07	27.03	5.84
regulation of cytokine secretion	14.42	5.48E-07	18.18	4.81
acute inflammatory response	14.40	5.59E-07	21.43	4.58
positive regulation of protein secretion	14.25	6.46E-07	17.95	4.85
negative regulation of leukocyte apoptotic process	14.25	6.48E-07	26.32	5.06
cellular chemical homeostasis	14.24	6.54E-07	9.63	5.27
immunoglobulin mediated immune response	14.24	6.57E-07	43.75	4.11
regulation of protein modification process	14.23	6.61E-07	7.27	4.90
regulation of cellular metabolic process	14.22	6.70E-07	5.19	4.68
regulation of anatomical structure morphogenesis	14.20	6.78E-07	8.25	4.76
pattern recognition receptor	14.17	6.99E-07	30.00	4.75

signaling pathway				
response to alcohol	14.17	7.00E-07	9.60	5.14
positive regulation of MAPK cascade	14.06	7.86E-07	10.66	5.00
eosinophil migration	13.98	8.46E-07	54.55	5.41
response to drug	13.91	9.12E-07	8.99	5.27
regulation of endocytosis	13.90	9.16E-07	15.38	4.80
regulation of catalytic activity	13.90	9.22E-07	6.60	4.76
regulation of MAPK cascade	13.83	9.81E-07	9.12	4.84
positive regulation of T cell proliferation	13.80	1.01E-06	20.34	5.65
cellular response to mechanical stimulus	13.80	1.01E-06	20.34	5.01
cellular response to external stimulus	13.78	1.03E-06	12.66	5.48
regulation of interleukin-8 production	13.73	1.09E-06	25.00	5.45
regulation of cytokine-mediated signaling pathway	13.73	1.09E-06	25.00	5.99
positive regulation of inflammatory response	13.61	1.23E-06	20.00	5.18
response to gamma radiation	13.59	1.26E-06	22.00	5.42
regulation of chemokine production	13.48	1.39E-06	24.39	5.39
immune response-activating signal transduction	13.47	1.42E-06	16.87	4.31
response to hormone	13.46	1.42E-06	7.45	4.61
cell motility	13.39	1.53E-06	8.41	4.92
regulation of transcription factor import into nucleus	13.38	1.55E-06	21.57	4.65
NIK/NF-kappaB signaling	13.30	1.67E-06	100.00	5.30
immune response-inhibiting cell surface receptor signaling pathway	13.30	1.67E-06	100.00	3.97
2'-5'-oligoadenylate synthetase activity	13.30	1.67E-06	100.00	6.23
positive regulation of protein metabolic process	13.29	1.70E-06	7.41	4.50
regulation of granulocyte chemotaxis	13.28	1.70E-06	32.00	5.42
positive regulation of transcription factor import into nucleus	13.28	1.70E-06	32.00	5.02
B cell mediated immunity	13.28	1.72E-06	38.89	4.11
secretion	13.22	1.82E-06	9.54	4.88
MAPK cascade	13.19	1.87E-06	15.46	4.59
positive regulation of angiogenesis	13.17	1.90E-06	16.47	5.09
regulation of cell-cell adhesion	13.06	2.12E-06	19.05	5.07
positive regulation of stress-activated protein kinase signaling cascade	12.97	2.33E-06	20.75	5.56
regulation of metal ion transport	12.97	2.34E-06	11.60	4.95
aging	12.96	2.35E-06	11.22	5.94
exocytosis	12.92	2.44E-06	15.15	5.22
negative regulation of response to stimulus	12.89	2.53E-06	7.24	4.90
positive regulation of T cell differentiation	12.79	2.80E-06	22.73	4.63
cellular response to abiotic	12.77	2.83E-06	12.34	4.76

stimulus				
myeloid dendritic cell activation	12.73	2.95E-06	46.15	4.64
homeostatic process	12.62	3.29E-06	7.00	5.02
response to extracellular stimulus	12.57	3.47E-06	9.24	5.56
regulation of response to cytokine stimulus	12.57	3.49E-06	22.22	5.99
negative regulation of cell activation	12.54	3.58E-06	14.71	4.39
signal transduction by phosphorylation	12.54	3.59E-06	13.91	4.53
single-organism developmental process	12.51	3.67E-06	5.11	4.61
wound healing	12.46	3.86E-06	16.67	4.59
regulation of cellular process	12.41	4.09E-06	4.39	4.64
negative regulation of cell proliferation	12.32	4.48E-06	8.51	4.51
positive regulation of endocytosis	12.22	4.94E-06	19.30	5.01
regulation of monocyte chemotaxis	12.20	5.01E-06	42.86	5.16
positive regulation of NF-kappaB import into nucleus	12.20	5.01E-06	42.86	5.66
positive regulation of cellular component organization	12.15	5.31E-06	7.97	4.70
lymphocyte migration	12.07	5.70E-06	33.33	6.70
leukocyte mediated cytotoxicity	12.07	5.70E-06	33.33	3.26
regulation of cell shape	12.07	5.76E-06	17.39	4.93
positive regulation of cell-cell adhesion	12.03	5.95E-06	27.59	5.61
positive regulation of reactive oxygen species metabolic process	12.03	5.95E-06	27.59	4.25
anion transport	11.99	6.21E-06	9.77	4.02
negative regulation of leukocyte activation	11.95	6.49E-06	14.89	4.41
positive regulation of antigen processing and presentation	11.92	6.69E-06	55.56	5.73
regulation of interferon-alpha production	11.92	6.69E-06	55.56	6.34
positive regulation of programmed cell death	11.83	7.31E-06	9.45	5.17
positive regulation of hydrolase activity	11.83	7.31E-06	9.45	4.70
cellular cation homeostasis	11.78	7.62E-06	9.65	5.27
negative regulation of lymphocyte activation	11.75	7.86E-06	15.66	4.53
regulation of interleukin-1 production	11.75	7.86E-06	26.67	4.31
cytokine metabolic process	11.72	8.10E-06	40.00	4.74
regulation of T cell apoptotic process	11.72	8.10E-06	31.82	5.22
positive regulation of natural killer cell chemotaxis	11.72	8.12E-06	80.00	6.34
ISG15-protein conjugation	11.72	8.12E-06	80.00	6.20
immune response-inhibiting signal transduction	11.72	8.12E-06	80.00	3.97
response to mechanical stimulus	11.69	8.40E-06	11.92	5.02
regulation of homeostatic process	11.60	9.18E-06	10.04	5.10
response to toxic substance	11.55	9.60E-06	12.32	4.56
cellular homeostasis	11.51	1.00E-05	8.31	5.25

T cell proliferation	11.49	1.03E-05	25.81	5.06
regulation of intracellular transport	11.48	1.03E-05	9.72	5.11
positive regulation of lymphocyte differentiation	11.37	1.15E-05	19.61	4.63
positive regulation of cellular protein metabolic process	11.34	1.19E-05	7.28	4.56
negative regulation of NF-kappaB transcription factor activity	11.33	1.20E-05	21.95	4.71
regulation of vesicle-mediated transport	11.31	1.23E-05	10.45	4.41
positive regulation of monocyte chemotaxis	11.25	1.30E-05	50.00	5.53
response to inorganic substance	11.25	1.31E-05	8.21	5.67
negative regulation of defense response	11.23	1.33E-05	14.94	5.09
positive regulation of cell death	11.20	1.37E-05	8.94	5.08
positive regulation of stress-activated MAPK cascade	11.19	1.38E-05	19.23	5.78
regulation of stress-activated protein kinase signaling cascade	11.15	1.44E-05	13.16	5.43
cellular ion homeostasis	11.13	1.47E-05	9.29	5.27
response to peptide	11.07	1.56E-05	9.06	4.80
positive regulation of apoptotic process	11.00	1.67E-05	9.23	5.23
positive regulation of interleukin-1 beta production	10.88	1.88E-05	35.29	4.52
positive regulation of type I interferon production	10.88	1.88E-05	35.29	6.16
regulation of cell differentiation	10.85	1.93E-05	6.36	4.23
negative regulation of programmed cell death	10.80	2.05E-05	7.80	5.09
organ development	10.79	2.06E-05	6.35	4.86
positive regulation of interferon-gamma production	10.75	2.15E-05	23.53	5.42
positive regulation of JNK cascade	10.72	2.21E-05	20.45	5.94
chemical homeostasis	10.71	2.22E-05	7.39	5.16
cytokine biosynthetic process	10.68	2.31E-05	45.45	5.00
integrin-mediated signaling pathway	10.67	2.31E-05	18.18	3.98
positive regulation of homeostatic process	10.67	2.31E-05	18.18	5.86
T-helper 1 type immune response	10.65	2.37E-05	66.67	5.11
cellular extravasation	10.65	2.37E-05	66.67	3.70
antigen processing and presentation via MHC class Ib	10.65	2.37E-05	66.67	5.25
phosphorylation	10.61	2.48E-05	7.53	4.37
negative regulation of cell death	10.56	2.59E-05	7.52	4.97
positive regulation of protein phosphorylation	10.54	2.64E-05	7.92	4.73
response to nutrient levels	10.53	2.67E-05	8.78	5.67
T cell activation involved in immune response	10.52	2.70E-05	22.86	5.00
macrophage activation	10.51	2.74E-05	33.33	5.52
positive regulation of interleukin-1 production	10.51	2.74E-05	33.33	4.52
cation homeostasis	10.51	2.74E-05	8.60	5.37

regulation of myeloid leukocyte mediated immunity	10.50	2.76E-05	26.92	4.18
regulation of interleukin-1 beta production	10.50	2.76E-05	26.92	4.44
inflammatory response to antigenic stimulus	10.50	2.76E-05	26.92	4.63
immune response-regulating cell surface receptor signaling pathway	10.46	2.87E-05	16.18	3.92
negative regulation of locomotion	10.45	2.90E-05	11.33	5.34
developmental process	10.44	2.92E-05	4.89	4.59
response to steroid hormone	10.42	2.99E-05	7.99	4.55
regulation of JNK cascade	10.38	3.12E-05	13.83	5.67
negative regulation of apoptotic process	10.34	3.24E-05	7.73	5.10
positive regulation of intracellular transport	10.33	3.26E-05	12.30	5.39
cell killing	10.23	3.61E-05	25.93	3.26
regulation of ion transport	10.21	3.68E-05	8.80	5.04
regulation of reactive oxygen species metabolic process	10.19	3.74E-05	17.24	4.71
inorganic anion transport	10.18	3.79E-05	15.71	4.47
myeloid dendritic cell differentiation	10.17	3.84E-05	41.67	4.10
positive regulation of interleukin-1 secretion	10.17	3.84E-05	41.67	4.78
positive regulation of interleukin-1 beta secretion	10.17	3.84E-05	41.67	4.78
positive regulation of calcium ion import	10.17	3.84E-05	41.67	4.46
positive regulation of toll-like receptor signaling pathway	10.17	3.84E-05	41.67	5.06
positive regulation of cytokine biosynthetic process	10.16	3.86E-05	19.15	4.88
regulation of cytokine biosynthetic process	10.04	4.35E-05	15.49	4.72
interspecies interaction between organisms	10.04	4.35E-05	15.49	5.25
positive regulation of smooth muscle cell proliferation	9.99	4.60E-05	18.75	4.18
negative regulation of mononuclear cell proliferation	9.99	4.60E-05	18.75	4.63
negative regulation of lymphocyte proliferation	9.99	4.60E-05	18.75	4.63
apoptotic signaling pathway	9.99	4.60E-05	10.53	5.49
negative regulation of protein metabolic process	9.98	4.64E-05	8.06	4.95
alpha-beta T cell activation	9.97	4.66E-05	25.00	4.49
regulation of interleukin-12 production	9.97	4.66E-05	25.00	4.61
response to type I interferon	9.97	4.67E-05	100.00	7.61
antigen processing and presentation of endogenous peptide antigen via MHC class Ib	9.97	4.67E-05	100.00	5.88
antigen processing and presentation of endogenous peptide antigen via MHC class I	9.97	4.67E-05	100.00	7.54
regulation of T cell differentiation	9.91	4.97E-05	15.28	4.85
cell death	9.90	4.99E-05	8.31	4.77
tolerance induction	9.83	5.37E-05	57.14	4.72

response to platelet-derived growth factor	9.83	5.37E-05	57.14	4.54
cellular response to platelet-derived growth factor stimulus	9.83	5.37E-05	57.14	4.54
negative regulation of interleukin-12 production	9.83	5.37E-05	57.14	3.66
activated T cell proliferation	9.83	5.37E-05	57.14	3.99
negative regulation of viral transcription	9.83	5.38E-05	30.00	6.71
regulation of stress-activated MAPK cascade	9.82	5.46E-05	12.39	5.58
cellular response to endogenous stimulus	9.77	5.70E-05	7.16	4.56
endothelial cell migration	9.73	5.96E-05	24.14	4.47
response to temperature stimulus	9.72	6.02E-05	12.28	4.88
regulation of myeloid cell differentiation	9.72	6.02E-05	12.28	4.38
regulation of interleukin-1 beta secretion	9.71	6.06E-05	38.46	4.78
cellular process	9.70	6.15E-05	4.07	4.61
regulation of peptidyl-tyrosine phosphorylation	9.67	6.30E-05	11.11	4.78
negative regulation of leukocyte proliferation	9.65	6.44E-05	18.00	4.63
chloride transport	9.65	6.44E-05	18.00	4.32
cellular developmental process	9.64	6.54E-05	5.43	4.33
regulation of proteolysis	9.59	6.83E-05	8.16	4.89
programmed cell death	9.59	6.85E-05	8.47	4.89
intracellular signal transduction	9.55	7.13E-05	6.34	4.68
death	9.54	7.19E-05	8.13	4.77
myeloid cell activation involved in immune response	9.52	7.30E-05	28.57	4.70
regulation of protein processing	9.51	7.43E-05	7.98	4.96
modification of morphology or physiology of other organism involved in symbiotic interaction	9.50	7.52E-05	20.00	4.46
response to exogenous dsRNA	9.49	7.53E-05	23.33	5.03
cellular component movement	9.41	8.22E-05	6.70	4.60
cellular response to nitrogen compound	9.37	8.52E-05	8.36	5.00
regulation of response to biotic stimulus	9.33	8.87E-05	17.31	4.86
negative regulation of multicellular organismal process	9.32	8.93E-05	8.51	4.52
negative regulation of sequence-specific DNA binding transcription factor activity	9.32	9.00E-05	13.33	4.94
intracellular pH reduction	9.30	9.14E-05	35.71	5.58
regulation of membrane protein ectodomain proteolysis	9.30	9.14E-05	35.71	4.93
regulation of viral entry into host cell	9.30	9.14E-05	35.71	5.97
regulation of secretion	9.29	9.24E-05	7.63	4.42
dendritic cell differentiation	9.24	9.74E-05	27.27	3.97
positive regulation of ion transmembrane transport	9.24	9.74E-05	27.27	4.31
response to lipoteichoic acid	9.17	1.04E-04	50.00	4.85

cellular response to lipoteichoic acid	9.17	1.04E-04	50.00	4.85
I-kappaB phosphorylation	9.17	1.04E-04	50.00	5.25
response to peptidoglycan	9.17	1.04E-04	50.00	4.31
response to muramyl dipeptide	9.17	1.04E-04	50.00	4.38
negative regulation of natural killer cell mediated immunity	9.17	1.04E-04	50.00	6.24
negative regulation of natural killer cell mediated cytotoxicity	9.17	1.04E-04	50.00	6.24
basic amino acid transport	9.17	1.04E-04	50.00	3.96
response to oxidative stress	9.14	1.07E-04	8.80	5.06
response to progesterone	9.13	1.08E-04	19.05	4.92
modification of morphology or physiology of other organism	9.13	1.08E-04	19.05	4.46
ion homeostasis	9.09	1.13E-04	7.92	5.37
response to metal ion	9.08	1.14E-04	8.76	5.96
regulation of intracellular pH	8.97	1.28E-04	26.09	5.11
regulation of interferon-beta production	8.97	1.28E-04	26.09	5.95
regulation of natural killer cell mediated immunity	8.97	1.28E-04	26.09	5.57
regulation of natural killer cell mediated cytotoxicity	8.97	1.28E-04	26.09	5.57
regulation of interleukin-1 secretion	8.92	1.33E-04	33.33	4.78
positive regulation of cytokine-mediated signaling pathway	8.92	1.33E-04	33.33	5.76
pH reduction	8.92	1.33E-04	33.33	5.58
regulation of myeloid leukocyte differentiation	8.92	1.33E-04	14.93	4.38
negative regulation of cytokine production	8.89	1.38E-04	12.77	4.79
positive regulation of secretion	8.87	1.41E-04	9.09	4.76
positive regulation of multi-organism process	8.85	1.44E-04	21.21	5.48
regulation of protein localization to nucleus	8.82	1.48E-04	11.93	4.54
regulation of wound healing	8.80	1.51E-04	14.71	4.55
negative regulation of phosphorylation	8.74	1.60E-04	9.27	4.82
regulation of hydrolase activity	8.73	1.61E-04	6.80	4.63
positive regulation of acute inflammatory response	8.71	1.65E-04	25.00	5.59
positive regulation of transmembrane transport	8.71	1.65E-04	25.00	4.31
positive regulation of production of molecular mediator of immune response	8.71	1.65E-04	25.00	4.64
regulation of cellular response to stress	8.68	1.69E-04	8.53	5.27
regulation of cytokine production involved in immune response	8.65	1.76E-04	20.59	4.16
regulation of ion homeostasis	8.63	1.78E-04	11.71	5.09
regulation of type IIa hypersensitivity	8.61	1.82E-04	75.00	4.37
positive regulation of type IIa hypersensitivity	8.61	1.82E-04	75.00	4.37
regulation of type II hypersensitivity	8.61	1.82E-04	75.00	4.37

positive regulation of type II hypersensitivity	8.61	1.82E-04	75.00	4.37
regulation of dendritic cell antigen processing and presentation	8.61	1.82E-04	75.00	5.22
positive regulation of dendritic cell antigen processing and presentation	8.61	1.82E-04	75.00	5.22
positive regulation of integrin activation	8.61	1.82E-04	75.00	4.80
positive regulation of RIG-I signaling pathway	8.61	1.82E-04	75.00	5.82
antigen processing and presentation of peptide antigen via MHC class Ib	8.61	1.82E-04	75.00	5.88
protection from natural killer cell mediated cytotoxicity	8.61	1.82E-04	75.00	7.07
negative regulation of immune response	8.60	1.85E-04	15.79	5.77
cell differentiation	8.59	1.87E-04	5.74	4.37
positive regulation of leukocyte apoptotic process	8.58	1.88E-04	31.25	5.31
granulocyte activation	8.58	1.88E-04	31.25	4.28
positive regulation of response to cytokine stimulus	8.58	1.88E-04	31.25	5.76
positive regulation of biosynthetic process	8.57	1.90E-04	5.85	4.42
regulation of cell adhesion	8.55	1.93E-04	9.13	4.87
regulation of nucleocytoplasmic transport	8.55	1.94E-04	11.02	5.13
nitrogen compound transport	8.47	2.11E-04	8.40	4.65
negative regulation of lymphocyte apoptotic process	8.46	2.11E-04	24.00	5.48
membrane docking	8.46	2.11E-04	24.00	4.51
vesicle fusion	8.46	2.11E-04	24.00	4.92
regulation of cellular pH	8.46	2.11E-04	24.00	5.11
positive regulation of nitrogen compound metabolic process	8.46	2.12E-04	5.92	4.61
lymphocyte activation involved in immune response	8.46	2.12E-04	15.52	4.88
positive regulation of myeloid leukocyte differentiation	8.46	2.13E-04	20.00	4.58
regulation of extrinsic apoptotic signaling pathway	8.39	2.27E-04	12.12	5.81
positive regulation of protein modification process	8.34	2.38E-04	6.88	4.78
positive regulation of myeloid cell differentiation	8.33	2.42E-04	15.25	4.70
cell adhesion	8.32	2.44E-04	7.13	4.42
negative regulation of response to external stimulus	8.30	2.49E-04	10.77	4.57
regulation of nitrogen compound metabolic process	8.29	2.52E-04	4.99	4.59
biological adhesion	8.28	2.54E-04	7.11	4.42
response to dsRNA	8.27	2.56E-04	19.44	5.03
regulation of T cell migration	8.26	2.58E-04	29.41	6.12
positive regulation of neutrophil migration	8.26	2.58E-04	29.41	5.43
regulation of apoptotic signaling pathway	8.16	2.87E-04	8.62	5.31
response to reactive oxygen	8.14	2.92E-04	10.61	5.31

species				
response to ethanol	8.14	2.92E-04	10.61	5.91
positive regulation of macrophage differentiation	8.13	2.95E-04	40.00	3.79
T cell migration	8.13	2.95E-04	40.00	5.66
chaperone mediated protein folding requiring cofactor	8.13	2.95E-04	40.00	4.88
negative regulation of viral entry into host cell	8.13	2.95E-04	40.00	5.71
oligopeptide transport	8.13	2.95E-04	40.00	5.46
leukocyte differentiation	8.12	2.99E-04	9.39	4.11
regulation of protein import into nucleus	8.11	3.00E-04	11.76	4.70
response to peptide hormone	8.09	3.05E-04	8.18	4.88
regulation of gliogenesis	8.07	3.14E-04	14.75	4.10
positive regulation of cellular biosynthetic process	8.03	3.24E-04	5.79	4.43
positive regulation of lipid metabolic process	7.97	3.46E-04	12.36	5.12
regulation of vascular endothelial growth factor production	7.97	3.47E-04	27.78	5.48
T cell mediated immunity	7.97	3.47E-04	27.78	4.11
regulation of production of molecular mediator of immune response	7.94	3.55E-04	14.52	4.41
positive regulation of peptidyl-tyrosine phosphorylation	7.93	3.60E-04	11.54	4.60
regulation of cellular component organization	7.88	3.78E-04	5.62	4.59
negative regulation of phosphorus metabolic process	7.88	3.79E-04	8.44	4.78
negative regulation of phosphate metabolic process	7.88	3.79E-04	8.44	4.78
cellular metal ion homeostasis	7.88	3.79E-04	8.44	5.40
single-organism membrane fusion	7.87	3.83E-04	16.00	4.59
intrinsic apoptotic signaling pathway in response to DNA damage	7.87	3.83E-04	16.00	4.92
regulation of smooth muscle cell proliferation	7.87	3.84E-04	13.16	4.02
response to nutrient	7.86	3.86E-04	9.19	5.75
angiogenesis	7.83	3.97E-04	10.29	4.34
negative regulation of T cell activation	7.82	4.02E-04	14.29	4.80
regulation of lymphocyte differentiation	7.77	4.20E-04	12.09	4.85
organic substance transport	7.76	4.26E-04	5.70	4.55
positive regulation of myeloid leukocyte mediated immunity	7.72	4.42E-04	60.00	4.37
T cell chemotaxis	7.72	4.42E-04	60.00	5.89
positive regulation of cell-cell adhesion mediated by integrin	7.72	4.42E-04	60.00	6.67
endothelial cell chemotaxis	7.72	4.42E-04	60.00	5.10
hydrogen peroxide biosynthetic process	7.72	4.42E-04	60.00	5.58
antigen processing and presentation of exogenous peptide antigen via MHC class I	7.72	4.42E-04	60.00	4.81

membrane to membrane docking	7.72	4.42E-04	60.00	5.87
type I interferon biosynthetic process	7.72	4.42E-04	60.00	6.03
toll-like receptor 4 signaling pathway	7.72	4.42E-04	60.00	4.65
arginine transport	7.72	4.42E-04	60.00	4.36
positive regulation of humoral immune response	7.70	4.51E-04	36.36	4.71
microglial cell activation	7.70	4.51E-04	36.36	5.81
monocyte chemotaxis	7.70	4.51E-04	36.36	4.40
macrophage chemotaxis	7.70	4.51E-04	36.36	4.40
myeloid leukocyte mediated immunity	7.70	4.51E-04	36.36	3.15
regulation of B cell apoptotic process	7.69	4.57E-04	26.32	4.41
regulation of neutrophil migration	7.69	4.57E-04	26.32	5.43
regulation of calcium ion import	7.69	4.57E-04	26.32	4.46
positive regulation of phosphatidylinositol 3-kinase activity	7.69	4.57E-04	26.32	4.82
protein trimerization	7.60	5.00E-04	20.69	5.75
protein kinase B signaling	7.60	5.00E-04	20.69	5.95
regulation of viral transcription	7.60	5.00E-04	20.69	6.71
cellular monovalent inorganic cation homeostasis	7.60	5.00E-04	20.69	5.11
activation of cysteine-type endopeptidase activity involved in apoptotic process	7.59	5.04E-04	15.38	4.60
cytokine production	7.59	5.04E-04	15.38	5.47
regulation of hemostasis	7.59	5.04E-04	15.38	4.67
regulation of blood coagulation	7.59	5.04E-04	15.38	4.67
cellular response to organonitrogen compound	7.59	5.04E-04	7.89	4.91
regulation of tyrosine phosphorylation of STAT protein	7.59	5.05E-04	17.50	4.51
epithelial cell migration	7.59	5.05E-04	17.50	4.47
leukocyte proliferation	7.58	5.09E-04	13.85	4.94
protein processing	7.58	5.09E-04	6.83	5.39
peptidyl-tyrosine phosphorylation	7.55	5.26E-04	12.66	4.08
negative regulation of cell migration	7.54	5.32E-04	10.48	5.23
negative regulation of cellular protein metabolic process	7.53	5.38E-04	7.55	5.03
positive regulation of endopeptidase activity	7.49	5.56E-04	11.70	5.12
response to vitamin	7.49	5.56E-04	11.70	5.80
single organism cell adhesion	7.48	5.63E-04	9.20	4.85
carboxylic acid transport	7.46	5.73E-04	9.93	3.97
positive regulation of protein import into nucleus	7.46	5.75E-04	15.09	5.02
regulation of acute inflammatory response	7.44	5.90E-04	17.07	5.83
regulation of humoral immune response	7.43	5.92E-04	25.00	5.22
response to ATP	7.43	5.92E-04	25.00	5.44
positive regulation of lipid kinase	7.43	5.92E-04	25.00	4.82

activity				
antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	7.43	5.92E-04	25.00	4.52
cytokine secretion	7.43	5.92E-04	25.00	4.55
regulation of astrocyte differentiation	7.43	5.92E-04	25.00	4.08
cellular response to osmotic stress	7.43	5.92E-04	25.00	4.61
metal ion homeostasis	7.40	6.11E-04	7.77	5.51
organic acid transport	7.39	6.16E-04	9.86	3.97
negative regulation of signal transduction	7.37	6.30E-04	6.46	4.81
positive regulation of cysteine-type endopeptidase activity involved in apoptotic process	7.35	6.43E-04	12.35	4.96
peptidyl-tyrosine modification	7.35	6.43E-04	12.35	4.08
regulation of coagulation	7.33	6.54E-04	14.81	4.67
negative regulation of immune effector process	7.33	6.54E-04	14.81	5.32
alpha-beta T cell activation involved in immune response	7.33	6.57E-04	33.33	4.63
positive regulation of B cell mediated immunity	7.33	6.57E-04	33.33	4.02
positive regulation of immunoglobulin mediated immune response	7.33	6.57E-04	33.33	4.02
negative regulation of leukocyte mediated cytotoxicity	7.33	6.57E-04	33.33	6.24
negative regulation of cell killing	7.33	6.57E-04	33.33	6.24
positive regulation of transferase activity	7.32	6.61E-04	7.89	4.90
response to hydrogen peroxide	7.31	6.66E-04	11.46	5.02
organic anion transport	7.31	6.66E-04	8.76	3.83
regulation of biosynthetic process	7.30	6.77E-04	4.90	4.51
organelle fusion	7.28	6.86E-04	16.67	4.72
multicellular organismal homeostasis	7.28	6.86E-04	16.67	6.19
response to oxygen levels	7.27	6.94E-04	7.87	5.05
protein maturation	7.26	7.06E-04	6.70	5.39
regulation of cellular biosynthetic process	7.25	7.10E-04	4.91	4.52
regulation of G-protein coupled receptor protein signaling pathway	7.25	7.13E-04	13.24	4.86
regulation of actin filament length	7.25	7.13E-04	13.24	4.40
regulation of actin polymerization or depolymerization	7.25	7.13E-04	13.24	4.40
negative regulation of cell motility	7.23	7.21E-04	10.16	5.23
response to glucocorticoid	7.23	7.22E-04	8.99	4.73
protein activation cascade	7.22	7.29E-04	19.35	6.52
positive regulation of actin filament polymerization	7.22	7.29E-04	19.35	4.82
response to osmotic stress	7.21	7.41E-04	14.55	4.90
response to estrogen	7.20	7.48E-04	8.67	4.95
alpha-beta T cell differentiation	7.19	7.54E-04	23.81	4.38
regulation of platelet activation	7.19	7.54E-04	23.81	3.70

positive regulation of response to biotic stimulus	7.19	7.54E-04	23.81	5.38
cellular response to fibroblast growth factor stimulus	7.14	7.95E-04	16.28	5.81
protein secretion	7.14	7.95E-04	16.28	4.55
activation of cysteine-type endopeptidase activity	7.08	8.38E-04	14.29	4.60
myeloid leukocyte differentiation	7.08	8.38E-04	14.29	4.27
regulation of actin filament polymerization	7.08	8.38E-04	14.29	4.47
astrocyte cell migration	7.06	8.60E-04	50.00	4.72
cellular response to lipoprotein particle stimulus	7.06	8.60E-04	50.00	6.87
superoxide anion generation	7.06	8.60E-04	50.00	5.58
regulation of viral-induced cytoplasmic pattern recognition receptor signaling pathway	7.06	8.60E-04	50.00	5.82
regulation of RIG-I signaling pathway	7.06	8.60E-04	50.00	5.82
fever generation	7.06	8.60E-04	50.00	5.89
positive regulation of tolerance induction	7.06	8.60E-04	50.00	4.03
MyD88-dependent toll-like receptor signaling pathway	7.06	8.60E-04	50.00	3.63
oligopeptide transmembrane transport	7.06	8.60E-04	50.00	5.73
cellular response to exogenous dsRNA	7.06	8.60E-04	50.00	5.64
positive regulation of kinase activity	7.05	8.67E-04	7.91	4.89
response to cold	7.05	8.70E-04	18.75	5.52
regulation of calcium-mediated signaling	7.05	8.70E-04	18.75	3.89
response to radiation	7.03	8.81E-04	7.72	5.14
regulation of gene expression	7.03	8.84E-04	4.79	4.64
response to decreased oxygen levels	7.03	8.85E-04	8.09	5.20
negative regulation of cellular component movement	7.02	8.97E-04	9.92	5.23
drug transport	6.99	9.22E-04	30.77	3.75
phagocytosis, engulfment	6.99	9.22E-04	30.77	3.21
chronic inflammatory response	6.99	9.22E-04	30.77	7.33
antigen processing and presentation of exogenous peptide antigen via MHC class II	6.99	9.22E-04	30.77	4.13
regulation of phosphatidylinositol 3-kinase activity	6.96	9.47E-04	22.73	4.82
chemokine-mediated signaling pathway	6.96	9.47E-04	22.73	5.54
positive regulation of peptidase activity	6.89	1.02E-03	10.89	5.12
regulation of cell morphogenesis	6.88	1.03E-03	7.98	4.45
positive regulation of cysteine-type endopeptidase activity	6.88	1.03E-03	11.63	4.96
negative regulation of signaling	6.86	1.05E-03	6.22	4.89
response to fibroblast growth factor	6.86	1.05E-03	15.56	5.81
regulation of protein kinase activity	6.78	1.14E-03	6.93	4.97

response to corticosteroid	6.76	1.16E-03	8.60	4.73
regulation of endopeptidase activity	6.76	1.16E-03	8.11	5.06
modification by host of symbiont morphology or physiology	6.75	1.17E-03	21.74	4.87
chaperone-mediated protein folding	6.75	1.17E-03	21.74	4.69
regulation of leukocyte degranulation	6.75	1.17E-03	21.74	3.95
regulation of extrinsic apoptotic signaling pathway via death domain receptors	6.75	1.17E-03	21.74	5.98
response to heat	6.73	1.20E-03	12.33	4.49
positive regulation of epithelial cell proliferation	6.72	1.20E-03	10.68	4.35
response to organophosphorus	6.71	1.22E-03	10.08	4.57
positive regulation of alpha-beta T cell activation	6.71	1.22E-03	17.65	4.57
establishment of endothelial barrier	6.68	1.25E-03	28.57	4.30
positive regulation vascular endothelial growth factor production	6.68	1.25E-03	28.57	5.60
negative regulation of I-kappaB kinase/NF-kappaB signaling	6.68	1.25E-03	28.57	4.78
regulation of macrophage differentiation	6.68	1.25E-03	28.57	3.79
glial cell migration	6.68	1.25E-03	28.57	4.73
neutrophil activation	6.68	1.25E-03	28.57	4.64
regulation of lipopolysaccharide-mediated signaling pathway	6.68	1.25E-03	28.57	4.08
single organismal cell-cell adhesion	6.66	1.28E-03	9.15	4.84
lymphocyte proliferation	6.62	1.33E-03	13.33	5.06
regulation of transferase activity	6.61	1.35E-03	6.67	5.07
regulation of nucleobase-containing compound metabolic process	6.60	1.36E-03	4.81	4.63
regulation of kinase activity	6.60	1.36E-03	6.75	5.07
regulation of calcium ion transport into cytosol	6.59	1.37E-03	14.89	5.40
positive regulation of tyrosine phosphorylation of STAT protein	6.56	1.42E-03	17.14	4.16
regulation of mast cell activation	6.54	1.44E-03	20.83	3.79
regulation of cysteine-type endopeptidase activity involved in apoptotic process	6.54	1.45E-03	9.42	4.76
positive regulation of hypersensitivity	6.53	1.46E-03	42.86	4.37
interleukin-1 beta production	6.53	1.46E-03	42.86	6.81
interleukin-1 production	6.53	1.46E-03	42.86	6.81
cellular response to ATP	6.53	1.46E-03	42.86	4.70
regulation of cell-cell adhesion mediated by integrin	6.53	1.46E-03	42.86	6.67
regulation of integrin activation	6.53	1.46E-03	42.86	4.80
negative regulation of type I interferon production	6.53	1.46E-03	42.86	6.48
positive regulation of interferon-alpha production	6.53	1.46E-03	42.86	6.04
positive regulation of chemokine	6.53	1.46E-03	42.86	6.50

biosynthetic process				
regulation of tolerance induction	6.53	1.46E-03	42.86	4.03
positive regulation of nucleocytoplasmic transport	6.51	1.49E-03	13.11	5.02
mononuclear cell proliferation	6.51	1.49E-03	13.11	5.06
negative regulation of protein phosphorylation	6.50	1.51E-03	8.67	4.89
apoptotic process	6.49	1.52E-03	7.55	4.66
cellular response to drug	6.47	1.55E-03	14.58	6.42
regeneration	6.43	1.62E-03	9.76	4.38
myeloid cell differentiation	6.41	1.64E-03	10.28	3.97
positive regulation of cytosolic calcium ion concentration	6.41	1.64E-03	10.28	4.47
membrane fusion	6.41	1.65E-03	12.90	4.59
regulation of smooth muscle cell migration	6.40	1.66E-03	16.67	4.80
positive regulation of gliogenesis	6.40	1.66E-03	16.67	4.36
membrane invagination	6.40	1.66E-03	26.67	3.21
positive regulation of T cell migration	6.40	1.66E-03	26.67	6.73
CD4-positive, alpha-beta T cell differentiation	6.40	1.66E-03	26.67	4.62
regulation of homotypic cell-cell adhesion	6.40	1.66E-03	26.67	5.05
execution phase of apoptosis	6.40	1.66E-03	26.67	6.64
antigen processing and presentation of peptide antigen via MHC class II	6.40	1.66E-03	26.67	4.13
positive regulation of interferon-beta production	6.40	1.66E-03	26.67	5.93
cellular divalent inorganic cation homeostasis	6.38	1.70E-03	8.29	4.99
response to hypoxia	6.36	1.73E-03	7.83	5.09
positive regulation of calcium ion transport into cytosol	6.35	1.75E-03	20.00	6.10
interaction with symbiont	6.35	1.75E-03	20.00	4.87
positive regulation of protein complex assembly	6.35	1.75E-03	11.69	4.81
amino acid transport	6.35	1.75E-03	11.69	4.43
response to alkaloid	6.34	1.77E-03	10.19	5.58
regulation of peptidase activity	6.31	1.81E-03	7.79	5.06
establishment of localization in cell	6.29	1.86E-03	5.62	4.86
intrinsic apoptotic signaling pathway	6.27	1.88E-03	10.75	5.16
proteolysis	6.25	1.93E-03	6.78	5.61
negative regulation of cell communication	6.17	2.09E-03	6.02	4.81
regulation of lipid storage	6.17	2.10E-03	19.23	4.40
regulation of lipid kinase activity	6.17	2.10E-03	19.23	4.82
regulation of cell adhesion mediated by integrin	6.17	2.10E-03	19.23	5.90
regulation of defense response to virus	6.17	2.10E-03	19.23	5.49
positive regulation of neutrophil chemotaxis	6.14	2.15E-03	25.00	5.39
'de novo' posttranslational protein	6.14	2.15E-03	25.00	4.88

folding				
'de novo' protein folding	6.14	2.15E-03	25.00	4.88
mast cell activation	6.14	2.15E-03	25.00	4.11
negative regulation of protein modification process	6.12	2.19E-03	7.66	5.12
positive regulation of cell adhesion	6.12	2.21E-03	9.91	5.16
chloride transmembrane transport	6.12	2.21E-03	15.79	4.43
regulation of lipid metabolic process	6.11	2.23E-03	8.33	5.04
zymogen activation	6.10	2.25E-03	12.31	4.60
peptide metabolic process	6.10	2.25E-03	12.31	5.65
positive regulation of acute inflammatory response to antigenic stimulus	6.08	2.28E-03	37.50	4.37
regulation of cellular extravasation	6.08	2.28E-03	37.50	5.10
positive regulation of interferon-gamma biosynthetic process	6.08	2.28E-03	37.50	5.65
positive regulation of interleukin-2 biosynthetic process	6.08	2.28E-03	37.50	5.34
respiratory burst	6.08	2.28E-03	37.50	5.01
neutrophil activation involved in immune response	6.08	2.28E-03	37.50	3.02
multicellular organismal iron ion homeostasis	6.08	2.28E-03	37.50	7.10
negative regulation of nitric oxide biosynthetic process	6.08	2.28E-03	37.50	4.56
regulation of cysteine-type endopeptidase activity	6.03	2.41E-03	8.90	4.76
regulation of intracellular protein transport	6.03	2.41E-03	8.90	5.05
spleen development	5.99	2.50E-03	18.52	5.43
negative regulation of T cell proliferation	5.98	2.53E-03	15.38	5.17
regulation of pH	5.98	2.53E-03	15.38	5.11
phosphate-containing compound metabolic process	5.92	2.67E-03	5.22	4.35
cell communication	5.90	2.73E-03	6.92	4.89
CD4-positive, alpha-beta T cell activation	5.90	2.74E-03	23.53	4.62
regulation of necrotic cell death	5.90	2.74E-03	23.53	5.54
positive regulation of cytokine production involved in immune response	5.90	2.74E-03	23.53	4.38
membrane organization	5.90	2.75E-03	7.69	4.06
positive regulation of protein kinase activity	5.90	2.75E-03	7.50	4.89
cellular calcium ion homeostasis	5.89	2.75E-03	8.15	4.87
ion transmembrane transport	5.87	2.82E-03	6.78	4.46
regulation of ossification	5.83	2.94E-03	9.09	4.01
regulation of interleukin-10 production	5.82	2.96E-03	17.86	5.13
interaction with host	5.82	2.96E-03	17.86	5.13
reproductive process	5.79	3.05E-03	5.67	4.75
monovalent inorganic cation homeostasis	5.77	3.11E-03	12.96	5.05
response to purine-containing compound	5.77	3.12E-03	9.02	4.57

regulation of hypersensitivity	5.70	3.33E-03	33.33	4.37
cellular response to ethanol	5.70	3.33E-03	33.33	8.78
regulation of interferon-gamma biosynthetic process	5.70	3.33E-03	33.33	5.65
positive regulation of myeloid leukocyte cytokine production involved in immune response	5.70	3.33E-03	33.33	4.13
T cell mediated cytotoxicity	5.70	3.33E-03	33.33	3.34
acute inflammatory response to antigenic stimulus	5.70	3.33E-03	33.33	3.95
negative regulation of astrocyte differentiation	5.70	3.33E-03	33.33	3.57
heat generation	5.70	3.33E-03	33.33	5.89
negative regulation of viral release from host cell	5.70	3.33E-03	33.33	6.08
cellular response to oxidative stress	5.70	3.34E-03	9.40	5.42
transmembrane transport	5.69	3.39E-03	6.18	4.48
response to insulin	5.68	3.42E-03	8.55	5.06
positive regulation of granulocyte chemotaxis	5.68	3.42E-03	22.22	5.39
regulation of neutrophil chemotaxis	5.68	3.42E-03	22.22	5.39
positive regulation of osteoclast differentiation	5.68	3.42E-03	22.22	5.23
regulation of mast cell activation involved in immune response	5.68	3.42E-03	22.22	4.11
regulation of mast cell degranulation	5.68	3.42E-03	22.22	4.11
divalent inorganic cation homeostasis	5.67	3.44E-03	7.73	4.99
immune response-activating cell surface receptor signaling pathway	5.67	3.45E-03	12.73	4.00
positive regulation of gene expression	5.67	3.45E-03	5.47	4.55
response to increased oxygen levels	5.66	3.47E-03	17.24	5.03
response to hyperoxia	5.66	3.47E-03	17.24	5.03
phosphorus metabolic process	5.62	3.61E-03	5.13	4.34
cation transport	5.62	3.63E-03	6.22	4.76
regulation of glial cell differentiation	5.59	3.72E-03	14.29	4.00
cell proliferation	5.58	3.77E-03	6.63	4.63
negative regulation of lymphocyte chemotaxis	5.57	3.80E-03	66.67	5.65
negative regulation of lymphocyte migration	5.57	3.80E-03	66.67	5.65
vacuolar acidification	5.57	3.80E-03	66.67	7.15
positive regulation of MDA-5 signaling pathway	5.57	3.80E-03	66.67	6.04
regulation of type III hypersensitivity	5.57	3.80E-03	66.67	3.09
positive regulation of type III hypersensitivity	5.57	3.80E-03	66.67	3.09
type I interferon production	5.57	3.80E-03	66.67	4.78
ciliary body morphogenesis	5.57	3.80E-03	66.67	4.15
innate immune response activating cell surface receptor signaling pathway	5.57	3.80E-03	66.67	3.48

platelet degranulation	5.57	3.80E-03	66.67	3.60
positive regulation of oligodendrocyte progenitor proliferation	5.57	3.80E-03	66.67	4.04
nucleotide-binding domain, leucine rich repeat containing receptor signaling pathway	5.57	3.80E-03	66.67	5.03
nucleotide-binding oligomerization domain containing signaling pathway	5.57	3.80E-03	66.67	5.03
nucleotide-binding oligomerization domain containing 2 signaling pathway	5.57	3.80E-03	66.67	5.03
RNA import into mitochondrion	5.57	3.80E-03	66.67	4.81
rRNA import into mitochondrion	5.57	3.80E-03	66.67	4.81
antigen processing and presentation of exogenous peptide antigen via MHC class Ib	5.57	3.80E-03	66.67	5.40
antigen processing and presentation of exogenous protein antigen via MHC class Ib, TAP-dependent	5.57	3.80E-03	66.67	5.40
toll-like receptor 2 signaling pathway	5.57	3.80E-03	66.67	5.21
cellular response to peptidoglycan	5.57	3.80E-03	66.67	5.21
antibiotic transport	5.57	3.80E-03	66.67	4.13
intracellular transport of viral protein in host cell	5.57	3.80E-03	66.67	7.19
symbiont intracellular protein transport in host	5.57	3.80E-03	66.67	7.19
intracellular protein transport in other organism involved in symbiotic interaction	5.57	3.80E-03	66.67	7.19
extracellular transport	5.57	3.80E-03	66.67	7.19
positive regulation of apoptotic signaling pathway	5.50	4.07E-03	10.34	4.96
positive regulation of protein polymerization	5.47	4.19E-03	13.95	4.82
positive regulation of JAK-STAT cascade	5.47	4.19E-03	13.95	4.16
response to interleukin-6	5.47	4.21E-03	21.05	4.31
response to sterol	5.47	4.21E-03	21.05	5.18
response to cholesterol	5.47	4.21E-03	21.05	5.18
bone resorption	5.47	4.21E-03	21.05	4.23
positive regulation of natural killer cell mediated immunity	5.47	4.21E-03	21.05	4.76
negative regulation of leukocyte mediated immunity	5.47	4.21E-03	21.05	6.24
negative regulation of lymphocyte mediated immunity	5.47	4.21E-03	21.05	6.24
regulation of fat cell differentiation	5.47	4.23E-03	12.28	4.75
positive regulation of protein kinase B signaling	5.47	4.23E-03	12.28	4.77
cellular response to stress	5.42	4.43E-03	5.59	5.00
transmembrane receptor protein tyrosine kinase signaling pathway	5.42	4.45E-03	8.00	4.49
calcium ion homeostasis	5.40	4.53E-03	7.73	4.87
CD4-positive, alpha-beta T cell differentiation involved in immune	5.38	4.63E-03	30.00	4.45

response				
T-helper cell differentiation	5.38	4.63E-03	30.00	4.45
negative regulation by host of viral transcription	5.38	4.63E-03	30.00	5.67
positive regulation of fever generation	5.38	4.63E-03	30.00	6.82
positive T cell selection	5.38	4.63E-03	30.00	3.90
positive thymic T cell selection	5.38	4.63E-03	30.00	3.90
regulation of macrophage cytokine production	5.38	4.63E-03	30.00	4.02
positive regulation of membrane protein ectodomain proteolysis	5.38	4.63E-03	30.00	5.13
regulation of chemokine biosynthetic process	5.38	4.63E-03	30.00	6.50
cellular response to hormone stimulus	5.37	4.65E-03	6.99	4.29
negative regulation of molecular function	5.37	4.66E-03	5.93	4.89
amide transport	5.37	4.66E-03	12.07	4.72
regulation of JAK-STAT cascade	5.37	4.66E-03	12.07	4.51
response to amphetamine	5.37	4.68E-03	16.13	4.46
natural killer cell activation	5.37	4.68E-03	16.13	3.44
T cell differentiation	5.29	5.06E-03	10.81	4.12
cellular response to steroid hormone stimulus	5.28	5.10E-03	10.00	4.34
release of sequestered calcium ion into cytosol	5.28	5.11E-03	20.00	4.60
negative regulation of sequestering of calcium ion	5.28	5.11E-03	20.00	4.60
negative regulation of developmental process	5.26	5.19E-03	6.06	4.25
regulation of JUN kinase activity	5.25	5.27E-03	13.33	5.22
regulation of macromolecule biosynthetic process	5.24	5.30E-03	4.68	4.51
regulation of actin cytoskeleton organization	5.24	5.30E-03	8.45	4.24
acute-phase response	5.22	5.38E-03	15.63	4.99
cytosolic calcium ion homeostasis	5.20	5.52E-03	8.80	4.47
regulation of MAP kinase activity	5.19	5.56E-03	8.07	4.38
single organism reproductive process	5.18	5.61E-03	5.70	4.81
negative regulation of cell differentiation	5.17	5.67E-03	6.41	4.17
response to estradiol	5.14	5.85E-03	8.73	4.70
leukocyte homeostasis	5.14	5.88E-03	13.04	4.13
regulation of alpha-beta T cell activation	5.14	5.88E-03	13.04	4.57
regulation of protein polymerization	5.12	5.95E-03	10.53	4.47
organonitrogen compound metabolic process	5.09	6.14E-03	5.25	4.86
endothelial cell development	5.09	6.14E-03	19.05	4.30
negative regulation of chemotaxis	5.09	6.14E-03	19.05	5.34
regulation of sequestering of calcium ion	5.09	6.14E-03	19.05	4.60
positive regulation of alpha-beta T cell differentiation	5.09	6.14E-03	19.05	5.05

negative regulation of smooth muscle cell migration	5.08	6.20E-03	27.27	3.95
T cell differentiation involved in immune response	5.08	6.20E-03	27.27	4.45
alpha-beta T cell differentiation involved in immune response	5.08	6.20E-03	27.27	4.45
regulation of necroptotic process	5.08	6.20E-03	27.27	5.80
regulation of triglyceride biosynthetic process	5.08	6.20E-03	27.27	4.74
leukocyte migration involved in inflammatory response	5.08	6.20E-03	27.27	3.78
regulation of fever generation	5.08	6.20E-03	27.27	6.82
regulation of toll-like receptor 4 signaling pathway	5.08	6.20E-03	27.27	4.00
cellular response to dsRNA	5.08	6.20E-03	27.27	5.64
regulation of viral release from host cell	5.08	6.20E-03	27.27	6.08
response to ketone	5.07	6.25E-03	8.28	5.71
cellular iron ion homeostasis	4.96	7.01E-03	14.71	6.97
positive regulation of organelle organization	4.94	7.15E-03	7.35	4.49
cellular glucose homeostasis	4.93	7.25E-03	12.50	5.72
regulation of oxidoreductase activity	4.93	7.25E-03	12.50	4.85
positive regulation of phosphatase activity	4.92	7.29E-03	18.18	3.93
cellular response to morphine	4.90	7.42E-03	50.00	6.29
T-helper 2 cell differentiation	4.90	7.42E-03	50.00	3.38
negative regulation of tumor necrosis factor biosynthetic process	4.90	7.42E-03	50.00	4.08
regulation of apoptotic cell clearance	4.90	7.42E-03	50.00	4.29
negative regulation of macrophage apoptotic process	4.90	7.42E-03	50.00	6.71
modulation by symbiont of host cellular process	4.90	7.42E-03	50.00	3.83
regulation of interleukin-10 secretion	4.90	7.42E-03	50.00	7.32
regulation of MDA-5 signaling pathway	4.90	7.42E-03	50.00	6.04
hyaluronan biosynthetic process	4.90	7.42E-03	50.00	5.17
negative regulation of membrane protein ectodomain proteolysis	4.90	7.42E-03	50.00	4.64
chronic inflammatory response to antigenic stimulus	4.90	7.42E-03	50.00	6.03
regulation of toll-like receptor 2 signaling pathway	4.90	7.42E-03	50.00	4.56
positive regulation of regulatory T cell differentiation	4.90	7.42E-03	50.00	4.40
hypotonic response	4.90	7.42E-03	50.00	4.86
cellular hypotonic response	4.90	7.42E-03	50.00	4.86
neutrophil mediated killing of bacterium	4.90	7.42E-03	50.00	3.67
extracellular matrix-cell signaling	4.90	7.42E-03	50.00	5.04
positive regulation of platelet activation	4.90	7.42E-03	50.00	3.75
phagosome acidification	4.90	7.42E-03	50.00	5.25
manganese ion transport	4.90	7.42E-03	50.00	5.31

manganese ion transmembrane transport	4.90	7.42E-03	50.00	5.31
positive regulation of interferon-beta biosynthetic process	4.90	7.42E-03	50.00	6.79
cellular amide metabolic process	4.86	7.75E-03	9.38	5.32
positive regulation of JUN kinase activity	4.84	7.94E-03	14.29	5.00
response to hydroperoxide	4.82	8.04E-03	25.00	5.47
positive regulation of inflammatory response to antigenic stimulus	4.82	8.04E-03	25.00	4.37
positive regulation of nitric-oxide synthase biosynthetic process	4.82	8.04E-03	25.00	4.02
positive regulation of heat generation	4.82	8.04E-03	25.00	6.82
positive regulation of lymphocyte apoptotic process	4.82	8.04E-03	25.00	6.59
positive regulation of cAMP-mediated signaling	4.82	8.04E-03	25.00	8.13
response to L-ascorbic acid	4.82	8.04E-03	25.00	7.04
developmental process involved in reproduction	4.82	8.06E-03	6.40	4.57
regulation of epithelial cell migration	4.82	8.08E-03	10.00	5.47
positive regulation of nucleobase-containing compound metabolic process	4.81	8.16E-03	5.19	4.60
regulation of catabolic process	4.79	8.31E-03	5.91	4.57
positive regulation of smooth muscle cell migration	4.76	8.58E-03	17.39	5.01
regulation of inflammatory response to antigenic stimulus	4.76	8.58E-03	17.39	4.38
calcium ion transport into cytosol	4.76	8.58E-03	17.39	4.60
positive regulation of calcium-mediated signaling	4.76	8.58E-03	17.39	4.02
peptide transport	4.73	8.85E-03	12.00	5.05
cellular response to glucocorticoid stimulus	4.73	8.85E-03	12.00	4.36
negative regulation of leukocyte differentiation	4.73	8.85E-03	12.00	4.04
negative regulation of G-protein coupled receptor protein signaling pathway	4.72	8.96E-03	13.89	4.85
regulation of release of sequestered calcium ion into cytosol	4.72	8.96E-03	13.89	6.34
amine metabolic process	4.66	9.45E-03	10.61	5.17
regulation of protein complex assembly	4.66	9.45E-03	7.84	4.46
cellular response to corticosteroid stimulus	4.63	9.73E-03	11.76	4.36
positive regulation of endothelial cell proliferation	4.63	9.73E-03	11.76	3.78
negative regulation of protein processing	4.63	9.73E-03	11.76	5.27
single-organism membrane organization	4.62	9.85E-03	7.29	4.24
regulation of actin filament-based process	4.61	9.92E-03	7.79	4.24
cellular response to alkaloid	4.60	1.00E-02	16.67	6.33
regulation of myeloid cell apoptotic process	4.60	1.00E-02	16.67	5.11

cytosolic calcium ion transport	4.60	1.00E-02	16.67	4.60
positive chemotaxis	4.60	1.00E-02	16.67	4.92
negative regulation of tumor necrosis factor production	4.60	1.00E-02	16.67	4.25
regulation of protein kinase B signaling	4.60	1.00E-02	9.64	4.72
regulation of osteoclast differentiation	4.60	1.01E-02	13.51	5.01
metabolic process	4.59	1.01E-02	4.12	4.76
ruffle organization	4.59	1.02E-02	23.08	3.73
positive regulation of tissue remodeling	4.59	1.02E-02	23.08	3.58
release of cytochrome c from mitochondria	4.59	1.02E-02	23.08	6.69
positive regulation of release of cytochrome c from mitochondria	4.59	1.02E-02	23.08	5.37
negative regulation of B cell apoptotic process	4.59	1.02E-02	23.08	4.76
I-kappaB kinase/NF-kappaB signaling	4.59	1.02E-02	23.08	4.75
regulation of acute inflammatory response to antigenic stimulus	4.59	1.02E-02	23.08	4.37
response to lipoprotein particle	4.59	1.02E-02	23.08	6.87
cellular response to interleukin-6	4.59	1.02E-02	23.08	4.10
positive regulation of cell adhesion mediated by integrin	4.59	1.02E-02	23.08	6.67
negative regulation of T cell apoptotic process	4.59	1.02E-02	23.08	6.20
regulation of interleukin-2 biosynthetic process	4.59	1.02E-02	23.08	5.34
protein destabilization	4.59	1.02E-02	23.08	5.13
positive regulation of intracellular protein transport	4.53	1.07E-02	9.52	5.02
monocarboxylic acid transport	4.50	1.11E-02	10.29	3.28
metal ion transport	4.50	1.11E-02	6.31	4.97
positive regulation of macromolecule biosynthetic process	4.49	1.12E-02	5.11	4.22
positive regulation of lipid transport	4.49	1.12E-02	13.16	4.57
regulation of lipid biosynthetic process	4.48	1.13E-02	8.82	5.28
anatomical structure development	4.48	1.14E-02	4.57	4.63
positive regulation of fat cell differentiation	4.46	1.16E-02	16.00	4.16
response to morphine	4.46	1.16E-02	16.00	5.88
actin filament bundle assembly	4.46	1.16E-02	16.00	5.04
actin filament bundle organization	4.46	1.16E-02	16.00	5.04
regulation of mitochondrial membrane potential	4.46	1.16E-02	16.00	5.52
regulation of sensory perception of pain	4.46	1.16E-02	16.00	4.32
regulation of sensory perception	4.46	1.16E-02	16.00	4.32
positive regulation of oxidoreductase activity	4.46	1.16E-02	16.00	4.49
negative regulation of gliogenesis	4.46	1.16E-02	16.00	3.87
defense response to Gram-negative bacterium	4.46	1.16E-02	16.00	6.02

iron ion homeostasis	4.45	1.17E-02	11.32	7.22
organ regeneration	4.43	1.19E-02	10.14	3.92
tryptophan catabolic process to kynurenine	4.42	1.21E-02	40.00	4.55
cellular response to isoquinoline alkaloid	4.42	1.21E-02	40.00	6.29
DNA dealkylation involved in DNA repair	4.42	1.21E-02	40.00	3.80
regulation of interleukin-1 alpha production	4.42	1.21E-02	40.00	4.73
positive regulation of cellular extravasation	4.42	1.21E-02	40.00	5.80
response to high density lipoprotein particle	4.42	1.21E-02	40.00	7.24
sarcoplasmic reticulum calcium ion transport	4.42	1.21E-02	40.00	3.39
regulation of macrophage apoptotic process	4.42	1.21E-02	40.00	6.71
positive regulation of homotypic cell-cell adhesion	4.42	1.21E-02	40.00	6.39
negative regulation of wound healing	4.42	1.21E-02	40.00	4.66
comma-shaped body morphogenesis	4.42	1.21E-02	40.00	3.33
regulation of response to interferon-gamma	4.42	1.21E-02	40.00	4.77
regulation of interferon-gamma-mediated signaling pathway	4.42	1.21E-02	40.00	4.77
positive regulation of granulocyte macrophage colony-stimulating factor production	4.42	1.21E-02	40.00	5.11
positive regulation of immature T cell proliferation	4.42	1.21E-02	40.00	6.91
negative regulation of toll-like receptor 4 signaling pathway	4.42	1.21E-02	40.00	4.56
positive regulation of T cell tolerance induction	4.42	1.21E-02	40.00	4.40
regulation of Fc receptor mediated stimulatory signaling pathway	4.42	1.21E-02	40.00	2.94
regulation of oligodendrocyte progenitor proliferation	4.42	1.21E-02	40.00	4.04
response to molecule of fungal origin	4.42	1.21E-02	40.00	3.58
neutrophil mediated cytotoxicity	4.42	1.21E-02	40.00	3.67
neutrophil mediated killing of symbiont cell	4.42	1.21E-02	40.00	3.67
cytoplasmic pattern recognition receptor signaling pathway	4.42	1.21E-02	40.00	5.03
glomerular capillary formation	4.42	1.21E-02	40.00	3.83
neutrophil degranulation	4.42	1.21E-02	40.00	2.73
regulation of interferon-beta biosynthetic process	4.42	1.21E-02	40.00	6.79
response to monosaccharide	4.41	1.21E-02	8.26	5.83
homeostasis of number of cells	4.40	1.23E-02	9.30	4.15
lymphocyte homeostasis	4.38	1.25E-02	12.82	4.27
negative regulation of protein catabolic process	4.38	1.25E-02	12.82	4.68
regulation of heat generation	4.37	1.26E-02	21.43	6.82
positive regulation of alpha-beta T cell proliferation	4.37	1.26E-02	21.43	4.67

purine nucleobase metabolic process	4.37	1.26E-02	21.43	4.67
transepithelial transport	4.37	1.26E-02	21.43	3.90
negative regulation of toll-like receptor signaling pathway	4.37	1.26E-02	21.43	4.14
regulation of cellular component size	4.36	1.28E-02	8.65	4.40
regulation of endothelial cell proliferation	4.35	1.29E-02	10.00	3.86
protein phosphorylation	4.35	1.29E-02	6.12	4.29
response to isoquinoline alkaloid	4.32	1.33E-02	15.38	5.88
blood vessel remodeling	4.32	1.33E-02	15.38	3.35
complement activation	4.32	1.33E-02	15.38	6.75
response to copper ion	4.32	1.33E-02	15.38	6.13
response to fluid shear stress	4.32	1.33E-02	15.38	4.03
vesicle-mediated transport	4.29	1.38E-02	5.73	4.99
positive regulation of protein processing	4.28	1.38E-02	9.86	4.71
negative regulation of protein transport	4.27	1.40E-02	9.09	4.69
divalent metal ion transport	4.25	1.42E-02	8.06	4.93
regulation of anatomical structure size	4.24	1.44E-02	7.41	4.53
response to carbohydrate	4.24	1.45E-02	7.69	5.60
negative regulation of transport	4.21	1.48E-02	6.46	4.62
response to ammonium ion	4.19	1.51E-02	10.71	5.00
regulation of tumor necrosis factor biosynthetic process	4.18	1.54E-02	20.00	3.93
positive regulation of lipid storage	4.18	1.54E-02	20.00	5.28
positive regulation of G-protein coupled receptor protein signaling pathway	4.18	1.54E-02	20.00	5.42
modulation by host of viral transcription	4.18	1.54E-02	20.00	5.67
modulation of transcription in other organism involved in symbiotic interaction	4.18	1.54E-02	20.00	5.67
modulation by host of symbiont transcription	4.18	1.54E-02	20.00	5.67
regulation of macrophage activation	4.18	1.54E-02	20.00	3.28
positive regulation of monooxygenase activity	4.18	1.54E-02	20.00	4.76
regulation of defense response to virus by host	4.18	1.54E-02	20.00	5.12
catabolic process	4.14	1.59E-02	4.91	4.90
endocytosis	4.13	1.60E-02	7.07	4.59
negative regulation of intracellular signal transduction	4.13	1.61E-02	6.86	5.19
regulation of protein serine/threonine kinase activity	4.12	1.62E-02	6.53	4.49
regulation of neuron differentiation	4.12	1.63E-02	6.17	4.05
negative regulation of ion transport	4.11	1.64E-02	10.53	4.97
response to acid	4.11	1.64E-02	6.27	5.42
signal transduction	4.09	1.68E-02	4.25	4.53
response to transition metal	4.08	1.68E-02	8.79	6.40

nanoparticle				
tissue homeostasis	4.08	1.68E-02	8.79	4.27
positive regulation of extrinsic apoptotic signaling pathway	4.08	1.70E-02	11.90	5.65
regulation of neurological system process	4.08	1.70E-02	11.90	4.40
cellular response to glucose stimulus	4.08	1.70E-02	11.90	6.38
negative regulation of proteolysis	4.08	1.70E-02	11.90	4.88
cellular response to cAMP	4.08	1.70E-02	11.90	4.21
negative regulation of neurogenesis	4.07	1.71E-02	9.46	4.30
regulation of monooxygenase activity	4.06	1.72E-02	14.29	4.45
lymphocyte differentiation	4.05	1.74E-02	7.81	4.19
indole-containing compound catabolic process	4.04	1.77E-02	33.33	4.55
indolalkylamine catabolic process	4.04	1.77E-02	33.33	4.55
tryptophan catabolic process	4.04	1.77E-02	33.33	4.55
kynurenine metabolic process	4.04	1.77E-02	33.33	4.55
cellular response to hydroperoxide	4.04	1.77E-02	33.33	6.29
regulation of protein activation cascade	4.04	1.77E-02	33.33	7.08
regulation of complement activation	4.04	1.77E-02	33.33	7.08
regulation of glial cell apoptotic process	4.04	1.77E-02	33.33	5.65
negative regulation of glial cell apoptotic process	4.04	1.77E-02	33.33	5.65
bradykinin catabolic process	4.04	1.77E-02	33.33	6.16
chondrocyte proliferation	4.04	1.77E-02	33.33	4.80
S-shaped body morphogenesis	4.04	1.77E-02	33.33	3.33
cellular response to interferon-alpha	4.04	1.77E-02	33.33	6.31
response to ozone	4.04	1.77E-02	33.33	6.32
ectopic germ cell programmed cell death	4.04	1.77E-02	33.33	6.32
regulation of calcidiol 1-monooxygenase activity	4.04	1.77E-02	33.33	5.29
regulation of T-helper 1 cell differentiation	4.04	1.77E-02	33.33	5.20
positive regulation of chemokine secretion	4.04	1.77E-02	33.33	5.48
negative regulation of lipopolysaccharide-mediated signaling pathway	4.04	1.77E-02	33.33	4.19
pulmonary valve morphogenesis	4.04	1.77E-02	33.33	4.15
regulation of T cell tolerance induction	4.04	1.77E-02	33.33	4.40
regulation of regulatory T cell differentiation	4.04	1.77E-02	33.33	4.40
rRNA transport	4.04	1.77E-02	33.33	4.81
positive regulation of defense response to virus by host	4.04	1.77E-02	33.33	4.98
positive regulation of necrotic cell death	4.04	1.77E-02	33.33	4.23
cellular amine metabolic process	4.03	1.77E-02	10.34	4.74
cellular biogenic amine metabolic process	4.03	1.77E-02	10.34	4.74

regulation of endothelial cell migration	4.03	1.77E-02	10.34	5.48
actin filament organization	4.00	1.83E-02	9.33	4.78
leukocyte degranulation	4.00	1.84E-02	18.75	2.77
thymic T cell selection	4.00	1.84E-02	18.75	3.90
regulation of macrophage derived foam cell differentiation	4.00	1.84E-02	18.75	3.90
regulation of cAMP-mediated signaling	4.00	1.84E-02	18.75	8.13
lymph node development	4.00	1.84E-02	18.75	4.79
negative regulation of response to biotic stimulus	4.00	1.84E-02	18.75	4.90
regulation of growth of symbiont in host	4.00	1.84E-02	18.75	3.86
negative regulation of growth of symbiont in host	4.00	1.84E-02	18.75	3.86
modulation of growth of symbiont involved in interaction with host	4.00	1.84E-02	18.75	3.86
negative regulation of growth of symbiont involved in interaction with host	4.00	1.84E-02	18.75	3.86
regulation of interleukin-17 production	4.00	1.84E-02	18.75	3.45
regulation of T-helper 1 type immune response	4.00	1.84E-02	18.75	5.73
tumor necrosis factor-mediated signaling pathway	4.00	1.84E-02	18.75	5.07
positive regulation of interleukin-12 production	4.00	1.84E-02	18.75	5.90
negative regulation of neuron differentiation	3.98	1.86E-02	11.63	4.41
regulation of protein catabolic process	3.96	1.91E-02	7.38	4.67
negative regulation of extrinsic apoptotic signaling pathway	3.96	1.91E-02	10.17	5.38
cellular modified amino acid metabolic process	3.95	1.92E-02	7.69	4.41
anatomical structure homeostasis	3.95	1.92E-02	7.69	4.86
signal transduction in absence of ligand	3.94	1.94E-02	13.79	5.83
extrinsic apoptotic signaling pathway in absence of ligand	3.94	1.94E-02	13.79	5.83
regulation of alpha-beta T cell differentiation	3.94	1.94E-02	13.79	5.05
RNA phosphodiester bond hydrolysis	3.94	1.94E-02	13.79	5.06
cellular response to alcohol	3.93	1.95E-02	9.21	6.62
calcium ion transport	3.92	1.99E-02	8.04	4.72
regulation of cellular catabolic process	3.92	1.99E-02	5.72	4.58
cellular response to hexose stimulus	3.89	2.04E-02	11.36	6.38
signaling	3.89	2.05E-02	6.49	4.59
single organism signaling	3.89	2.05E-02	6.49	4.59
proteolysis involved in cellular protein catabolic process	3.87	2.09E-02	6.64	5.88
regulation of neurogenesis	3.86	2.11E-02	5.76	4.09
divalent inorganic cation transport	3.86	2.11E-02	7.58	4.93
regulation of purine nucleotide metabolic process	3.83	2.17E-02	6.08	5.04

regulation of triglyceride metabolic process	3.83	2.18E-02	17.65	4.74
regulation of nitric-oxide synthase biosynthetic process	3.83	2.18E-02	17.65	4.02
negative regulation of myeloid cell apoptotic process	3.83	2.18E-02	17.65	5.68
modification by symbiont of host morphology or physiology	3.83	2.18E-02	17.65	3.77
regulation of alpha-beta T cell proliferation	3.83	2.18E-02	17.65	4.67
positive regulation of release of sequestered calcium ion into cytosol	3.83	2.18E-02	17.65	8.13
heart valve morphogenesis	3.83	2.18E-02	17.65	3.91
positive regulation of MAP kinase activity	3.81	2.21E-02	7.89	4.60
negative regulation of catabolic process	3.81	2.22E-02	8.97	4.99
positive regulation of epithelial cell migration	3.80	2.23E-02	11.11	4.65
response to amine	3.80	2.23E-02	11.11	4.46
cellular response to monosaccharide stimulus	3.80	2.23E-02	11.11	6.38
positive regulation of cytoskeleton organization	3.79	2.25E-02	8.33	4.51
negative regulation of cell development	3.79	2.25E-02	8.33	4.15
cell-cell adhesion	3.79	2.25E-02	8.33	4.24
response to cAMP	3.79	2.25E-02	8.33	4.22
activation of protein kinase activity	3.76	2.32E-02	7.46	4.54
regulation of nucleotide metabolic process	3.74	2.37E-02	6.02	5.04
protein metabolic process	3.74	2.37E-02	4.40	5.11
positive regulation of proteolysis	3.73	2.39E-02	9.68	4.34
regulation of cytoskeleton organization	3.73	2.41E-02	6.51	4.26
negative regulation of cellular metabolic process	3.72	2.41E-02	4.78	4.79
drug transmembrane transport	3.72	2.42E-02	28.57	3.36
response to UV-C	3.72	2.42E-02	28.57	3.44
mature B cell differentiation involved in immune response	3.72	2.42E-02	28.57	3.85
germinal center formation	3.72	2.42E-02	28.57	5.42
macrophage differentiation	3.72	2.42E-02	28.57	4.46
negative regulation of leukocyte chemotaxis	3.72	2.42E-02	28.57	5.65
positive regulation of T cell apoptotic process	3.72	2.42E-02	28.57	7.68
positive regulation of macrophage cytokine production	3.72	2.42E-02	28.57	4.39
positive regulation of triglyceride biosynthetic process	3.72	2.42E-02	28.57	3.66
auditory receptor cell differentiation	3.72	2.42E-02	28.57	3.34
positive regulation of tyrosine phosphorylation of Stat1 protein	3.72	2.42E-02	28.57	2.80
negative regulation of glutamate secretion	3.72	2.42E-02	28.57	6.03
regulation of vitamin D	3.72	2.42E-02	28.57	5.29

biosynthetic process				
positive regulation of T-helper cell differentiation	3.72	2.42E-02	28.57	5.20
regulation of chemokine secretion	3.72	2.42E-02	28.57	5.48
interleukin-1-mediated signaling pathway	3.72	2.42E-02	28.57	2.94
positive regulation of type I interferon-mediated signaling pathway	3.72	2.42E-02	28.57	6.96
response to yeast	3.72	2.42E-02	28.57	3.25
neutrophil mediated immunity	3.72	2.42E-02	28.57	3.67
disruption by host of symbiont cells	3.72	2.42E-02	28.57	3.67
killing by host of symbiont cells	3.72	2.42E-02	28.57	3.67
GTP biosynthetic process	3.72	2.42E-02	28.57	5.18
toxin transport	3.72	2.42E-02	28.57	4.13
hematopoietic or lymphoid organ development	3.72	2.43E-02	7.41	5.35
negative regulation of myeloid cell differentiation	3.72	2.43E-02	10.87	3.51
protein import into nucleus, translocation	3.67	2.54E-02	16.67	4.60
regulation of type 2 immune response	3.67	2.54E-02	16.67	4.63
negative regulation of interleukin-6 production	3.67	2.54E-02	16.67	4.07
positive regulation of natural killer cell mediated cytotoxicity	3.67	2.54E-02	16.67	4.07
leukotriene metabolic process	3.67	2.54E-02	16.67	3.42
regulation of cellular macromolecule biosynthetic process	3.67	2.55E-02	4.45	4.45
tissue remodeling	3.66	2.56E-02	9.52	3.57
steroid biosynthetic process	3.66	2.56E-02	9.52	5.25
response to axon injury	3.63	2.64E-02	10.64	4.72
positive regulation of lipid biosynthetic process	3.63	2.64E-02	10.64	5.05
regulation of B cell proliferation	3.63	2.64E-02	10.64	4.88
carbohydrate homeostasis	3.62	2.69E-02	7.63	5.14
glucose homeostasis	3.62	2.69E-02	7.63	5.14
protein autophosphorylation	3.62	2.69E-02	7.63	4.01
positive regulation of glucose transport	3.61	2.70E-02	12.50	5.97
regulation of B cell mediated immunity	3.61	2.70E-02	12.50	4.02
regulation of immunoglobulin mediated immune response	3.61	2.70E-02	12.50	4.02
regulation of interleukin-2 production	3.61	2.70E-02	12.50	5.70
regulation of RNA biosynthetic process	3.61	2.72E-02	4.48	4.59
regulation of RNA metabolic process	3.60	2.73E-02	4.47	4.62
extrinsic apoptotic signaling pathway	3.60	2.75E-02	9.38	6.19
cellular response to extracellular stimulus	3.58	2.80E-02	8.00	6.17
organic substance catabolic process	3.57	2.81E-02	4.84	4.97

negative regulation of apoptotic signaling pathway	3.57	2.82E-02	7.56	5.47
single-organism metabolic process	3.56	2.85E-02	4.31	4.61
positive regulation of cell projection organization	3.54	2.90E-02	7.19	4.16
positive regulation of protein serine/threonine kinase activity	3.53	2.92E-02	6.92	4.45
developmental programmed cell death	3.53	2.94E-02	15.79	5.25
positive regulation of collagen biosynthetic process	3.53	2.94E-02	15.79	3.97
T cell selection	3.53	2.94E-02	15.79	3.90
regulation of activated T cell proliferation	3.53	2.94E-02	15.79	4.75
positive regulation of GTPase activity	3.53	2.94E-02	7.92	4.40
negative regulation of behavior	3.51	2.99E-02	12.12	5.34
apoptotic mitochondrial changes	3.51	2.99E-02	12.12	6.62
response to glucose	3.47	3.10E-02	7.84	5.52
positive regulation of neurogenesis	3.47	3.10E-02	7.84	4.17
coagulation	3.47	3.10E-02	10.20	6.40
blood coagulation	3.47	3.10E-02	10.20	6.40
tryptophan metabolic process	3.46	3.15E-02	25.00	4.55
indolalkylamine metabolic process	3.46	3.15E-02	25.00	4.55
angiogenesis involved in wound healing	3.46	3.15E-02	25.00	3.59
negative regulation of necroptotic process	3.46	3.15E-02	25.00	6.84
positive regulation of macrophage activation	3.46	3.15E-02	25.00	3.49
retina vasculature morphogenesis in camera-type eye	3.46	3.15E-02	25.00	6.22
dichotomous subdivision of an epithelial terminal unit	3.46	3.15E-02	25.00	4.61
Fc receptor signaling pathway	3.46	3.15E-02	25.00	3.09
cellular response to iron ion	3.46	3.15E-02	25.00	5.88
central nervous system myelination	3.46	3.15E-02	25.00	3.67
axon ensheathment in central nervous system	3.46	3.15E-02	25.00	3.67
neuronal stem cell maintenance	3.46	3.15E-02	25.00	3.34
regulation of granulocyte macrophage colony-stimulating factor production	3.46	3.15E-02	25.00	5.11
positive regulation of interleukin-17 production	3.46	3.15E-02	25.00	3.36
regulation of vitamin metabolic process	3.46	3.15E-02	25.00	5.29
regulation of immature T cell proliferation	3.46	3.15E-02	25.00	6.91
response to stilbenoid	3.46	3.15E-02	25.00	6.27
regulation of cholesterol storage	3.46	3.15E-02	25.00	3.64
disruption of cells of other organism involved in symbiotic interaction	3.46	3.15E-02	25.00	3.67
killing of cells in other organism involved in symbiotic interaction	3.46	3.15E-02	25.00	3.67
urate metabolic process	3.46	3.15E-02	25.00	5.02

stress fiber assembly	3.46	3.15E-02	25.00	6.42
gamma-aminobutyric acid transport	3.46	3.15E-02	25.00	3.62
stress-activated protein kinase signaling cascade	3.41	3.30E-02	11.76	3.92
negative regulation of inflammatory response	3.40	3.34E-02	8.96	4.06
transition metal ion transport	3.40	3.34E-02	8.96	6.44
hemostasis	3.40	3.35E-02	10.00	6.40
negative regulation of angiogenesis	3.40	3.35E-02	10.00	4.51
positive regulation of cAMP metabolic process	3.40	3.35E-02	10.00	6.92
membrane protein proteolysis	3.39	3.37E-02	15.00	4.97
B cell homeostasis	3.39	3.37E-02	15.00	3.44
intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress	3.39	3.37E-02	15.00	5.12
negative regulation of cytokine biosynthetic process	3.39	3.37E-02	15.00	3.90
vesicle docking	3.39	3.37E-02	15.00	3.50
positive regulation of collagen metabolic process	3.39	3.37E-02	15.00	3.97
superoxide metabolic process	3.39	3.37E-02	15.00	5.58
negative regulation of glial cell differentiation	3.39	3.37E-02	15.00	3.57
regulation of GTPase activity	3.36	3.47E-02	6.52	4.46
aromatic compound catabolic process	3.33	3.59E-02	5.51	5.08
regulation of epithelial cell proliferation	3.32	3.60E-02	6.49	4.35
regulation of cell cycle G1/S phase transition	3.32	3.60E-02	9.80	4.10
regulation of G1/S transition of mitotic cell cycle	3.32	3.60E-02	9.80	4.10
cellular transition metal ion homeostasis	3.32	3.60E-02	9.80	6.97
regulation of cellular response to insulin stimulus	3.32	3.62E-02	11.43	5.52
regulation of GTP catabolic process	3.29	3.73E-02	6.45	4.46
regulation of nucleotide catabolic process	3.29	3.73E-02	6.28	4.31
regulation of purine nucleotide catabolic process	3.29	3.73E-02	6.28	4.31
cellular response to insulin stimulus	3.28	3.75E-02	8.05	5.01
ameboidal cell migration	3.28	3.75E-02	8.05	4.47
negative regulation of metabolic process	3.27	3.78E-02	4.61	4.80
pyrimidine nucleotide metabolic process	3.26	3.83E-02	14.29	4.87
regulation of oligodendrocyte differentiation	3.26	3.83E-02	14.29	3.66
appendage development	3.26	3.83E-02	14.29	3.65
limb development	3.26	3.83E-02	14.29	3.65
regulation of glucose transport	3.25	3.87E-02	9.62	6.19
cellular response to carbohydrate stimulus	3.25	3.87E-02	9.62	6.38
regulation of cell development	3.25	3.89E-02	5.25	4.04

cellular biogenic amine catabolic process	3.23	3.95E-02	22.22	4.55
amine catabolic process	3.23	3.95E-02	22.22	4.55
mature B cell differentiation	3.23	3.95E-02	22.22	3.85
necroptotic process	3.23	3.95E-02	22.22	6.24
regulation of platelet aggregation	3.23	3.95E-02	22.22	3.70
regulation of circadian sleep/wake cycle, non-REM sleep	3.23	3.95E-02	22.22	4.61
positive regulation of vascular permeability	3.23	3.95E-02	22.22	3.92
regulation of T cell chemotaxis	3.23	3.95E-02	22.22	8.23
positive regulation of T cell chemotaxis	3.23	3.95E-02	22.22	8.23
peptide hormone processing	3.23	3.95E-02	22.22	5.73
positive regulation of receptor recycling	3.23	3.95E-02	22.22	6.24
nucleobase catabolic process	3.23	3.95E-02	22.22	3.49
regulation of granulocyte differentiation	3.23	3.95E-02	22.22	3.92
hair cell differentiation	3.23	3.95E-02	22.22	3.34
iron ion transmembrane transport	3.23	3.95E-02	22.22	7.25
positive regulation of immunoglobulin production	3.23	3.95E-02	22.22	4.99
regulation of tyrosine phosphorylation of Stat1 protein	3.23	3.95E-02	22.22	2.80
positive regulation of astrocyte differentiation	3.23	3.95E-02	22.22	4.84
negative regulation of NF-kappaB import into nucleus	3.23	3.95E-02	22.22	3.62
positive regulation of glial cell proliferation	3.23	3.95E-02	22.22	4.05
cellular defense response	3.23	3.95E-02	22.22	3.92
regulation of inositol phosphate biosynthetic process	3.23	3.95E-02	22.22	3.91
positive regulation of T-helper 1 type immune response	3.23	3.95E-02	22.22	6.79
cellular response to follicle-stimulating hormone stimulus	3.23	3.95E-02	22.22	5.44
bicarbonate transport	3.23	3.95E-02	22.22	3.50
multi-organism intracellular transport	3.23	3.95E-02	22.22	7.19
multi-organism transport	3.23	3.95E-02	22.22	7.19
transport of virus	3.23	3.95E-02	22.22	7.19
intracellular transport of virus	3.23	3.95E-02	22.22	7.19
negative regulation of protein secretion	3.23	3.96E-02	11.11	3.96
ethanolamine-containing compound metabolic process	3.23	3.96E-02	11.11	4.83
phagocytosis	3.23	3.96E-02	11.11	4.50
iron ion transport	3.23	3.96E-02	11.11	6.51
regulation of membrane potential	3.22	3.99E-02	6.38	4.45
enzyme linked receptor protein signaling pathway	3.22	4.02E-02	5.84	4.25
memory	3.21	4.02E-02	8.57	5.51
fat cell differentiation	3.21	4.02E-02	8.57	5.07
tissue morphogenesis	3.19	4.11E-02	5.93	4.46

fatty acid derivative metabolic process	3.18	4.16E-02	9.43	3.81
icosanoid metabolic process	3.18	4.16E-02	9.43	3.81
regulation of nucleoside metabolic process	3.16	4.25E-02	6.16	4.31
DNA damage response, signal transduction by p53 class mediator	3.14	4.32E-02	13.64	5.33
negative regulation of myeloid leukocyte differentiation	3.14	4.32E-02	13.64	3.45
adrenal gland development	3.14	4.32E-02	13.64	5.21
protein polymerization	3.14	4.32E-02	10.81	4.05
insulin receptor signaling pathway	3.14	4.32E-02	10.81	4.97
response to hexose	3.14	4.33E-02	7.34	5.52
morphogenesis of an epithelium	3.13	4.38E-02	6.13	4.22
cellular response to hydrogen peroxide	3.11	4.45E-02	9.26	5.44
hormone transport	3.11	4.45E-02	9.26	4.68
response to activity	3.11	4.45E-02	9.26	6.48
epithelial cell development	3.10	4.51E-02	8.33	4.02
regulation of protein binding	3.08	4.60E-02	7.69	4.09
cellular response to growth factor stimulus	3.07	4.66E-02	6.07	4.96
protein oligomerization	3.06	4.69E-02	5.63	5.32
positive regulation of neuron apoptotic process	3.06	4.70E-02	10.53	4.90
tissue regeneration	3.06	4.70E-02	10.53	5.08
cellular response to reactive oxygen species	3.04	4.78E-02	8.22	5.95
humoral immune response	3.04	4.78E-02	8.22	5.89
transition metal ion homeostasis	3.04	4.78E-02	8.22	7.22
vagina development	3.03	4.82E-02	20.00	4.06
programmed necrotic cell death	3.03	4.82E-02	20.00	6.24
protein autoprocessing	3.03	4.82E-02	20.00	5.53
negative regulation of bone mineralization	3.03	4.82E-02	20.00	3.40
positive regulation of macrophage derived foam cell differentiation	3.03	4.82E-02	20.00	4.21
positive regulation of mast cell activation involved in immune response	3.03	4.82E-02	20.00	5.09
positive regulation of mast cell degranulation	3.03	4.82E-02	20.00	5.09
phagocytosis, recognition	3.03	4.82E-02	20.00	3.07
response to aluminum ion	3.03	4.82E-02	20.00	4.93
negative regulation of stem cell differentiation	3.03	4.82E-02	20.00	3.34
astrocyte differentiation	3.03	4.82E-02	20.00	2.79
negative regulation of oligodendrocyte differentiation	3.03	4.82E-02	20.00	3.67
inner ear receptor cell differentiation	3.03	4.82E-02	20.00	3.34
positive regulation of prostaglandin secretion	3.03	4.82E-02	20.00	6.32
killing of cells of other organism	3.03	4.82E-02	20.00	3.67
disruption of cells of other organism	3.03	4.82E-02	20.00	3.67

negative regulation of lipid storage	3.03	4.82E-02	20.00	3.07
nitric oxide metabolic process	3.03	4.82E-02	20.00	3.30
leukocyte tethering or rolling	3.03	4.82E-02	20.00	5.43
benzene-containing compound metabolic process	3.03	4.84E-02	13.04	3.87
positive regulation of glial cell differentiation	3.03	4.84E-02	13.04	4.43
anatomical structure formation involved in morphogenesis	3.00	4.99E-02	5.16	4.24

8.2.1.2 Significantly enriched biological processes by Panther

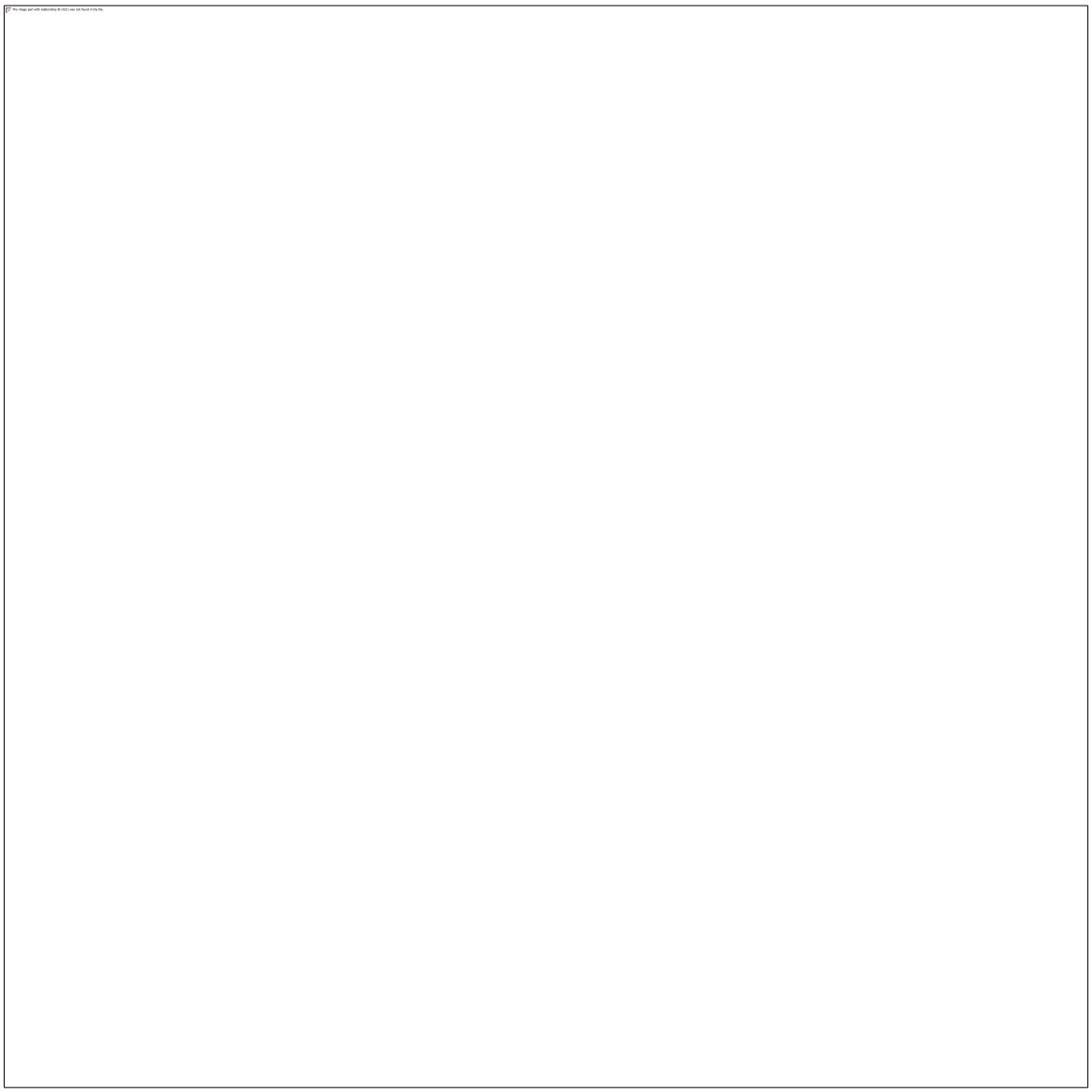

Figure 4.4.1a: Significantly enriched biological processes*. Using Panther software, gene ontology clustering was performed on the genes differentially expressed by ≥ 1.4-fold in the cultures treated with mAb O4compared to isotype cultures alone. The co-expression of functionally-related gene clusters was rated in order of significance*

8.2.1.3 *Table 4.4.1b: Enriched molecular functions by Partek Genomic Suite.* *Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the antibody in comparison to isotype treated cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	O4- vs IgM-score
cytokine receptor binding	37.07	7.96E-17	20.61	5.17
chemokine receptor binding	33.36	3.24E-15	44.74	5.82
cytokine activity	27.90	7.67E-13	20.00	5.42
binding	26.53	3.02E-12	4.58	4.75
receptor binding	25.06	1.30E-11	8.14	4.90
CCR chemokine receptor binding	20.85	8.79E-10	56.25	6.01
protein binding	20.44	1.33E-09	5.16	4.68
double-stranded RNA binding	18.52	9.09E-09	27.08	5.57
carbohydrate derivative binding	15.50	1.85E-07	6.18	4.99
G-protein coupled receptor binding	14.31	6.07E-07	12.15	5.45
CCR1 chemokine receptor binding	13.30	1.67E-06	100.00	6.34
growth factor receptor binding	12.74	2.92E-06	15.91	4.30
antigen binding	12.22	4.91E-06	17.65	5.65
MHC protein binding	12.20	5.01E-06	42.86	5.56
MHC class I protein binding	11.92	6.69E-06	55.56	5.64
CCR2 chemokine receptor binding	11.72	8.12E-06	80.00	5.90
CXCR3 chemokine receptor binding	11.72	8.12E-06	80.00	7.85
anion transmembrane transporter activity	10.74	2.16E-05	10.73	3.78
interleukin-1 receptor binding	10.17	3.84E-05	41.67	5.18
MHC class Ib protein binding	9.97	4.67E-05	100.00	6.01
TAP binding	9.97	4.67E-05	100.00	7.54
TAP1 binding	9.97	4.67E-05	100.00	7.54
TAP2 binding	9.97	4.67E-05	100.00	7.54
CXCR chemokine receptor binding	9.83	5.37E-05	57.14	7.85
oligopeptide transporter activity	9.83	5.37E-05	57.14	5.46
anion binding	9.77	5.69E-05	5.33	4.93
protein homodimerization activity	9.58	6.89E-05	7.34	4.80
nucleoside binding	9.58	6.93E-05	5.74	4.99
peptide binding	9.47	7.74E-05	10.49	5.19
purine nucleotide binding	9.46	7.78E-05	5.70	4.98
purine ribonucleoside triphosphate binding	9.41	8.16E-05	5.73	4.97
purine ribonucleotide binding	9.27	9.39E-05	5.68	4.93
purine ribonucleoside binding	9.26	9.50E-05	5.70	4.97
amide binding	9.24	9.73E-05	10.30	5.19
purine nucleoside binding	9.21	9.99E-05	5.70	4.97
ribonucleoside binding	9.19	1.02E-04	5.69	4.97
peptide transporter activity	9.17	1.04E-04	50.00	5.46
ribonucleotide binding	8.98	1.26E-04	5.63	4.93
amide transmembrane transporter activity	8.61	1.82E-04	44.44	4.99
cytokine receptor activity	8.60	1.85E-04	15.79	4.89

protein dimerization activity	8.59	1.86E-04	6.13	4.56
guanyl nucleotide binding	8.52	2.00E-04	8.24	5.03
guanyl ribonucleotide binding	8.52	2.00E-04	8.24	5.03
transporter activity	8.40	2.25E-04	6.05	4.22
active transmembrane transporter activity	8.30	2.48E-04	9.23	4.36
GTP binding	8.25	2.61E-04	8.27	5.14
organic anion transmembrane transporter activity	8.11	3.00E-04	11.76	3.41
secondary active transmembrane transporter activity	8.11	3.02E-04	11.11	3.83
transmembrane transporter activity	8.07	3.14E-04	6.35	4.18
single-stranded RNA binding	8.01	3.31E-04	22.22	5.32
adenylyltransferase activity	7.97	3.47E-04	27.78	6.25
substrate-specific transmembrane transporter activity	7.78	4.18E-04	6.42	4.21
carboxylic acid transmembrane transporter activity	7.55	5.26E-04	12.66	3.39
substrate-specific transporter activity	7.55	5.26E-04	6.14	4.18
carbohydrate binding	7.52	5.43E-04	8.65	4.29
organic acid transmembrane transporter activity	7.45	5.82E-04	12.50	3.39
identical protein binding	7.10	8.26E-04	5.88	4.91
amino acid transmembrane transporter activity	7.08	8.38E-04	14.29	3.43
CCR5 chemokine receptor binding	7.06	8.60E-04	50.00	5.67
oligopeptide transmembrane transporter activity	7.06	8.60E-04	50.00	5.73
basic amino acid transmembrane transporter activity	7.06	8.60E-04	50.00	3.02
chloride transmembrane transporter activity	6.85	1.06E-03	13.79	4.29
hydrolase activity	6.74	1.18E-03	5.00	5.14
phospholipase activator activity	6.53	1.46E-03	42.86	6.21
IgG binding	6.53	1.46E-03	42.86	3.53
glycosaminoglycan binding	6.50	1.51E-03	9.84	5.33
drug transporter activity	6.40	1.66E-03	26.67	3.54
hydrolase activity, acting on acid anhydrides	6.38	1.70E-03	6.27	5.25
inorganic anion transmembrane transporter activity	6.35	1.75E-03	11.69	4.20
cell adhesion molecule binding	6.22	1.98E-03	14.00	4.25
heparin binding	6.19	2.04E-03	10.64	5.68
immunoglobulin binding	6.14	2.15E-03	25.00	3.51
nucleotide binding	6.12	2.20E-03	4.96	4.84
nucleoside phosphate binding	6.12	2.20E-03	4.96	4.84
protein tyrosine kinase activity	6.11	2.21E-03	10.53	4.18
ion transmembrane transporter activity	6.08	2.28E-03	6.10	4.04
deaminase activity	5.90	2.74E-03	23.53	5.21
cysteine-type endopeptidase activity	5.85	2.89E-03	15.00	5.80
lipase activator activity	5.70	3.33E-03	33.33	6.21

protease binding	5.67	3.45E-03	12.73	5.81
small molecule binding	5.62	3.63E-03	4.79	4.85
double-stranded RNA adenosine deaminase activity	5.57	3.80E-03	66.67	5.56
CCR10 chemokine receptor binding	5.57	3.80E-03	66.67	6.75
lipoteichoic acid binding	5.57	3.80E-03	66.67	4.58
lipopeptide binding	5.57	3.80E-03	66.67	3.50
MHC class I receptor activity	5.57	3.80E-03	66.67	4.21
superoxide-generating NADPH oxidase activity	5.57	3.80E-03	66.67	4.12
arginine transmembrane transporter activity	5.57	3.80E-03	66.67	3.14
oligopeptide-transporting ATPase activity	5.57	3.80E-03	66.67	7.19
peptide-transporting ATPase activity	5.57	3.80E-03	66.67	7.19
catalytic activity	5.46	4.25E-03	4.30	4.88
antiporter activity	5.37	4.68E-03	16.13	3.90
transferase activity, transferring pentosyl groups	5.09	6.16E-03	15.15	5.58
drug transmembrane transporter activity	5.08	6.20E-03	27.27	3.17
voltage-gated chloride channel activity	5.08	6.20E-03	27.27	4.96
ion binding	4.93	7.21E-03	4.27	4.81
monocarboxylic acid transmembrane transporter activity	4.92	7.29E-03	18.18	3.42
molybdopterin cofactor binding	4.90	7.42E-03	50.00	3.93
IgE binding	4.90	7.42E-03	50.00	3.53
3'-5'-exodeoxyribonuclease activity	4.90	7.42E-03	50.00	7.66
CARD domain binding	4.90	7.42E-03	50.00	5.26
manganese ion transmembrane transporter activity	4.90	7.42E-03	50.00	5.31
gamma-aminobutyric acid:sodium symporter activity	4.90	7.42E-03	50.00	3.62
molecular_function	4.85	7.85E-03	3.77	4.73
hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in cyclic amidines	4.76	8.58E-03	17.39	4.25
transition metal ion transmembrane transporter activity	4.76	8.58E-03	17.39	5.67
symporter activity	4.75	8.69E-03	9.88	3.57
phosphotyrosine binding	4.59	1.02E-02	23.08	4.65
NAD+ ADP-ribosyltransferase activity	4.59	1.02E-02	23.08	5.55
protein complex binding	4.45	1.17E-02	5.55	4.60
iron ion transmembrane transporter activity	4.42	1.21E-02	40.00	6.04
gamma-aminobutyric acid transmembrane transporter activity	4.42	1.21E-02	40.00	3.62
voltage-gated anion channel activity	4.37	1.26E-02	21.43	4.96
transferase activity, transferring phosphorus-containing groups	4.23	1.45E-02	5.34	4.61

ATP binding	4.23	1.46E-02	4.99	4.90
non-membrane spanning protein tyrosine kinase activity	4.19	1.52E-02	14.81	4.29
phosphatidylinositol-3,4-bisphosphate binding	4.18	1.54E-02	20.00	3.61
protein kinase activity	4.10	1.66E-02	5.82	4.28
receptor antagonist activity	4.04	1.77E-02	33.33	7.26
immunoglobulin receptor activity	4.04	1.77E-02	33.33	3.09
semaphorin receptor activity	4.04	1.77E-02	33.33	4.18
metalloenzyme inhibitor activity	4.04	1.77E-02	33.33	3.77
metalloendopeptidase inhibitor activity	4.04	1.77E-02	33.33	3.77
adenyl nucleotide binding	4.01	1.82E-02	4.90	4.97
chemoattractant activity	4.00	1.84E-02	18.75	5.26
sulfur compound binding	3.95	1.92E-02	7.69	5.68
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen	3.94	1.94E-02	13.79	4.74
SNARE binding	3.89	2.04E-02	11.36	4.78
transmembrane receptor protein tyrosine kinase activity	3.89	2.04E-02	11.36	4.14
adenyl ribonucleotide binding	3.86	2.12E-02	4.88	4.90
UDP-N-acetylmuramate dehydrogenase activity	3.72	2.42E-02	28.57	3.93
low-density lipoprotein receptor activity	3.72	2.42E-02	28.57	5.65
proteasome binding	3.72	2.42E-02	28.57	4.67
bicarbonate transmembrane transporter activity	3.72	2.42E-02	28.57	3.50
sodium:amino acid symporter activity	3.72	2.42E-02	28.57	3.62
protein heterodimerization activity	3.70	2.47E-02	5.81	4.13
enzyme inhibitor activity	3.69	2.51E-02	6.33	4.56
calcium ion binding	3.68	2.53E-02	5.53	4.16
chloride channel activity	3.63	2.64E-02	10.64	4.53
ubiquitin protein ligase binding	3.62	2.69E-02	7.63	3.68
small conjugating protein ligase binding	3.62	2.69E-02	7.63	3.68
GTPase activity	3.62	2.69E-02	7.63	5.78
kinase activity	3.61	2.70E-02	5.30	4.27
protein self-association	3.61	2.70E-02	12.50	5.97
peptidase regulator activity	3.58	2.79E-02	6.74	4.96
beta-amyloid binding	3.53	2.94E-02	15.79	5.22
protein phosphorylated amino acid binding	3.53	2.94E-02	15.79	4.65
cysteine-type peptidase activity	3.52	2.95E-02	7.50	5.45
adenosine deaminase activity	3.46	3.15E-02	25.00	5.56
complement binding	3.46	3.15E-02	25.00	5.04
receptor inhibitor activity	3.46	3.15E-02	25.00	7.26
exodeoxyribonuclease activity, producing 5'-phosphomonoesters	3.46	3.15E-02	25.00	7.66

exodeoxyribonuclease activity	3.46	3.15E-02	25.00	7.66
platelet-derived growth factor receptor binding	3.46	3.15E-02	25.00	3.53
metalloenzyme regulator activity	3.46	3.15E-02	25.00	3.77
enzyme binding	3.44	3.20E-02	4.82	4.56
monooxygenase activity	3.42	3.26E-02	7.77	4.14
scavenger receptor activity	3.41	3.30E-02	11.76	5.94
extracellular ligand-gated ion channel activity	3.40	3.35E-02	10.00	3.61
epidermal growth factor receptor binding	3.39	3.37E-02	15.00	3.06
ADP binding	3.39	3.37E-02	15.00	6.62
exonuclease activity, active with either ribo- or deoxyribonucleic acids and producing 5'-phosphomonoesters	3.39	3.37E-02	15.00	7.10
threonine-type endopeptidase activity	3.39	3.37E-02	15.00	7.30
threonine-type peptidase activity	3.39	3.37E-02	15.00	7.30
anion channel activity	3.32	3.60E-02	9.80	4.53
phosphotransferase activity, alcohol group as acceptor	3.27	3.80E-02	5.26	4.28
oxidoreductase activity, acting on NAD(P)H, oxygen as acceptor	3.23	3.95E-02	22.22	4.12
N,N-dimethylaniline monooxygenase activity	3.23	3.95E-02	22.22	3.83
protein binding, bridging	3.18	4.16E-02	9.43	4.71
phosphoprotein binding	3.18	4.16E-02	9.43	4.09
transmembrane receptor protein kinase activity	3.18	4.16E-02	9.43	4.14
iron ion binding	3.16	4.26E-02	6.51	4.39
tumor necrosis factor receptor binding	3.14	4.32E-02	13.64	5.68
SNAP receptor activity	3.14	4.32E-02	13.64	6.18
syntaxin binding	3.14	4.32E-02	10.81	4.10
cytokine binding	3.11	4.45E-02	9.26	4.51
ribonuclease activity	3.11	4.45E-02	9.26	4.58
endopeptidase regulator activity	3.10	4.51E-02	6.67	5.15
BH domain binding	3.03	4.82E-02	20.00	3.50
D1 dopamine receptor binding	3.03	4.82E-02	20.00	3.96
nucleoside diphosphate kinase activity	3.03	4.82E-02	20.00	4.89
macrolide binding	3.03	4.82E-02	20.00	3.51
FK506 binding	3.03	4.82E-02	20.00	3.51
neurotrophin receptor binding	3.03	4.82E-02	20.00	3.25
insulin-like growth factor receptor binding	3.03	4.82E-02	20.00	5.12
cation:amino acid symporter activity	3.03	4.82E-02	20.00	3.62
pattern recognition receptor activity	3.03	4.82E-02	20.00	3.96
signaling pattern recognition receptor activity	3.03	4.82E-02	20.00	3.96
nucleotidyltransferase activity	3.03	4.83E-02	7.61	6.20
steroid hydroxylase activity	3.03	4.84E-02	13.04	5.45

8.2.1.4 *Table 4.4.1c: Significantly enriched cellular components by Partek Genomic Suite. Gene ontology clustering was performed on the genes differentially regulated by fold change or 1.4 or greater in the antibody in comparison to isotype treated cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	O4- vs. IgM-score
plasma membrane part	35.19	5.20E-16	8.38	4.41
extracellular region part	23.22	8.25E-11	6.39	5.06
external side of plasma membrane	22.19	2.31E-10	15.17	4.76
extracellular space	21.60	4.14E-10	8.30	4.99
side of membrane	19.71	2.75E-09	13.21	4.70
cell surface	17.99	1.54E-08	10.66	4.58
MHC protein complex	16.94	4.40E-08	29.73	5.31
cytoplasm	15.49	1.87E-07	5.33	4.73
receptor complex	13.15	1.95E-06	11.73	4.44
vesicle	12.09	5.60E-06	5.67	5.10
MHC class I protein complex	10.78	2.08E-05	28.00	5.63
intracellular	10.15	3.92E-05	6.86	5.06
NLRP1 inflammasome complex	9.97	4.67E-05	100.00	5.71
AIM2 inflammasome complex	9.97	4.67E-05	100.00	6.85
TAP complex	9.97	4.67E-05	100.00	7.54
membrane-bounded vesicle	9.96	4.75E-05	5.48	5.02
extracellular vesicular exosome	9.40	8.23E-05	5.76	5.14
extracellular organelle	9.38	8.45E-05	5.75	5.14
extracellular membrane-bounded organelle	9.38	8.45E-05	5.75	5.14
integral component of plasma membrane	9.38	8.46E-05	8.94	3.87
blood microparticle	8.93	1.33E-04	13.75	5.62
I-kappaB/NF-kappaB complex	8.61	1.82E-04	75.00	4.71
NLRP3 inflammasome complex	8.61	1.82E-04	75.00	6.85
symbiont-containing vacuole membrane	8.61	1.82E-04	75.00	6.44
late endosome	8.27	2.55E-04	12.79	5.26
endosome	7.91	3.66E-04	7.90	5.16
host cell part	7.72	4.42E-04	60.00	6.44
other organism part	7.72	4.42E-04	60.00	6.44
spermatoproteasome complex	7.72	4.42E-04	60.00	7.30
membrane	7.72	4.43E-04	4.41	4.42
integrin complex	7.43	5.92E-04	25.00	3.86
MHC class II protein complex	7.33	6.57E-04	33.33	4.75
lamellipodium	7.25	7.09E-04	12.20	3.70
vacuolar part	7.23	7.24E-04	9.32	5.13
extracellular region	7.06	8.59E-04	6.18	5.01
basal part of cell	7.06	8.60E-04	50.00	5.43
immunological synapse	6.96	9.47E-04	22.73	4.95
vacuolar membrane	6.92	9.92E-04	9.40	5.23
lysosomal membrane	6.67	1.27E-03	9.56	5.17
lysosome	6.56	1.42E-03	8.72	4.25

lytic vacuole	6.50	1.51E-03	8.67	4.25
cytosol	6.42	1.63E-03	5.62	4.59
vacuole	6.32	1.79E-03	8.25	4.46
stress fiber	6.26	1.92E-03	16.22	4.95
actin filament bundle	5.98	2.53E-03	15.38	4.95
perinuclear region of cytoplasm	5.81	3.00E-03	6.65	4.99
membrane region	5.78	3.09E-03	5.79	4.11
actomyosin	5.59	3.72E-03	14.29	4.95
MHC class I peptide loading complex	5.57	3.80E-03	66.67	6.94
plasma membrane region	5.41	4.46E-03	6.75	3.92
organelle membrane	5.11	6.05E-03	4.95	4.71
IPAF inflammasome complex	4.90	7.42E-03	50.00	6.60
ripoptosome	4.90	7.42E-03	50.00	4.31
NADPH oxidase complex	4.90	7.42E-03	50.00	4.12
external encapsulating structure part	4.90	7.42E-03	50.00	4.73
cytoplasmic vesicle	4.85	7.86E-03	5.66	5.04
cell	4.68	9.31E-03	6.96	4.37
microvillus membrane	4.59	1.02E-02	23.08	4.69
apical part of cell	4.58	1.02E-02	10.45	5.64
SNARE complex	4.46	1.16E-02	16.00	5.36
lipopolysaccharide receptor complex	4.42	1.21E-02	40.00	4.71
proteasome activator complex	4.42	1.21E-02	40.00	6.99
cell body	4.37	1.26E-02	6.23	4.11
cytoplasmic part	4.30	1.36E-02	4.13	4.72
membrane part	4.28	1.39E-02	4.09	4.47
chloride channel complex	4.19	1.52E-02	14.81	4.92
cell projection	4.11	1.63E-02	5.02	4.13
basolateral plasma membrane	4.05	1.74E-02	7.81	3.97
bounding membrane of organelle	4.01	1.82E-02	4.96	4.53
multivesicular body	4.00	1.84E-02	18.75	5.22
early endosome	3.96	1.90E-02	8.60	5.52
proteasome complex	3.94	1.94E-02	13.79	7.00
apical plasma membrane	3.71	2.46E-02	6.67	3.81
endosome membrane	3.63	2.66E-02	7.30	4.76
proteasome core complex	3.53	2.94E-02	15.79	7.30
recycling endosome	3.47	3.10E-02	10.20	5.28
cytoplasmic vesicle membrane	3.46	3.16E-02	6.83	4.85
late endosome membrane	3.41	3.30E-02	11.76	5.39
organelle envelope lumen	3.40	3.35E-02	10.00	4.88
organelle envelope	3.34	3.55E-02	8.14	4.23
envelope	3.34	3.55E-02	8.14	4.23
plasma membrane	3.28	3.76E-02	4.20	4.33
membrane raft	3.22	3.99E-02	6.38	4.61
endosomal part	3.14	4.35E-02	6.71	4.76
membrane-enclosed lumen	3.11	4.45E-02	5.98	4.70

endoplasmic reticulum lumen	3.11	4.45E-02	9.26	5.03
secretory granule membrane	3.06	4.70E-02	10.53	4.13
filopodium	3.06	4.70E-02	10.53	4.38
acrosomal membrane	3.03	4.82E-02	20.00	3.54
podosome	3.03	4.82E-02	20.00	5.98

8.2.2 Gene ontology clustering of cultures treated with serum alone

8.2.2.1 *Table 4.4.2a: Enriched biological processes by Partek Genomic Suite.* *Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the serum treated cultures in comparison to control cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	C+ vs C- score
response to external stimulus	75.67	1.38E-33	15.83	4.44
single-organism process	67.09	7.31E-30	8.17	4.17
response to external biotic stimulus	66.77	1.00E-29	19.81	4.36
response to biotic stimulus	63.36	3.05E-28	19.05	4.36
response to stress	62.87	4.95E-28	12.57	4.29
positive regulation of biological process	54.45	2.26E-24	10.09	4.25
response to organic substance	53.13	8.45E-24	12.02	4.31
single-organism cellular process	53.04	9.27E-24	8.4	4.22
defense response	52.92	1.04E-23	18.16	4.75
response to lipopolysaccharide	51.94	2.77E-23	24.42	4.53
response to molecule of bacterial origin	51.23	5.62E-23	23.79	4.52
immune system process	51.19	5.89E-23	15.34	4.48
response to cytokine	51.19	5.90E-23	20.83	4.72
positive regulation of response to stimulus	49.57	2.97E-22	13.84	4.16
positive regulation of cellular process	48.24	1.12E-21	10.16	4.25
cellular response to chemical stimulus	47.31	2.85E-21	12.86	4.48
response to lipid	44.77	3.59E-20	15.24	4.30
regulation of response to stimulus	41.36	1.09E-18	10.73	4.22
response to oxygen-containing compound	41.03	1.52E-18	12.78	4.32
regulation of cell communication	39.36	8.09E-18	11	4.18
regulation of signaling	38.72	1.53E-17	10.96	4.17
regulation of immune system process	38.18	2.63E-17	14.84	4.24
cellular response to organic substance	37.14	7.40E-17	12.84	4.47
response to wounding	36.67	1.19E-16	18.23	4.97
response to organic cyclic compound	36.48	1.44E-16	14.04	4.21
positive regulation of signal transduction	36.41	1.54E-16	13.79	4.11
multi-organism process	36.32	1.69E-16	15.66	4.21
regulation of multicellular organismal process	36.19	1.92E-16	10.89	4.21
positive regulation of cell communication	34.81	7.63E-16	13.33	4.13
positive regulation of signaling	34.81	7.63E-16	13.33	4.13
negative regulation of biological process	33.26	3.60E-15	9.39	4.13
single-organism developmental process	33.04	4.50E-15	9.06	4.32

developmental process	32.76	5.90E-15	8.9	4.32
positive regulation of intracellular signal transduction	32.5	7.66E-15	15.21	4.07
positive regulation of immune system process	32.32	9.17E-15	16.89	4.31
cell chemotaxis	32.17	1.07E-14	30.93	4.93
regulation of signal transduction	31.99	1.28E-14	10.83	4.14
immune response	31.3	2.54E-14	16.54	4.67
cellular response to cytokine stimulus	30.54	5.44E-14	19.01	4.76
negative regulation of cellular process	30.3	6.95E-14	9.44	4.10
response to other organism	30.03	9.12E-14	17.57	4.24
inflammatory response	29.26	1.96E-13	20.57	5.34
response to drug	29.22	2.04E-13	16.14	4.48
aging	28.54	4.04E-13	20.92	4.67
regulation of intracellular signal transduction	28.39	4.68E-13	12.31	4.09
taxis	28.31	5.09E-13	23.08	4.98
chemotaxis	28.31	5.09E-13	23.08	4.98
regulation of cell proliferation	28.15	5.96E-13	11.74	4.33
regulation of response to external stimulus	28.13	6.10E-13	16.08	4.28
regulation of developmental process	27.97	7.12E-13	10.74	4.28
response to virus	27.56	1.08E-12	24.26	4.22
cellular response to lipopolysaccharide	27.53	1.11E-12	25.62	4.66
positive regulation of transport	26.83	2.23E-12	14.5	4.30
cellular response to molecule of bacterial origin	26.59	2.83E-12	24.8	4.66
organ development	26.26	3.94E-12	11.58	4.26
leukocyte chemotaxis	26.08	4.72E-12	32.39	4.70
response to nitrogen compound	26.04	4.92E-12	12.85	4.12
response to endogenous stimulus	25.8	6.24E-12	11.27	4.12
positive regulation of developmental process	25.79	6.28E-12	13.11	4.09
regulation of response to stress	24.84	1.63E-11	13.38	4.22
anatomical structure development	24.74	1.79E-11	9.35	4.30
regulation of localization	24.7	1.87E-11	10.47	4.26
response to organonitrogen compound	24.58	2.12E-11	12.88	4.17
cellular response to biotic stimulus	24.23	3.00E-11	22.79	4.66
leukocyte migration	24.01	3.72E-11	25.47	4.57
response to alcohol	23.78	4.69E-11	15.79	4.64
response to stimulus	23.74	4.89E-11	7.49	4.35
response to hormone	23.69	5.14E-11	12.42	4.21
regulation of locomotion	23.68	5.21E-11	14.22	4.38
response to extracellular stimulus	23.65	5.35E-11	15.92	4.57
response to steroid hormone	23.49	6.30E-11	14.69	4.32
cellular response to oxygen-containing compound	23.39	6.93E-11	13.16	4.45

regulation of leukocyte migration	23.32	7.47E-11	27.59	4.13
response to abiotic stimulus	23.22	8.22E-11	11.8	4.38
regulation of cytokine production	22.94	1.09E-10	15.81	4.21
biological_process	22.84	1.21E-10	6.37	4.16
positive regulation of locomotion	22.6	1.53E-10	17.52	4.28
regulation of programmed cell death	22.33	2.01E-10	11.19	4.21
regulation of apoptotic process	22.23	2.21E-10	11.22	4.20
regulation of cell death	22.04	2.67E-10	10.95	4.27
response to interferon-gamma	22.01	2.77E-10	36.96	4.98
regulation of biological quality	21.95	2.93E-10	9.49	4.30
cellular response to lipid	21.86	3.21E-10	16.6	4.60
regulation of multicellular organismal development	21.57	4.29E-10	10.7	4.22
regulation of phosphorus metabolic process	21.27	5.80E-10	10.53	4.14
positive regulation of response to external stimulus	21.25	5.91E-10	20.39	4.28
regulation of cell motility	21.15	6.55E-10	14.1	4.34
response to nutrient levels	21.04	7.30E-10	15.54	4.67
positive regulation of leukocyte migration	20.92	8.21E-10	29.41	4.19
myeloid leukocyte migration	20.91	8.30E-10	32.73	4.48
regulation of phosphate metabolic process	20.76	9.63E-10	10.47	4.16
regulation of cell migration	20.69	1.03E-09	14.21	4.32
response to tumor necrosis factor	20.66	1.07E-09	26.51	4.93
positive regulation of cell motility	20.49	1.26E-09	17.19	4.23
cellular developmental process	20.21	1.67E-09	9.34	4.29
negative regulation of viral process	20.13	1.81E-09	35.56	3.97
positive regulation of cell migration	19.62	3.01E-09	16.97	4.17
positive regulation of cellular component movement	19.59	3.11E-09	16.67	4.23
response to interleukin-1	19.54	3.26E-09	27.4	5.28
regulation of transport	19.39	3.80E-09	10.67	4.21
regulation of cellular component movement	19.23	4.44E-09	13.27	4.32
regulation of I-kappaB kinase/NF-kappaB signaling	19.21	4.53E-09	20.29	3.78
regulation of chemotaxis	18.74	7.25E-09	23.23	4.44
single-organism transport	18.65	7.91E-09	9.27	4.22
cellular process	18.65	7.95E-09	6.63	4.20
defense response to other organism	18.61	8.27E-09	16.37	4.13
regulation of ERK1 and ERK2 cascade	18.23	1.21E-08	21.19	4.19
positive regulation of chemotaxis	18.05	1.45E-08	25.32	4.43
negative regulation of multi-organism process	17.96	1.59E-08	26.39	4.01
granulocyte chemotaxis	17.93	1.64E-08	35.9	5.01
regulation of anatomical structure morphogenesis	17.83	1.81E-08	12.27	4.34

positive regulation of behavior	17.72	2.01E-08	22.92	4.32
granulocyte migration	17.55	2.39E-08	35	5.01
establishment of localization	17.53	2.45E-08	8.65	4.25
regulation of molecular function	17.45	2.65E-08	9.39	4.22
cell differentiation	17.44	2.66E-08	9.89	4.23
regulation of defense response	17.37	2.85E-08	15.18	4.17
negative regulation of response to stimulus	17.17	3.48E-08	11.08	4.31
negative regulation of programmed cell death	17.13	3.65E-08	12.61	4.41
response to interferon-beta	16.83	4.89E-08	66.67	4.34
regulation of cellular localization	16.75	5.30E-08	11.33	4.13
regulation of leukocyte chemotaxis	16.57	6.39E-08	28.57	4.23
negative regulation of cell death	16.53	6.63E-08	12.11	4.50
regulation of lymphocyte migration	16.36	7.89E-08	42.31	4.78
biological adhesion	16.22	9.03E-08	12.39	4.19
cellular response to interleukin-1	16.04	1.08E-07	29.41	5.20
negative regulation of apoptotic process	16.01	1.11E-07	12.41	4.39
single-organism metabolic process	15.92	1.23E-07	8.19	4.14
positive regulation of I-kappaB kinase/NF-kappaB signaling	15.83	1.34E-07	20.75	3.77
positive regulation of cell differentiation	15.81	1.36E-07	12.44	4.07
cell migration	15.71	1.50E-07	12.63	4.51
positive regulation of multicellular organismal process	15.58	1.72E-07	12.35	4.07
immune effector process	15.52	1.82E-07	15.57	4.21
regulation of vasculature development	15.52	1.82E-07	17.65	4.45
cellular response to interferon-gamma	15.44	1.97E-07	39.29	5.05
metabolic process	15.43	1.99E-07	7.18	4.18
response to peptide	15.41	2.03E-07	13.94	4.29
cell adhesion	15.41	2.03E-07	12.18	4.16
positive regulation of metabolic process	15.38	2.09E-07	8.81	4.36
eosinophil chemotaxis	15.34	2.18E-07	70	5.48
regulation of behavior	15.33	2.21E-07	17.93	4.28
negative regulation of viral genome replication	15.29	2.29E-07	43.48	3.91
defense response to virus	15.28	2.30E-07	22.62	3.88
regulation of natural killer cell chemotaxis	15.22	2.46E-07	85.71	5.65
regulation of phosphorylation	14.99	3.08E-07	10.41	4.09
positive regulation of leukocyte chemotaxis	14.95	3.22E-07	29.17	4.25
transport	14.89	3.41E-07	8.43	4.26
positive regulation of ion transport	14.8	3.73E-07	19.01	4.64
response to progesterone	14.75	3.95E-07	30.95	3.93
regulation of angiogenesis	14.71	4.08E-07	17.86	4.57

regulation of catalytic activity	14.56	4.77E-07	9.56	4.31
regulation of immune effector process	14.55	4.78E-07	15.79	4.36
biological regulation	14.48	5.15E-07	6.75	4.26
eosinophil migration	14.38	5.69E-07	63.64	5.48
positive regulation of cytokine production	14.33	5.97E-07	16.67	4.02
regulation of protein metabolic process	14.31	6.09E-07	9.14	4.12
locomotion	14.17	6.99E-07	11.38	4.56
regulation of symbiosis, encompassing mutualism through parasitism	14.14	7.21E-07	21.11	4.06
cellular response to tumor necrosis factor	14.09	7.61E-07	24.24	5.04
positive regulation of molecular function	13.97	8.54E-07	10.28	4.00
response to nutrient	13.96	8.63E-07	15.68	4.73
cellular response to interferon-beta	13.88	9.34E-07	75	4.17
response to estrogen	13.88	9.42E-07	15.31	4.29
regulation of lymphocyte chemotaxis	13.78	1.03E-06	50	5.19
positive regulation of phosphorus metabolic process	13.78	1.04E-06	10.95	4.07
positive regulation of phosphate metabolic process	13.78	1.04E-06	10.95	4.07
response to organophosphorus	13.73	1.09E-06	18.49	4.19
cell communication	13.67	1.16E-06	12.89	3.87
positive regulation of protein metabolic process	13.66	1.16E-06	10.49	4.10
regulation of viral process	13.66	1.17E-06	23.53	3.97
negative regulation of cell proliferation	13.65	1.19E-06	12.11	4.08
positive regulation of ERK1 and ERK2 cascade	13.52	1.34E-06	21.18	3.99
regulation of metabolic process	13.49	1.39E-06	7.51	4.26
single-multicellular organism process	13.4	1.51E-06	8.46	4.10
positive regulation of cellular protein metabolic process	13.34	1.61E-06	10.75	4.09
regulation of primary metabolic process	13.34	1.61E-06	7.67	4.25
positive regulation of MAPK cascade	13.31	1.67E-06	13.93	3.86
wound healing	13.27	1.72E-06	21.79	4.20
response to mechanical stimulus	13.25	1.76E-06	16.56	4.40
response to ethanol	13.21	1.84E-06	17.42	5.27
regulation of cellular metabolic process	13.21	1.84E-06	7.66	4.24
organonitrogen compound metabolic process	13.17	1.90E-06	9.6	4.44
response to decreased oxygen levels	13.13	1.99E-06	14.04	4.27
small molecule metabolic process	13.06	2.13E-06	9.07	4.19
response to peptide hormone	13.04	2.18E-06	13.38	4.31
cellular response to organic cyclic compound	12.95	2.37E-06	14.16	4.77

multicellular organismal process	12.91	2.46E-06	8.36	4.08
regulation of cellular component organization	12.87	2.59E-06	9.18	4.19
lymphocyte chemotaxis	12.83	2.67E-06	53.85	5.53
cellular component movement	12.7	3.06E-06	10.37	4.24
regulation of multi-organism process	12.65	3.20E-06	15.66	3.98
response to hypoxia	12.57	3.46E-06	13.91	4.20
regulation of cell differentiation	12.56	3.50E-06	9.49	4.22
cell motility	12.55	3.53E-06	11.36	4.51
cellular response to lipoprotein particle stimulus	12.52	3.66E-06	83.33	5.60
response to ionizing radiation	12.49	3.77E-06	18.35	5.15
cellular response to external stimulus	12.41	4.09E-06	15.82	4.11
positive chemotaxis	12.4	4.14E-06	37.5	4.71
negative regulation of cell communication	12.39	4.15E-06	10.5	4.24
cell proliferation	12.32	4.48E-06	12.1	3.96
positive regulation of calcium ion transport	12.3	4.53E-06	25.49	4.97
response to oxygen levels	12.24	4.83E-06	13.11	4.22
regulation of biological process	12.15	5.27E-06	6.7	4.26
positive regulation of cell proliferation	12.05	5.87E-06	10.63	4.30
secretion	12.03	5.99E-06	12.5	4.17
response to vitamin	12	6.13E-06	19.15	4.71
regulation of innate immune response	11.85	7.16E-06	18.95	4.41
response to radiation	11.82	7.32E-06	12.87	4.57
response to purine-containing compound	11.82	7.37E-06	16.54	4.19
negative regulation of signaling	11.8	7.52E-06	10.36	4.21
positive regulation of phosphorylation	11.77	7.72E-06	10.82	3.89
regulation of immune response	11.77	7.72E-06	12.23	4.24
neutrophil migration	11.74	8.00E-06	31.25	4.67
neutrophil chemotaxis	11.74	8.00E-06	31.25	4.67
regulation of viral genome replication	11.74	8.00E-06	31.25	3.91
negative regulation of viral transcription	11.71	8.20E-06	40	4.49
cellular amide metabolic process	11.69	8.34E-06	18.75	4.65
regulation of secretion	11.63	8.93E-06	11.45	3.74
response to ketone	11.57	9.44E-06	15.86	4.09
innate immune response	11.57	9.44E-06	15.86	4.55
positive regulation of protein modification process	11.54	9.74E-06	10.73	4.06
regulation of protein modification process	11.52	9.93E-06	9.69	4.03
cellular response to nitrogen compound	11.51	9.99E-06	12.37	4.27
NIK/NF-kappaB signaling	11.41	1.11E-05	100	4.26
positive regulation of catalytic activity	11.32	1.21E-05	10.32	4.06

lipopolysaccharide-mediated signaling pathway	11.28	1.26E-05	38.1	4.50
positive regulation of cellular metabolic process	11.27	1.28E-05	8.38	4.27
anion transport	11.27	1.28E-05	12.89	4.13
cellular response to organonitrogen compound	11.26	1.28E-05	12.54	4.32
regulation of MAPK cascade	11.26	1.29E-05	11.6	4.00
monocyte chemotaxis	11.23	1.32E-05	54.55	3.85
organic acid biosynthetic process	11.22	1.34E-05	14.21	4.39
carboxylic acid biosynthetic process	11.22	1.34E-05	14.21	4.39
leukocyte cell-cell adhesion	11.13	1.46E-05	29.41	4.42
regulation of tumor necrosis factor production	11.03	1.62E-05	21.54	4.34
response to inorganic substance	11.03	1.63E-05	11.28	5.09
regulation of protein phosphorylation	10.96	1.73E-05	10.08	4.06
negative regulation of neuron apoptotic process	10.96	1.73E-05	17.82	4.15
regulation of cAMP metabolic process	10.94	1.77E-05	20.27	4.90
regulation of ion transport	10.88	1.89E-05	12.32	4.55
organic substance metabolic process	10.88	1.89E-05	6.97	4.16
regulation of cellular protein metabolic process	10.85	1.95E-05	9.04	4.08
regulation of cellular process	10.83	1.98E-05	6.69	4.26
positive regulation of programmed cell death	10.67	2.31E-05	12.36	3.88
cellular response to stimulus	10.66	2.34E-05	7.09	4.37
regulation of myeloid leukocyte differentiation	10.66	2.34E-05	20.9	4.23
regeneration	10.61	2.46E-05	16.26	4.29
positive regulation of macromolecule metabolic process	10.61	2.47E-05	8.38	4.28
antigen processing and presentation	10.61	2.47E-05	19.74	4.45
positive regulation of angiogenesis	10.6	2.49E-05	18.82	4.74
ensheathment of neurons	10.59	2.51E-05	22.03	5.04
axon ensheathment	10.59	2.51E-05	22.03	5.04
response to interferon-alpha	10.59	2.52E-05	50	4.46
positive regulation of gliogenesis	10.58	2.55E-05	27.78	4.01
regulation of cell adhesion	10.56	2.59E-05	13.46	4.32
central nervous system myelination	10.38	3.10E-05	62.5	4.70
axon ensheathment in central nervous system	10.38	3.10E-05	62.5	4.70
rhythmic process	10.37	3.12E-05	13.57	3.87
response to cAMP	10.35	3.21E-05	17.71	4.32
negative regulation of signal transduction	10.33	3.27E-05	10.15	4.17
regulation of cell shape	10.31	3.32E-05	20.29	4.78
response to glucocorticoid	10.29	3.39E-05	14.04	4.89
cellular response to endogenous	10.27	3.45E-05	10.25	4.22

stimulus				
negative regulation of neuron death	10.26	3.51E-05	16.38	4.29
regulation of gliogenesis	10.22	3.65E-05	21.31	3.98
regulation of neuron apoptotic process	10.2	3.71E-05	14.97	4.17
regulation of cellular response to stress	10.19	3.75E-05	12.4	4.36
regulation of mononuclear cell proliferation	10.16	3.86E-05	15.33	4.38
positive regulation of protein phosphorylation	10.16	3.87E-05	10.89	4.07
intracellular signal transduction	10.1	4.10E-05	9.25	4.35
positive regulation of apoptotic process	10.09	4.13E-05	12.18	3.91
gland development	10.07	4.23E-05	17.35	3.66
positive regulation of lipid transport	10.06	4.27E-05	26.32	3.47
positive regulation of tumor necrosis factor production	10.03	4.42E-05	29.03	4.02
chronic inflammatory response	10.02	4.45E-05	46.15	5.28
response to lipoprotein particle	10.02	4.45E-05	46.15	5.34
regulation of purine nucleotide metabolic process	10	4.53E-05	11.82	4.51
cytokine-mediated signaling pathway	9.91	4.97E-05	15.5	4.75
cellular response to stress	9.89	5.04E-05	9.46	4.16
positive regulation of natural killer cell chemotaxis	9.84	5.31E-05	80	5.94
membrane to membrane docking	9.84	5.31E-05	80	5.08
ISG15-protein conjugation	9.84	5.31E-05	80	4.55
antigen processing and presentation of endogenous peptide antigen	9.84	5.31E-05	80	4.45
response to metal ion	9.84	5.35E-05	12.35	5.23
one-carbon metabolic process	9.82	5.43E-05	32	3.49
regulation of granulocyte chemotaxis	9.82	5.43E-05	32	3.73
cellular modified amino acid metabolic process	9.8	5.56E-05	15.38	4.44
regulation of nucleotide metabolic process	9.79	5.58E-05	11.71	4.51
regulation of calcium ion transport	9.78	5.68E-05	15.83	4.59
positive regulation of defense response	9.78	5.68E-05	15.83	4.47
response to growth hormone	9.76	5.77E-05	36.84	4.54
negative regulation of developmental process	9.76	5.79E-05	10.39	3.94
regulation of leukocyte proliferation	9.73	5.96E-05	14.89	4.38
cell death	9.68	6.27E-05	11.38	4.54
positive regulation of immune effector process	9.66	6.38E-05	15.7	4.45
response to bacterium	9.65	6.42E-05	14.11	4.65
regulation of mononuclear cell migration	9.62	6.64E-05	55.56	4.87
positive regulation of antigen processing and presentation	9.62	6.64E-05	55.56	3.39

positive regulation of transferase activity	9.59	6.81E-05	12.03	4.10
positive regulation of cell death	9.59	6.85E-05	11.59	3.88
myelination	9.59	6.85E-05	21.43	5.18
acute inflammatory response	9.59	6.85E-05	21.43	5.43
regulation of cytokine-mediated signaling pathway	9.58	6.89E-05	25	4.65
response to corticosteroid	9.55	7.09E-05	13.44	4.89
positive regulation of neurogenesis	9.55	7.14E-05	16.67	4.06
positive regulation of secretion	9.54	7.16E-05	12.73	3.85
peptide metabolic process	9.52	7.37E-05	20	4.96
purine nucleobase metabolic process	9.51	7.41E-05	42.86	4.42
drug metabolic process	9.5	7.45E-05	30.77	3.37
regulation of leukocyte differentiation	9.48	7.62E-05	14.29	4.19
cellular response to peptide	9.47	7.73E-05	15.04	4.33
regulation of inflammatory response	9.46	7.77E-05	13.94	4.23
positive regulation of immune response	9.43	8.05E-05	12.86	4.39
cellular component organization	9.42	8.14E-05	7.51	4.22
positive regulation of leukocyte differentiation	9.39	8.37E-05	17.86	4.41
response to protozoan	9.38	8.44E-05	35	3.89
regulation of astrocyte differentiation	9.38	8.44E-05	35	3.69
regulation of cyclic nucleotide metabolic process	9.32	8.93E-05	17.02	4.75
negative regulation of molecular function	9.3	9.10E-05	9.94	4.21
response to temperature stimulus	9.3	9.16E-05	15.79	4.63
regulation of transferase activity	9.24	9.66E-05	10.48	4.14
death	9.23	9.80E-05	11.14	4.54
ion transport	9.22	9.86E-05	9.41	4.25
regulation of neuron death	9.18	1.03E-04	13.69	4.28
homeostatic process	9.16	1.05E-04	9.15	4.54
regulation of lymphocyte proliferation	9.15	1.06E-04	14.71	4.41
response to estradiol	9.1	1.12E-04	15.08	4.46
regulation of leukocyte apoptotic process	9.05	1.17E-04	20.34	4.80
lymphocyte migration	9.03	1.20E-04	33.33	5.53
developmental growth	9.02	1.22E-04	14.19	4.25
regulation of metal ion transport	9.01	1.22E-04	13.26	4.65
primary metabolic process	9.01	1.22E-04	6.88	4.17
positive regulation of myeloid leukocyte differentiation	8.98	1.26E-04	25.71	4.37
negative regulation of cellular metabolic process	8.98	1.26E-04	8.52	4.16
negative regulation by host of viral transcription	8.98	1.26E-04	50	3.98
regulation of antigen processing and presentation	8.98	1.26E-04	50	3.39
positive regulation of cellular	8.97	1.27E-04	10.13	4.23

component organization				
regulation of establishment of protein localization	8.97	1.27E-04	11.5	3.94
small molecule biosynthetic process	8.96	1.29E-04	11.79	4.37
regulation of kinase activity	8.94	1.32E-04	10.5	4.12
apoptotic signaling pathway	8.91	1.35E-04	13.45	4.56
regulation of interleukin-6 production	8.87	1.40E-04	18.84	3.92
negative regulation of metabolic process	8.87	1.40E-04	8.35	4.19
negative regulation of locomotion	8.82	1.47E-04	14	5.02
positive regulation of kinase activity	8.82	1.48E-04	11.86	4.07
cellular metabolic process	8.8	1.50E-04	6.87	4.10
positive regulation of macrophage chemotaxis	8.79	1.52E-04	66.67	4.67
response to low-density lipoprotein particle	8.79	1.52E-04	66.67	4.63
antigen processing and presentation of endogenous antigen	8.79	1.52E-04	66.67	4.45
ovulation cycle process	8.71	1.65E-04	16.85	3.85
adrenal gland development	8.69	1.68E-04	31.82	3.19
regulation of growth	8.68	1.70E-04	10.55	3.81
negative regulation of response to external stimulus	8.68	1.70E-04	14.62	4.55
protein kinase B signaling	8.65	1.75E-04	27.59	4.29
protein trimerization	8.65	1.75E-04	27.59	3.94
regulation of viral transcription	8.65	1.75E-04	27.59	4.49
carboxylic acid transport	8.65	1.76E-04	14.18	3.99
defense response to protozoan	8.63	1.79E-04	37.5	3.93
cellular response to extracellular stimulus	8.57	1.89E-04	16	4.07
regulation of nervous system development	8.55	1.93E-04	10.24	4.39
negative regulation of lymphocyte chemotaxis	8.55	1.93E-04	100	4.93
negative regulation of lymphocyte migration	8.55	1.93E-04	100	4.93
response to type I interferon	8.55	1.93E-04	100	5.76
cellular response to low-density lipoprotein particle stimulus	8.55	1.93E-04	100	4.83
antigen processing and presentation of endogenous peptide antigen via MHC class I	8.55	1.93E-04	100	4.72
organic acid transport	8.55	1.94E-04	14.08	3.99
regulation of response to cytokine stimulus	8.52	1.99E-04	22.22	4.65
regulation of neurogenesis	8.52	2.00E-04	10.47	4.38
regulation of cell activation	8.49	2.05E-04	11.35	4.29
chemical homeostasis	8.46	2.11E-04	9.73	4.52
reproductive process	8.46	2.11E-04	9.08	4.10
organic acid metabolic process	8.46	2.12E-04	9.41	4.28
programmed cell death	8.45	2.14E-04	11.19	4.55
regulation of intracellular	8.4	2.24E-04	11.74	4.59

transport				
regulation of lipid biosynthetic process	8.34	2.39E-04	15.69	3.63
negative regulation of phosphorus metabolic process	8.26	2.58E-04	11.81	4.24
negative regulation of phosphate metabolic process	8.26	2.58E-04	11.81	4.24
organonitrogen compound biosynthetic process	8.26	2.58E-04	10.84	4.20
integrin-mediated signaling pathway	8.25	2.61E-04	20	4.17
single-organism biosynthetic process	8.25	2.62E-04	9.16	4.16
regulation of cAMP biosynthetic process	8.24	2.63E-04	18.75	4.51
response to chemical	8.23	2.65E-04	7.1	4.42
regulation of protein kinase B signaling	8.23	2.67E-04	16.87	4.15
regulation of protein kinase activity	8.2	2.76E-04	10.4	4.05
regulation of cell morphogenesis	8.19	2.77E-04	11.76	4.67
cellular component organization or biogenesis	8.14	2.90E-04	7.34	4.22
regulation of lipid metabolic process	8.14	2.91E-04	12.78	3.74
glial cell differentiation	8.14	2.91E-04	21.28	4.74
positive regulation of protein kinase activity	8.05	3.18E-04	11.67	3.95
carboxylic acid metabolic process	8.01	3.31E-04	9.44	4.25
cellular amino acid biosynthetic process	8.01	3.33E-04	17.33	4.49
positive regulation of NF-kappaB transcription factor activity	8.01	3.33E-04	17.33	3.86
epithelial cell differentiation	8	3.34E-04	12.2	4.40
regulation of adaptive immune response	8	3.36E-04	15.24	4.11
lung development	7.97	3.45E-04	15.79	4.44
organic anion transport	7.95	3.53E-04	12.37	4.10
negative regulation of adenylate cyclase activity	7.93	3.61E-04	41.67	4.90
positive regulation of toll-like receptor signaling pathway	7.93	3.61E-04	41.67	4.12
response to fatty acid	7.92	3.63E-04	19.3	3.45
positive regulation of protein kinase B signaling	7.92	3.63E-04	19.3	4.20
regulation of nucleocytoplasmic transport	7.91	3.68E-04	14.17	4.04
acute-phase response	7.91	3.68E-04	25	5.40
negative regulation of cyclic nucleotide biosynthetic process	7.89	3.75E-04	33.33	4.65
negative regulation of cAMP biosynthetic process	7.89	3.75E-04	33.33	4.65
growth	7.86	3.85E-04	11.37	4.11
regulation of T cell proliferation	7.86	3.87E-04	15.63	4.34
endocytosis	7.82	4.00E-04	12.5	4.15
positive regulation of phagocytosis	7.81	4.06E-04	28	4.76
regulation of multicellular organism growth	7.76	4.24E-04	18.97	4.03

regulation of JAK-STAT cascade	7.76	4.24E-04	18.97	3.80
MAPK cascade	7.74	4.34E-04	15.46	4.27
regulation of protein serine/threonine kinase activity	7.72	4.45E-04	11.43	3.91
apoptotic mitochondrial changes	7.68	4.62E-04	24.24	5.21
regulation of wound healing	7.66	4.72E-04	17.65	4.07
negative regulation of cell adhesion	7.66	4.72E-04	17.65	4.73
transmembrane receptor protein tyrosine kinase signaling pathway	7.62	4.90E-04	12.57	4.09
intrinsic apoptotic signaling pathway in response to DNA damage	7.61	4.94E-04	20	4.53
response to gamma radiation	7.61	4.94E-04	20	5.98
cellular chemical homeostasis	7.59	5.06E-04	10.56	4.40
gamma-aminobutyric acid signaling pathway	7.56	5.21E-04	31.58	5.30
positive regulation of lymphocyte migration	7.56	5.21E-04	31.58	5.09
negative regulation of cyclic nucleotide metabolic process	7.56	5.21E-04	31.58	4.65
negative regulation of cAMP metabolic process	7.56	5.21E-04	31.58	4.65
temperature homeostasis	7.55	5.28E-04	26.92	4.35
regulation of NF-kappaB import into nucleus	7.55	5.28E-04	26.92	3.65
regulation of toll-like receptor signaling pathway	7.55	5.28E-04	26.92	4.27
regulation of macromolecule metabolic process	7.54	5.29E-04	7.12	4.23
multicellular organismal homeostasis	7.5	5.51E-04	21.43	4.54
negative regulation of cyclase activity	7.49	5.58E-04	38.46	4.90
positive regulation of mononuclear cell proliferation	7.49	5.59E-04	15.73	4.64
regulation of cyclic nucleotide biosynthetic process	7.49	5.61E-04	16.46	4.35
regulation of endocrine process	7.46	5.74E-04	23.53	3.25
multicellular organismal reproductive process	7.46	5.74E-04	10.11	3.98
positive regulation of inflammatory response	7.46	5.75E-04	18.33	4.69
enzyme linked receptor protein signaling pathway	7.44	5.85E-04	10.95	3.97
regulation of body fluid levels	7.44	5.85E-04	13.29	3.83
cellular response to peptide hormone stimulus	7.44	5.88E-04	14.05	4.34
cell-cell signaling	7.43	5.94E-04	11.94	3.83
positive regulation of adaptive immune response	7.39	6.20E-04	17.14	4.29
regulation of protein transport	7.35	6.39E-04	11.03	3.96
regulation of macrophage chemotaxis	7.35	6.46E-04	50	4.67
response to lipoteichoic acid	7.35	6.46E-04	50	4.76
cellular response to lipoteichoic acid	7.35	6.46E-04	50	4.76
regulation of endothelial cell chemotaxis	7.35	6.46E-04	50	4.50

regulation of cellular senescence	7.35	6.46E-04	50	3.55
positive regulation of intracellular transport	7.34	6.47E-04	13.93	4.75
digestive tract development	7.32	6.62E-04	20.93	3.92
regulation of leukocyte mediated cytotoxicity	7.32	6.65E-04	18.03	4.39
behavior	7.31	6.72E-04	10.11	4.24
secretion by tissue	7.29	6.85E-04	19.23	3.45
oxoacid metabolic process	7.25	7.08E-04	9.09	4.26
response to ATP	7.25	7.09E-04	30	4.15
negative regulation of purine nucleotide biosynthetic process	7.25	7.09E-04	30	4.65
negative regulation of nucleotide biosynthetic process	7.25	7.09E-04	30	4.65
negative regulation of amine transport	7.25	7.09E-04	30	4.60
toll-like receptor signaling pathway	7.25	7.09E-04	30	4.63
positive regulation of growth	7.25	7.09E-04	13.43	3.82
positive regulation of sequence-specific DNA binding transcription factor activity	7.25	7.09E-04	13.43	3.80
positive regulation of protein transport	7.22	7.33E-04	12.74	3.91
regulation of dendritic cell antigen processing and presentation	7.21	7.39E-04	75	3.12
positive regulation of dendritic cell antigen processing and presentation	7.21	7.39E-04	75	3.12
positive regulation of RIG-I signaling pathway	7.21	7.39E-04	75	2.90
chronic inflammatory response to antigenic stimulus	7.21	7.39E-04	75	5.40
protection from natural killer cell mediated cytotoxicity	7.21	7.39E-04	75	4.19
regulation of cellular response to growth factor stimulus	7.2	7.48E-04	14.71	3.83
regulation of lipid transport	7.17	7.67E-04	17.74	3.85
myeloid leukocyte activation	7.17	7.67E-04	17.74	4.14
negative regulation of cell migration	7.16	7.81E-04	13.71	5.02
regulation of protein localization	7.15	7.86E-04	10.2	3.96
positive regulation of leukocyte proliferation	7.15	7.86E-04	15.22	4.64
positive regulation of cytokine secretion	7.13	8.01E-04	18.87	3.65
learning or memory	7.1	8.24E-04	12.93	3.94
purinergic receptor signaling pathway	7.1	8.27E-04	35.71	4.35
glial cell migration	7.1	8.27E-04	35.71	3.95
negative regulation of lyase activity	7.1	8.27E-04	35.71	4.90
positive regulation of epithelial cell proliferation	7.09	8.30E-04	14.56	4.34
regulation of myeloid cell differentiation	7.07	8.52E-04	14.04	4.21
cognition	7.06	8.61E-04	12.58	3.89
positive regulation of protein	7.06	8.61E-04	12.58	4.03

serine/threonine kinase activity				
activation of innate immune response	7.06	8.63E-04	22.22	4.32
monocarboxylic acid metabolic process	7.05	8.65E-04	10.82	3.94
ossification	7.04	8.78E-04	15.05	3.63
signaling	7.03	8.81E-04	11.26	3.81
single organism signaling	7.03	8.81E-04	11.26	3.81
regulation of cell-cell adhesion	7.03	8.82E-04	17.46	4.43
secretion by cell	7.02	8.96E-04	11.42	4.52
regulation of purine nucleotide biosynthetic process	7	9.08E-04	15.66	4.35
regulation of nucleotide biosynthetic process	7	9.08E-04	15.66	4.35
regulation of nitrogen compound metabolic process	7	9.10E-04	7.31	4.27
negative regulation of cell differentiation	7	9.14E-04	10.03	3.97
positive regulation of peptidyl-tyrosine phosphorylation	6.99	9.20E-04	14.42	3.96
regulation of hormone levels	6.98	9.32E-04	12.5	4.19
positive regulation of epithelial cell migration	6.97	9.37E-04	20	3.90
signal transduction by phosphorylation	6.97	9.38E-04	13.91	4.20
endothelial cell development	6.97	9.44E-04	28.57	4.16
negative regulation of chemotaxis	6.97	9.44E-04	28.57	4.94
negative regulation of purine nucleotide metabolic process	6.97	9.44E-04	28.57	4.65
regulation of neuron differentiation	6.95	9.61E-04	10.39	4.42
regulation of JNK cascade	6.93	9.78E-04	14.89	4.28
response to toxic substance	6.9	1.00E-03	13.04	4.88
positive regulation of innate immune response	6.9	1.01E-03	17.19	4.21
regulation of protein secretion	6.88	1.03E-03	13.79	3.73
negative regulation of neurogenesis	6.87	1.03E-03	16.22	3.86
lactation	6.86	1.05E-03	21.62	3.74
regulation of phagocytosis	6.86	1.05E-03	21.62	4.91
regulation of osteoclast differentiation	6.86	1.05E-03	21.62	4.15
regulation of adenylate cyclase activity	6.83	1.08E-03	24.14	4.37
response to increased oxygen levels	6.83	1.08E-03	24.14	4.48
response to hyperoxia	6.83	1.08E-03	24.14	4.48
positive regulation of cell-cell adhesion	6.83	1.08E-03	24.14	4.28
body fluid secretion	6.83	1.08E-03	18.18	3.45
positive regulation of homeostatic process	6.83	1.08E-03	18.18	5.22
positive regulation of cell activation	6.82	1.09E-03	12.35	4.73
cellular response to hormone stimulus	6.82	1.09E-03	10.66	4.54
positive regulation of lymphocyte	6.82	1.09E-03	12.95	4.70

activation				
defense response to bacterium	6.82	1.09E-03	12.95	4.42
organic hydroxy compound metabolic process	6.81	1.10E-03	10.93	3.76
heat generation	6.8	1.11E-03	44.44	4.24
cellular response to ethanol	6.8	1.11E-03	44.44	7.07
positive regulation of astrocyte differentiation	6.8	1.11E-03	44.44	3.53
regulation of interferon-alpha production	6.8	1.11E-03	44.44	3.76
single organism cell adhesion	6.8	1.11E-03	12.07	4.17
negative regulation of cell motility	6.79	1.12E-03	13.28	5.02
regulation of cell killing	6.76	1.15E-03	16.92	4.39
execution phase of apoptosis	6.74	1.18E-03	33.33	4.86
modulation by host of viral transcription	6.74	1.18E-03	33.33	3.98
modulation of transcription in other organism involved in symbiotic interaction	6.74	1.18E-03	33.33	3.98
modulation by host of symbiont transcription	6.74	1.18E-03	33.33	3.98
nitrogen compound metabolic process	6.71	1.22E-03	7.19	4.14
negative regulation of secretion	6.71	1.22E-03	13.18	3.86
regulation of T cell apoptotic process	6.7	1.23E-03	27.27	5.08
response to insulin	6.69	1.24E-03	12.5	3.98
response to acid	6.69	1.24E-03	10.45	4.13
negative regulation of leukocyte apoptotic process	6.68	1.26E-03	21.05	4.75
positive regulation of cell development	6.65	1.29E-03	12.77	4.07
positive regulation of hydrolase activity	6.65	1.30E-03	10.55	4.00
cation homeostasis	6.63	1.32E-03	10.19	4.43
pattern recognition receptor signaling pathway	6.62	1.33E-03	23.33	4.38
positive regulation of T cell activation	6.62	1.34E-03	14.43	4.58
cell activation	6.58	1.39E-03	10.26	4.23
single-organism behavior	6.55	1.43E-03	10.61	4.08
regulation of cell cycle	6.55	1.43E-03	9.35	3.86
alpha-amino acid biosynthetic process	6.54	1.44E-03	17.54	4.46
positive regulation of endocytosis	6.54	1.44E-03	17.54	4.80
negative regulation of cellular component movement	6.54	1.45E-03	12.98	5.02
amino acid transport	6.52	1.48E-03	15.58	3.98
regulation of steroid metabolic process	6.51	1.49E-03	16.42	3.54
regulation of protein localization to nucleus	6.5	1.50E-03	13.76	4.09
regulation of pH	6.5	1.50E-03	20.51	4.00
antigen processing and presentation of peptide antigen	6.49	1.52E-03	18.75	3.81
positive regulation of leukocyte activation	6.46	1.56E-03	12.26	4.77

positive regulation of lymphocyte proliferation	6.45	1.58E-03	14.77	4.70
modification by host of symbiont morphology or physiology	6.44	1.59E-03	26.09	4.11
regulation of extrinsic apoptotic signaling pathway via death domain receptors	6.44	1.59E-03	26.09	5.25
regulation of leukocyte mediated immunity	6.42	1.62E-03	13.22	4.30
regulation of extrinsic apoptotic signaling pathway	6.42	1.63E-03	14.14	4.45
semaphorin-plexin signaling pathway	6.41	1.64E-03	31.25	5.77
positive regulation of protein secretion	6.4	1.66E-03	15.38	3.75
regulation of nucleobase-containing compound metabolic process	6.36	1.72E-03	7.24	4.26
positive regulation of lipid metabolic process	6.35	1.76E-03	14.61	3.67
negative regulation of catalytic activity	6.34	1.76E-03	9.59	4.37
T cell migration	6.34	1.76E-03	40	5.37
regulation of macrophage cytokine production	6.34	1.76E-03	40	3.32
DNA replication initiation	6.34	1.76E-03	40	2.99
positive regulation of prostaglandin secretion	6.34	1.76E-03	40	3.66
purinergic nucleotide receptor signaling pathway	6.34	1.76E-03	40	3.67
defense response to Gram-positive bacterium	6.34	1.77E-03	18.37	4.28
negative regulation of platelet aggregation	6.34	1.77E-03	60	3.92
negative regulation of neurotrophin TRK receptor signaling pathway	6.34	1.77E-03	60	3.46
response to iron(III) ion	6.34	1.77E-03	60	4.09
T cell chemotaxis	6.34	1.77E-03	60	4.93
positive regulation of cell-cell adhesion mediated by integrin	6.34	1.77E-03	60	4.67
regulation of keratinocyte migration	6.34	1.77E-03	60	3.68
positive regulation of keratinocyte migration	6.34	1.77E-03	60	3.68
radial glial cell differentiation	6.34	1.77E-03	60	4.82
positive regulation of endothelial cell chemotaxis	6.34	1.77E-03	60	5.02
toll-like receptor 4 signaling pathway	6.34	1.77E-03	60	5.22
modification of morphology or physiology of other organism involved in symbiotic interaction	6.33	1.79E-03	20	3.88
positive regulation of myeloid cell differentiation	6.27	1.89E-03	16.95	4.40
regulation of sequence-specific DNA binding transcription factor activity	6.26	1.90E-03	10.82	3.83
organ regeneration	6.26	1.91E-03	15.94	4.31
positive regulation of adaptive immune response based on somatic recombination of	6.26	1.91E-03	15.94	4.33

immune receptors built from immunoglobulin superfamily domains				
regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	6.22	1.98E-03	13.86	4.07
regulation of excitatory postsynaptic membrane potential	6.22	1.99E-03	21.88	3.82
response to cold	6.22	1.99E-03	21.88	4.75
innate immune response-activating signal transduction	6.22	1.99E-03	21.88	4.38
positive regulation of transmembrane transport	6.21	2.02E-03	25	4.29
positive regulation of production of molecular mediator of immune response	6.21	2.02E-03	25	3.76
negative regulation of nucleotide metabolic process	6.21	2.02E-03	25	4.65
neutral amino acid transport	6.21	2.02E-03	25	3.98
negative regulation of angiogenesis	6.19	2.05E-03	18	4.30
cellular response to glucocorticoid stimulus	6.19	2.05E-03	18	5.46
regulation of epithelial cell migration	6.18	2.07E-03	15	4.13
regulation of T cell activation	6.16	2.10E-03	11.95	4.33
regulation of nitric oxide biosynthetic process	6.16	2.11E-03	19.51	5.35
regulation of chemokine production	6.16	2.11E-03	19.51	3.73
memory	6.14	2.15E-03	15.71	4.64
fat cell differentiation	6.14	2.15E-03	15.71	3.55
activation of immune response	6.14	2.15E-03	13.27	4.85
regulation of protein import into nucleus	6.13	2.18E-03	13.73	4.01
angiogenesis	6.13	2.19E-03	12.5	4.33
cellular cation homeostasis	6.12	2.21E-03	10.42	4.41
regulation of leukocyte activation	6.12	2.21E-03	10.42	4.40
regulation of necrotic cell death	6.11	2.21E-03	29.41	4.89
positive regulation of neutrophil migration	6.11	2.21E-03	29.41	3.00
positive regulation of type I interferon production	6.11	2.21E-03	29.41	3.76
biosynthetic process	6.07	2.31E-03	7.27	4.04
cellular response to corticosteroid stimulus	6.05	2.36E-03	17.65	5.46
regulation of transcription factor import into nucleus	6.05	2.36E-03	17.65	3.67
positive regulation of endothelial cell proliferation	6.05	2.36E-03	17.65	4.61
negative regulation of behavior	6.03	2.41E-03	21.21	4.71
regulation of cytokine biosynthetic process	6.03	2.41E-03	15.49	4.07
interspecies interaction between organisms	6.03	2.41E-03	15.49	4.18
regulation of MAP kinase activity	6.02	2.43E-03	11.8	3.79

regulation of cell development	6.01	2.47E-03	9.03	4.31
regulation of neurological system process	6	2.48E-03	19.05	3.76
regulation of glial cell differentiation	6	2.48E-03	19.05	3.61
regulation of lymphocyte apoptotic process	6	2.48E-03	19.05	4.92
modification of morphology or physiology of other organism	6	2.48E-03	19.05	3.88
regulation of T cell mediated cytotoxicity	6	2.48E-03	19.05	4.01
interaction with symbiont	5.98	2.53E-03	24	4.11
cell projection organization	5.97	2.55E-03	9.31	4.55
nitrogen compound transport	5.95	2.60E-03	10.31	4.55
ion homeostasis	5.95	2.61E-03	9.68	4.41
regulation of spindle organization	5.93	2.65E-03	36.36	4.61
regulation of necroptotic process	5.93	2.65E-03	36.36	5.12
regulation of triglyceride biosynthetic process	5.93	2.65E-03	36.36	4.22
macrophage chemotaxis	5.93	2.65E-03	36.36	4.60
negative regulation of amino acid transport	5.93	2.65E-03	36.36	5.27
regulation of prostaglandin secretion	5.93	2.65E-03	36.36	3.66
regulation of lymphocyte activation	5.93	2.65E-03	10.71	4.48
phosphorylation	5.93	2.67E-03	9	4.06
positive regulation of leukocyte mediated immunity	5.92	2.70E-03	15.28	4.54
regulation of hemostasis	5.91	2.71E-03	17.31	4.17
regulation of blood coagulation	5.91	2.71E-03	17.31	4.17
response to oxidative stress	5.9	2.73E-03	10.4	4.64
positive regulation of gene expression	5.9	2.73E-03	8.14	4.27
regulation of system process	5.9	2.74E-03	10.03	3.73
negative regulation of transport	5.9	2.74E-03	10.27	3.98
regulation of fibroblast proliferation	5.89	2.76E-03	16.13	3.74
positive regulation of cell cycle	5.86	2.84E-03	14.46	3.72
hormone metabolic process	5.86	2.86E-03	13.33	4.11
antigen processing and presentation of peptide antigen via MHC class I	5.85	2.88E-03	20.59	4.02
protein secretion	5.85	2.89E-03	18.6	4.66
positive regulation of osteoclast differentiation	5.84	2.92E-03	27.78	4.30
cellular homeostasis	5.79	3.06E-03	9.35	4.42
phosphate-containing compound metabolic process	5.78	3.08E-03	7.74	4.19
regulation of lipid storage	5.77	3.13E-03	23.08	4.77
positive regulation of chemokine production	5.77	3.13E-03	23.08	3.81
response to copper ion	5.77	3.13E-03	23.08	4.86
monocarboxylic acid biosynthetic process	5.77	3.13E-03	13.21	4.29
single organism reproductive process	5.74	3.21E-03	8.56	4.10

positive regulation of JNK cascade	5.7	3.36E-03	18.18	3.63
cellular response to interferon-alpha	5.69	3.38E-03	50	4.17
T-helper 1 type immune response	5.69	3.38E-03	50	4.73
astrocyte cell migration	5.69	3.38E-03	50	4.25
cellular extravasation	5.69	3.38E-03	50	4.82
regulation of calcidiol 1-monooxygenase activity	5.69	3.38E-03	50	3.52
regulation of viral-induced cytoplasmic pattern recognition receptor signaling pathway	5.69	3.38E-03	50	2.90
regulation of RIG-I signaling pathway	5.69	3.38E-03	50	2.90
fever generation	5.69	3.38E-03	50	4.74
lipid metabolic process	5.68	3.43E-03	8.36	4.09
negative regulation of immune system process	5.67	3.43E-03	11.45	4.20
regulation of catabolic process	5.66	3.47E-03	8.97	4.38
response to hydrogen peroxide	5.66	3.48E-03	13.54	5.40
negative regulation of cell development	5.66	3.48E-03	13.54	3.87
regulation of homeostatic process	5.65	3.53E-03	10.48	4.35
regulation of coagulation	5.65	3.53E-03	16.67	4.17
monovalent inorganic cation homeostasis	5.65	3.53E-03	16.67	3.83
positive regulation of leukocyte mediated cytotoxicity	5.65	3.53E-03	16.67	4.49
establishment of localization in cell	5.59	3.72E-03	8.06	4.23
positive regulation of hormone secretion	5.59	3.74E-03	14.67	3.95
cellular ion homeostasis	5.59	3.75E-03	10.04	4.41
regulation of neutrophil migration	5.58	3.77E-03	26.32	3.00
positive regulation of phosphatidylinositol 3-kinase activity	5.58	3.77E-03	26.32	3.68
response to interleukin-6	5.58	3.77E-03	26.32	6.81
placenta blood vessel development	5.58	3.77E-03	26.32	3.69
estrous cycle phase	5.58	3.79E-03	33.33	3.81
positive regulation of calcium ion import	5.58	3.79E-03	33.33	4.48
negative regulation of osteoclast differentiation	5.58	3.79E-03	33.33	3.57
phosphorus metabolic process	5.57	3.81E-03	7.65	4.16
regulation of hydrolase activity	5.57	3.81E-03	8.64	4.43
regulation of catecholamine secretion	5.57	3.83E-03	22.22	3.96
cellular response to fatty acid	5.57	3.83E-03	22.22	3.53
spleen development	5.57	3.83E-03	22.22	4.72
regulation of type I interferon production	5.57	3.83E-03	22.22	3.77
response to reactive oxygen species	5.53	3.96E-03	12.12	4.98
regulation of peptidyl-tyrosine phosphorylation	5.52	3.99E-03	11.81	3.99

negative regulation of G-protein coupled receptor protein signaling pathway	5.51	4.04E-03	19.44	4.83
regulation of postsynaptic membrane potential	5.51	4.04E-03	19.44	3.82
positive regulation of interleukin-6 production	5.51	4.04E-03	19.44	3.81
positive regulation of nitrogen compound metabolic process	5.49	4.13E-03	7.89	4.34
cellular response to alcohol	5.49	4.15E-03	14.47	5.65
leukocyte activation	5.48	4.17E-03	10.08	4.20
cellular response to insulin stimulus	5.47	4.21E-03	13.79	3.91
myeloid leukocyte differentiation	5.39	4.54E-03	16.07	4.09
regulation of T cell mediated immunity	5.39	4.54E-03	16.07	3.83
regulation of cytokine secretion	5.38	4.59E-03	14.29	3.58
regulation of cyclase activity	5.35	4.74E-03	18.92	4.37
positive regulation of developmental growth	5.35	4.74E-03	18.92	4.52
cytokine secretion	5.34	4.79E-03	25	5.38
positive regulation of lipid kinase activity	5.34	4.79E-03	25	3.68
regulation of fibroblast growth factor receptor signaling pathway	5.34	4.79E-03	25	4.31
cellular hormone metabolic process	5.31	4.92E-03	14.93	4.50
negative regulation of catabolic process	5.28	5.08E-03	14.1	4.66
positive regulation of cytokine biosynthetic process	5.28	5.11E-03	17.02	4.29
positive regulation of T cell mediated immunity	5.28	5.11E-03	17.02	3.91
protein oligomerization	5.27	5.13E-03	9.6	4.16
regulation of norepinephrine secretion	5.25	5.23E-03	30.77	3.17
release of cytochrome c from mitochondria	5.25	5.23E-03	30.77	5.53
cellular response to interleukin-6	5.25	5.23E-03	30.77	6.56
regulation of interleukin-6 biosynthetic process	5.25	5.23E-03	30.77	3.46
JAK-STAT cascade	5.25	5.23E-03	30.77	4.22
fatty acid metabolic process	5.23	5.33E-03	10.61	4.08
organic substance transport	5.21	5.47E-03	7.73	4.23
regulation of G-protein coupled receptor protein signaling pathway	5.21	5.48E-03	14.71	5.48
tissue regeneration	5.2	5.53E-03	18.42	4.40
cellular modified amino acid biosynthetic process	5.2	5.53E-03	18.42	4.32
positive regulation of T cell mediated cytotoxicity	5.2	5.53E-03	18.42	4.13
liver development	5.19	5.55E-03	13.33	4.78
cellular response to steroid hormone stimulus	5.19	5.55E-03	13.33	5.47
positive regulation of reactive oxygen species metabolic process	5.19	5.56E-03	20.69	3.63
cellular monovalent inorganic cation homeostasis	5.19	5.56E-03	20.69	4.05

positive regulation of nitric oxide biosynthetic process	5.19	5.56E-03	20.69	5.32
nucleobase metabolic process	5.19	5.56E-03	20.69	4.42
adenylate cyclase-modulating G-protein coupled receptor signaling pathway	5.19	5.60E-03	13.92	4.20
cellular response to nutrient levels	5.19	5.60E-03	13.92	4.48
regulation of stress-activated MAPK cascade	5.19	5.60E-03	12.39	4.28
regulation of hormone secretion	5.18	5.64E-03	11.41	3.76
G-protein coupled purinergic receptor signaling pathway	5.17	5.67E-03	42.86	5.32
negative regulation of homotypic cell-cell adhesion	5.17	5.67E-03	42.86	3.92
protein K6-linked ubiquitination	5.17	5.67E-03	42.86	2.57
positive regulation of corticosteroid hormone secretion	5.17	5.67E-03	42.86	3.35
interleukin-1 beta production	5.17	5.67E-03	42.86	3.33
interleukin-1 production	5.17	5.67E-03	42.86	3.33
negative regulation of leukocyte chemotaxis	5.17	5.67E-03	42.86	4.93
cellular response to ATP	5.17	5.67E-03	42.86	5.27
regulation of cell-cell adhesion mediated by integrin	5.17	5.67E-03	42.86	4.67
positive regulation of macrophage cytokine production	5.17	5.67E-03	42.86	3.07
regulation of vitamin D biosynthetic process	5.17	5.67E-03	42.86	3.52
positive regulation of triglyceride biosynthetic process	5.17	5.67E-03	42.86	3.20
negative regulation of glutamate secretion	5.17	5.67E-03	42.86	4.68
positive regulation of interferon-alpha production	5.17	5.67E-03	42.86	3.68
regulation of reactive oxygen species metabolic process	5.16	5.76E-03	15.52	4.26
positive regulation of cell killing	5.16	5.76E-03	15.52	4.49
cellular response to drug	5.14	5.83E-03	16.67	4.95
apoptotic process	5.13	5.92E-03	9.81	4.53
regulation of platelet activation	5.12	6.00E-03	23.81	3.65
negative regulation of innate immune response	5.12	6.00E-03	23.81	4.68
negative regulation of protein metabolic process	5.12	6.00E-03	9.17	4.03
regulation of stress-activated protein kinase signaling cascade	5.11	6.05E-03	12.28	4.28
negative regulation of macromolecule metabolic process	5.1	6.08E-03	7.63	4.03
neuron differentiation	5.1	6.10E-03	10.86	4.12
regulation of lyase activity	5.05	6.41E-03	17.95	4.37
cellular response to mechanical stimulus	5.04	6.46E-03	15.25	4.17
regulation of anion transport	5.04	6.46E-03	15.25	4.65
positive regulation of T cell proliferation	5.04	6.46E-03	15.25	4.76
organic substance biosynthetic process	5.04	6.48E-03	7.11	4.00

negative regulation of nitrogen compound metabolic process	5.02	6.58E-03	8.04	4.09
regulation of interleukin-1 production	5.02	6.62E-03	20	3.86
chondrocyte differentiation	5.02	6.62E-03	20	3.44
positive regulation of lymphocyte mediated immunity	5	6.74E-03	14.29	4.33
inorganic anion transport	5	6.74E-03	14.29	4.23
regulation of endocytosis	4.97	6.91E-03	12.5	4.81
establishment of endothelial barrier	4.96	6.98E-03	28.57	4.32
negative regulation of systemic arterial blood pressure	4.96	6.98E-03	28.57	4.69
regulation of membrane protein ectodomain proteolysis	4.96	6.98E-03	28.57	4.18
regulation of cell aging	4.96	6.98E-03	28.57	3.55
negative regulation of endothelial cell apoptotic process	4.96	6.98E-03	28.57	5.58
regulation of viral entry into host cell	4.96	6.98E-03	28.57	4.26
positive regulation of fatty acid transport	4.96	6.98E-03	28.57	3.66
positive regulation of icosanoid secretion	4.96	6.98E-03	28.57	3.66
regulation of peptide transport	4.96	7.05E-03	12.07	3.95
intrinsic apoptotic signaling pathway	4.93	7.22E-03	12.9	4.69
single organismal cell-cell adhesion	4.92	7.33E-03	11.11	4.13
positive regulation of phosphatidylinositol 3-kinase signaling	4.91	7.39E-03	17.5	4.06
regulation of tyrosine phosphorylation of STAT protein	4.91	7.39E-03	17.5	4.21
cell aging	4.91	7.39E-03	17.5	4.31
cyclic-nucleotide-mediated signaling	4.91	7.40E-03	22.73	4.36
positive regulation of ion transmembrane transport	4.91	7.40E-03	22.73	4.49
regulation of phosphatidylinositol 3-kinase activity	4.91	7.40E-03	22.73	3.68
negative regulation of myeloid leukocyte differentiation	4.91	7.40E-03	22.73	3.36
negative regulation of intracellular signal transduction	4.9	7.46E-03	10.29	3.90
positive regulation of organelle organization	4.9	7.46E-03	10.29	3.95
branching morphogenesis of an epithelial tube	4.9	7.48E-03	12.38	4.85
positive regulation of cAMP metabolic process	4.89	7.50E-03	16	5.32
multi-organism reproductive process	4.88	7.59E-03	11.63	3.90
kidney development	4.88	7.59E-03	11.97	4.06
cellular response to oxidative stress	4.88	7.59E-03	11.97	5.35
nerve development	4.85	7.82E-03	19.35	4.08
negative regulation of cytokine production	4.85	7.85E-03	12.77	3.98
negative regulation of	4.84	7.87E-03	10.24	4.18

phosphorylation				
cellular biosynthetic process	4.83	7.95E-03	7.09	4.02
heart development	4.83	8.01E-03	11.27	4.93
positive regulation of cyclic nucleotide metabolic process	4.82	8.05E-03	14.75	4.97
epithelial cell development	4.8	8.21E-03	13.89	3.79
female pregnancy	4.8	8.21E-03	13.89	3.91
regulation of apoptotic signaling pathway	4.8	8.23E-03	9.91	4.22
single-organism nuclear import	4.77	8.45E-03	15.69	3.95
regulation of amine transport	4.77	8.45E-03	15.69	4.23
protein import into nucleus	4.77	8.45E-03	15.69	3.95
positive regulation of cell division	4.77	8.45E-03	15.69	3.91
positive regulation of fibroblast proliferation	4.77	8.48E-03	17.07	3.34
regulation of neurotrophin TRK receptor signaling pathway	4.75	8.68E-03	37.5	3.46
purine nucleobase biosynthetic process	4.75	8.68E-03	37.5	3.87
negative regulation of necroptotic process	4.75	8.68E-03	37.5	5.78
regulation of cellular extravasation	4.75	8.68E-03	37.5	5.15
regulation of cholesterol storage	4.75	8.68E-03	37.5	3.65
diterpenoid biosynthetic process	4.75	8.68E-03	37.5	4.51
retinoic acid biosynthetic process	4.75	8.68E-03	37.5	4.51
regulation of vitamin metabolic process	4.75	8.68E-03	37.5	3.52
regulation of immature T cell proliferation	4.75	8.68E-03	37.5	3.86
mammary gland involution	4.75	8.68E-03	37.5	3.75
luteinization	4.75	8.68E-03	37.5	4.38
cellular response to iron ion	4.75	8.68E-03	37.5	4.35
negative regulation of JAK-STAT cascade	4.75	8.68E-03	37.5	2.53
positive regulation of interleukin-2 biosynthetic process	4.75	8.68E-03	37.5	4.01
mitotic metaphase plate congression	4.75	8.68E-03	37.5	3.78
maintenance of fidelity involved in DNA-dependent DNA replication	4.75	8.68E-03	37.5	2.99
serine transport	4.75	8.68E-03	37.5	3.43
rRNA transcription	4.75	8.68E-03	37.5	4.73
cellular response to growth hormone stimulus	4.75	8.68E-03	37.5	5.25
regulation of mitotic spindle organization	4.75	8.68E-03	37.5	4.04
negative regulation of natural killer cell mediated immunity	4.75	8.68E-03	37.5	4.19
negative regulation of natural killer cell mediated cytotoxicity	4.75	8.68E-03	37.5	4.19
negative regulation of cellular biosynthetic process	4.74	8.70E-03	7.91	4.04
positive regulation of cytosolic calcium ion concentration	4.74	8.74E-03	12.15	3.66
regulation of mitotic cell cycle	4.74	8.76E-03	10.14	3.71

developmental process involved in reproduction	4.73	8.80E-03	9.15	4.35
regulation of interferon-gamma production	4.72	8.94E-03	14.52	4.26
regulation of fatty acid biosynthetic process	4.71	9.01E-03	21.74	3.46
positive regulation of protein tyrosine kinase activity	4.71	9.01E-03	21.74	3.79
positive regulation of glial cell differentiation	4.71	9.01E-03	21.74	3.44
mammary gland development	4.71	9.01E-03	21.74	4.12
regulation of intracellular pH	4.71	9.01E-03	21.74	4.37
regulation of homotypic cell-cell adhesion	4.7	9.09E-03	26.67	4.65
response to glucagon	4.7	9.09E-03	26.67	4.87
positive regulation of cytokine-mediated signaling pathway	4.7	9.09E-03	26.67	4.46
positive regulation of lipid storage	4.7	9.09E-03	26.67	5.52
regulation of glial cell proliferation	4.7	9.09E-03	26.67	3.64
cytokine metabolic process	4.7	9.09E-03	26.67	4.51
collagen catabolic process	4.7	9.09E-03	26.67	2.62
adenylate cyclase-activating G-protein coupled receptor signaling pathway	4.69	9.16E-03	18.75	4.03
cyclic nucleotide metabolic process	4.69	9.16E-03	18.75	4.03
positive regulation of endothelial cell migration	4.69	9.16E-03	18.75	4.01
purine-containing compound biosynthetic process	4.68	9.25E-03	12.5	3.91
cell maturation	4.68	9.25E-03	12.5	4.50
regulation of biosynthetic process	4.68	9.28E-03	6.96	4.20
morphogenesis of a branching epithelium	4.66	9.43E-03	11.67	4.67
nuclear import	4.66	9.50E-03	15.38	3.95
cytokine production	4.66	9.50E-03	15.38	3.96
positive regulation of stress-activated MAPK cascade	4.66	9.50E-03	15.38	3.63
regulation of steroid biosynthetic process	4.66	9.50E-03	15.38	3.40
regulation of cellular biosynthetic process	4.65	9.60E-03	6.97	4.19
alcohol metabolic process	4.65	9.60E-03	10.59	3.96
establishment of blood-nerve barrier	4.64	9.65E-03	66.67	3.85
positive regulation of somatostatin secretion	4.64	9.65E-03	66.67	2.77
embryonic placenta morphogenesis	4.64	9.65E-03	66.67	4.74
positive regulation of monocyte differentiation	4.64	9.65E-03	66.67	3.61
positive regulation of MDA-5 signaling pathway	4.64	9.65E-03	66.67	2.97
central nervous system myelin maintenance	4.64	9.65E-03	66.67	5.64
negative regulation of gamma-aminobutyric acid secretion	4.64	9.65E-03	66.67	5.37
negative regulation of pro-B cell	4.64	9.65E-03	66.67	3.77

differentiation				
negative regulation of inner ear receptor cell differentiation	4.64	9.65E-03	66.67	3.77
forebrain radial glial cell differentiation	4.64	9.65E-03	66.67	3.77
auditory receptor cell fate determination	4.64	9.65E-03	66.67	3.77
negative regulation of mechanoreceptor differentiation	4.64	9.65E-03	66.67	3.77
positive regulation of cell aging	4.64	9.65E-03	66.67	3.24
response to linoleic acid	4.64	9.65E-03	66.67	3.74
virion attachment to host cell	4.64	9.65E-03	66.67	5.57
adhesion of symbiont to host	4.64	9.65E-03	66.67	5.57
adhesion of symbiont to host cell	4.64	9.65E-03	66.67	5.57
receptor-mediated virion attachment to host cell	4.64	9.65E-03	66.67	5.57
NLS-bearing protein import into nucleus	4.64	9.65E-03	66.67	4.30
creatinine metabolic process	4.64	9.65E-03	66.67	4.28
cellular lactam metabolic process	4.64	9.65E-03	66.67	4.28
positive regulation of receptor binding	4.64	9.65E-03	66.67	3.03
negative regulation of transcription from RNA polymerase III promoter	4.64	9.65E-03	66.67	4.54
nucleotide-binding domain, leucine rich repeat containing receptor signaling pathway	4.64	9.65E-03	66.67	4.52
nucleotide-binding oligomerization domain containing signaling pathway	4.64	9.65E-03	66.67	4.52
nucleotide-binding oligomerization domain containing 2 signaling pathway	4.64	9.65E-03	66.67	4.52
anterior/posterior axon guidance	4.64	9.65E-03	66.67	4.39
G-protein coupled purinergic nucleotide receptor signaling pathway	4.64	9.65E-03	66.67	4.44
RNA import into mitochondrion	4.64	9.65E-03	66.67	4.95
rRNA import into mitochondrion	4.64	9.65E-03	66.67	4.95
antigen processing and presentation of endogenous peptide antigen via MHC class Ib	4.64	9.65E-03	66.67	3.66
succinate transport	4.64	9.65E-03	66.67	4.51
citrate transport	4.64	9.65E-03	66.67	4.51
succinate transmembrane transport	4.64	9.65E-03	66.67	4.51
intracellular transport of viral protein in host cell	4.64	9.65E-03	66.67	4.48
symbiont intracellular protein transport in host	4.64	9.65E-03	66.67	4.48
intracellular protein transport in other organism involved in symbiotic interaction	4.64	9.65E-03	66.67	4.48
extracellular transport	4.64	9.65E-03	66.67	4.48
positive regulation of extrinsic apoptotic signaling pathway	4.64	9.68E-03	16.67	3.98
tissue remodeling	4.61	9.91E-03	14.29	4.41

response to amino acid	4.59	1.01E-02	11.57	4.43
multi-multicellular organism process	4.59	1.02E-02	11.93	3.64
regulation of GTPase activity	4.58	1.03E-02	10.33	4.31
regulation of cellular catabolic process	4.57	1.03E-02	8.71	4.54
regulation of intracellular protein transport	4.57	1.03E-02	10.96	4.03
negative regulation of nucleobase-containing compound metabolic process	4.56	1.04E-02	7.91	4.04
cellular metal ion homeostasis	4.56	1.05E-02	9.7	4.39
terpenoid metabolic process	4.54	1.06E-02	15.09	3.60
positive regulation of protein import into nucleus	4.54	1.06E-02	15.09	4.07
positive regulation of stress-activated protein kinase signaling cascade	4.54	1.06E-02	15.09	3.63
activation of protein kinase activity	4.54	1.06E-02	11.19	3.88
response to dexamethasone	4.54	1.07E-02	18.18	4.67
substantia nigra development	4.54	1.07E-02	18.18	5.59
regulation of developmental growth	4.52	1.08E-02	12.24	4.18
behavioral defense response	4.52	1.09E-02	20.83	3.36
regulation of tyrosine phosphorylation of Stat3 protein	4.52	1.09E-02	20.83	3.58
fatty acid biosynthetic process	4.52	1.09E-02	13.33	4.37
positive regulation of JAK-STAT cascade	4.51	1.10E-02	16.28	4.54
negative regulation of neuron differentiation	4.51	1.10E-02	16.28	3.88
cellular response to DNA damage stimulus	4.49	1.12E-02	8.98	3.91
morphogenesis of an epithelium	4.48	1.13E-02	9.91	4.54
hematopoietic or lymphoid organ development	4.48	1.14E-02	11.11	4.49
G-protein coupled receptor signaling pathway, coupled to cyclic nucleotide second messenger	4.47	1.14E-02	12.64	4.20
negative regulation of defense response	4.47	1.14E-02	12.64	3.93
regulation of GTP catabolic process	4.47	1.14E-02	10.22	4.31
ERK1 and ERK2 cascade	4.46	1.16E-02	25	4.02
positive regulation of response to cytokine stimulus	4.46	1.16E-02	25	4.46
intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	4.46	1.16E-02	25	3.81
positive regulation of neutrophil chemotaxis	4.46	1.16E-02	25	2.78
regulation of macrophage derived foam cell differentiation	4.46	1.16E-02	25	3.81
regulation of cellular response to oxidative stress	4.46	1.16E-02	25	5.24
regulation of peptidyl-threonine phosphorylation	4.46	1.16E-02	25	5.86
regulation of cAMP-mediated	4.46	1.16E-02	25	5.49

signaling				
response to food	4.46	1.16E-02	25	4.11
peripheral nervous system development	4.46	1.16E-02	25	3.84
regulation of glutamate secretion	4.46	1.16E-02	25	5.27
morphogenesis of a branching structure	4.46	1.16E-02	11.38	4.67
regulation of lymphocyte mediated immunity	4.45	1.17E-02	12.12	4.10
positive regulation of cell adhesion	4.44	1.17E-02	11.71	3.95
cellular response to hydrogen peroxide	4.43	1.19E-02	14.81	5.52
positive regulation of peptide secretion	4.43	1.19E-02	14.81	4.35
cellular amino acid metabolic process	4.42	1.21E-02	9.36	4.50
positive regulation of purine nucleotide metabolic process	4.42	1.21E-02	13.85	4.97
positive regulation of nucleotide metabolic process	4.42	1.21E-02	13.85	4.97
response to zinc ion	4.4	1.23E-02	17.65	5.57
regulation of cytokine production involved in immune response	4.4	1.23E-02	17.65	3.71
negative regulation of protein transport	4.39	1.24E-02	12.5	3.73
response to starvation	4.39	1.24E-02	12.5	3.87
regulation of platelet aggregation	4.38	1.25E-02	33.33	3.92
necroptotic process	4.38	1.25E-02	33.33	5.36
positive regulation of steroid hormone secretion	4.38	1.25E-02	33.33	3.35
negative regulation of NF-kappaB import into nucleus	4.38	1.25E-02	33.33	3.54
positive regulation of myeloid leukocyte cytokine production involved in immune response	4.38	1.25E-02	33.33	3.07
positive regulation of glial cell proliferation	4.38	1.25E-02	33.33	3.92
negative regulation of astrocyte differentiation	4.38	1.25E-02	33.33	3.90
regulation of carbohydrate metabolic process	4.37	1.26E-02	12	3.77
regulation of membrane potential	4.36	1.27E-02	10.11	4.13
negative regulation of multicellular organismal process	4.36	1.28E-02	9.22	3.63
cellular lipid metabolic process	4.36	1.28E-02	8.25	4.03
positive regulation of calcium ion transport into cytosol	4.35	1.29E-02	20	5.69
brown fat cell differentiation	4.35	1.29E-02	20	3.78
regulation of sensory perception of pain	4.35	1.29E-02	20	3.43
regulation of sensory perception	4.35	1.29E-02	20	3.43
termination of G-protein coupled receptor signaling pathway	4.35	1.29E-02	20	4.84
negative regulation of lymphocyte apoptotic process	4.35	1.29E-02	20	5.24
regulation of cellular pH	4.35	1.29E-02	20	4.37
negative regulation of gliogenesis	4.35	1.29E-02	20	3.69

positive regulation of protein complex assembly	4.34	1.30E-02	12.99	3.66
steroid metabolic process	4.33	1.32E-02	10.67	4.06
response to osmotic stress	4.33	1.32E-02	14.55	4.38
membrane depolarization	4.32	1.33E-02	13.64	3.67
amine metabolic process	4.32	1.33E-02	13.64	4.43
response to amine	4.27	1.40E-02	15.56	4.69
regulation of organic acid transport	4.27	1.40E-02	15.56	4.62
protein complex assembly	4.26	1.41E-02	7.97	4.02
cofactor metabolic process	4.26	1.41E-02	10	3.83
anatomical structure morphogenesis	4.26	1.41E-02	7.58	4.40
negative regulation of kinase activity	4.26	1.42E-02	11.11	4.01
positive regulation of tyrosine phosphorylation of STAT protein	4.26	1.42E-02	17.14	4.51
glial cell development	4.26	1.42E-02	17.14	5.37
L-amino acid transport	4.26	1.42E-02	17.14	3.75
positive regulation of nucleobase-containing compound metabolic process	4.24	1.44E-02	7.55	4.19
regulation of triglyceride metabolic process	4.24	1.45E-02	23.53	4.22
positive regulation of interleukin-1 beta production	4.24	1.45E-02	23.53	3.17
regulation of T cell migration	4.24	1.45E-02	23.53	4.20
regulation of response to oxidative stress	4.24	1.45E-02	23.53	5.24
positive regulation of cytokine production involved in immune response	4.24	1.45E-02	23.53	2.88
prostanoid metabolic process	4.24	1.45E-02	23.53	3.70
prostaglandin metabolic process	4.24	1.45E-02	23.53	3.70
regulation of icosanoid secretion	4.24	1.45E-02	23.53	3.66
negative regulation of protein kinase activity	4.24	1.45E-02	11.4	4.06
regulation of peptide secretion	4.24	1.45E-02	11.4	4.00
positive regulation of MAP kinase activity	4.24	1.45E-02	11.4	3.83
cellular nitrogen compound metabolic process	4.23	1.45E-02	6.87	4.06
negative regulation of sequence-specific DNA binding transcription factor activity	4.23	1.45E-02	12.22	4.06
negative regulation of biosynthetic process	4.23	1.45E-02	7.7	4.04
negative regulation of cell activation	4.22	1.46E-02	11.76	3.53
coenzyme metabolic process	4.21	1.48E-02	10.53	3.79
termination of signal transduction	4.18	1.53E-02	19.23	4.84
positive regulation of multicellular organism growth	4.18	1.53E-02	19.23	3.25
response to vitamin D	4.18	1.53E-02	19.23	4.11
regulation of interleukin-1 beta production	4.18	1.53E-02	19.23	3.44
regulation of lipid kinase activity	4.18	1.53E-02	19.23	3.68

negative regulation of ERK1 and ERK2 cascade	4.18	1.53E-02	19.23	4.02
inflammatory response to antigenic stimulus	4.18	1.53E-02	19.23	5.53
cellular response to acid	4.17	1.55E-02	10.71	3.86
response to transition metal nanoparticle	4.15	1.57E-02	12.09	5.28
regulation of bone mineralization	4.15	1.57E-02	15.22	3.22
signal transduction by p53 class mediator	4.15	1.57E-02	15.22	4.16
negative regulation of myeloid cell differentiation	4.15	1.57E-02	15.22	3.59
ethanolamine-containing compound metabolic process	4.12	1.62E-02	16.67	4.23
regulation of smooth muscle cell migration	4.12	1.62E-02	16.67	4.36
response to dsRNA	4.12	1.62E-02	16.67	4.04
cellular divalent inorganic cation homeostasis	4.11	1.64E-02	9.84	4.01
protein metabolic process	4.11	1.64E-02	6.85	4.16
cellular response to metal ion	4.09	1.67E-02	12.5	4.19
regulation of nucleotide catabolic process	4.07	1.70E-02	9.66	4.23
regulation of purine nucleotide catabolic process	4.07	1.70E-02	9.66	4.23
divalent inorganic cation homeostasis	4.07	1.70E-02	9.66	3.97
programmed necrotic cell death	4.07	1.71E-02	30	5.36
regulation of corticosteroid hormone secretion	4.07	1.71E-02	30	3.35
positive regulation of circadian rhythm	4.07	1.71E-02	30	3.38
positive regulation of macrophage differentiation	4.07	1.71E-02	30	4.45
positive regulation of monocyte chemotaxis	4.07	1.71E-02	30	6.62
positive regulation of fever generation	4.07	1.71E-02	30	4.87
positive regulation of macrophage derived foam cell differentiation	4.07	1.71E-02	30	3.90
serine family amino acid biosynthetic process	4.07	1.71E-02	30	3.89
regulation of transcription from RNA polymerase III promoter	4.07	1.71E-02	30	3.93
myelin maintenance	4.07	1.71E-02	30	4.80
negative regulation of catecholamine secretion	4.07	1.71E-02	30	4.51
negative regulation of oligodendrocyte differentiation	4.07	1.71E-02	30	4.10
astrocyte differentiation	4.07	1.71E-02	30	5.62
negative regulation of cellular response to oxidative stress	4.07	1.71E-02	30	6.02
negative regulation of response to oxidative stress	4.07	1.71E-02	30	6.02
negative regulation of viral entry into host cell	4.07	1.71E-02	30	4.55
negative regulation of membrane potential	4.07	1.71E-02	30	4.37
oligopeptide transport	4.07	1.71E-02	30	4.63

negative regulation of protein serine/threonine kinase activity	4.05	1.75E-02	13.04	3.67
polyol metabolic process	4.04	1.76E-02	14.89	3.82
regulation of phosphatidylinositol 3-kinase signaling	4.04	1.76E-02	14.89	4.06
positive regulation of lipid biosynthetic process	4.04	1.76E-02	14.89	3.70
protein import into nucleus, translocation	4.03	1.78E-02	22.22	4.02
regulation of vascular endothelial growth factor production	4.03	1.78E-02	22.22	5.30
positive regulation of interleukin-1 production	4.03	1.78E-02	22.22	3.17
macrophage activation	4.03	1.78E-02	22.22	3.45
positive regulation of granulocyte chemotaxis	4.03	1.78E-02	22.22	2.78
regulation of neutrophil chemotaxis	4.03	1.78E-02	22.22	2.78
decidualization	4.03	1.78E-02	22.22	4.45
negative regulation of organic acid transport	4.03	1.78E-02	22.22	5.27
regulation of glycolytic process	4.03	1.78E-02	22.22	5.04
positive regulation of Notch signaling pathway	4.03	1.78E-02	22.22	4.34
positive regulation of interleukin-8 production	4.03	1.78E-02	22.22	4.93
multicellular organismal catabolic process	4.03	1.78E-02	22.22	2.62
positive regulation of axon extension	4.03	1.78E-02	22.22	4.22
intrinsic apoptotic signaling pathway by p53 class mediator	4.02	1.79E-02	18.52	3.97
positive regulation of organic acid transport	4.02	1.79E-02	18.52	4.33
regulation of endothelial cell migration	4.02	1.79E-02	13.79	4.14
amide transport	4.02	1.79E-02	13.79	4.59
positive regulation of biosynthetic process	4.02	1.80E-02	7.39	4.31
choline metabolic process	3.99	1.86E-02	50	4.16
regulation of monocyte chemotactic protein-1 production	3.99	1.86E-02	50	2.83
positive regulation of circadian sleep/wake cycle, non-REM sleep	3.99	1.86E-02	50	3.60
regulation of apoptotic cell clearance	3.99	1.86E-02	50	5.33
response to macrophage colony-stimulating factor	3.99	1.86E-02	50	4.43
cellular response to macrophage colony-stimulating factor stimulus	3.99	1.86E-02	50	4.43
regulation of chemokine-mediated signaling pathway	3.99	1.86E-02	50	5.73
negative regulation of chemokine-mediated signaling pathway	3.99	1.86E-02	50	5.73
regulation of interleukin-10 secretion	3.99	1.86E-02	50	3.97
mammary duct terminal end bud growth	3.99	1.86E-02	50	5.59
hydrogen sulfide metabolic process	3.99	1.86E-02	50	3.14

positive regulation of integrin activation	3.99	1.86E-02	50	3.59
9-cis-retinoic acid biosynthetic process	3.99	1.86E-02	50	3.94
9-cis-retinoic acid metabolic process	3.99	1.86E-02	50	3.94
regulation of MDA-5 signaling pathway	3.99	1.86E-02	50	2.97
locomotion involved in locomotory behavior	3.99	1.86E-02	50	4.33
negative regulation of epinephrine secretion	3.99	1.86E-02	50	5.24
positive regulation of cortisol secretion	3.99	1.86E-02	50	3.86
creatine metabolic process	3.99	1.86E-02	50	5.11
glutamate biosynthetic process	3.99	1.86E-02	50	5.19
response to triglyceride	3.99	1.86E-02	50	4.30
regulation of pro-B cell differentiation	3.99	1.86E-02	50	3.77
regulation of forebrain neuron differentiation	3.99	1.86E-02	50	3.77
renal interstitial cell development	3.99	1.86E-02	50	2.63
metanephric nephron tubule morphogenesis	3.99	1.86E-02	50	3.77
macropinocytosis	3.99	1.86E-02	50	3.88
cellular response to cell-matrix adhesion	3.99	1.86E-02	50	2.44
replication fork processing	3.99	1.86E-02	50	2.54
regulation of hyaluronan biosynthetic process	3.99	1.86E-02	50	2.96
D-amino acid transport	3.99	1.86E-02	50	3.67
antigen processing and presentation of peptide antigen via MHC class Ib	3.99	1.86E-02	50	3.66
phagosome acidification	3.99	1.86E-02	50	6.05
tricarboxylic acid transport	3.99	1.86E-02	50	4.51
JAK-STAT cascade involved in growth hormone signaling pathway	3.99	1.86E-02	50	5.85
negative regulation of membrane protein ectodomain proteolysis	3.99	1.86E-02	50	4.64
developmental maturation	3.97	1.88E-02	11.02	4.46
carbohydrate homeostasis	3.97	1.88E-02	11.02	4.62
glucose homeostasis	3.97	1.88E-02	11.02	4.62
negative regulation of cell projection organization	3.96	1.91E-02	12.86	4.85
receptor-mediated endocytosis	3.96	1.91E-02	12.86	3.67
isoprenoid metabolic process	3.96	1.91E-02	12.86	3.71
regulation of endothelial cell proliferation	3.96	1.91E-02	12.86	4.61
adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	3.93	1.96E-02	14.58	4.44
positive regulation of smooth muscle cell proliferation	3.93	1.96E-02	14.58	4.12
regulation of extent of cell	3.93	1.96E-02	14.58	4.22

growth				
cellular glucose homeostasis	3.93	1.96E-02	14.58	4.49
regulation of binding	3.93	1.96E-02	10.19	3.97
multicellular organismal response to stress	3.93	1.97E-02	13.56	3.75
tissue development	3.92	1.99E-02	8.47	4.33
organic cyclic compound metabolic process	3.91	2.00E-02	6.78	4.03
regulation of nucleoside metabolic process	3.89	2.05E-02	9.48	4.23
regulation of ossification	3.89	2.05E-02	10.61	3.56
myeloid cell differentiation	3.88	2.07E-02	11.22	3.94
response to iron ion	3.88	2.07E-02	17.86	4.63
positive regulation of mitosis	3.88	2.07E-02	17.86	3.65
adaptive immune response	3.87	2.08E-02	12.68	4.55
regulation of protein tyrosine kinase activity	3.87	2.08E-02	15.79	4.15
retinoid metabolic process	3.87	2.08E-02	15.79	3.85
regulation of epidermis development	3.87	2.08E-02	15.79	3.67
immune response-activating signal transduction	3.86	2.12E-02	12.05	4.61
regulation of epithelial cell proliferation	3.85	2.13E-02	9.73	4.26
tissue morphogenesis	3.85	2.14E-02	9.09	4.46
cAMP metabolic process	3.84	2.15E-02	21.05	4.05
response to X-ray	3.84	2.15E-02	21.05	3.95
neuron recognition	3.84	2.15E-02	21.05	4.42
regulation of calcium ion import	3.84	2.15E-02	21.05	4.48
lipid storage	3.84	2.15E-02	21.05	3.36
cranial nerve development	3.84	2.15E-02	21.05	4.10
regulation of biomineral tissue development	3.83	2.18E-02	14.29	3.22
coagulation	3.83	2.18E-02	14.29	4.80
blood coagulation	3.83	2.18E-02	14.29	4.80
regulation of DNA metabolic process	3.82	2.20E-02	9.55	3.81
response to alkaloid	3.81	2.21E-02	11.11	4.93
cAMP biosynthetic process	3.8	2.25E-02	27.27	4.67
nucleobase biosynthetic process	3.8	2.25E-02	27.27	3.87
microglial cell activation	3.8	2.25E-02	27.27	3.40
regulation of fever generation	3.8	2.25E-02	27.27	4.87
nitric oxide mediated signal transduction	3.8	2.25E-02	27.27	3.75
positive regulation of peptidyl-threonine phosphorylation	3.8	2.25E-02	27.27	6.15
terpenoid biosynthetic process	3.8	2.25E-02	27.27	4.51
peptide catabolic process	3.8	2.25E-02	27.27	5.14
cytokine biosynthetic process	3.8	2.25E-02	27.27	4.43
organ growth	3.8	2.25E-02	27.27	4.13
negative regulation of protein phosphorylation	3.78	2.28E-02	9.83	3.90
DNA replication	3.78	2.28E-02	11.9	3.25

metal ion homeostasis	3.76	2.32E-02	8.83	4.52
regulation of neuronal synaptic plasticity	3.75	2.34E-02	15.38	4.06
negative regulation of nucleocytoplasmic transport	3.75	2.34E-02	15.38	3.63
positive regulation of nucleocytoplasmic transport	3.75	2.36E-02	13.11	4.07
signal transduction in absence of ligand	3.73	2.39E-02	17.24	5.44
extrinsic apoptotic signaling pathway in absence of ligand	3.73	2.39E-02	17.24	5.44
lung alveolus development	3.73	2.39E-02	17.24	4.09
catabolic process	3.73	2.40E-02	7.26	4.15
regulation of vesicle-mediated transport	3.73	2.41E-02	9.45	4.60
peptide transport	3.73	2.41E-02	14	4.83
hemostasis	3.73	2.41E-02	14	4.80
negative regulation of leukocyte differentiation	3.73	2.41E-02	14	3.28
chloride transport	3.73	2.41E-02	14	4.23
negative regulation of transferase activity	3.71	2.44E-02	10.37	4.01
cellular response to reactive oxygen species	3.71	2.45E-02	12.33	5.95
response to heat	3.71	2.45E-02	12.33	4.34
protein heterooligomerization	3.71	2.46E-02	11.76	4.27
ovulation	3.66	2.57E-02	20	3.62
positive regulation of tyrosine phosphorylation of Stat3 protein	3.66	2.57E-02	20	3.87
positive regulation of epidermis development	3.66	2.57E-02	20	3.50
negative regulation of axon extension	3.66	2.57E-02	20	4.29
negative regulation of glial cell differentiation	3.66	2.57E-02	20	3.63
axon development	3.66	2.57E-02	20	5.55
developmental growth involved in morphogenesis	3.66	2.58E-02	12.9	4.15
regulation of production of molecular mediator of immune response	3.66	2.58E-02	12.9	4.12
long-chain fatty acid metabolic process	3.64	2.63E-02	15	3.49
regulation of alcohol biosynthetic process	3.64	2.63E-02	15	3.60
response to antibiotic	3.64	2.63E-02	15	5.26
regulation of interleukin-8 production	3.64	2.63E-02	15	4.82
regulation of axon extension	3.64	2.63E-02	15	4.09
positive regulation of lymphocyte differentiation	3.63	2.66E-02	13.73	4.52
positive regulation of peptide hormone secretion	3.63	2.66E-02	13.73	4.44
negative regulation of axonogenesis	3.6	2.74E-02	16.67	4.36
response to exogenous dsRNA	3.6	2.74E-02	16.67	4.36
positive regulation of apoptotic signaling pathway	3.56	2.84E-02	11.49	3.63
alpha-amino acid metabolic	3.55	2.86E-02	9.93	4.47

process				
positive regulation of fatty acid biosynthetic process	3.55	2.87E-02	25	3.97
activation of NF-kappaB-inducing kinase activity	3.55	2.87E-02	25	4.76
midgut development	3.55	2.87E-02	25	4.44
glutamine family amino acid biosynthetic process	3.55	2.87E-02	25	5.47
negative regulation of necrotic cell death	3.55	2.87E-02	25	5.78
necrotic cell death	3.55	2.87E-02	25	5.36
positive regulation of interleukin-1 secretion	3.55	2.87E-02	25	3.06
positive regulation of interleukin-1 beta secretion	3.55	2.87E-02	25	3.06
positive regulation of nitric-oxide synthase biosynthetic process	3.55	2.87E-02	25	5.22
positive regulation of heat generation	3.55	2.87E-02	25	4.87
positive regulation of cAMP-mediated signaling	3.55	2.87E-02	25	6.59
phosphatidylinositol 3-kinase signaling	3.55	2.87E-02	25	4.10
labyrinthine layer blood vessel development	3.55	2.87E-02	25	2.89
response to L-ascorbic acid	3.55	2.87E-02	25	5.57
metaphase plate congression	3.55	2.87E-02	25	3.78
negative regulation of leukocyte mediated cytotoxicity	3.55	2.87E-02	25	4.19
negative regulation of cell killing	3.55	2.87E-02	25	4.19
cytosolic calcium ion homeostasis	3.55	2.88E-02	10.4	3.66
positive regulation of protein ubiquitination	3.53	2.93E-02	13.46	4.62
activation of cysteine-type endopeptidase activity involved in apoptotic process	3.53	2.93E-02	13.46	3.92
cellular response to carbohydrate stimulus	3.53	2.93E-02	13.46	4.57
positive regulation of carbohydrate metabolic process	3.53	2.93E-02	14.63	3.82
negative regulation of NF-kappaB transcription factor activity	3.53	2.93E-02	14.63	4.00
positive regulation of DNA metabolic process	3.52	2.96E-02	11	3.95
single-organism membrane organization	3.52	2.96E-02	9.38	4.55
adenylate cyclase-inhibiting dopamine receptor signaling pathway	3.51	2.97E-02	40	5.83
astrocyte activation	3.51	2.97E-02	40	3.27
diaphragm development	3.51	2.97E-02	40	5.94
negative regulation of protein export from nucleus	3.51	2.97E-02	40	4.14
regulation of short-term neuronal synaptic plasticity	3.51	2.97E-02	40	3.56
positive regulation of histone H3-K9 methylation	3.51	2.97E-02	40	2.91
complement activation, alternative pathway	3.51	2.97E-02	40	4.80
regulation of interleukin-1 alpha production	3.51	2.97E-02	40	4.36

regulation of somatostatin secretion	3.51	2.97E-02	40	2.77
positive regulation of cellular extravasation	3.51	2.97E-02	40	6.15
response to high density lipoprotein particle	3.51	2.97E-02	40	6.76
maternal process involved in parturition	3.51	2.97E-02	40	5.23
positive regulation of cholesterol storage	3.51	2.97E-02	40	3.97
copper ion transmembrane transport	3.51	2.97E-02	40	6.09
hydrogen peroxide biosynthetic process	3.51	2.97E-02	40	6.61
positive regulation of transcription from RNA polymerase I promoter	3.51	2.97E-02	40	3.53
Schwann cell differentiation	3.51	2.97E-02	40	4.56
post-embryonic hemopoiesis	3.51	2.97E-02	40	3.77
positive regulation of interleukin-6 biosynthetic process	3.51	2.97E-02	40	3.40
skeletal muscle acetylcholine-gated channel clustering	3.51	2.97E-02	40	4.43
regulation of epinephrine secretion	3.51	2.97E-02	40	5.24
positive regulation of glucocorticoid secretion	3.51	2.97E-02	40	3.86
regulation of cortisol secretion	3.51	2.97E-02	40	3.86
regulation of inner ear receptor cell differentiation	3.51	2.97E-02	40	3.77
regulation of mechanoreceptor differentiation	3.51	2.97E-02	40	3.77
comma-shaped body morphogenesis	3.51	2.97E-02	40	3.77
metanephric tubule morphogenesis	3.51	2.97E-02	40	3.77
negative regulation of glycolytic process	3.51	2.97E-02	40	5.30
positive regulation of immature T cell proliferation	3.51	2.97E-02	40	4.43
type I interferon biosynthetic process	3.51	2.97E-02	40	5.05
SMAD protein import into nucleus	3.51	2.97E-02	40	3.77
negative regulation of lamellipodium organization	3.51	2.97E-02	40	5.12
positive regulation of myelination	3.51	2.97E-02	40	5.38
cytoplasmic pattern recognition receptor signaling pathway	3.51	2.97E-02	40	4.52
glycine biosynthetic process	3.51	2.97E-02	40	4.56
lactate transport	3.51	2.97E-02	40	5.08
lactate transmembrane transport	3.51	2.97E-02	40	5.08
plasma membrane lactate transport	3.51	2.97E-02	40	5.08
growth hormone receptor signaling pathway	3.51	2.97E-02	40	4.93
bud elongation involved in lung branching	3.51	2.97E-02	40	3.95
positive regulation of steroid metabolic process	3.5	3.03E-02	19.05	3.10
alpha-beta T cell differentiation	3.5	3.03E-02	19.05	4.82

regulation of oligodendrocyte differentiation	3.5	3.03E-02	19.05	3.84
positive regulation of cellular component biogenesis	3.5	3.03E-02	19.05	3.95
positive regulation of nervous system development	3.5	3.03E-02	19.05	3.95
positive regulation of synapse assembly	3.5	3.03E-02	19.05	3.95
G2 DNA damage checkpoint	3.5	3.03E-02	19.05	3.05
negative regulation of transcription factor import into nucleus	3.5	3.03E-02	19.05	3.37
osteoclast differentiation	3.5	3.03E-02	19.05	4.45
response to immobilization stress	3.5	3.03E-02	19.05	5.17
regulation of fatty acid transport	3.5	3.03E-02	19.05	3.66
positive regulation of alpha-beta T cell differentiation	3.5	3.03E-02	19.05	4.87
regulation of cell growth	3.49	3.04E-02	9.09	3.52
cell cycle process	3.49	3.04E-02	8	3.73
cation transport	3.49	3.04E-02	8	4.28
extrinsic apoptotic signaling pathway	3.49	3.06E-02	12.5	4.84
protein import	3.48	3.08E-02	11.84	3.75
regulation of smooth muscle cell proliferation	3.48	3.08E-02	11.84	3.90
protein activation cascade	3.47	3.11E-02	16.13	5.77
positive regulation of actin filament polymerization	3.47	3.11E-02	16.13	4.26
response to amphetamine	3.47	3.11E-02	16.13	4.56
T cell proliferation	3.47	3.11E-02	16.13	4.20
positive regulation of GTPase activity	3.46	3.15E-02	10.89	4.02
membrane organization	3.45	3.16E-02	9.05	4.62
negative regulation of protein modification process	3.45	3.18E-02	8.94	3.82
negative regulation of cellular response to growth factor stimulus	3.44	3.21E-02	13.21	3.57
fatty acid derivative metabolic process	3.44	3.21E-02	13.21	3.51
icosanoid metabolic process	3.44	3.21E-02	13.21	3.51
calcium ion homeostasis	3.43	3.24E-02	9.28	3.99
regulation of morphogenesis of a branching structure	3.42	3.26E-02	14.29	3.78
cellular response to cAMP	3.42	3.26E-02	14.29	5.59
positive regulation of DNA replication	3.42	3.26E-02	14.29	3.93
cellular response to glucose stimulus	3.42	3.26E-02	14.29	4.83
regulation of cell projection organization	3.42	3.29E-02	8.71	4.85
cellular response to abiotic stimulus	3.4	3.33E-02	9.74	4.20
leukocyte differentiation	3.4	3.34E-02	9.39	4.17
macromolecule glycosylation	3.38	3.40E-02	10.43	4.20
protein glycosylation	3.38	3.40E-02	10.43	4.20
vesicle-mediated transport	3.38	3.40E-02	7.93	4.33

protein processing	3.38	3.40E-02	7.93	4.19
response to activity	3.35	3.51E-02	12.96	5.43
hormone transport	3.35	3.51E-02	12.96	4.13
negative regulation of immune effector process	3.35	3.51E-02	12.96	4.70
regulation of phospholipase activity	3.35	3.52E-02	15.63	4.78
placenta development	3.35	3.52E-02	15.63	4.22
regulation of calcium-mediated signaling	3.35	3.52E-02	15.63	2.99
glutathione metabolic process	3.35	3.52E-02	15.63	5.28
regulation of interleukin-2 production	3.35	3.52E-02	15.63	4.30
cyclic purine nucleotide metabolic process	3.34	3.54E-02	18.18	4.06
cyclic nucleotide biosynthetic process	3.34	3.54E-02	18.18	4.06
response to vitamin A	3.34	3.54E-02	18.18	3.56
negative regulation of cell-substrate adhesion	3.34	3.54E-02	18.18	4.62
regulation of endothelial cell apoptotic process	3.34	3.54E-02	18.18	5.58
apoptotic cell clearance	3.33	3.57E-02	23.08	3.57
myeloid dendritic cell activation	3.33	3.57E-02	23.08	4.30
I-kappaB kinase/NF-kappaB signaling	3.33	3.57E-02	23.08	4.66
positive regulation of release of cytochrome c from mitochondria	3.33	3.57E-02	23.08	2.85
regulation of steroid hormone secretion	3.33	3.57E-02	23.08	3.35
regulation of interleukin-1 beta secretion	3.33	3.57E-02	23.08	3.06
negative regulation of leukocyte migration	3.33	3.57E-02	23.08	4.93
positive regulation of cell adhesion mediated by integrin	3.33	3.57E-02	23.08	4.67
negative regulation of T cell apoptotic process	3.33	3.57E-02	23.08	5.76
positive regulation of triglyceride metabolic process	3.33	3.57E-02	23.08	3.20
regulation of interleukin-2 biosynthetic process	3.33	3.57E-02	23.08	4.01
establishment of chromosome localization	3.33	3.57E-02	23.08	3.78
positive regulation of sterol transport	3.33	3.57E-02	23.08	3.35
positive regulation of cholesterol transport	3.33	3.57E-02	23.08	3.35
regulation of potassium ion transport	3.32	3.61E-02	13.95	4.28
regulation of protein complex disassembly	3.32	3.61E-02	13.95	4.97
cellular response to fibroblast growth factor stimulus	3.32	3.61E-02	13.95	5.35
positive regulation of protein polymerization	3.32	3.61E-02	13.95	4.12
cellular component morphogenesis	3.32	3.62E-02	8.33	4.16
purine nucleotide biosynthetic process	3.29	3.72E-02	10.99	4.05
response to carbohydrate	3.29	3.74E-02	9.79	4.55

cellular calcium ion homeostasis	3.27	3.81E-02	9.24	4.03
peptidyl-tyrosine phosphorylation	3.26	3.83E-02	11.39	4.54
plasma membrane organization	3.26	3.83E-02	12.73	4.61
stem cell differentiation	3.26	3.83E-02	12.73	3.80
regulation of DNA binding	3.26	3.83E-02	12.73	3.82
positive regulation of cellular biosynthetic process	3.25	3.87E-02	7.16	4.31
central nervous system neuron differentiation	3.25	3.88E-02	11.94	4.12
brain development	3.24	3.93E-02	9.36	4.53
positive regulation of lipase activity	3.23	3.96E-02	15.15	4.09
acid secretion	3.23	3.96E-02	15.15	3.54
positive regulation of histone modification	3.23	3.96E-02	15.15	3.51
positive regulation of multi-organism process	3.23	3.96E-02	15.15	3.11
negative regulation of MAP kinase activity	3.22	3.98E-02	13.64	3.59
diterpenoid metabolic process	3.22	3.98E-02	13.64	3.85
positive regulation of T cell differentiation	3.22	3.98E-02	13.64	4.47
cellular response to hexose stimulus	3.22	3.98E-02	13.64	4.83
insulin secretion	3.2	4.09E-02	17.39	4.98
behavioral fear response	3.2	4.09E-02	17.39	3.36
positive regulation of calcium-mediated signaling	3.2	4.09E-02	17.39	3.15
positive regulation of smooth muscle cell migration	3.2	4.09E-02	17.39	3.95
antigen processing and presentation of exogenous antigen	3.2	4.09E-02	17.39	3.87
regulation of interferon-beta production	3.2	4.09E-02	17.39	3.50
regulation of natural killer cell mediated immunity	3.2	4.09E-02	17.39	4.34
regulation of natural killer cell mediated cytotoxicity	3.2	4.09E-02	17.39	4.34
neurological system process	3.19	4.11E-02	7.81	4.21
system development	3.19	4.12E-02	8.06	4.06
activation of cysteine-type endopeptidase activity	3.18	4.17E-02	12.5	3.92
unsaturated fatty acid metabolic process	3.18	4.17E-02	12.5	3.51
monocarboxylic acid transport	3.17	4.18E-02	11.76	4.26
lipid biosynthetic process	3.16	4.23E-02	8.49	4.26
neuron projection development	3.16	4.26E-02	10.08	5.09
negative regulation of apoptotic signaling pathway	3.16	4.26E-02	10.08	4.53
extracellular matrix organization	3.15	4.27E-02	10.38	4.36
adenosine receptor signaling pathway	3.15	4.29E-02	33.33	5.07
response to oleic acid	3.15	4.29E-02	33.33	4.49
skeletal muscle organ development	3.15	4.29E-02	33.33	5.94
cellular response to glucagon stimulus	3.15	4.29E-02	33.33	6.19

cleavage furrow formation	3.15	4.29E-02	33.33	4.27
positive regulation of natural killer cell differentiation	3.15	4.29E-02	33.33	4.80
amino-acid betaine biosynthetic process	3.15	4.29E-02	33.33	3.63
regulation of protein activation cascade	3.15	4.29E-02	33.33	7.17
regulation of complement activation	3.15	4.29E-02	33.33	7.17
cerebellar granule cell differentiation	3.15	4.29E-02	33.33	4.89
regulation of glial cell apoptotic process	3.15	4.29E-02	33.33	5.08
negative regulation of glial cell apoptotic process	3.15	4.29E-02	33.33	5.08
cellular response to hydroperoxide	3.15	4.29E-02	33.33	4.86
nucleoside diphosphate phosphorylation	3.15	4.29E-02	33.33	2.80
regulation of monocyte differentiation	3.15	4.29E-02	33.33	3.61
short-term memory	3.15	4.29E-02	33.33	3.55
post-embryonic organ development	3.15	4.29E-02	33.33	3.77
NADPH oxidation	3.15	4.29E-02	33.33	3.46
cytoplasm organization	3.15	4.29E-02	33.33	3.19
positive regulation of potassium ion transmembrane transporter activity	3.15	4.29E-02	33.33	4.71
receptor guanylyl cyclase signaling pathway	3.15	4.29E-02	33.33	2.81
neuron fate determination	3.15	4.29E-02	33.33	3.77
S-shaped body morphogenesis	3.15	4.29E-02	33.33	3.77
heterochromatin assembly	3.15	4.29E-02	33.33	3.24
mitotic G2 DNA damage checkpoint	3.15	4.29E-02	33.33	2.94
negative regulation of intrinsic apoptotic signaling pathway in response to oxidative stress	3.15	4.29E-02	33.33	6.47
response to ozone	3.15	4.29E-02	33.33	3.63
ectopic germ cell programmed cell death	3.15	4.29E-02	33.33	3.63
regulation of T-helper 1 cell differentiation	3.15	4.29E-02	33.33	4.51
positive regulation of chemokine secretion	3.15	4.29E-02	33.33	3.70
positive regulation of corticotropin secretion	3.15	4.29E-02	33.33	3.69
negative regulation of peptidyl-threonine phosphorylation	3.15	4.29E-02	33.33	5.06
mesenchymal-epithelial cell signaling	3.15	4.29E-02	33.33	3.75
cellular response to zinc ion	3.15	4.29E-02	33.33	4.01
negative regulation of macroautophagy	3.15	4.29E-02	33.33	5.99
negative regulation of norepinephrine secretion	3.15	4.29E-02	33.33	3.25
cellular response to granulocyte macrophage colony-stimulating factor stimulus	3.15	4.29E-02	33.33	3.48

response to granulocyte macrophage colony-stimulating factor	3.15	4.29E-02	33.33	3.48
rRNA transport	3.15	4.29E-02	33.33	4.95
antigen processing and presentation via MHC class Ib	3.15	4.29E-02	33.33	3.66
positive regulation of necrotic cell death	3.15	4.29E-02	33.33	3.55
L-serine metabolic process	3.15	4.29E-02	33.33	4.56
negative regulation of Ras GTPase activity	3.15	4.29E-02	33.33	3.95
oligopeptide transmembrane transport	3.15	4.29E-02	33.33	4.48
cellular response to exogenous dsRNA	3.15	4.29E-02	33.33	3.39
organic substance catabolic process	3.14	4.33E-02	7.15	4.23
nucleobase-containing small molecule metabolic process	3.14	4.33E-02	7.85	4.30
protein maturation	3.14	4.34E-02	7.78	4.19
negative regulation of growth	3.14	4.34E-02	9.59	3.75
inositol phosphate metabolic process	3.13	4.35E-02	21.43	3.81
ovulation cycle	3.13	4.35E-02	21.43	4.34
mechanoreceptor differentiation	3.13	4.35E-02	21.43	3.39
positive regulation vascular endothelial growth factor production	3.13	4.35E-02	21.43	4.84
negative regulation of I-kappaB kinase/NF-kappaB signaling	3.13	4.35E-02	21.43	4.16
regulation of macrophage differentiation	3.13	4.35E-02	21.43	4.45
positive regulation of NF-kappaB import into nucleus	3.13	4.35E-02	21.43	3.19
regulation of monocyte chemotaxis	3.13	4.35E-02	21.43	6.62
regulation of heat generation	3.13	4.35E-02	21.43	4.87
protein homotrimerization	3.13	4.35E-02	21.43	5.41
hexose catabolic process	3.13	4.35E-02	21.43	3.33
transepithelial transport	3.13	4.35E-02	21.43	4.65
regulation of catecholamine metabolic process	3.13	4.35E-02	21.43	4.23
negative regulation of GTPase activity	3.13	4.35E-02	21.43	4.72
intracellular pH reduction	3.13	4.35E-02	21.43	5.23
regulation of JUN kinase activity	3.13	4.38E-02	13.33	4.70
response to fibroblast growth factor	3.13	4.38E-02	13.33	5.35
pyridine-containing compound metabolic process	3.13	4.38E-02	13.33	4.24
cellular response to monosaccharide stimulus	3.13	4.38E-02	13.33	4.83
peptidyl-tyrosine modification	3.13	4.39E-02	11.11	4.54
positive regulation of cysteine-type endopeptidase activity involved in apoptotic process	3.13	4.39E-02	11.11	4.17
adenylate cyclase-inhibiting G-protein coupled receptor signaling pathway	3.12	4.43E-02	14.71	5.22

positive regulation of interferon-gamma production	3.12	4.43E-02	14.71	4.34
positive regulation of alpha-beta T cell activation	3.12	4.43E-02	14.71	4.63
regulation of Notch signaling pathway	3.12	4.43E-02	14.71	4.18
stem cell proliferation	3.12	4.43E-02	14.71	4.77
negative regulation of leukocyte activation	3.1	4.49E-02	10.64	3.76
positive regulation of endopeptidase activity	3.1	4.49E-02	10.64	4.15
positive regulation of cell cycle process	3.1	4.49E-02	10	3.89
cellular response to inorganic substance	3.1	4.52E-02	10.28	4.27
extracellular structure organization	3.1	4.52E-02	10.28	4.36
cell recognition	3.1	4.52E-02	12.28	4.03
regulation of fat cell differentiation	3.1	4.52E-02	12.28	4.67
regulation of neuron projection development	3.09	4.54E-02	8.91	4.62
learning	3.06	4.69E-02	10.98	3.57
negative regulation of cell-cell adhesion	3.06	4.69E-02	16.67	4.68
positive regulation of acute inflammatory response	3.06	4.69E-02	16.67	5.46
positive regulation of Ras protein signal transduction	3.06	4.69E-02	16.67	4.17
ERBB signaling pathway	3.06	4.69E-02	16.67	3.92
pituitary gland development	3.06	4.69E-02	16.67	3.59
response to monosaccharide	3.05	4.74E-02	9.92	4.82
negative regulation of cellular protein metabolic process	3.05	4.76E-02	8.18	3.91
regulation of cellular carbohydrate metabolic process	3.04	4.77E-02	10.53	3.94
leukocyte homeostasis	3.04	4.80E-02	13.04	4.73
regulation of neural precursor cell proliferation	3.04	4.80E-02	13.04	4.03
DNA metabolic process	3.03	4.81E-02	7.86	3.48
regulation of anatomical structure size	3.03	4.85E-02	9.26	4.10
negative regulation of RNA biosynthetic process	3.02	4.90E-02	7.42	4.00
cellular amine metabolic process	3.02	4.90E-02	12.07	4.29
cellular biogenic amine metabolic process	3.02	4.90E-02	12.07	4.29
positive regulation of JUN kinase activity	3.01	4.93E-02	14.29	4.69
regulation of cellular response to insulin stimulus	3.01	4.93E-02	14.29	3.34
purine-containing compound metabolic process	3	4.96E-02	8.08	4.36

8.2.2.2 Significantly altered biological processes by Panther

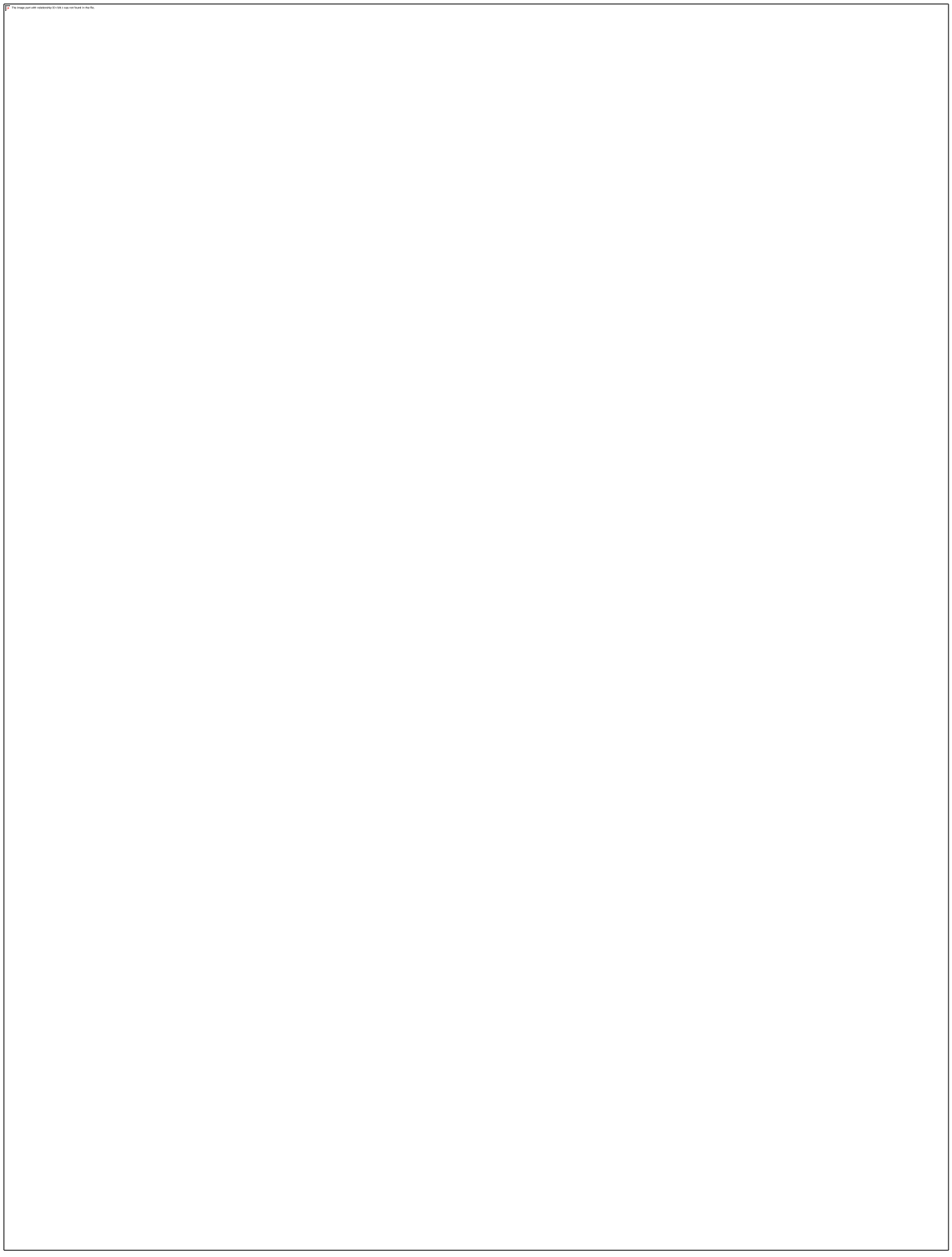

Figure 4.4.2a: Significantly altered biological processes*. Panther analysis of differentially expressed gene list in serum alone treated cultures compared to control.*

8.2.2.3 *Table 4.4.2b: Enriched molecular function by Partek Genomic Suite. Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the serum treated cultures in comparison to control cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	C+ vs C- score
chemokine receptor binding	33.97	1.76E-15	52.63	5.38
chemokine activity	33.05	4.45E-15	58.06	5.32
cytokine receptor binding	24.98	1.42E-11	21.21	4.80
binding	24.53	2.23E-11	6.95	4.21
protein binding	24.06	3.54E-11	7.90	4.16
CCR chemokine receptor binding	19.88	2.32E-09	62.50	5.08
receptor binding	19.31	4.11E-09	10.49	4.14
identical protein binding	17.95	1.60E-08	10.85	4.01
G-protein coupled receptor binding	15.64	1.62E-07	16.57	4.78
cytokine activity	14.82	3.68E-07	18.46	4.95
CCR5 chemokine receptor binding	12.52	3.66E-06	83.33	5.20
CCR1 chemokine receptor binding	11.41	1.11E-05	100.00	5.94
2'-5'-oligoadenylate synthetase activity	11.41	1.11E-05	100.00	3.55
carbohydrate derivative binding	10.77	2.11E-05	8.31	4.32
protein homodimerization activity	10.41	3.02E-05	10.58	4.05
catalytic activity	9.89	5.09E-05	7.07	4.22
CXCR3 chemokine receptor binding	9.84	5.31E-05	80.00	5.68
glycosaminoglycan binding	9.55	7.15E-05	15.57	4.68
anion transmembrane transporter activity	9.37	8.55E-05	13.56	4.46
ion binding	9.03	1.20E-04	7.03	4.29
chemoattractant activity	8.63	1.79E-04	37.50	4.10
protein dimerization activity	8.61	1.82E-04	8.89	4.02
anion binding	8.55	1.93E-04	7.74	4.25
TAP binding	8.55	1.93E-04	100.00	4.72
TAP1 binding	8.55	1.93E-04	100.00	4.72
TAP2 binding	8.55	1.93E-04	100.00	4.72
low-density lipoprotein receptor activity	7.99	3.38E-04	57.14	4.40
CXCR chemokine receptor binding	7.99	3.38E-04	57.14	5.68
double-stranded RNA binding	7.96	3.49E-04	20.83	3.51
secondary active transmembrane transporter activity	7.84	3.95E-04	14.53	4.48
peptide binding	7.75	4.32E-04	12.96	3.98
amide binding	7.50	5.53E-04	12.73	3.98
carboxylic acid transmembrane transporter activity	7.49	5.61E-04	16.46	4.14
organic acid transmembrane transporter activity	7.36	6.35E-04	16.25	4.14
metalloenzyme regulator activity	7.35	6.46E-04	50.00	3.87
organic anion transmembrane transporter activity	7.20	7.48E-04	14.71	4.18
collagen binding	7.06	8.63E-04	22.22	3.12
carbohydrate binding	6.94	9.68E-04	11.54	4.25

purinergic nucleotide receptor activity	6.74	1.18E-03	33.33	3.43
nucleotide receptor activity	6.74	1.18E-03	33.33	3.43
nucleoside binding	6.53	1.45E-03	7.82	4.21
hyaluronic acid binding	6.41	1.64E-03	31.25	4.44
CCR2 chemokine receptor binding	6.34	1.77E-03	60.00	6.57
ribonucleoside binding	6.33	1.79E-03	7.78	4.19
symporter activity	6.07	2.30E-03	14.81	5.08
nucleotidyltransferase activity	6.04	2.38E-03	14.13	4.06
transferase activity, transferring pentosyl groups	6.03	2.41E-03	21.21	4.59
anion:cation symporter activity	6.03	2.41E-03	21.21	5.19
purine ribonucleoside binding	6.02	2.42E-03	7.72	4.20
purine nucleoside binding	5.98	2.54E-03	7.71	4.20
retinal binding	5.93	2.65E-03	36.36	4.01
lipoprotein particle receptor activity	5.93	2.65E-03	36.36	4.40
transition metal ion binding	5.88	2.78E-03	7.96	4.31
heparin binding	5.85	2.88E-03	13.83	4.63
adenylyltransferase activity	5.84	2.92E-03	27.78	4.08
transferase activity, transferring phosphorus-containing groups	5.81	3.00E-03	8.48	4.07
cargo receptor activity	5.70	3.36E-03	18.18	4.38
adenyl ribonucleotide binding	5.70	3.36E-03	7.88	4.05
adenyl nucleotide binding	5.69	3.37E-03	7.87	4.06
intermediate filament binding	5.69	3.38E-03	50.00	3.59
metalloenzyme inhibitor activity	5.69	3.38E-03	50.00	4.41
metalloendopeptidase inhibitor activity	5.69	3.38E-03	50.00	4.41
sulfur compound binding	5.68	3.40E-03	12.31	4.73
purinergic receptor activity	5.58	3.77E-03	26.32	3.43
interleukin-1 receptor binding	5.58	3.79E-03	33.33	5.06
protease binding	5.52	4.01E-03	16.36	5.39
purine nucleotide binding	5.51	4.06E-03	7.57	4.20
purine ribonucleotide binding	5.50	4.09E-03	7.58	4.20
transferase activity	5.47	4.22E-03	7.54	4.12
active transmembrane transporter activity	5.41	4.47E-03	10.77	4.47
amino acid transmembrane transporter activity	5.39	4.54E-03	16.07	4.07
peptide antigen binding	5.35	4.74E-03	18.92	3.85
phosphoric diester hydrolase activity	5.27	5.12E-03	15.79	4.07
ribonucleotide binding	5.23	5.36E-03	7.50	4.20
growth factor binding	5.19	5.55E-03	13.33	3.73
L-amino acid transmembrane transporter activity	5.19	5.56E-03	20.69	3.92
hydroxymethyl-, formyl- and related transferase activity	5.17	5.67E-03	42.86	4.99
phospholipase activator activity	5.17	5.67E-03	42.86	4.30
polypeptide N-acetylgalactosaminyltransferase activity	5.17	5.67E-03	42.86	4.31

oligopeptide transporter activity	5.17	5.67E-03	42.86	4.63
ATP binding	5.10	6.08E-03	7.75	4.07
transferase activity, transferring glycosyl groups	5.10	6.10E-03	10.86	4.27
purine ribonucleoside triphosphate binding	5.09	6.14E-03	7.50	4.22
drug binding	5.02	6.62E-03	13.04	3.74
small molecule binding	4.98	6.86E-03	7.13	4.28
MHC protein binding	4.96	6.98E-03	28.57	3.79
cell adhesion molecule binding	4.89	7.50E-03	16.00	3.91
neuropeptide binding	4.75	8.68E-03	37.50	4.61
G-protein coupled nucleotide receptor activity	4.75	8.68E-03	37.50	3.78
G-protein coupled purinergic nucleotide receptor activity	4.75	8.68E-03	37.50	3.78
peptide transporter activity	4.75	8.68E-03	37.50	4.63
purine nucleobase binding	4.64	9.65E-03	66.67	4.71
CCR10 chemokine receptor binding	4.64	9.65E-03	66.67	2.57
calcium-dependent protein kinase activity	4.64	9.65E-03	66.67	2.56
calcium-dependent protein kinase C activity	4.64	9.65E-03	66.67	2.56
calcium-dependent protein serine/threonine kinase activity	4.64	9.65E-03	66.67	2.56
oleic acid binding	4.64	9.65E-03	66.67	3.20
oxidoreductase activity, acting on the CH-OH group of donors, quinone or similar compound as acceptor	4.64	9.65E-03	66.67	6.52
nuclear localization sequence binding	4.64	9.65E-03	66.67	4.14
citrate transmembrane transporter activity	4.64	9.65E-03	66.67	4.51
succinate transmembrane transporter activity	4.64	9.65E-03	66.67	4.51
oligopeptide-transporting ATPase activity	4.64	9.65E-03	66.67	4.48
peptide-transporting ATPase activity	4.64	9.65E-03	66.67	4.48
MHC class Ib protein binding	4.64	9.65E-03	66.67	4.48
enzyme inhibitor activity	4.56	1.05E-02	9.70	4.29
cytokine binding	4.43	1.19E-02	14.81	3.61
solute:cation symporter activity	4.43	1.19E-02	14.81	5.36
transmembrane receptor protein tyrosine kinase activity	4.39	1.24E-02	15.91	4.36
adenylate cyclase binding	4.38	1.25E-02	33.33	5.25
lipase activator activity	4.38	1.25E-02	33.33	4.30
MHC class I protein binding	4.38	1.25E-02	33.33	4.19
amide transmembrane transporter activity	4.38	1.25E-02	33.33	3.95
inorganic anion transmembrane transporter activity	4.34	1.30E-02	12.99	5.10
pyridoxal phosphate binding	4.26	1.42E-02	17.14	3.88
acetylgalactosaminyltransferase activity	4.24	1.45E-02	23.53	4.02
fibronectin binding	4.24	1.45E-02	23.53	3.36

cofactor binding	4.24	1.45E-02	9.68	4.57
kinase inhibitor activity	4.18	1.53E-02	19.23	3.53
cytokine receptor activity	4.12	1.62E-02	14.04	4.02
protein heterodimerization activity	4.11	1.64E-02	8.72	3.63
oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	4.10	1.66E-02	10.39	3.97
UDP-galactosyltransferase activity	4.03	1.78E-02	22.22	3.93
protein phosphatase inhibitor activity	4.03	1.78E-02	22.22	3.16
single-stranded RNA binding	4.02	1.79E-02	18.52	4.12
iron ion binding	3.99	1.85E-02	10.06	4.44
nucleobase binding	3.99	1.86E-02	50.00	4.71
immunoglobulin receptor binding	3.99	1.86E-02	50.00	5.27
5'-deoxyribose-5-phosphate lyase activity	3.99	1.86E-02	50.00	3.24
3'-5'-exodeoxyribonuclease activity	3.99	1.86E-02	50.00	4.45
CARD domain binding	3.99	1.86E-02	50.00	4.33
tricarboxylic acid transmembrane transporter activity	3.99	1.86E-02	50.00	4.51
secondary active monocarboxylate transmembrane transporter activity	3.99	1.86E-02	50.00	5.08
tumor necrosis factor-activated receptor activity	3.99	1.86E-02	50.00	4.55
transporter activity	3.87	2.09E-02	7.50	4.32
DNA-directed DNA polymerase activity	3.84	2.15E-02	21.05	3.69
phosphatase inhibitor activity	3.84	2.15E-02	21.05	3.16
neutral amino acid transmembrane transporter activity	3.84	2.15E-02	21.05	3.70
phosphatidylinositol phospholipase C activity	3.80	2.25E-02	27.27	4.39
low-density lipoprotein particle binding	3.80	2.25E-02	27.27	3.72
signal sequence binding	3.80	2.25E-02	27.27	3.49
protein complex binding	3.76	2.32E-02	7.87	3.83
vitamin binding	3.73	2.41E-02	14.00	4.36
small GTPase binding	3.68	2.51E-02	10.91	4.63
cadherin binding	3.66	2.57E-02	20.00	3.51
threonine-type endopeptidase activity	3.66	2.57E-02	20.00	5.08
threonine-type peptidase activity	3.66	2.57E-02	20.00	5.08
substrate-specific transporter activity	3.65	2.60E-02	7.60	4.35
metalloendopeptidase activity	3.63	2.65E-02	12.16	3.97
hydrolase activity	3.58	2.78E-02	6.86	4.27
phospholipase C activity	3.55	2.87E-02	25.00	4.39
snRNA binding	3.55	2.87E-02	25.00	4.40
nucleotide binding	3.53	2.92E-02	6.89	4.23
nucleoside phosphate binding	3.53	2.92E-02	6.89	4.23
opsonin binding	3.51	2.97E-02	40.00	4.62
cAMP response element binding	3.51	2.97E-02	40.00	2.83
sphingosine-1-phosphate receptor	3.51	2.97E-02	40.00	5.71

activity				
C4-dicarboxylate transmembrane transporter activity	3.51	2.97E-02	40.00	4.51
iron ion transmembrane transporter activity	3.51	2.97E-02	40.00	6.51
death receptor activity	3.51	2.97E-02	40.00	4.55
organic acid:sodium symporter activity	3.50	3.03E-02	19.05	5.50
antiporter activity	3.47	3.11E-02	16.13	3.49
regulatory region nucleic acid binding	3.47	3.12E-02	8.59	4.11
regulatory region DNA binding	3.47	3.12E-02	8.59	4.11
transmembrane receptor protein kinase activity	3.44	3.21E-02	13.21	4.36
kinase activity	3.40	3.34E-02	7.77	4.12
lipid binding	3.38	3.41E-02	8.10	4.29
retinoid binding	3.34	3.54E-02	18.18	4.01
DNA polymerase activity	3.34	3.54E-02	18.18	3.69
NF-kappaB binding	3.34	3.54E-02	18.18	4.34
monocarboxylic acid transmembrane transporter activity	3.34	3.54E-02	18.18	4.80
calmodulin binding	3.33	3.57E-02	11.54	4.01
Ras GTPase binding	3.33	3.57E-02	10.68	4.51
GABA receptor binding	3.33	3.57E-02	23.08	4.90
carboxylic acid binding	3.30	3.67E-02	9.62	4.36
phosphotransferase activity, alcohol group as acceptor	3.22	4.01E-02	7.79	4.14
organic acid binding	3.21	4.04E-02	9.49	4.36
protein kinase inhibitor activity	3.20	4.09E-02	17.39	3.80
steroid hydroxylase activity	3.20	4.09E-02	17.39	3.37
RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity	3.19	4.10E-02	11.25	3.59
transcription regulatory region DNA binding	3.18	4.18E-02	8.42	4.07
metallopeptidase activity	3.16	4.26E-02	10.08	4.00
calcium- and calmodulin-responsive adenylate cyclase activity	3.15	4.29E-02	33.33	5.57
receptor antagonist activity	3.15	4.29E-02	33.33	6.90
GTP-Rho binding	3.15	4.29E-02	33.33	4.36
axon guidance receptor activity	3.15	4.29E-02	33.33	6.24
N-methyl-D-aspartate selective glutamate receptor activity	3.15	4.29E-02	33.33	5.43
glucose binding	3.15	4.29E-02	33.33	3.39
3',5'-cyclic-GMP phosphodiesterase activity	3.15	4.29E-02	33.33	3.25
cation:chloride symporter activity	3.15	4.29E-02	33.33	4.88
sodium:dicarboxylate symporter activity	3.15	4.29E-02	33.33	4.51
oligopeptide transmembrane transporter activity	3.15	4.29E-02	33.33	4.48
enzyme binding	3.15	4.30E-02	7.17	4.34

glutamate receptor activity	3.13	4.35E-02	21.43	4.59
sequence-specific DNA binding RNA polymerase II transcription factor activity	3.13	4.36E-02	8.96	4.19
transmembrane transporter activity	3.12	4.41E-02	7.43	4.31
scavenger receptor activity	3.12	4.43E-02	14.71	5.19
calcium ion binding	3.11	4.45E-02	7.83	4.34
UDP-glycosyltransferase activity	3.10	4.50E-02	11.59	4.12
hydrolase activity, acting on acid anhydrides	3.09	4.54E-02	7.65	4.21
nucleoside-triphosphatase activity	3.06	4.69E-02	7.73	4.16
GTPase binding	3.05	4.74E-02	9.92	4.63
chloride transmembrane transporter activity	3.02	4.90E-02	12.07	5.14
solute:sodium symporter activity	3.01	4.93E-02	14.29	5.45
zinc ion binding	3.01	4.94E-02	7.29	4.04

8.2.2.4 *Table 4.4.2c: Cellular components by Partek Genomic Suite. Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the serum treated cultures in comparison to control cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	C+ vs C- score
extracellular region part	32.34	9.02E-15	9.93	4.55
extracellular space	30.40	6.29E-14	12.78	4.60
cytoplasm	22.74	1.33E-10	8.46	4.14
receptor complex	15.89	1.26E-07	16.76	4.66
vesicle	15.03	2.96E-07	8.69	4.69
plasma membrane part	13.99	8.43E-07	9.13	4.39
membrane-bounded vesicle	13.47	1.41E-06	8.58	4.72
extracellular vesicular exosome	11.86	7.07E-06	8.85	4.80
extracellular organelle	11.82	7.34E-06	8.84	4.80
extracellular membrane-bounded organelle	11.82	7.34E-06	8.84	4.80
cell surface	10.91	1.83E-05	11.91	4.16
intracellular part	9.66	6.41E-05	6.43	4.10
myelin sheath	8.63	1.79E-04	37.50	5.23
AIM2 inflammasome complex	8.55	1.93E-04	100.00	4.32
TAP complex	8.55	1.93E-04	100.00	4.72
membrane-bounded organelle	8.55	1.93E-04	6.62	4.19
membrane raft	8.42	2.19E-04	12.77	4.66
extracellular region	8.19	2.77E-04	9.27	4.01
organelle	7.71	4.47E-04	6.49	4.18
blood microparticle	7.36	6.35E-04	16.25	6.04
cellular_component	7.25	7.13E-04	6.00	4.16
I-kappaB/NF-kappaB complex	7.21	7.39E-04	75.00	4.90
NLRP3 inflammasome complex	7.21	7.39E-04	75.00	4.32
symbiont-containing vacuole membrane	7.21	7.39E-04	75.00	3.69
cell pole	6.80	1.11E-03	44.44	3.60
MCM complex	6.80	1.11E-03	44.44	2.65
basolateral plasma membrane	6.79	1.12E-03	13.28	4.31
side of membrane	6.67	1.26E-03	11.32	4.23
external side of plasma membrane	6.51	1.48E-03	11.80	4.26
host cell part	6.34	1.77E-03	60.00	3.69
other organism part	6.34	1.77E-03	60.00	3.69
spermatoproteasome complex	6.34	1.77E-03	60.00	5.65
perinuclear region of cytoplasm	6.26	1.91E-03	9.70	4.17
neuron part	5.79	3.06E-03	8.25	4.21
basal plasma membrane	5.77	3.13E-03	23.08	3.89
cell projection	5.63	3.59E-03	7.98	4.26
cytoplasmic part	5.48	4.17E-03	6.56	4.15
plasma membrane region	5.48	4.19E-03	9.65	4.04
leading edge membrane	5.28	5.08E-03	14.10	5.04
membrane region	5.28	5.08E-03	8.29	4.06

cell tip	5.17	5.67E-03	42.86	3.62
intracellular membrane-bounded organelle	4.87	7.70E-03	6.42	4.08
BRCA1-BARD1 complex	4.64	9.65E-03	66.67	2.69
NLRP1 inflammasome complex	4.64	9.65E-03	66.67	3.30
astrocyte projection	4.64	9.65E-03	66.67	3.73
MHC class I peptide loading complex	4.64	9.65E-03	66.67	4.45
extracellular matrix	4.63	9.72E-03	10.05	4.15
dendrite	4.44	1.17E-02	9.73	4.53
ruffle	4.33	1.32E-02	14.55	3.39
intracellular organelle	4.30	1.36E-02	6.29	4.08
proteinaceous extracellular matrix	4.26	1.42E-02	11.11	4.08
neuron projection membrane	4.18	1.53E-02	19.23	5.52
integral component of plasma membrane	4.14	1.59E-02	9.35	4.72
podosome	4.07	1.71E-02	30.00	4.53
cytoplasmic vesicle	4.01	1.82E-02	7.96	4.52
IPAF inflammasome complex	3.99	1.86E-02	50.00	4.55
ripoptosome	3.99	1.86E-02	50.00	3.77
intracellular	3.94	1.94E-02	7.81	4.51
cell body	3.92	1.98E-02	8.72	4.46
filopodium	3.87	2.08E-02	15.79	4.29
proteasome core complex	3.84	2.15E-02	21.05	5.08
collagen	3.73	2.41E-02	14.00	2.78
integrin complex	3.66	2.57E-02	20.00	4.05
replication fork	3.66	2.57E-02	20.00	3.33
DNA polymerase complex	3.55	2.87E-02	25.00	3.72
lipopolysaccharide receptor complex	3.51	2.97E-02	40.00	5.66
glial cell projection	3.51	2.97E-02	40.00	3.73
proteasome activator complex	3.51	2.97E-02	40.00	4.62
synaptic membrane	3.45	3.17E-02	9.80	4.37
nucleus	3.40	3.32E-02	6.49	3.98
neuron projection	3.40	3.34E-02	7.77	4.39
synapse part	3.36	3.47E-02	8.50	4.07
cytosol	3.35	3.52E-02	7.30	4.10
microvillus membrane	3.33	3.57E-02	23.08	4.25
perikaryon	3.18	4.17E-02	12.50	4.88
basal part of cell	3.15	4.29E-02	33.33	4.75
nucleotide-excision repair complex	3.15	4.29E-02	33.33	3.37
cell-cell junction	3.14	4.31E-02	8.64	3.91
organelle membrane	3.13	4.39E-02	6.91	4.50
cell part	3.01	4.93E-02	5.98	4.15

8.2.3 Gene ontology clustering of cultures treated with O4 antibody in the presence of serum

8.2.3.1 *Table 4.4.3a: Significantly enriched biological processes by Partek Genomic Suite. Gene ontology clustering was performed on the genes differentially regulated by fold change of 1.4 or greater in the mAb O4 and serum treated cultures in comparison to serum only cultures.*

Function	Enrichment Score	Enrichment p-value	% genes present in group	O4+ vs C+ score
regulation of toll-like receptor signaling pathway	13.09	2.07E-06	11.54	4.66
central nervous system myelin maintenance	12.78	2.81E-06	66.67	5.96
ensheathment of neurons	10.58	2.53E-05	5.08	5.13
axon ensheathment	10.58	2.53E-05	5.08	5.13
myelin maintenance	10.08	4.19E-05	20.00	5.96
positive regulation of toll-like receptor signaling pathway	9.70	6.14E-05	16.67	4.78
oligodendrocyte development	9.10	1.11E-04	12.50	4.26
glial cell development	7.51	5.46E-04	5.71	4.26
epidermal cell differentiation	6.84	1.07E-03	4.08	6.12
plasma membrane organization	6.61	1.35E-03	3.64	5.96
myelination	6.57	1.40E-03	3.57	5.43
sphingolipid metabolic process	5.92	2.69E-03	2.56	7.24
propionate metabolic process	5.81	3.01E-03	33.33	4.41
N-terminal protein lipidation	5.81	3.01E-03	33.33	6.86
negative regulation of lamellipodium assembly	5.81	3.01E-03	33.33	7.15
developmental process	5.68	3.42E-03	0.27	5.78
short-chain fatty acid catabolic process	5.52	4.01E-03	25.00	4.41
regulation of toll-like receptor 2 signaling pathway	5.52	4.01E-03	25.00	4.41
cellular response to progesterone stimulus	5.52	4.01E-03	25.00	4.41
galactolipid metabolic process	5.52	4.01E-03	25.00	6.72
paranodal junction assembly	5.52	4.01E-03	25.00	6.72
galactosylceramide metabolic process	5.52	4.01E-03	25.00	6.72
membrane lipid metabolic process	5.41	4.47E-03	1.98	7.24
negative regulation of toll-like receptor 4 signaling pathway	5.30	5.01E-03	20.00	4.41
positive regulation of myelination	5.30	5.01E-03	20.00	4.14
negative regulation of lamellipodium organization	5.30	5.01E-03	20.00	7.15
positive regulation of immune system process	5.19	5.55E-03	0.80	4.66
calcium-independent cell-cell adhesion	5.11	6.01E-03	16.67	4.54
negative regulation of lipopolysaccharide-mediated signaling pathway	5.11	6.01E-03	16.67	4.41
cellular response to lipopolysaccharide	5.06	6.35E-03	1.65	4.26
cellular response to molecule of bacterial origin	5.00	6.76E-03	1.60	4.26

peripheral nervous system myelin maintenance	4.96	7.01E-03	14.29	7.77
tolerance induction	4.96	7.01E-03	14.29	4.41
negative regulation of type I interferon production	4.96	7.01E-03	14.29	4.41
positive regulation of neurological system process	4.96	7.01E-03	14.29	4.14
cellular response to biotic stimulus	4.83	7.96E-03	1.47	4.26
cellular response to interferon-beta	4.83	8.00E-03	12.50	4.41
central nervous system myelination	4.83	8.00E-03	12.50	4.14
axon ensheathment in central nervous system	4.83	8.00E-03	12.50	4.14
glycosylceramide metabolic process	4.83	8.00E-03	12.50	6.72
cell adhesion	4.77	8.49E-03	0.69	5.83
biological adhesion	4.76	8.54E-03	0.69	5.83
short-chain fatty acid metabolic process	4.61	1.00E-02	10.00	4.41
glycosphingolipid biosynthetic process	4.61	1.00E-02	10.00	6.72
positive regulation of oligodendrocyte differentiation	4.61	1.00E-02	10.00	4.37
positive regulation of humoral immune response	4.51	1.10E-02	9.09	4.41
regulation of toll-like receptor 4 signaling pathway	4.51	1.10E-02	9.09	4.41
keratinization	4.51	1.10E-02	9.09	4.47
response to interferon-beta	4.42	1.20E-02	8.33	4.41
N-terminal protein amino acid modification	4.42	1.20E-02	8.33	6.86
regulation of multicellular organismal process	4.37	1.27E-02	0.33	4.96
peptidyl-cysteine modification	4.34	1.30E-02	7.69	6.86
cellular lipid metabolic process	4.34	1.30E-02	0.59	6.30
regulation of lipopolysaccharide-mediated signaling pathway	4.27	1.40E-02	7.14	4.41
negative regulation of toll-like receptor signaling pathway	4.27	1.40E-02	7.14	4.41
negative regulation of GTPase activity	4.27	1.40E-02	7.14	7.15
cellular developmental process	4.21	1.48E-02	0.32	5.50
regulation of hair cycle	4.20	1.50E-02	6.67	7.77
negative regulation of microtubule depolymerization	4.20	1.50E-02	6.67	6.86
single-organism membrane organization	4.17	1.54E-02	1.04	5.96
negative regulation of response to biotic stimulus	4.14	1.60E-02	6.25	4.41
semaphorin-plexin signaling pathway	4.14	1.60E-02	6.25	7.15
fatty acid metabolic process	4.12	1.63E-02	1.01	6.09
regulation of lamellipodium assembly	4.08	1.69E-02	5.88	7.15
epithelial cell differentiation	4.05	1.74E-02	0.98	6.12
regulation of microtubule depolymerization	4.02	1.79E-02	5.56	6.86
positive regulation of interleukin-8 production	4.02	1.79E-02	5.56	4.11

single organism reproductive process	3.92	1.99E-02	0.50	4.35
regulation of humoral immune response	3.92	1.99E-02	5.00	4.41
protein palmitoylation	3.92	1.99E-02	5.00	6.86
regulation of myelination	3.92	1.99E-02	5.00	4.14
oligodendrocyte differentiation	3.92	1.99E-02	5.00	4.14
membrane organization	3.91	2.01E-02	0.90	5.96
positive regulation of response to biotic stimulus	3.87	2.09E-02	4.76	4.41
negative regulation of innate immune response	3.87	2.09E-02	4.76	4.41
negative regulation of microtubule polymerization or depolymerization	3.87	2.09E-02	4.76	6.86
peptide cross-linking	3.87	2.09E-02	4.76	4.47
regulation of oligodendrocyte differentiation	3.87	2.09E-02	4.76	4.37
regulation of lamellipodium organization	3.82	2.19E-02	4.55	7.15
regulation of immune system process	3.81	2.21E-02	0.48	4.66
positive regulation of glial cell differentiation	3.78	2.29E-02	4.35	4.37
positive chemotaxis	3.74	2.38E-02	4.17	7.15
ceramide biosynthetic process	3.70	2.48E-02	4.00	6.72
glycosphingolipid metabolic process	3.70	2.48E-02	4.00	6.72
positive regulation of cellular process	3.70	2.48E-02	0.24	4.94
negative regulation of protein depolymerization	3.66	2.58E-02	3.85	6.86
regulation of type I interferon production	3.62	2.68E-02	3.70	4.41
axon cargo transport	3.62	2.68E-02	3.70	6.72
response to lipopolysaccharide	3.62	2.68E-02	0.78	4.26
cellular response to lipid	3.61	2.70E-02	0.77	4.26
lipid metabolic process	3.61	2.70E-02	0.45	6.30
cellular response to interferon-gamma	3.58	2.78E-02	3.57	4.41
negative regulation of protein complex disassembly	3.58	2.78E-02	3.57	6.86
positive regulation of reactive oxygen species metabolic process	3.55	2.87E-02	3.45	4.41
positive regulation of nitric oxide biosynthetic process	3.55	2.87E-02	3.45	4.11
glycolipid biosynthetic process	3.55	2.87E-02	3.45	6.72
monocarboxylic acid metabolic process	3.55	2.88E-02	0.75	6.09
microtubule-based process	3.55	2.88E-02	0.75	6.79
response to molecule of bacterial origin	3.54	2.90E-02	0.74	4.26
lipid biosynthetic process	3.53	2.94E-02	0.74	7.24
reproductive process	3.48	3.08E-02	0.43	4.35
anatomical structure development	3.47	3.12E-02	0.27	5.46
positive regulation of multi-organism process	3.42	3.26E-02	3.03	4.41
substantia nigra development	3.42	3.26E-02	3.03	7.51

keratinocyte differentiation	3.42	3.26E-02	3.03	4.47
cell-cell junction assembly	3.42	3.26E-02	3.03	6.72
negative regulation of cellular component organization	3.41	3.30E-02	0.69	7.01
sphingolipid biosynthetic process	3.36	3.46E-02	2.86	6.72
positive regulation of gliogenesis	3.34	3.56E-02	2.78	4.37
embryo implantation	3.28	3.75E-02	2.63	4.41
regulation of protein depolymerization	3.28	3.75E-02	2.63	6.86
regulation of cytokine production	3.28	3.76E-02	0.65	4.26
regulation of microtubule polymerization or depolymerization	3.26	3.85E-02	2.56	6.86
regulation of interleukin-8 production	3.23	3.94E-02	2.50	4.11
negative regulation of NF-kappaB transcription factor activity	3.21	4.04E-02	2.44	4.41
regulation of nitric oxide biosynthetic process	3.21	4.04E-02	2.44	4.11
response to progesterone	3.19	4.14E-02	2.38	4.41
regulation of neurological system process	3.19	4.14E-02	2.38	4.14
regulation of glial cell differentiation	3.19	4.14E-02	2.38	4.37
single-organism developmental process	3.18	4.18E-02	0.22	5.26
cellular response to ketone	3.16	4.23E-02	2.33	4.41
regulation of protein complex disassembly	3.16	4.23E-02	2.33	6.86
positive regulation of biological process	3.15	4.27E-02	0.22	4.94
response to interferon-gamma	3.10	4.52E-02	2.17	4.41
glial cell differentiation	3.08	4.62E-02	2.13	4.14
glycolipid metabolic process	3.08	4.62E-02	2.13	6.72
regulation of nitrogen compound metabolic process	3.04	4.78E-02	0.24	4.84
protein lipidation	3.03	4.81E-02	2.04	6.86
ceramide metabolic process	3.03	4.81E-02	2.04	6.72