Sterol biosynthesis pathway is part of the interferon host defence response

Mathieu Blanc

A thesis submitted in fulfilment of requirements for the degree of Doctor of Philosophy

Division of Pathway Medicine, School of Biomedical Sciences

The University of Edinburgh

April 2010

Declaration

I hereby declare that this thesis is of my own composition, and that it contains no material previously submitted for the award of any other degree. The work reported in this thesis has been executed by myself, except where due acknowledgement is made in the text.

Mathieu Blanc

Abstract

Recently, cholesterol metabolism has been shown to modulate the infection of several viruses and there is growing evidence that inflammatory response to infection also modulates lipid metabolism.

However little is known about the role of inflammatory processes in modulating lipid metabolism and their consequences for the viral infection.

This study investigates host-lipid viral interaction pathways using mouse cytomegalovirus, a large double-stranded DNA genome, which represents one of the few models for a natural infection of its natural host.

In this study, transcriptomic and lipidomic profiling of macrophages shows that there is a specific coordinated regulation of the sterol pathways upon viral infection or treatment with IFNγ or β (but not TNFα, IL1β or IL6) resulting in the decrease of free cellular cholesterol.

Furthermore, we show that pharmacological and RNAi inhibition of the sterol pathway augments protection against infection in vitro and in vivo and we identified that the prenylation branch of the sterol metabolic network was involved in the protective response.

Finally, we show that genetic knock out of IFNβ results in a partial reduction while genetic knock out of Ifnar1 completely abolishes the reduction of the sterol biosynthetic activity upon infection.

Overall these results support a role for part of the sterol metabolic network in protective immunity and show that type 1 IFN signalling is both necessary and sufficient for reducing the sterol metabolic network upon infection; thereby linking the sterol pathway with IFN defence responses.

Acknowledgements

A considerable number of people helped me during this project.
First of all I would like to thank my supervisor Prof Peter Ghazal for giving me the opportunity of starting this project in his laboratory and for his guidance all along the difficult ways of my PhD.
Thanks to Prof Rudolph Riemersma who has taken a considerable amount of his time to teach and advise me during the PhD.
I am also grateful to Dr John McLauchlan for advises and Dr Ana Angulo for helping me during my time in Barcelona and for her helpful comments.
I would also like to thank all the past and present members of the DPM for their help and especially, Garwin (who made me see science in a different way), Paul for our helpful discussions, Kai and Donal for their help with the writing process but also for the coffee time.
Also a special thanks to Sara alias Dr Rodriguez Martin who has been here since I started and helped me to pass the difficult times inside and outside of the lab.
I also would like to thank my friend Sanjay (alias Dr Shrew), who helped me with the corrections of the manuscript but also for all the good and bad times that we went through together.
I am also very grateful to the British heart foundation and the Wellcome trust for funding my PhD.
Of course, I would like to thanks my family: My parents who gave an unconditional support during my studies, my grand- parents who are a model for me and to whom I dedicate this thesis and finally my two brothers Damien and Hugo.
And last but not least, I would like to thank Laura for her kindness, her patient and all the other qualities she needed to support me during this PhD, certainly I would not have finished without her.

List of Abbreviations

%	Percentage
~	Approximately
°C	Degrees Celsius
µ	Micro
µg	Micrograms
µl	Microlitre
A	Absorbance
Ad	Adenovirus
AIDs	Acquired immunodeficiency syndrome
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
BAC	Bacterial artificial chromosome
BALB/c	Laboratory mouse strain
BMDMs	Bone marrow derived macrophages
bp	Base pairs
BSA	Bovine serum albumin
C57Bl/6	laboratory mouse strain
CBP	CREB-binding protein
cm^2	Centimeters square
CO_2	Carbon dioxide
CPE	Cytopathogenic effect
CS	Calf serum
DC	Denditric cell
dH2O	Distilled water
DMEM	Dulbecco's modified essential medium
DMSO	Dimethyl sulfoxide
DNA	deoxyribonucleic acid
EBV	Epstein-Barr virus
FACS	fluorescent-activated cell sorter
FAM	Carboxyfluorescein
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
g	gravitational force or grams, depending on context
h.p.i	hrs post infection
hCMV	Human cytomegalovirus
HCV	Hepatitis C virus
hour	hr
HRP	horseradish peroxidase
HSV1	Herpes simplex virus 1
i.p	Intraperitoneal route
IE	Immediate early

IE3	Immediate early 3 protein
IFN	Interferon
IFNAR	Type I interferon receptor
IFNGR I	Type II interferon receptor
Ig	Immunoglobulin
IL	Interleukin
IPA	Ingenuity Pathway Analysis
kb	kilo bases
kg	kilogram
LPS	lipopolysaccharide
LT	Lymphotoxin
M	Molar
MAPK	Mitogen-activated protein kinases
mCMV	murine cytomegalovirus
mg	milligram
MHC	Major histocompatibility complex
MIEP	Major Immediate Early Promoter
min	Minute
ml	Milliliter
mM	millimolar
MOI	Multiplicity of infection
mRNA	Messenger ribonucleic acid
NIH 3T3	murine fibroblast cell line
NK	Natural Killer
nm	nanometer
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
pfu	Plaque forming units
RNA	Ribonucleic acid
RT	Reverse transcriptase
rt	Room temperature
Q-PCR	Quantitative polymerase chain reaction
SD	Standard deviation
SFV1	semliki forest virus 1
Th	T helper
TNF	Tumor necrosis factor
tyk2	Tyrosine kinase 2
U	Units
UV	Ultraviolet
VV	Vaccinia virus
w/vol	Weight per volume

Table of contents

List of figures

List of tables

1 Chapter 1: Introduction

1.1 Characteristics of Cytomegalovirus

1.1.1 Overview of the Herpes virus family

The family of the Herpesviridae contains 8 human-pathogenic members, e.g. the Herpes Simplex viruses 1 and 2 (HSV1 and HSV2), the Varizella Zoster virus (VZV), the cytomegalovirus (CMV), the Epstein-Barr virus (EBV), and the Karposi-Sarcoma virus (KHSV). The illnesses caused by Herpes virus infections have been known since antiquity, mainly that of the HSV1, an infection which causes small skin lesions known as cold sores. The term “Herpes” is based on the Greek expression for 'to creep' and reflects that Herpes viral infections spread mainly from cell to cell by direct cell contact.

The Herpesviridae family (www.ictvonline.org/virusTaxonomy.asp?version=2009) contains in total more than 100 members, which infect a large spectrum of different species such as mammals, birds, reptiles and molluscs. Most of these viruses show a strict species specificity (Davison, Eberle *et al.* 2009). Members of the Herpesviridae share many structural properties, while differences in their biological properties allow a classification into three subfamilies, the *alpha (*α), *beta*- (β) and *gamma*- (γ) Herpesviridae. The classification of the families depends on their structural and genetic characteristics (Roizman, Carmichael *et al.* 1981; Pellet 2007).

Structural properties common to all Herpes viruses:

- The virion consists of a nucleoprotein core, which contains a linear double stranded DNA genome (125–290 kbp), protected by an icosahedral T-16 type capsid composed of 162 caspomeres. The size of the virion varies among species, being between 130 and 300 nm.
- The capsid is then surrounded by an amorphous layer of viral proteins, called the tegument. So far, more than 30 different viral proteins are known to form the tegument.
- The virion is surrounded by a lipid bilayer envelope, derived from cellular membranes spiked with viral proteins.

All Herpes viruses (except γ-herpes viruses) share also certain biological properties:

- The genome of herpes viruses is composed of a unique long (Ul) and a unique short (Us) region, bounded by inverted repeats.
- Around 70 core genes are functionally conserved between all viruses.
- They encode a large number of enzymes involved in nucleic acid metabolism and protein processing.
- The DNA synthesis and assembly of the viral capsid occurs in the cell nucleus.
- Production of progeny virus results in host cell destruction
- Herpes viruses establish a lifelong latency in their hosts. (Ds: Double stranded)

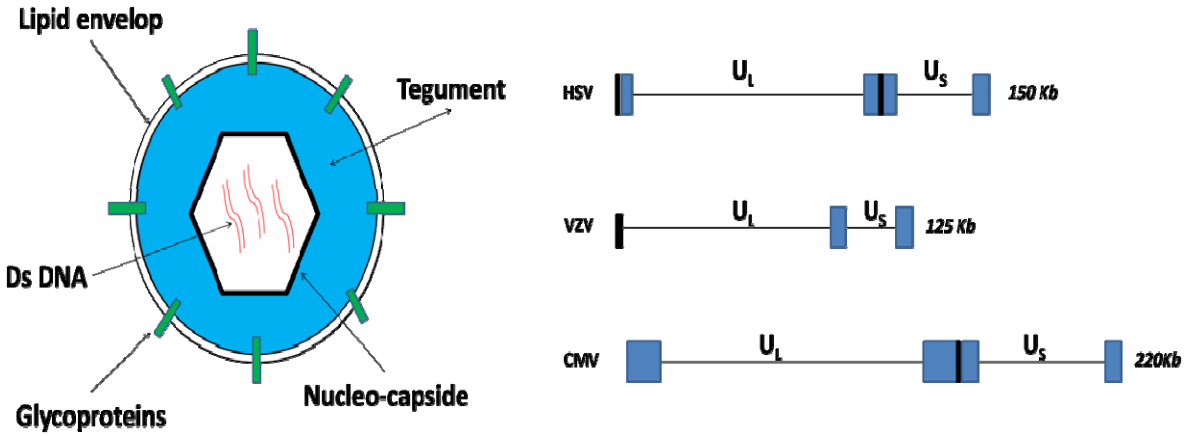


Figure 1-1: Structure of herpes virion and genomes

Ds: Double stranded, HSV: Herpes simplex virus, VZV: Varizella Zoster virus, CMV: cytomegalovirus, U_L: Unique Long, U_S: Unique Short.

However, millions of years of co-evolution with their host have led to specific properties of each Herpes virus-host system. The three subfamilies of the Herpesviridae are distinguished by their specific biological properties in e.g. cell tropism, replication time and host spectrum.

The *α*-Herpes viruses display a broad host spectrum and a short replication time in cell culture. This subfamily contains the pathogens HSV1 and HSV2 and VZV.
HSV1 and HSV2 are ubiquitously present in the human population, HSV1 is transmitted by contact during an acute infection and causes oral Herpes and usually

infects the mucosa in the mouth causing cold sores. HSV2 or genital Herpes infections occur mainly as the results of sexual contact and are the cause of congenital Herpes infections in newborns. Both HSV1 and 2 establish latency in ganglia, HSV1 preferably in sensory ganglia of the face and HSV2 in the genital tract. VZV infection causes Varicella or Chicken Pox. During outbreaks, the virus is transmitted by contact or airborne droplets of respiratory mucosal fluids and finally VZV establishes latency in the dorsal root ganglia. The reactivation of latent virus then can cause Herpes Zoster or Shingles.

The *β*-Herpes viruses are defined by a strict species specificity and cell tropism. In contrast to α and *γ* Herpes viruses, they show a slow replication rate in cell culture. Infection with *β*-Herpes viruses is characterised by enlargement of the infected cells. The beta Herpes virus subfamily includes the mouse and human cytomegalovirus (mCMV; hCMV) and human Herpesviruses 6 and 7 (HHV6 and HHV7). HHV6 and HHV7 are ubiquitous in the human population. HHV7 has been shown to cause exanthema subitum, whereas so far no disease has been linked with HHV6.

The *γ*-Herpes viruses also display species specificity and are lymphotropic as they mainly infect B and T-cells (However they also have been showed to infect endothelial and epithelia cells). Furthermore γ-Herpes viruses are characterised by their short replication cycle in cell culture. This subfamily include the lymphocryptoviruses (γ-1) e.g. EBV and the Rhadinoviruses (γ-2) e.g. (Karposi's sarcoma associated virus (KSHV), Rhesus monkey Rhadinovirus (RRV), Equine Herpesevirus 2 (EHV-2) and Murine Herpes virus 68 (MHV68).
EBV is transmitted mainly through the exchange of saliva and is the causative agent for Mononucleosis. One of the main characteristics of the γ-Herpes viruses is that their infection is oncogenic. EBV has been shown to be associated with Hodgkin's Lymphoma, Nasopharyngeal carcinoma and some forms of gastric cancer arising from latent infected cells.

1.1.2 Characteristics of the Herpes virus life cycle

All Herpes viruses have very similar life cycles which can be divided in three phases; 1: virus attachment and entry, and transportation of the capsid to the nucleus; 2: viral gene transcription and translation and synthesis of viral DNA; 3: assembly of new virion, enveloping and egress.

1. Attachment, penetration and transport to the nucleus.

The attachment of the virion to a cellular receptor mediated by viral glycoprotein complexes gH/gL/gO and gB/gB, triggers the fusion of the virus to the cell membrane and the penetration of the nucleocapsid inside the cytoplasm. Tegument proteins associate with the cytoskeleton and transport the capsid to nuclear pores, where it penetrates and releases the linear viral DNA-genome by a so far unknown mechanism.

2. Viral gene transcription, translation and synthesis of viral DNA.

In the nucleus, viral DNA utilises the host RNA polymerase II to initiate the transcription of viral genes. The expression of Herpes virus genes is tightly regulated and can be divided in three temporal phases: immediate early, early and late.

The transcription of the viral immediate early genes starts within minutes after penetration of the virus and is independent of protein synthesis, for it occurs even under a cycloheximide-block of protein synthesis. The major function of the immediate early genes is to promote and enhance the expression of early and late genes. The induction of the transcription of the early genes is dependent on the cooperation of viral immediate early (IE) proteins and cellular transcription factors. Most of the early genes encode proteins with enzymatic properties involved in the replication of the viral genome. All Herpes viruses encode for an early gene which serves as their own DNA polymerase. This viral protein replicates the viral genome by a rolling circle-mechanism. This is made possible by circularisation of the linear viral genome in the nucleus forming a structure called episome. This allows the synthesis of new viral genomes as concatemers that are cleaved upon integration into new assembled capsids. The late genes encode structural proteins, like capsid proteins and the glycoproteins and their expression is only triggered by onset of the

viral DNA synthesis. New viral capsids are assembled in the nucleus with components imported from the cytoplasm (Sears and Roizman 1990).

3. Assembly of new virions, enveloping and egress.

When the temporal expression cascade of viral genes is completed, the newly synthesized genomes are complexed with core-protein while they are packed inside the newly assembled capsids, receive their tegument and are then transported out of the host cell. The mechanisms involved in the egress of the viruses are still not fully understood. Exactly how capsids leave the nucleus and gain their tegument is not fully characterized. Two models of viral egress of HSV 1 capsids from the perinuclear space are discussed in the literature (Mettenleiter 2002):

In the first model called the de-envelopment re-envelopment process, Herpes viruses acquire a first envelope from the nuclear inner membrane while entering the perinuclear space. Virions then translocate to the ER lumen where it loses its first envelope by fusion with the outer nuclear or the ER membrane. Viral capsids associate then with the tegument compounds while they cross the cytoplasmic space and bud then into large cytoplasmic vesicles. During that process they receive their final viral envelope. The viral glycoproteins that are necessary to form the final envelope are transported from the ER to the trans-Golgi network and the cytoplasmic vesicles, where they reside until virion formation. The assembly of fully enveloped virions with functional glycoproteins occurs in these vesicles while they are transported to the cell surface. The viral particles are then released by exocytosis.

Recently a modification of this model, named the enlarged nuclear pore model, has been proposed: in this model the newly assembled capsids reach the cytoplasm by direct transport through enlarged nuclear pores and fuse directly to the Golgi apparatus to receive their envelope (Wild, Engels *et al.* 2005).

In the second classical model, the nuclear virions attain their full tegument and envelope in the nucleus and then translocate directly to the Golgi apparatus through the ER where they acquire a second membrane. During the transit, the envelope proteins are modified in situ

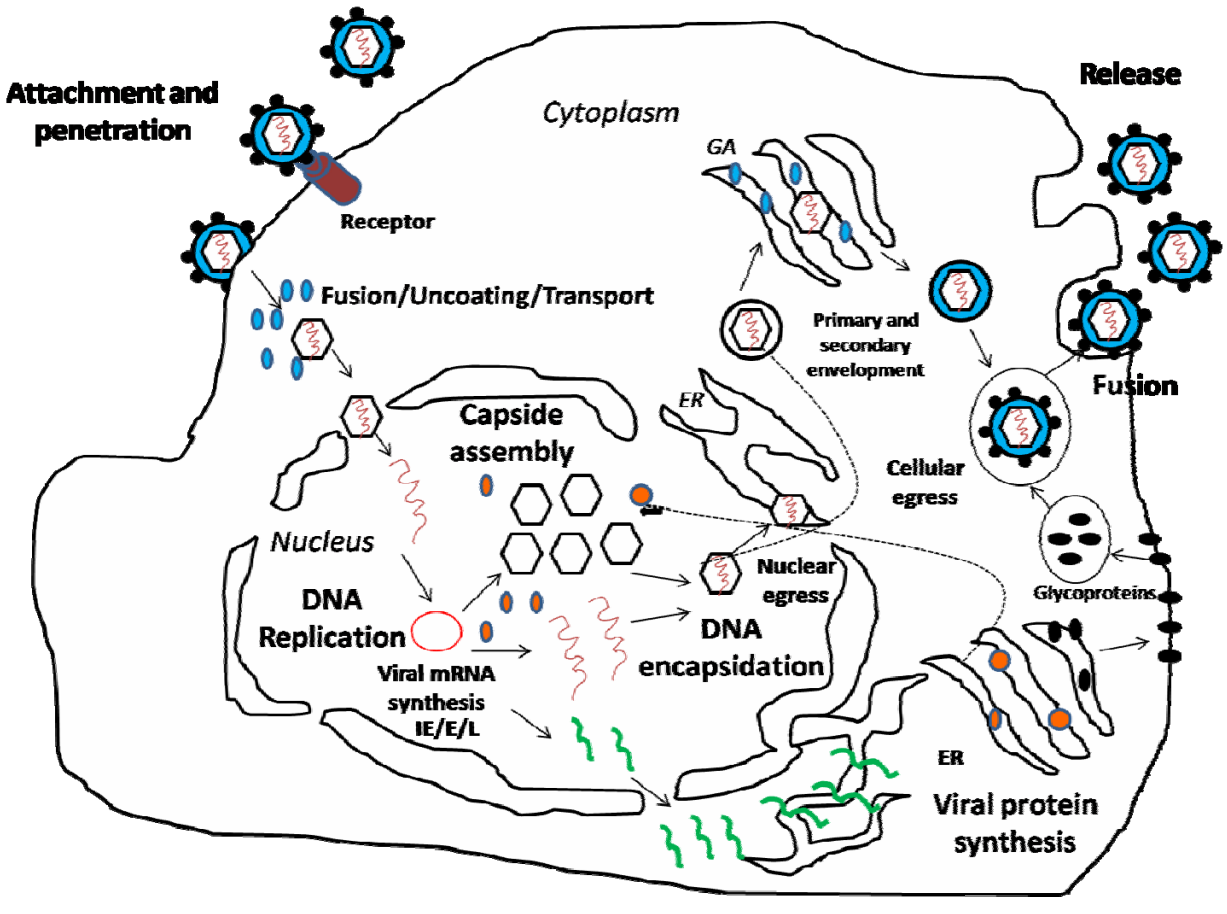


Figure 1-2: Herpes virus life cycle

All Herpes viruses have very similar life cycles which can be divided in three phases; 1: virus attachment and entry, and transportation of the capsid to the nucleus; 2: viral gene transcription and translation and synthesis of viral DNA; 3: assembly of new virions, enveloping and egress. IE: Immediate Early, E: Early, L: Late, ER: Endoplasmic reticulum, GA: Golgi apparatus.

1.1.3 Latency

All Herpes viruses have the ability to establish latency in their hosts (Chakravarty 2008). Latency is defined as the maintenance without assemblage of infectious virions of the viral genome in the absence of productive infection (Reddehase, Podlech et al. 2002) . During latency no viral replication and no infectious particles are detectable and consequently, viruses evade the immune surveillance. Latent infections occur in most organs but only in specific cell types that form reservoirs. For most Herpes viruses these cell types are still not identified. This is because the cell types that allow establishment of latency are normally different from those that support the primary productive infection. Reactivation mechanisms are still not understood. It is thought that external stimuli drive the virus out of latency, most likely in periodical manner. It was shown that general inflammatory signals or cellular differentiation events trigger reactivation of certain Herpes viruses such as CMV (Reddehase, Podlech et al. 2002). Latency is to be differentiated from persistent infection which is defined by at long-lasting virus production, which may occur at a low level (Reddehase, Simon et al. 2008).

1.1.4 Immune evasion

Another prominent biological property of the Herpes viruses is their highly developed ability to counteract the immune response. Their replication cycle and their ability to establish latency make it necessary to escape the immune system of their host. First, Herpes viruses have to overcome the intrinsic defence mechanisms of the host cell, such as shut down of viral gene expression or apoptosis. In human cytomegalovirus infections the viral tegument protein pp71 (UL82) and the 71kD IE1 protein prevent formation of a closed chromatin structure of the viral genome and the viral UL36 and UL37 gene products are strong inhibitors of apoptosis (McCormick, Skaletskaya *et al.* 2003).

Each Herpes virus has adapted to its host and developed specific mechanisms to evade their innate and adaptive immune responses. Viral gene products and microRNAs interact with different aspects of the host immune response and are able

to control, block or inhibit immune signalling, release of cytokines, and recognition by immune cells.

1.1.5 Biology of Cytomegalovirus

The etymology of the name Cytomegalovirus comes from the Greek "cyto*":* cell and "megalo": large. Indeed CMV infection induces cytopathic effects that are characterised by nuclear and cytoplasmic inclusions and cell rounding. In 1904, Ribbert was the first to specifically describe the pathology of the cytomegalovirus inclusion disease (CID) in a stillborn foetus.

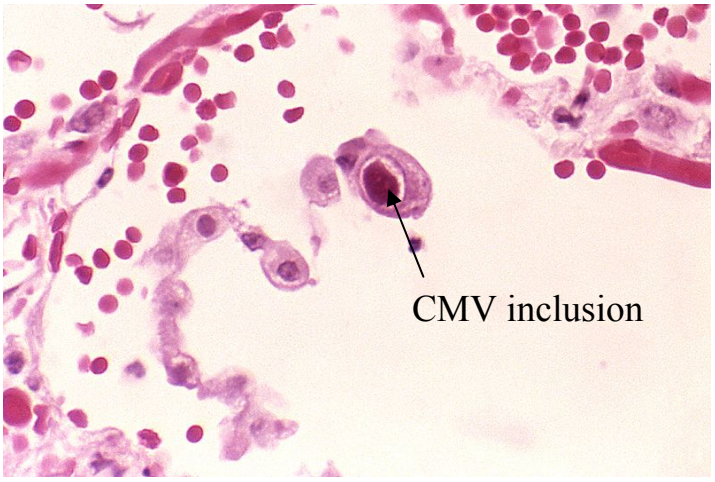


Figure 1-3: Typical Cytomegalovirus inclusion in monocytes in the lung.

The central cell displays the dramatically enlarged nuclei characteristic of CMV. Histopathology of lung shows cytomegalic pneumocyte containing characteristic intranuclear inclusion. Source: Wikipedia, this is a file from the Wikimedia Commons. Commons is a freely licensed media file repository

1.1.5.1 Pathogenesis of cytomegalovirus infection

hCMV infection is ubiquitous and affects 50 to 80% of the population depending of the socio economic and geographic conditions (Western vs. developing country).The primary infection usually occurs during early age, and requires an exchange of body fluids (mainly saliva) (Wong, Tan *et al.* 2000). Epidemiology studies suggest that the entry of the virus is consecutive to contact with mucosal surfaces (Sinzger and Jahn 1996). Circulating leukocytes and endothelial cells are believed to disseminate the virus in the body of the host (Dankner, McCutchan *et al.* 1990; Gerna, Percivalle *et al.* 2000). hCMV has been shown to infect ubiquitously distributed cell types such as epithelial cells, fibroblasts, and endothelial cells. hCMV also infects leukocytes such as macrophages, denditric cells, T cells and also specialized parenchymal cells such as smooth muscle cells and hepatocytes. As a consequence, a wide range of organs is affected by CMV infection, such as salivary glands, lungs, kidneys, the spleen, the liver, but also neurons and astrocytes in the brain (Ho 1995; Campbell, Cavanaugh *et al.* 2008). hCMV infection in immunocompetent individuals is normally asymptomatic. In some cases a primary infection may induce mononucleosis like symptoms, but in rare cases it may lead to pneumonia, myocarditis, haemolytic anaemia and other complications (Landolfo, 2003). hCMV infection becomes a life threatening disease for immunodeficient patients. It is the most common congenital viral infection in developed countries affecting 1% of all new born babies (Demmler 1991). Foetuses are mainly infected *in utero* or congenitally when the mother has either had a primary infection during pregnancy or a reactivation of the latent virus. 5-10% of the infected newborns display CMV related pathology such as hearing loss, mental retardation or other neuronal abnormalities and around 0.25% of all new born baby will die from CMV infection during infancy (Bale, Blackman *et al.* 1990). Postnatal infection of newborns, originate most frequently from breast milk and, although of higher incidence, does not display the same severity as the congenital infection (Demmler 1991).

hCMV infection is a life threatening disease for immunosuppressed adults: hCMV infection is the most frequent opportunistic infection associated with Acquired Immunodeficiency Syndrome (AIDS) although hCMV pathologies in AIDS patient have been reduced following the use of the “Highly Active Antiretroviral Therapy”

(HAART). However acute infection can lead to the development of pneumonia in the lung, gastrointestinal and brain damage as well as retinitis leading to blindness (Ostrowski, Krakauer *et al.* 1998; Kovacs, Schluchter *et al.* 1999). hCMV has become a frequent infection in transplanted patients (Boeckh and Bowden 1995; Sagedal, Hartmann *et al.* 2005). Ultimately, patients may develop pathologies associated with hCMV infection, but rejection of the transplant caused by the inflammatory processes and the immune response to the hCMV infection or reactivation in the graft also occurs. hCMV infection has been associated with the development of atherosclerosis, although the contribution of hCMV remains controversial. Epidemiological and pathological studies suggested a link between hCMV infection and atherosclerosis (Adam, Melnick *et al.* 1987). Studies in cardiac transplant patients implicate hCMV infection as a significant factor in the development of coronary artery disease following transplantation (Danesh, Collins *et al.* 1997; Danesh and Appleby 1998). Since atherosclerosis is an inflammatory disease, it is difficult to differentiate if the effects of inflammation and disease cause the reactivation of hCMV or if hCMV infection triggers the inflammatory response and by doing so, cause the disease.

1.1.5.2 Diagnosis and therapy for hCMV infection

hCMV can be detected in blood, bronchial and throat washing, cerebrospinal fluid and urine. Several methods of detection exist to monitor hCMV particle numbers. The most accurate detection is the serological test using CMV specific pp65 antibodies. PCR is also frequently used to monitor DNAemia, i.e. to detect viral genomes in the bloodstream. At this time there is no cure for hCMV infection, but antiviral drugs have been developed to slow the infection. Ganciclovir and foscarnet are the two main antiviral substances used nowadays against HCMV infections. Gancyclovir is phosphorylated by a viral kinase and inhibits the incorporation of dGTP by the viral DNA polymerase disrupting DNA synthesis. Foscarnet inhibits viral replication by blocking the docking site of the viral DNA polymerase. Several HCMV vaccines are in various stages of pre-clinical testing but none have been proved safe and successful enough to eradicate hCMV infection so far (Schleiss and Heineman 2005).

1.1.5.3 mCMV has a model for studying hCMV

hCMV does not infect laboratory animals and human studies have obvious ethical limitations. mCMV, which shares 70 % homology of its genome as well as biological similarities (virion structure, life cycle and pathologies in the host), represents one of the few suitable mammalian models for an infection in its natural host. The mouse model has been extensively used to study the pathogenesis of acute, latent and recurrent viral infections since both mCMV and hCMV cause acute infection in immunocompromised hosts triggering the same clinical symptoms (Hamilton and Seaworth 1985; Reddehase, Balthesen *et al.* 1994). There are also many similarities in the host immune response to mCMV and hCMV infection (Mocarski and Kemble 1996).

1.1.5.4 mCMV characteristics

mCMV share many characteristics with other Herpes viruses (See section 1). Mature virions have a diameter of 150 to 200 nm and are composed of a core, capsid, tegument and lipid membrane. More than 30 proteins have been purified and identified from the mCMV virion. The tegument is constituted of about 20 proteins with M83 (homolog of hCMV pp65) as major constitutive. The envelope includes two major Herpes virus conserved glycoprotein complexes composed of the glycoproteins gB, gH, gL and gO. mCMV has one of the longest genomes of the Herpes family composed of a linear double stranded DNA of 235 kb (Rawlinson, Farrell *et al.* 1996). mCMV virions contain a single unique sequence flanked by terminal repeats in contrast to the hCMV which has two unique regions (U_L and U_S) (Chee, Bankier *et al.* 1990).

To date no specific cellular receptor for CMV virions has been clearly identified. However, proteoglycan heparin sulphate has been shown to be important in the first step of the infection. Moreover glycoproteins gB and gH in combination with the epidermal growth factors receptor (EGFR) and α and β integrins have been shown to be important in the fusion of the viral membrane to the host (Wang, Huong *et al.* 2003; Kinzler and Compton 2005).

Like all Herpes viruses, after viral penetration and uncoating, mCMV nucleocapsids migrate to the nucleus and inject the viral genome into the nucleus, which then

circularises. The mCMV gene expression is also regulated in a strict temporal cascade composed of the three phases: Immediate early (IE), early (E) and late (L). The IE1 and IE3 gene products act as transcriptional activators of the viral and cellular gene expression (Keil, Ebeling-Keil *et al.* 1984).

The IE gene expression is controlled by the major immediate early (MIE) enhancer. This enhancer contains binding sites for transcription factors such as CREB, AP1, NFKB and retinoic acid receptor (RAR) (Stinski and Isomura 2008). It is required for viral replication *in vivo* and *in vitro.* The enhancer is also thought to play a crucial role in reaction from latency (Angulo, Messerle *et al.* 1998; Ghazal, Messerle *et al.* 2003).

1.2 Innate immune response to CMV infection

1.2.1 Cells of the innate immune response to CMV infection

After the physical barrier, innate immunity is the first defence against path. The innate immune system is found in fungi, plants and animals. It consists in the cells and mechanisms that recognize and provide immediate defence against infection with broad specificity. But, unlike the adaptive immune system, it does not confer long-lasting protective immunity against secondary infections to the host. The innate immune system in vertebrates assures several functions, such as the recruitment of immune cells to the sites of infection through the production of chemical factors (toxic proteins, chemokines, interleukin, cytokines etc..), the identification and removal of foreign substances, the activation of cells to trigger the activation of cellular defence mechanisms and also the promotion of clearance of dead cells and antibody complexes. Finally the innate immune system is able to activate the adaptive immune system though a process called antigen presentation. The cells of the innate immune system use receptors, such as the Toll like receptors (TLRs), that recognise molecular patterns present on a large spectrum of pathogens (pathogen associated patterns or PAMPs) (Gordon 2002; Akira and Takeda 2004; Beutler 2009). Cells of the innate immune system comprise mast cells, phagocytic cells (macrophages, neutrophils and denditric cells (DC)), basophils, eosinophils and natural killer (NK) cells. These cells permanently sense the environment and are subdivided in many sub-cell types which have distinct functions and (micro) anatomical distribution.

Three cell types of the innate immune system have been shown to be central for the control of CMV infection: NK cells, macrophages and DCs.

NK cells are specialised cytotoxic cells involved in the elimination of viral infected or tumor cells (Gazit, Gruda *et al.* 2006). NK cells sense the neighbouring cells through inhibitory NK cell receptors which are specific for a cell-surface marker called MHC class I (major histocompatibility complex). When a compromised host cell presents decreased levels of MHC I, (such as tumor cells or virus-infected cells), it can activate NK cells due to decreased inhibitory signals consecutive to decreased

engagement of inhibitory receptors. Nk cells then kill the abnormal cells by secreting perforin and granzymes which trigger apoptosis. NK cells can also be activated and migrate to the site of infection in response to cytokines (IFNα/β, IL-2, IL-12, IL-15 and IL-18) induced by infected cells. NK cells, when activated, are also able to secrete antiviral cytokines such as IFNγ and TNFα. NK cell functions are tightly regulated by the activities of both inhibitory and activating cell surface receptors that serve either to suppress or to activate their cytolytic function. NK cell inhibitory molecules include Ly49 receptors in rodents, killer cell Ig-like receptors (KIR) molecules in humans, and heterodimers of CD94 and NKG2A, which are found in both species. Whilst inhibitory NK cell receptors (NKR) are specific to MHC class I molecules, activating receptors use various ligand in addition to the MHC class I. These ligands are induced upon infection or cellular stress. NK cell activating receptors include KIRs in humans, Ly49H and Ly49D in mice, and NKGD, which is found in both species.

Patients with NK cell defects are more susceptible for herpes viruses infections and particularly CMV (Biron, Byron et al. 1989). Furthermore, NK cells have been shown to be important in mCMV resistance (Welsh, Brubaker et al. 1991). The importance of NK cell in the protection of mCMV infection has been linked to the variation in *Cmv1*, a highly variable host resistance locus that regulates the efficacy of NK cell responsiveness. *Cmv1* encodes for the receptor Ly49H, a C-type lectin-like receptor which has been shown to recognise a mCMV protein (m157) expressed at the surface of infected cells. In fact, Balb/C mice lacking the NK cell receptor Ly49H are much more susceptible to mCMV infection than the C57Bl/6 mice that have it (Scalzo, Fitzgerald *et al.* 1990). Mice deficient in NK cells function or depleted of NK cell show a higher susceptibility to mCMV infection (Bukowski et al, 1984, Shanley 1990). However, NK cells are necessary but not sufficient in the control of CMV infection.

Macrophages as well as DCs are professional antigen presenting cells (APC). Both cell types are able to display foreign antigens associated with major histocompatibility complex class I and II (MHC I and MHC II) on their surfaces. When an infection occurs, the ingestion of pathogens will result in the degradation of

the pathogens into peptides by specialised enzymes. The foreign peptides are then introduced in the MHC class II presentation pathway, which will trigger adaptive immune response. For the MHC class I presentation pathway, in most cells, peptides are generated by proteosomal degradation of endogenous cytosolic proteins and then transported to the ER where they are associated with the MHC class I complexes. For the MHC class II presentation pathway, peptides originate from endocytosed exogenous molecules and associate with MHC class II complexes in lysosomal compartments. MHC class I antigens are present in most cell and are recognised by CD8+ T cells while MHC class II antigens are recognized by CD4+ T cells which help to trigger the immune response.

Macrophages arise from the differentiation of monocytes which circulate in blood and tissues. In response to inflammatory stimuli, monocytes will migrate to the site of inflammation and differentiate into mature macrophages. Macrophages have many functions in innate and adaptive immunity. First of all, macrophages act as scavenger cells, removing the necrotic cellular debris and pathogens by phagocytosis. The phagocytic function is mediated through, in one hand the Fc receptors present at the surface of macrophages which are able to bind antibody-coated microorganisms and in another hand by the TLRs which recognize a large spectrum of pathogen associated receptors (PAMPs) (Gordon 1998). In addition, in response to infection and inflammation, macrophages will produce a wide array of powerful chemical substances including enzymes, complement proteins, and regulatory factors such as cytokines (IL1β, IL6, IL12, and IL8). Particularly, macrophages will secret pro-inflammatory cytokines such as TNFα and IL-1 which stimulate the activation of T cells and NK cells and trigger an antiviral response.

Macrophages play also a role in mCMV pathogenesis. Indeed, intraperitoneal (IP) mCMV-infected mice induce an inflammatory response consisting largely of macrophages (Heise and Virgin 1995). These macrophages are activated and there is an increase of the expression of Fc receptors (Price, Winter et al. 1987), as well as MHC class II, MHC class I, and ICAM-1 (Heise and Virgin 1995). Moreover, one of the primary mediators of macrophage activation during MCMV infection is IFNγ (Heise and Virgin 1995). Macrophages are also able to produce type I interferon upon mCMV infection, although they are not the major contributors in response to

infection. Type I interferons production in macrophages in response to mCMV infection has been shown to be important in the expression of MHC class II molecules (Heise, Pollock et al. 1998).

Macrophages and monocytes are believed to be important in CMV dissemination of the virus in tissues and latency (Mitchell, Leung et al. 1996; Hanson, Slater et al. 1999). Indeed monocytes are the predominant cell type infected in the blood but, when infected, monocytes are unable to start a proper viral replication cycle. Once activated by inflammatory signals, monocytes migrate to the loci of inflammation and differentiate into macrophages which allow the start of a productive infection.

DCs are cells specialised in antigen presentation and link innate and adaptive immune response. At an immature stage of development, DCs act as sentinels in peripheral tissues, continuously sampling the antigenic environment. DCs are divided in two subtypes: Classical DC (cDC) and Plasmacytoid DCs (PDCs) which differ by their life span, their locations and there functions. DCs have the ability to endocytose a large variety of exogenous antigens for presentation via MHC class I molecules (Wilson and Villadangos 2005). This phenomenon is called cross-presentation and allows DCs to present viral antigens to $CD8^+$ T cells without being infected themselves. Macrophages are also capable of cross presentation although not as efficient as DC. When infected or activated, DCs migration to the lymph nodes is increased and the adaptive immune response is triggered by the activation of T cells (Andrews, Andoniou *et al.* 2001). In the absence of microbial infections or other kind of stress, a small proportion of DCs migrate into lymphoid tissues. This helps to maintain a state of peripheral T-cell tolerance to self-antigens by elimination of the CD4+ and CD8+ cells expressing a self antigen. Activated DCs, mainly pDC, release antiviral cytokines such Type I interferon and IL12 (Dalod, Salazar-Mather *et al.* 2002). These cytokines play a key role in antiviral defence. DCs, mainly cDC, have also the ability to activate NK cells by direct interaction in the site of infection or in the lymph nodes. Furthermore IL-2 secretion in association with direct cell contacts is necessary for the promotion by DC of NK cell IFNγ production (Granucci, Zanoni et al. 2004).

DCs can be infected by CMV in vivo and in vitro (Dalod, Hamilton et al. 2003) and play a key role in the expansion of Ly49H NK cells (Andrews, Scalzo et al. 2003). Furthermore, during infection with mCMV, pDCs are the main producers of type I interferons and also contribute significantly to IL-12 and TNF-α production (Dalod, Hamilton et al. 2003; Zucchini, Bessou et al. 2008).

In addition, cytokines have also been show to play a key role in the control of CMV infection. Studies with neutralizing antibodies or exogenous cytokine treatments have demonstrated the importance of IFNγ, TNFα, IL-12, and IFNα/β in resistance to mCMV (Fennie, Lie et al. 1988; Heise and Virgin 1995; Orange, Wang et al. 1995; Cousens, Orange et al. 1997). The section 1.2.4 of this chapter describe specifically the role of type I interferon in CMV infection.

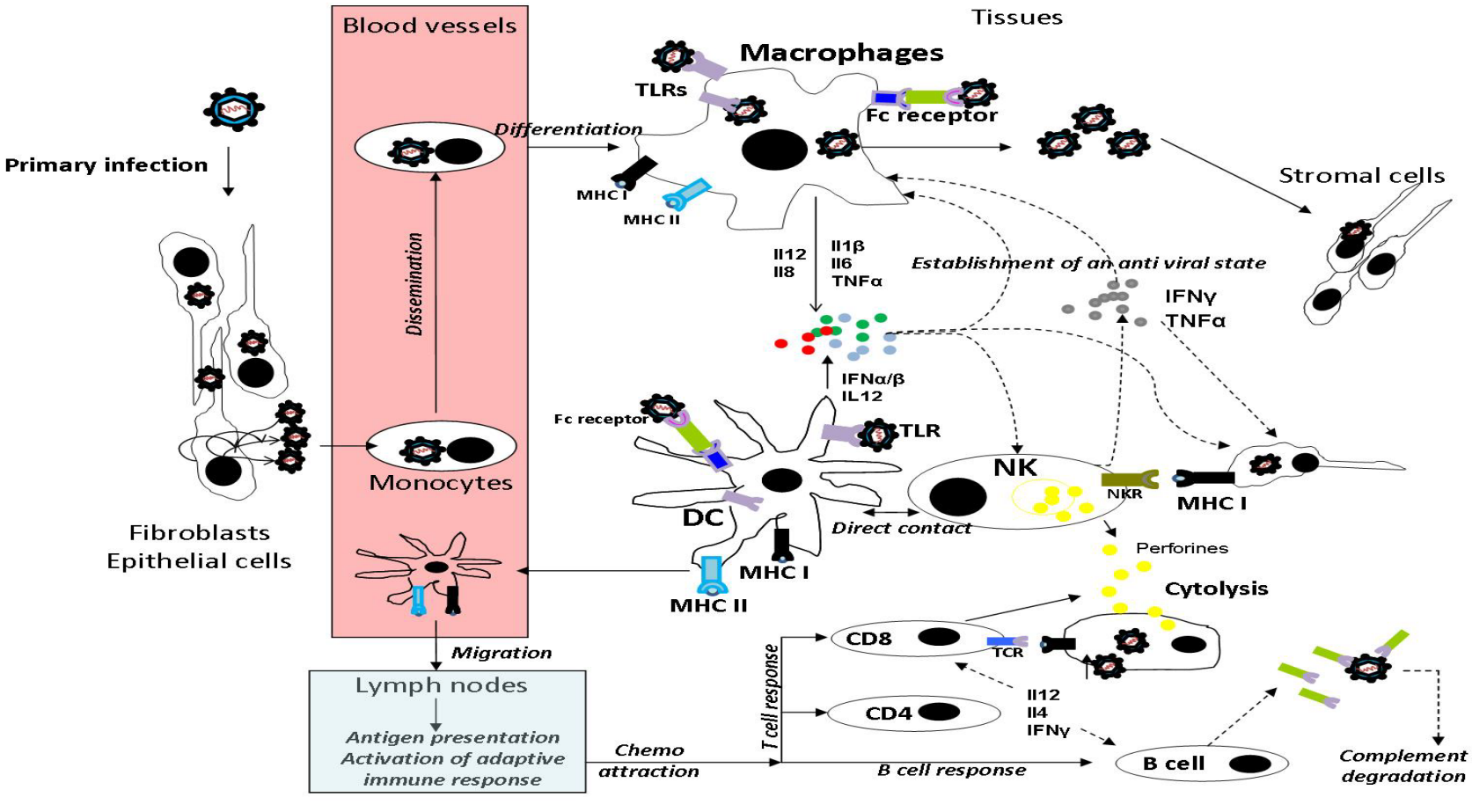


Figure 1-4: Initiation of the innate immune response to mCMV infection and major immune players involved in the control of mCMV infection.

After a primary infection, mCMV viruses diffuse to the blood stream where they infect monocytes. Dissemination of the virus to tissues occurs through the activation of monocyte migration and differentiation into mature macrophages. DCs and macrophages secrete cytokines which induce an antiviral state and help to activate NK cells. Activated DC induce cytokines, principally Type I interferons, and migrate to the lymph node to induce the adaptive immune response by activating B and T cells. NK cells directly lyse infected cells and also exert some antiviral activities by secreting cytokines.

NKR: NK cell receptor, TCR: T cell receptor

1.2.2 CMV interactions with innate immune cells

Although the innate immune system possesses efficient mechanisms to fight viral infections, it is not sufficient to suppress both mCMV and hCMV primary infection. Indeed, mCMV has evolved with its host and developed mechanisms to counteract and evade the immune response by expressing a great diversity of proteins able to alter the immune response. The interplay between innate immune response and the ability of the virus to escape it, determine the success of the infection.

mCMV virus has developed several immune evasion strategies against innate immune cells. Since antigen presentation is such a key aspect of the innate and adaptive immune response it is not surprising that many viral proteins interfere with this process. Both hMCV (UL18) and mCMV (m144) encodes for MHC class I homologs which act as ligand for NK cell receptors and inhibits their activation (Farrell, Vally *et al.* 1997; Reyburn, Mandelboim *et al.* 1997; Babic, Pyzik *et al.* 2010). In addition, mCMV (gp34) and hCMV (UL40, US2, US3, US6 and US11) down regulates specific components of the MHC class I processing and presentation pathway to evade both CD8+ T and NK cell control (Alcami and Koszinowski 2000; Reddehase 2002). In macrophages, down regulation of MHC class II molecules has also been observed (Heise, Connick et al. 1998). Furthermore mCMV also induces the secretion of IL-10 which decreases the expression of MHC class II proteins (Redpath, Angulo *et al.* 1999) and hCMV produce an IL-10 homolog with immunosuppressive properties (Chang, Baumgarth et al. 2004). mCMV encode for a chemokine (MCK) that increase the recruitment of leukocytes to the site of infection which facilitate virus dissemination (Mocarski 2002).

Moreover, CVM have developed in human and mouse many other mechanisms to alter the immune response to infection. For example, hCMV has been shown to perturb the DCs-T cells interaction by promoting the rapid disappearance of the co-stimulatory molecule B7-1 (CD80) from the cell surface of DCs (Mintern, Klemm *et al.* 2006). hCMV alters the cytokine secretion of activated DCs especially IL-12 (Chang, Baumgarth *et al.* 2004; Varani, Cederarv *et al.* 2007). hCMV protein UL16 has been shown to block the interaction between NKG2D and its activating ligand (Cosman, Mullberg et al. 2001) while mCMV glycoprotein gp40 down modulates

expression of NKG2D ligand (Arase, Mocarski et al. 2002; Krmpotic, Busch et al. 2002; Lenac, Arapovic et al. 2008). In addition, two hCMV proteins, gp68 and gp 34, have been shown to interact with the Fc receptors which prevents the binding of antigen bound IgG antibodies (Lilley, Ploegh et al. 2001; Atalay, Zimmermann et al. 2002).

1.2.3 Initiation of the innate immune response

Antigen presenting cells (APC) essentially express a range of pattern recognition receptors (PRRs) placed in different cellular locations and able to identify specific motifs of pathogens (Akira 2009). Once activated by the virus, these receptors initiate signalling cascade which in turn induces the transcription of inflammatory cytokines and chemokines, in particular type I interferon and TNFα (Akira and Takeda 2004). Coordination of intracellular, cytokines and chemokines response is essential for the early control of infection. In particular induction of type I interferon by DCs and macrophages stimulates and enhances the immune response and induces an antiviral state (Hornung, Rothenfusser et al. 2002).

Toll like receptors (TLRs) are membrane receptors capable of sensing the viral infection by recognizing pathogen-associated molecular patterns (PAMPs) (Medzhitov and Janeway 1998; Kumar, Kawai *et al.* 2009).

Among the TLRs receptors, TLR3, 7, 8 and 9, localized in endosomal vesicles, have been shown to recognize viral infections. TLR9 in particular is activated in response to DNA viruses such as Herpes viruses (Hemmi, Takeuchi *et al.* 2000). mCMV is detected by TLR9 in DCs (Krug, French et al. 2004; Tabeta, Georgel et al. 2004; Delale, Paquin et al. 2005). However TLR9 knockout mice still produce Ifnβ in response to mCMV infection, indicating that an alternative mechanism to sense the virus exists (Krug, French et al. 2004; Delale, Paquin et al. 2005). Several studies have recently shown that other TLRs (such as TLR2 and 3) play also a crucial role in the recognition of CMV infection but the mechanisms involved are not yet well identified (DeFilippis, Alvarado *et al.* ; Compton, Kurt-Jones *et al.* 2003; Tabeta, Georgel *et al.* 2004). It has also been recently demonstrated that TLR7 plays also a role in sensing mCMV in pDC in vivo (Zucchini, Bessou et al. 2008).

Specific activation of TLRs results in the activation of downstream proteins such as MyD88, IRAK1, IRAK4 or TRAF6. These proteins will then activate a complex composed of IRF3, NFκB, ATF2, CBP and p300. The complex will translocate to the nucleus where, in coordination with NFκB and IRF3, they will trigger an antiviral response by inducing cellular factors such as interferon inducible genes (ISGs) and the secretion of cytokines, in particular Ifnαs and β in an IRF3- and IRF7-dependent manner (Yoneyama, Suhara *et al.* 1998; Kawai and Akira 2006). If IRF3 is necessary for type I IFN responses in hCMV infected human fibroblasts (Sato, Suemori et al. 2000), the expression of a subset of ISGs, such viperin (Grandvaux, Servant et al. 2002) and ISG56 (Peters, Smith et al. 2002), can also take place in an IRF3-dependent manner in the absence of IFN-mediated signalling. However, in pDC the major inducer of type I interferon in response to CMV infection, TLR7 has been shown to directly induce type I interferon genes expression without IRF3 activation (Kawai and Akira 2006; Zucchini, Bessou et al. 2008).

Two cytoplasmic receptors: retinoic acid inducible I (RIG-I) and the melanoma differentiation associated 5 (MDA5) are also able to sense virus associated dsRNA. Once viral infection is recognized, these receptors activate the mitochondrial antiviral protein MAVS, through a CARD: CARD domain interaction. Although it recognises dsRNA, MAVS has been shown to be essential for IRF3 and IRF7 activation by Herpes viruses (Scott 2009). This can be explained by the involvement of RNA polymerase III (POL3) to activate RIG-I. Indeed, POL3 converts ds DNA into dsRNA which contains 5' triphosphate moiety (Chiu, Macmillan *et al.* 2009).
Recently, a cytoplasmic dsDNA binding protein ZBP1 has been identified (Deigendesch, Koch-Nolte *et al.* 2006). ZBP1 activates Ifnβ production in HSV1 infected cells (Chiu, Macmillan *et al.* 2009). However, induction of Ifnβ expression in response to infection can occur without the activation of ZBP1 (Lippmann, Rothenburg *et al.* 2008).

1.2.4 Type I Interferon response and CMV

Type I interferon family is composed of 14 IFNαs, one IFNβ (Pinto and Hill 2005). Ifnβ is secreted in most cell types while secretion of IFNα is restricted to DCs, leukocytes and macrophages (Colonna, Krug *et al.* 2002; Diebold, Montoya *et al.* 2003). Type I interferons plays a pivotal role in the control of innate and adaptive immune responses (Ruzek, Miller et al. 1997; Presti, Pollock et al. 1998; Redpath, Angulo et al. 1999; Andrews, Andoniou et al. 2001; Randolph-Habecker, Iwata et al. 2002; Gamadia, Remmerswaal et al. 2003; Tang-Feldman, Wojtowicz et al. 2006; Fodil-Cornu and Vidal 2008). The synthesis and secretion of type I interferon is probably the earliest host antiviral reaction (around 8 hours post infection for mCMV (Schneider, Loewendorf et al. 2008)). Host defence against viral infections is strongly dependent on the early production of type I interferon, which promotes an antiviral state in adjacent non-infected cells as well as the activation of cytotoxic lymphocytes (Biron 2001). pDCs, being the main producer of type I interferon in response to infection, play a major role in the establishment of a successful immune response against infection (Liu 2005; Swiecki, Gilfillan *et al.* 2010).

As mentioned earlier, acute CMV infection triggers through the TLR signalling pathway specific intracellular responses, which activate the expression of ISGs and the release of inflammatory cytokines and chemokines such as type I interferons. A second pathway induced by MCMV infection, the lymphotoxin β receptor (LTβR) has been also shown to lead to the production of type I interferon (Banks, Rickert et al. 2005). LTβR, expressed only by the stromal and myeloid cells binds LTαβ, a membrane bound heterodimer of lymphotoxin (LT) α and β. The expression of LTαβ is restricted to T and B cells and NK cells (Ware 2005). Basak and colleague have shown that LTβR signalling leads to the activation of NF-κB and type I IFN secretion (Basak, Kim et al. 2007). The secretion of type I interferon by the LTβR signalling has been shown to occur as early as 8 hours post infection and to be physiologically significant for the resistance to mCMV infection (Benedict, Banks et al. 2001; Schneider, Loewendorf et al. 2008). In contrast pDCs, major producer of type I interferons in response to mCMV, have been shown to produce large amount of type I interferon only starting around 36 hours after the initial start of the infection

(Dalod, Salazar-Mather *et al.* 2002; Delale, Paquin *et al.* 2005; Zucchini, Bessou *et al.* 2008; Swiecki, Gilfillan *et al.* 2010).

Type I interferon secretion induces a cascade of transduction signals after binding to the type I interferon receptor. Type I interferon receptor is a transmembrane receptor composed of 2 subunits IFNAR1 and IFNAR2. After being activated by interferon binding, the two subunits dimerise and the complex phosphorylates Janus kinase 1 (JAK1) and Tyrosine Kinase 2 (TYK2). Activation of JAK1/TYK2 results in tyrosine phosphorylation of signal transducer and activators of transcription 1 and 2 (STAT1 and STAT2). Subsequently STAT1–STAT2 form a complex with IFN regulatory factor 9 (IRF9) known as ISGF3 (IFN-stimulated gene (ISG) factor 3) complexes. ISGF3 translocates to the nucleus and binds IFN-stimulated response elements (ISREs) contained in the promoter of interferon stimulated genes (ISG) to initiate genes transcription (Platanias 2005).

There are more than one hundred ISGs identified. Once activated, ISG proteins will target many different stages of the virus life cycle such as cleavage of single stranded RNA (ssRNA), gene transcription, protein translation, trafficking and protein processing (Samuel 2001). The first wave of type I interferon response induces a positive feedback loop leading on to a second wave of type I interferon secretion, which contributes to the enhancement of the antiviral response (Tailor, Tamura *et al.* 2007). Furthermore, in response to interferon signalling STAT1 can form alternative combinations with other STAT proteins: STAT1, 3, 4, 5, or 6. These dimers initiate the gene transcription of ISRE but also of IFN-activated sites (GAS) (Platanias 2005). In addition to the classical JAK-STAT pathway, multiple other JAK-kinase-dependent signalling cascades are activated, like the IRS-PI 3-kinase pathway, the Crk-family proteins pathway (CRKL) or the phosphatidyl-inositol pathway (PI3K). Type I interferons also activate p38 mitogen-activated protein kinase (MAP kinase) to induce gene transcription in a STAT independent manner (Platanias 2005).

Moreover, type I IFN also enhances DC maturation, NK cell cytotoxicity, and differentiation of virus-specific cytotoxic T lymphocytes ($CD8^+$ T cells) (Dalod, Hamilton et al. 2003; Hahm, Trifilo et al. 2005), thus providing a link between innate and adaptive immune responses.

Type I interferon plays a very important role in the control of mCMV infection. mCMV infection increases IFNβ and α levels at very early time point post infection in vivo (Schneider, Loewendorf et al. 2008; Zucchini, Bessou et al. 2008). Pre-treatment of human fibroblasts with IFNβ limits plaque formation and mice lacking the Type I receptor or a downstream effecter (such asTYK2) are much more sensitive to mCMV infection (Presti, Pollock et al. 1998; Malmgaard, Salazar-Mather et al. 2002; Sainz, LaMarca et al. 2005; Strobl, Bubic et al. 2005). Treatment with subtypes of IFNα of mCMV infected mice led to a significant reduction in mCMV replication (Cull, Bartlett et al. 2002). However, in vitro studies reveal an only moderate reduction of mCMV replication following treatment with recombinant IFNα (Davignon, Castanie et al. 1996) which indicates that CMV interferes with the type I interferon response.

Since type I interferon response plays a key role in the control of CMV infection, it is not surprising that the virus has developed mechanisms to counter its antiviral effect.

First of all, there is evidence that CMV interfere with type I gene induction. hCMV infection selectively blocks the induction of ISGs including IFNβ (Browne, Wing et al. 2001), however the precise molecular mechanisms of this inhibition remain to be identified.

In addition, CMV has developed strategies to block the type I signalling pathway. hCMV blocks IFNα stimulated gene expression in fibroblasts and endothelial cells (Miller, Zhang *et al.* 1999; Paulus, Krauss *et al.* 2006) and induce the degradation of JAK1 and IRF9 proteins levels (Miller, Rahill *et al.* 1998). Human CMV IE1 protein binds to the ISGF3 complex to inhibit the interaction between STAT1 and STAT2 (Paulus, Krauss *et al.* 2006). mCMV protein M27 is necessary and sufficient to block the induction of type I and II interferon. Le and colleagues have shown that mCMV inhibits IFNβ transcription by perturbing the formation of the enhanceosome (Le, Trilling *et al.* 2008).

Both human and mouse CMV interferes with interferon induced effectors such as the activation of protein kinase R (PKR) and 2'-5' oligoadenylate synthetase (RNaseL) which block viral protein synthesis (Schneider and Mohr 2003).

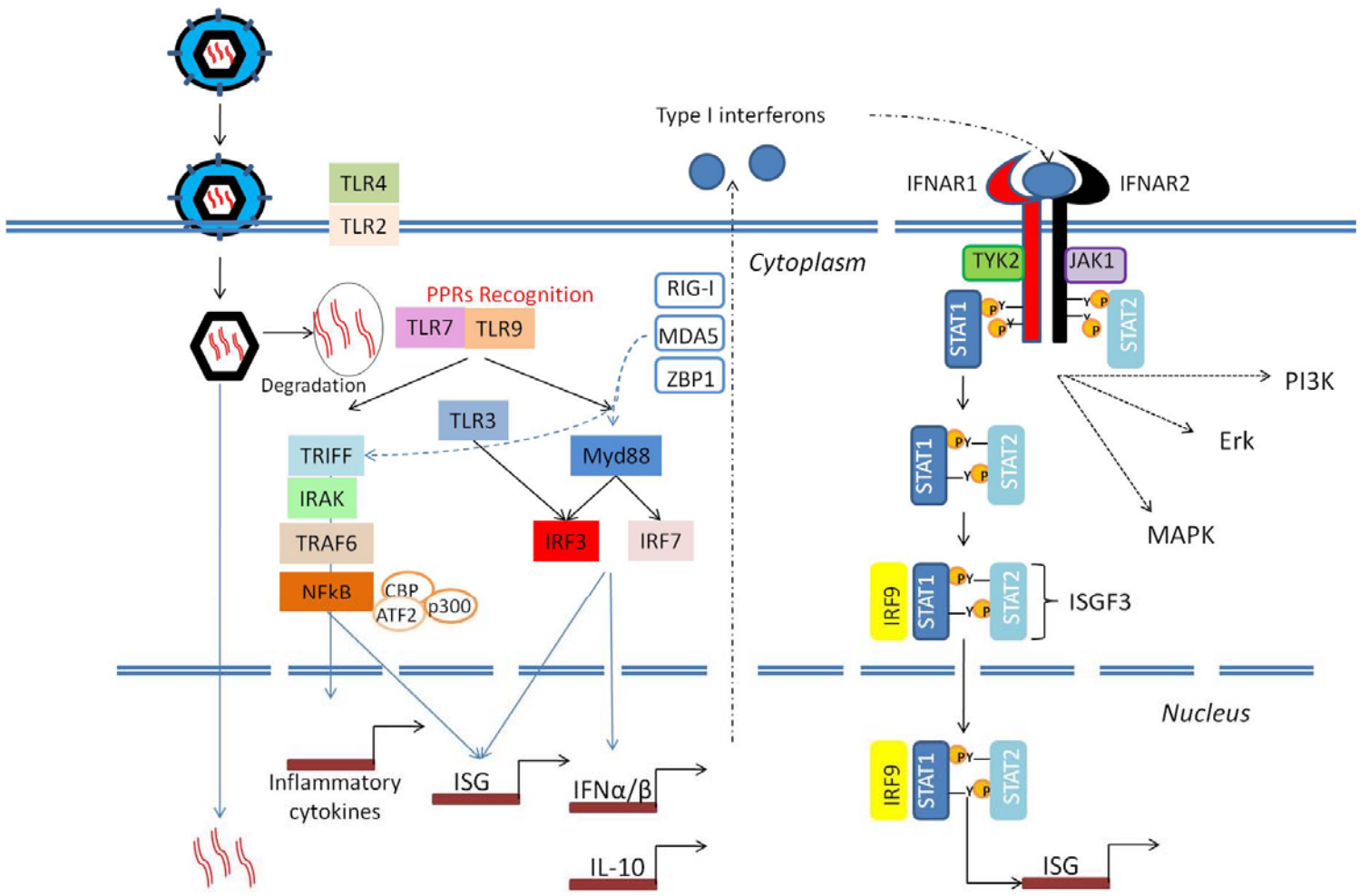


Figure 1-5: Induction of innate immune response by viral infection

Toll-like receptor (TLR) signalling. Activation of TLRs by mCMV leads to the recruitment of kinases which catalyse the phosphorylation of IRF3 and IRF7. TLRs engagement also results in the activation of nuclear factors such as NF-κB. Phosphorylated IRFs forms dimers, translocate into the nuclei and in collaboration with NF-κB and cofactors, bind to DNA and regulate the expression of type I interferons, inflammatory cytokines and ISG genes. **IFN signalling**. Endogenous IFNα/β bind to a common receptor expressed at the surface of target cells. Receptor engagement leads to the activation of STAT1 (signal transducer and activator of transcription 1) and STAT2, which, together with ISGF3G/IRF-9, bind IFN-stimulated response elements (ISREs) and activate ISG genes. PRRs: Pattern recognition receptors, ISG: Inteferons stimulated genes.

1.3 Adaptive immune response and CMV infection

The adaptive immune response provides the vertebrate immune system the ability to specifically recognize and remember viral infections. Both innate and adaptive immune responses play a role in the control of human and mouse CMV infection. While innate immune response can contain the primary infection, the adaptive immune response is capable of clearing it. However, CMV have developed mechanisms to evade the immune system by establishing latency. (Koszinowski, Reddehase *et al.* 1991)

The effector cells of the adaptive immune system are B lymphocytes, specialized in adaptive humoral response, CD8+ T lymphocytes (CD8+ T) or killer cells specialised in the elimination of the infected cells and CD4+T lymphocytes (CD4+ T) or helper cells which activate and maximize the immune response (Koszinowski, Reddehase *et al.* 1991).

The activators of the T and B cells are the antigen presenting cells (APC) such as DCs and macrophages. Once activated by the viral infection APCs, mainly DCs, migrate to the lymph nodes where they present viral antigen epitope to the naive CD4+ T cells through the MHC class II pathway and to the naïve CD8+ T cells through the MHC class I pathway. Activated and differentiated T and B cells proliferate and migrate outside the lymph node. Chemo-attraction recruits T and B cells to the site of infection. B cells secrete antibodies against CMV such as Gb, Gl or pp65. Once enveloped by antibodies, virions or infected cells are degraded by the proteins of the complement.

CD8+ T cells recognize infected cells through the antigen presentation of MHC class I and, in coordination with cytokines, when stimulated secrete perforin and granzymes to induce apoptosis of the infected cells. CD8+ T cells play a major role in the control of primary infection as well as latency (Reddehase, Weiland et al. 1985; Reddehase, Mutter et al. 1987; Jonjic, Pavic et al. 1990; Simon, Holtappels et al. 2006).

CD4+ T cell do not eliminate directly infected cells but their activation causes release of cytokines and other stimulatory signals such as Interleukins two and four

(Il2 and Il4) or Ifnγ, which stimulates the activity of macrophages, killer T and B cells.

CD4+ T cells play also a role in CMV clearance, especially in the salivary gland (Jonjic, Mutter *et al.* 1989).

B cell secreted antibodies were found to have a protective role in mCMV infected mice. However, experiments in irradiated mice where activated B cells were re-injected show that B cells are not sufficient to clear the infection although they contribute significantly (Klenovsek, Weisel *et al.* 2007). In humans, the transfer of antibodies from an hCMV seropositive mother to a newborn infant was shown to be protective against hCMV infection from seropositive blood transfusions (Wynn, Fulton et al. 2008). Jonjic and colleagues have shown that antibodies are not essential for the resolution of a primary infection but limit the dissemination of the virus (Jonjic, Pavic et al. 1994).

CMV has developed strategies to counteract the adaptive immune response.

Both human and mouse CMV interferes with the immunomodulatory functions of type I interferon response by decreasing the MHC class I expression and presentation as well as other proteins involved in the in antigen presentation (Mocarski 2002; Pinto and Hill 2005). Three mCMV proteins have been shown to interfere with MHC class I pathway. Gp34 forms complexes with MHC class I molecules in the ER that are then expressed at the cell surface (Kleijnen, Huppa et al. 1997). Gp34 co-operate with gp40 to prevent the recognition of infected cells by cytotoxic T cells (Kavanagh, Gold et al. 2001). Moreover, gp48 binds MHC class I complexes and induce their proteolytic destruction (Reusch, Muranyi et al. 1999).

CMV reduces the ability of CD4 T cells to amplify the immune response by interfering with their ability to enhance the MHC class II antigen presentation in professional antigen presenting cells (APC). For example, hCMV produces a protein cmvIl10 mimicking the effect of interleukin 10 (IL10), an inhibitor of MHC class II expression (Spencer, Lockridge *et al.* 2002).

1.3.1 Type II interferon response to mCMV infection

Type II interferon (IFNγ) is produced by activated T cells and NK cells. Ifnγ binds to the Ifnγ receptor IFNGR, which is composed of two subunits IFNARG1 and IFNARG2. When activated by IFNγ binding, the two subunits dimerize and activate kinase JAK1 and JAK. Activated JAK1 will phosphorylate STAT1s and forms homodimers. These complexes will translocate into the nucleus and bind to Ifnγ-activation site element (GAS), leading to the expression of Ifnγ inducible genes (Wesoly, Szweykowska-Kulinska *et al.* 2007).

IFNγ plays a major role in immune response against CMV infection in mice and humans such as the activation of macrophages during infection (Heise and Virgin 1995), the enhancement of MHC class I–dependent antigen presentation to CD8 T cells (Hengel, Lucin *et al.* 1994) and also the inhibition of mCMV replication and gene expression (Lucin, Jonjic *et al.* 1994). Furthermore it has been shown that injection of recombinant Ifnγ can protect against lethal infection (Fennie, Lie *et al.* 1988). In addition, IFNγ plays a role in the regulation of latent infection although the mechanisms are still not well understood (Presti, Pollock *et al.* 1998).

1.4 Cholesterol and host pathogen interaction

Cellular lipids were considered to have only a structural role for a long time. However during the last decades, it became clearer that lipids were involved in much more active biological processes such as cell signalling, membrane trafficking, inflammation and immune response (Wenk 2005).

It is not surprising, therefore, that lipids are involved in the life cycle of pathogens and especially that of viruses. Lipids play a key role in viral entry, viral replication and host pathogen interaction (Wenk 2006).

Lipid content is modulated by pathogens for their own benefit. For instance, Munger and colleagues (Munger, Bennett *et al.* 2008) have shown that pyruvate metabolism and fatty acid flux production were changed in response to HCV infection.

Among lipids, cholesterol has been identified as one of the most potent molecules. Not surprisingly cholesterol has been proven to be essential for almost every step of viral infection (from entry to egress).

Furthermore, cholesterol pathways have been shown to be modulated by pro-inflammatory signals and acute stress responses. In addition, several reports have shown that cytokines can modulate the serum cholesterol level. Changes in cholesterol pathways have also been shown to modulate the immune and inflammatory response.

In conclusion, a body of evidence points to the pivotal role of cholesterol in the interplay between the virus and the host to modulate the infection and the immune response.

However, the mechanisms involved in these processes are still not well understood.

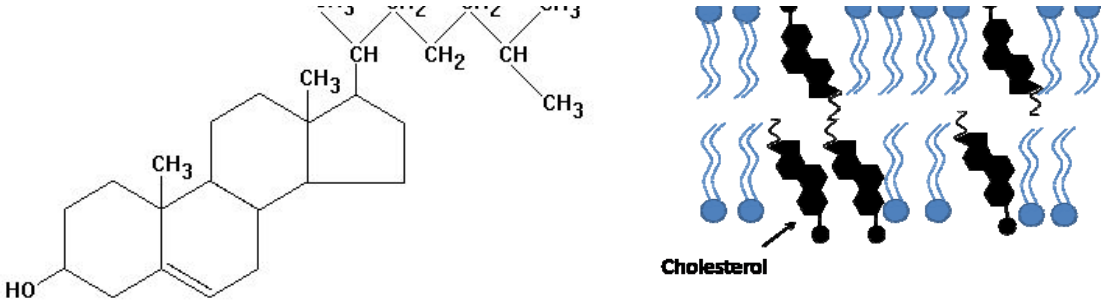
This section reviews the role of cholesterol in the life cycle of viruses and its relationship with the immune response.

1.4.1 Cholesterol metabolism

1.4.1.1 Structure of cholesterol

Cholesterol (Figure 1-6) is the most common steroid in mammalian biology. It is a complex molecule consisting of C27 H46 O with a molecular weight of 386.65 grams per mole. It has weak amphipathic properties due to the polar nature of the hydroxyl group and the hydrophobic characteristics of the ring structure. In the cell, cholesterol is inserted into lipid bilayers of cell membranes, with the polar region interacting with the phospholipids segment of the bilayer and the hydrophobic portions oriented inward. In cell membranes, cholesterol has a stabilizing effect, reducing permeability while making them more resistant to physical damage and temperature change.

Figure 1-6: molecular structure of cholesterol and its membrane organisation



1.4.1.2 Cholesterol synthesis and prenylation pathway

Cholesterol synthesis occurs in the cytoplasm and ER and involves a cascade of enzymatic transformations (Goldstein and Brown 1990) (Figure 1-7). Firstly, acetyl-CoAs are converted into 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) by HMG-CoA synthase 1 (*HMGCS1*). Then, HMG-CoA reductase (*HMGCR*), the rate-limiting enzyme of the pathway converts HMG-CoA into mevalonate. Mevalonate is then transformed by three successive enzymatic phosphorylations into 5-pyrophosphomevalonate (M5PP). After this step an ATP-dependent decarboxylation transforms the pyrophosphomevalonate in isopentenyl pyrophosphate, IsPP, an isoprenoid molecule. IsPP is in equilibrium with its isomer, dimethylallyl pyrophosphate (DMPP). Isopentenyl pyrophosphate (*IDI1*) condenses one molecule of IsPP with one molecule of DMPP to generate geranyl pyrophosphate (GPP). Farnesyl diphosphate synthase (FDPS) further condenses GPP with another IsPP molecule to become farnesyl pyrophosphate, FPP. Geranygeranyl pyrophosphate synthase (GGPS) convert one molecule of IPP and one of FPP into geranylgeranylpyrophsophate (GGPP). The main branch of these pathways leads to the formation of free cholesterol and is therefore named the sterol synthesis pathway. Squalene synthase (*FDFT1*) catalyzes the condensation of 2 molecules of FPP to form squalene. Squalene is then transformed into lanosterol by squalene epoxidase (*Sqle*) and lanosterol synthase (*LSS*) involving two-step cyclization. 19 additional reactions for lanosterol to be converted into free cholesterol are then required. The isoprenoid metabolites are not only metabolic precursors for the biosynthesis of cholesterol but also for alternative side pathways (Caraglia, Budillon *et al.* 2005). Protein prenylation is a posttranslational modification process occurring in the ER and involving the binding of a farnesyl or geranyl-geranyl molecules to the C terminal cysteine of a target protein. Ras and Ras-related small GTP-binding proteins (Rho, Rab, Rac and the γ subunit of the trimeric G proteins) are the main target for protein prenylation. Prenylated proteins play important roles in the regulation of apoptosis, signal transduction, cell differentiation, cell replication, cytoskeletal organization, vesicular trafficking and immune signalling. The enzymes that accomplish the prenylation of proteins in the cell are the farnesyltransferases type I (FNTA and FNTB), the geranylgeranyltransferase I (PGGT1B) and the Rab

geranylgeranyltransferase II (RABBGTA and RABGGTB). In addition, IsPPs are also the metabolic precursors of heme, ubiquinone, and dolichol.

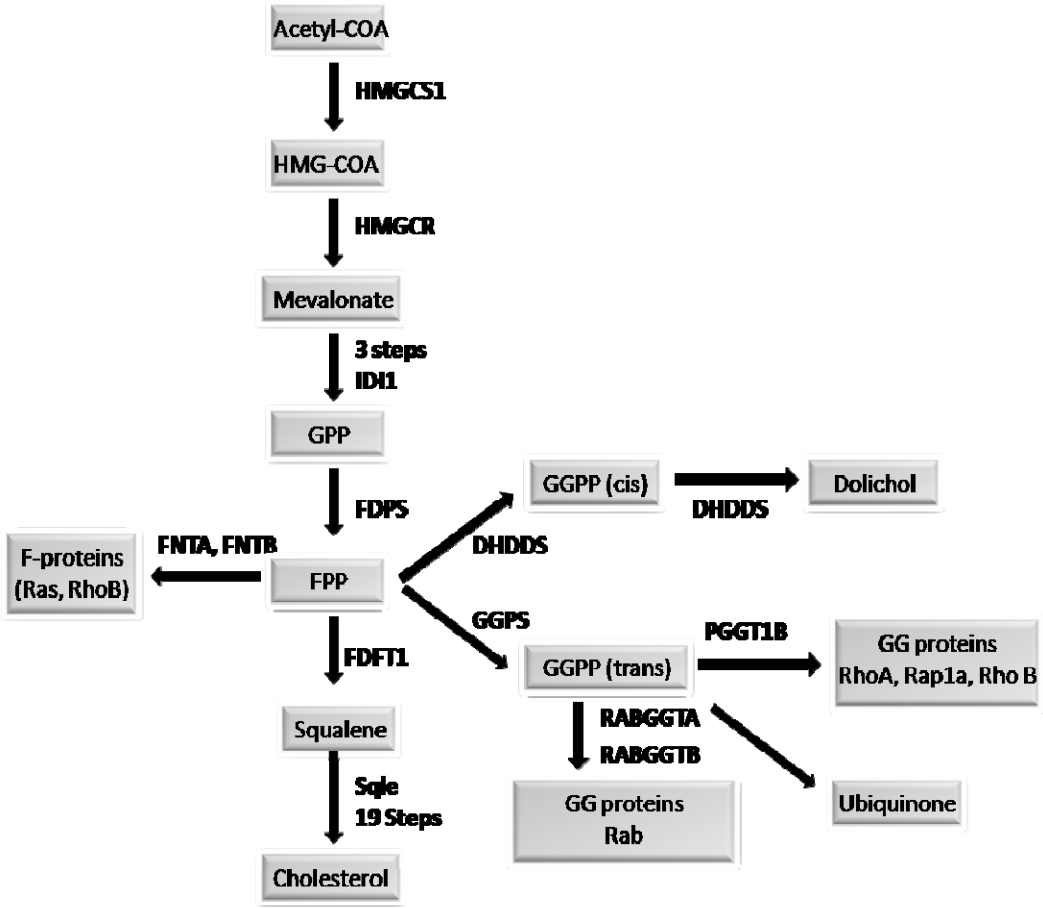


Figure 1-7: Schematic of the cholesterol synthesis pathway

Grey boxes representing metabolites and enzymes regulating the pathway are indicated. GPP: Geranyl Pyrophospahte, FPP: Farnesyl pyrophosphate, GGPP: Geranylgeranyl pyrophosphate, F-proteins: Farnesykated proteins, GG proteins: Geranylgeranylated proteins.

1.4.1.3 Regulation of the cholesterol synthesis

Cholesterol homeostasis is regulated in vertebrate cells by transcriptional factors, which sense intra cellular cholesterol levels (Brown and Goldstein 1997; Osborne 2000). These transcription factors are named sterol-regulatory element-binding proteins (SR*EBP*s). In the nucleus SR*EBP*s bind specifically to DNA sequences called sterol regulatory element (SRE) amplifying the expression of the genes containing these sequences. To date, more than 30 genes are known to contain SRE sequences including almost all the genes of the sterol synthesis pathway but also *Hmgcr*, *Ldlr* and Fasn (Horton 2002; Sun, Seemann *et al.* 2007). There are two genes encoding for three isoforms of SR*EBP*s protein. *Srefbf1* encodes for two splice variant proteins: SR*EBP*1a and SR*EBP*1c. SR*EBP*-1a and SR*EBP*-1c differ only by the first exon which encodes for a transactivator domain making SR*EBP*1c less potent than SR*EBP*-1a. SR*EBP*-1a and -1c both activate genes involved in the synthesis of fatty acids and triglycerides and phospholipids. *Srebf2* encodes for SR*EBP*2, which preferentially activates genes responsible for cholesterol synthesis and the LDL receptor (*Ldlr*).

The coordinate expression and translation of SR*EBP*s allows for the control of the intracellular lipid homeostasis (Horton, Goldstein *et al.* 2002).

SR*EBP*s are regulated at the transcriptional level by transcription factors although the exact mechanisms remain unclear (Raghow, Yellaturu *et al.* 2008). SR*EBP*s regulation also depends on the intra cellular cholesterol and oxysterol levels, which allow a feedback control mechanism for the cell to maintain and adjust the intracellular cholesterol concentration (see figure 1.7 and 1.8). Indeed, SR*EBP*s are associated in the ER membrane with SR*EBP* cleavage activating protein (SCAP) and Insulin induced gene 1 and 2 (Insig-1 and Insig-2). Cholesterol binds to the sterol-sensing domain of SCAP. When bound to cholesterol molecules, SCAP binds to INSIGs proteins. This association causes the SR*EBP*-SCAP complex to be retained within the ER. When the sterol concentration is low, SCAP and INSIGs do not interact together allowing the translocation of the complex SR*EBP*-SCAP to the Golgi Apparatus (GA). In the GA, Protease S1P and S2P cleave the transmembrane domain of SR*EBP*s precursor, releasing SR*EBP* to the cytosol which then translocates into the nucleus. Oxysterols can also regulate the cleavage of SR*EBP*,

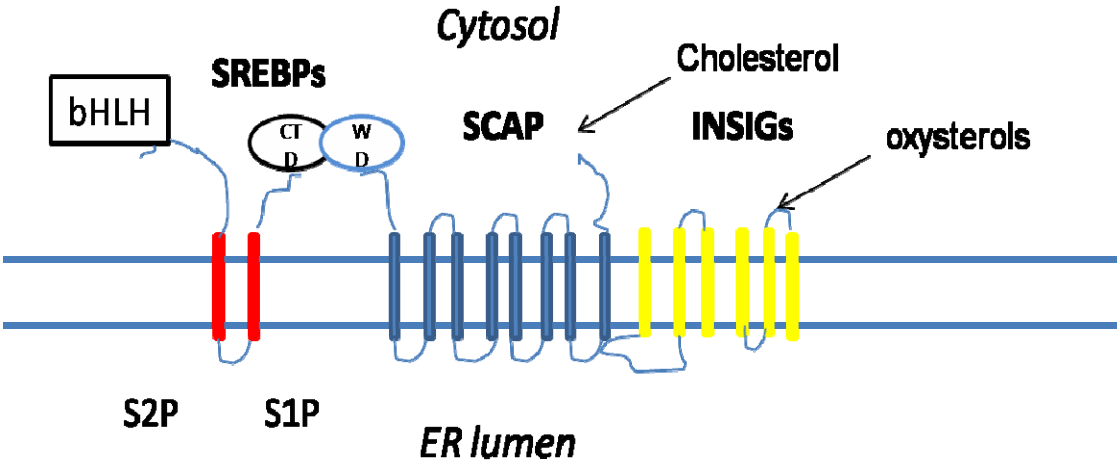


Figure 1-8: SREBP regulation by SCAP and INSIGs proteins

CT D: C terminus domain, W D: WD40 domain, bHLH: basic Helix Loop Helix.

25-hydroxycholesterol binds to Insig-2 and blocks the translocation of the SR*EBP*-SCAP complex (Radhakrishnan, Ikeda *et al.* 2007).

As mentioned before, *HMGCR* is the rate liming enzyme of the cholesterol synthesis pathway and its regulation is therefore crucial for the control of cholesterol synthesis. *HMGCR* activities are tightly controlled and involve several mechanisms apart from the SR*EBP*2 transcriptional regulation. Indeed, *HMGCR* activity also depends on its post transcriptional regulation, phosphorylation state and degradation rates. *HMGCR* is most active in the dephosphorylated state. Phosphorylation is catalyzed by AMP-activated protein kinase, AMPK, (used to be termed HMGR kinase), an enzyme whose activity is also regulated by phosphorylation. The phosphorylation state of *HMGCR* is additionally controlled by the cAMP signalling pathway. Increases in cAMP lead to dephosphorylation of *HMGCR* (Wang, Jones *et al.* 2006).

The degradation of *HMGCR* occurs within the proteosome, a complex specialized in protein degradation. The primary signal directing proteins to the proteosome involved multiple ubiquitination. Although the mechanisms are still not well understood, sterol levels are involved in *HMGCR* degradation. A high level of free cholesterol increases the rate of *HMGCR* degradation. Long-term regulation involves

control of synthesis and degradation, while short-term regulation involves the phosphorylation state of the enzyme (Ness, Lopez *et al.* 1998).

Hormones play also an important role in the regulation of *HMGCR*: insulin affects the post transational processing of *HMGCR* (Feramisco, Goldstein *et al.* 2004), thyroid hormones alter its expression and glucocorticoids act at the post-translational level (Geelen, Gibson *et al.* 1986; Ness, Lopez *et al.* 1998).

1.4.1.4 Cholesterol homeostasis

The concentration of intracellular cholesterol is tightly regulated by the cell. Apart from cholesterol synthesis, four mechanisms also regulate co-ordinately the intracellular concentration of cholesterol (Simons and Ikonen 2000): Firstly, cholesterol uptake, where lipoprotein receptor *Ldlr* plays an important role in the uptake of cholesterol as it binds and internalises Low Density Lipoprotein (LDL), which comprises cholesterol ester rich particles. When they bind to the *Ldlr*, LDL particles are internalised in endosomes followed by lysososomal degradation. In the lysosome, LDL-cholesterol ester is hydrolysed to free cholesterol, with a resultant increase in intracellular levels. Secondly, bile acid and steroids synthesis; free cholesterol is converted in downstream steroids in the bile acids synthesis pathway. There are two pathways characterised for the synthesis of bile acids. In the liver, bile synthesis is initiated by cholesterol 7α-hydroxylase (CYP7A1). In other tissues, bile acid synthesis can be initiated by alternative pathway by 27-hydroxylase (CYP27A1) or 25 cholesterol hydroxylase: (encoded by *Ch25h*). Thirdly, cholesterol storage; cholesterol is stored in the cell as an ester a non bioactive form, formed by ACAT: cholesterol acyltransferase enzyme encoded by *Soat1*. The accumulation of cholesterol esters will form lipid droplets in the cell. Fourthly, cholesterol efflux; ABCs proteins facilitate the efflux of cellular cholesterol from the cell. 2 proteins have been mainly involved in the regulation of the efflux: ABCA1 and ABCG1. *Abca1* KO mice have increased intracellular cholesterol concentrations. The cellular cholesterol is delivered to nascent HDL particles which then return cholesterol to the liver. One excreted lipoproteins rich in cholesterol are associated with APO proteins.*APOE* plays an important role in the reverse transport of cholesterol via *APOE* specific receptor. APOs are proteins present in lipoproteins such as VLDL,

HDL, and IDL. Among these *Apoe* has been shown to be important in the regulation of the cholesterol efflux, *Apoe* KO mice have an increase intracellular cholesterol concentration.

Nuclear hormones receptors (McEwan 2009) are a family that also plays an important role in the regulation of cholesterol metabolism. Members of this family are transcription factors characterized by their DNA binding domain and C terminal ligand binding. The main receptors of this family are the proliferator-activated receptors (PPARs), liver X receptors (LXRs), and farnesoid X receptors (FXR). Oxysterols, the oxidized form of cholesterol, act as ligands for these nuclear receptors. Hence receptors act as lipid sensors to coordinately regulating the changes in lipid metabolism (Chawla, Boisvert *et al.* 2001). All the nuclear receptors bind to the retinoid X receptors (RXRs) to form a dimer. LXRs regulate the cholesterol efflux (through the regulation of *Abca1*) and the synthesis of bile acid (through the regulation of Cyp27). LXRs have been directly linked to the regulation of SR*EBP*1a although its relative contribution to the expression of SR*EBP* is still in debate (Hegarty, Bobard *et al.* 2005).

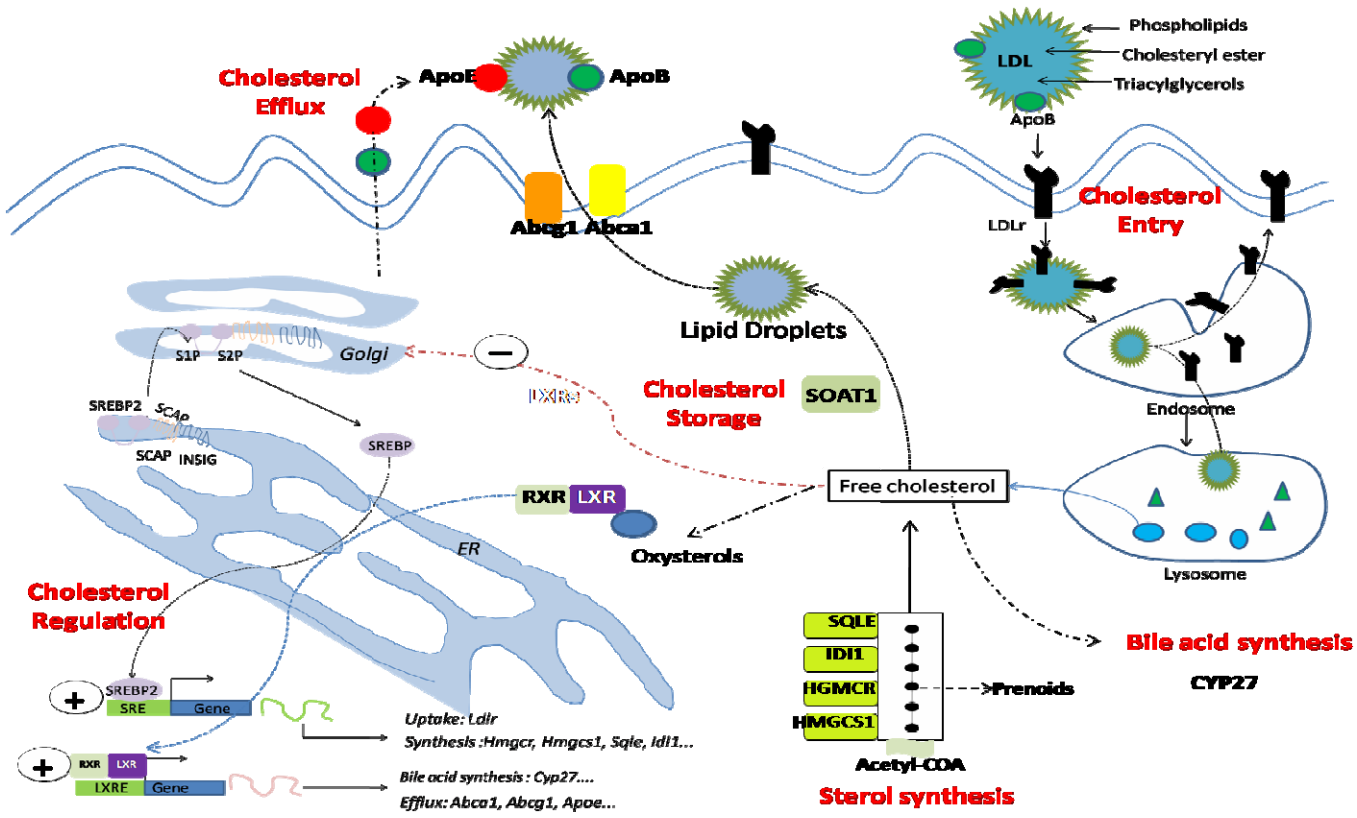


Figure 1-9: Schematic representation of the intra-cellular regulation of cholesterol homeostasis

Low cholesterol balance will trigger the up regulation of the cholesterol genes responsible for the entry and synthesis of cholesterol while the genes responsible of cholesterol storage and efflux will be down regulated. In the contrary excess level of cholesterol will decrease the expression of the genes controlling the entry and synthesis of cholesterol while the one responsible for the storage and efflux will be up regulated. ER: Endoplasmic Reticulum, SRE: Sterol regulatory elements, LXRE: LXR regulatory elements.

1.4.2 Involvement of cholesterol in viral lifecycle

1.4.2.1 Cholesterol is essential for viral entry, assembly and budding of viruses

The entry of many viruses in their host cell depends on cholesterol rich membrane structures called lipid rafts (LRs). LRs are specialised lipid-organised membrane domains rich in cholesterol, sphingolipids and marker proteins such as caveolin. The molecular composition of the lipid rafts confers a more rigid structure to the membrane that acts as an anchor for many proteins. In addition, LRs also contain viral receptors and co-receptors required for viral fusion and entry. Moreover, the entry of many enveloped and non-enveloped RNA and DNA viruses depends on LRs (Manie, de Breyne et al. 2000; Chawla, Boisvert et al. 2001; Ahn, Gibbons et al. 2002; Bavari, Bosio et al. 2002; Marjomaki, Pietiainen et al. 2002; Norkin, Anderson et al. 2002; Reynolds, Maurer et al. 2004; Nishi and Saigo 2007; Raghu, Sharma-Walia et al. 2007; Bader, Fazili et al. 2008; Desplanques, Nauwynck et al. 2008; Lee, Lin et al. 2008).

LRs have also been involved in the assembly and budding of the viral particles. LRs seem to serve as a platform for clustering of viral proteins. LRs are involved in assembly of Influenza virus and VZV (Chazal and Gerlier, 2003). LRs are also involved in viral budding for Influenza virus, Measles virus, HIV-1, and Ebola virus (Chazal and Gerlier 2003). Finally LRs also contribute to the properties of the viral envelope, and to their infectivity (Imhoff, von Messling *et al.* 2007).

1.4.2.2 Role of cholesterol synthesis in viral replication

The contribution of cholesterol synthesis to viral replication has been extensively studied using statins, drugs targeting the first enzyme *HMGCR* of the mevalonate pathway. Statin treatment of infected cells has antiviral properties *in vitro* and *in vivo* for RNA and DNA, enveloped and non enveloped viruses (except Novovirus), (Gower and Graham 2001; Horne, Muhlestein *et al.* 2003; Ye, Wang *et al.* 2003; del Real, Jimenez-Baranda *et al.* 2004; Potena, Frascaroli *et al.* 2004; Cohen 2005; Liu, Rodriguez *et al.* 2006; Ikeda and Kato 2007; Bader, Fazili *et al.* 2008; Mohan, Muller *et al.* 2008; Chang 2009; Robinzon, Dafa-Berger *et al.* 2009; Rothwell, Lebreton *et al.* 2009).

The mechanisms involved in the antiviral effects of statins are not well understood. It is still unclear whether this effect is due to the reduction in cellular cholesterol concentrations or a change in some sterol intermediaries. Indeed, the antiviral effect of statins *in vitro* and in mice models has been often linked to a reduction in prenylated proteins (Bordier, Ohkanda *et al.* 2003; Einav and Glenn 2003; Glenn 2006). For example, HCV has been shown to induce the prenylation of a cellular protein FBL2 critical for efficient replication (Wang, Gale *et al.* 2005). Although for most viruses, the mechanisms involving the reduction of viral replication and the prenylation pathway are not well understood, it is believed that apoptosis and cell cycle regulated by prenylated proteins may play a role in the antiviral properties of statins (McTaggart 2006).The antiviral effect of statins point at the crucial role of sterol synthesis in viral replication. Several trials have been started to evaluate the antiviral effect of statins in patients however the results so far are contradictory. Statins have an antiviral effect in HIV infected patients while they have no effect for HCV infection (O'Leary, Chan *et al.* 2007; Forde, Law *et al.* 2009; Montoya, Jaimes *et al.* 2009).

1.4.2.3 Viral infection manipulates intra cellular cholesterol metabolism

Since cholesterol plays such an important role in viral replication, it is not surprising that viruses have developed strategies to hijack the cellular cholesterol metabolism for their own benefit. Indeed, viruses have developed strategies to use cholesterol metabolism, its biosynthetic and regulatory pathways as well as transport machinery for their own efficient assembly and egress.

First of all, viruses exploit the cellular machinery involving the synthesis and transport of Lipid droplet (LD). LDs are ER-derived organelles composed of triacylglycerols and cholesterol esters surrounded by a phospholipids membrane. These organelles are specialised in storing intracellular neutral lipid and they dynamically move through the cytoplasm and interact with other organelles to facilitate the transport of lipids and proteins in the cell (Murphy 2001; Martin and Parton 2005). McLauchlan and others have demonstrated that HCV utilises the synthesis and the protein machinery of LD for assembly and egress of its own particles. (Miyanari, Atsuzawa *et al.* 2007; Boulant, Douglas *et al.* 2008; McLauchlan 2009). Furthermore, the Dengue virus capsid protein usurps lipid droplets for viral particle formation. These results may indicate that the hijacking of the LDs synthesis and machinery by viruses is a general viral strategy for assembly and egress. Pharmacological targeting of the LD formation is a promising therapeutic intervention as it drastically reduces the Dengue virus titre (Samsa, Mondotte *et al.* 2009).

Secondly, viruses have also developed mechanisms to modulate the transcription of the cholesterol metabolism regulatory genes and redistribute the intra cellular cholesterol pool.

For example, HCV viral protein, HCV core and NS4b, indirectly increase the expression of the sterol synthesis regulating genes through the up-regulation of the gene expression and the proteic cleavage of SR*EBP*S proteins (Waris, Felmlee *et al.* 2007; Park, Jun *et al.* 2009).

NEF, one of the HIV proteins, increases expression of the sterol biosynthesis pathways genes, as well as being able to bind directly to cholesterol. It is believed that NEF allows the redirection of cholesterol pool to LRs and newly synthesised viral particles (Zheng, Plemenitas *et al.* 2003).

Finally, West Nile virus infections increase cholesterol synthesis and redistribute intracellular cholesterol to newly formed viral membranes. This additionally disrupts the immune response to the infection by decreasing MHC class I and II presentation (Mackenzie, Khromykh *et al.* 2007).

Furthermore, a very recent study has demonstrated that acute Measles virus infection induces the down regulation of almost the entire sterol biosynthetic pathway (Robinzon, Dafa-Berger *et al.* 2009). The authors of this study suggest that the down regulation of the gene expression in response to the viral infection may reduce the viral budding without providing convincing evidence. Moreover, Dengue virus can manipulate the intracellular cholesterol content and direct it to the site of the viral replication (Rothwell, Lebreton *et al.* 2009).

1.4.2.4 Viral infection induced dyslipidemia in patient

Viral infections are well known to induce dyslipidemia in patients. For example hepatitis B and C infection induces liver steatosis (Rubbia-Brandt et al 2003) and decreases cholesterol (LDL and HDL) concentrations in blood (Cicognani C et al, 1997). Hypocholestemia is one of the symptoms of HIV infection (Zangerle et al, 1994). Furthermore, several studies suggest that the level of cholesterol in blood can influence the reactivation of viral infection (Del Pozo 2010)

The mechanisms leading to the dyslipidemia induced by viral infections are not well understood. In humans, a significant correlation has been made between the intensity of the acute phase response during sepsis and the reduction of cholesterol levels (Bentz and Magnette 1998; Chiarla, Giovannini *et al.* 2004) pointing at the involvement of a cytokine response in the reduction in cholesterol levels induced by infection.

1.4.2.5 Herpes viruses and cholesterol metabolism

The direct involvement of cholesterol on the life cycle of Herpes viruses has not been extensively studied and characterized. However there is some evidence that cholesterol plays an important role in Herpes virus infection. First of all, cholesterol has been shown to be critical for the viral entry of hCMV (Juckem, Boehme *et al.* 2008), HSV1 (Bender, Whitbeck *et al.* 2003; Hannah, Cairns *et al.* 2009),VZV

(Hambleton, Steinberg *et al.* 2007), EBV(Ikeda and Longnecker 2007) and human Herpesvirus-6 (Tang, Kawabata *et al.* 2008). Additionally, Kawabata and colleagues have shown that human Herpesvirus-6 envelope component was enriched in cholesterol rich lipid rafts (Kawabata, Tang *et al.* 2009) suggesting an important role for cholesterol in viral budding.

Furthermore, cholesterol synthesis has been shown to modulate Herpes virus gene expression. Indeed, pharmacological inhibition of the mevalonate pathway with statins have been reported to limit immediate early and late HCMV gene expression in endothelial cells (Potena, Frascaroli *et al.* 2004)

Herpes viruses also induce the level of total cholesterol in infected patients (Del Pozo, van de Beek *et al.*). HSV1 infection results in the reduction of HDL cholesterol concentrations in infected patients (Vilkuna-Rautiainen, Pussinen *et al.* 2006). CMV infections have been shown to directly increase the level of cholesterol and cholesterol ester in smooth muscle cells in vitro and in the arterial wall in vivo of chickens (Abrahamsen, Clay *et al.* 1996; Melnick, Adam *et al.* 1996). Furthermore, mCMV infection has been show to increase oxidised LDL uptake mRNA expression in vascular smooth muscle cells (Zhou, Guetta *et al.* 1996).

Hajjar and colleagues have shown that *MVD* infection of chicken smooth muscle cells increased sterol synthesis, decreased excretion of free cholesterol and increased cholesteryl ester synthetic activity (Hajjar, Falcone *et al.* 1985; Fabricant and Fabricant 1999). Infection of these cells also increased the internalisation of LDL and increased mRNA expression of *Ldlr* in the first hours post infection. The activity and expression of *Hmgcr* has been reported to be also increased by HSV infection in smooth muscle cells. In uninfected cells, mevalonic acid and 25-hydroxy cholesterol reduced the activity of *Hmgcr* but this effect was lost in the HSV-infected cells suggesting that the viruses interfere with the effects of oxysterols on cholesterol synthesis and uptake. Moreover this study suggests that a viral kinase (US3) is involved in this process (Hsu, Nicholson *et al.* 1995)

Interestingly there is indirect evidence suggesting that there is interplay between cholesterol regulation, CMV infection and inflammation response. Indeed, hCMV has been considered as a risk factor for the development of atherosclerosis. Atherosclerosis is an inflammatory disease characterized by the development of fatty

deposit in the arterial wall, which will eventually lead to thromboses or strokes. Macrophage disregulation of cholesterol metabolism leads to foam cells formation and this process is believed to be one of the factors involved in the development of fatty deposit or atheroma in the artery wall. Although the causal relationship is still in debate and the mechanisms are not well understood, it is believed that CMV infection enhances the formation of fatty plaques.

In fact, several studies have shown a significant relationship between Herpes virus infection, and an increasing risk of developing atherosclerosis, especially in hCMV infected patients (Alber, Powell *et al.* 2000; Georges, Rupprecht *et al.* 2003; Gomez, Laures *et al.* 2005). hCMV-seropositive individuals have endothelial dysfunction and an increased number of atherosclerotic plaques (Grahame-Clarke, Chan *et al.* 2003). Studies in apolipoprotein E-knockout mice also showed that mCMV infection increases lesion size (Hsich, Zhou *et al.* 2001). Moreover, mCMV infection increases the lesion T cell content and the levels of IFNγ and TNF in the arterial wall, which may contribute to the inflammatory process. Finally, mCMV reactivation has also been implicated in the development of atherosclerotic plaque (Vliegen, Duijvestijn *et al.* 2004).

In addition recently, HSV infection has been linked with an increasing risk of developing Alzheimer disease and it is believed that disregulation of cholesterol efflux in neurones by HSV infection contribute to the disease process (Itabashi, Arai *et al.* 1997; Itzhaki and Wozniak 2006; Carter 2008).

1.5 Innate immunity and cholesterol metabolism

1.5.1 Cytokines and chemokines modulate cholesterol metabolism

It is well documented that the acute phase response induces a decrease of total serum cholesterol levels in primates, and an increase in rodents' cholesterol levels by stimulating hepatic *de novo* cholesterol synthesis, decreasing lipoprotein clearance, and decreasing the conversion of cholesterol into bile acids (Khovidhunkit, Kim *et al.* 2004).

Pro-inflammatory cytokines and LPS treatments have been reported to alter sterol blood levels. LPS, TNF, IL-1, and Il-6 increase cholesterol levels in rodents.

At the transcriptional level, in rodents, TNF, IL-1 and Il-6 stimulate hepatic cholesterol synthesis by increasing the transcription and activities of *HMGCR* (Khovidhunkit, Kim *et al.* 2004). The effect on the *HMGCR* activities and transcription occur 16 hrs after treatment. Furthermore, in rodents, TNFα has been shown to decrease specifically the transcription of *SQLE*. It is interesting to note that TNF and IL-1 in combination have synergistic effect on serum cholesterol and HMG-CoA reductase mRNA levels (Hardardottir, Moser *et al.* 1994). TNF may also affect cholesterol metabolism and excretion by inhibiting the expression and activity of cholesterol-7a-hydroxylase (CYP7A1), the rate-limiting enzyme in the classic pathway of bile acid synthesis (De Fabiani, Mitro *et al.* 2001).

However, the effects of cytokines on cholesterol levels differ between rodents and primates. In primates, and humans TNFα, serum cholesterol levels do not change or decreases (Popa, Netea *et al.* 2007). In human hepatoma HepG2 cells IL-1 inhibits cholesterol synthesis and decreases cholesterol and apoB secretion. IL-6 increases cholesterol synthesis but decreases cholesterol secretion (Ettinger, Varma *et al.* 1994).

The role of IFN response in modulating the cholesterol synthesis is not clear and has not been extensively studied. In humans, high doses of IFNβ in Hella cells results in an increase of sterol synthesis (Pfeffer, Kwok *et al.* 1985) but IFNβ treatment decreases plasma cholesterol levels in multiple sclerosis patients (Morra, Coppola *et al.* 2004). Patients treated with IFNγ do not show changes in blood cholesterol level (Kurzrock, Rohde *et al.* 1986). Furthermore, IFNβ inhibits ApoE production at the

transcriptional level in a dose-dependent manner in patients (O'Toole and Love 2002). IFNγ treated macrophages develop an unbalanced cellular cholesterol regulation leading to the increase of intracellular cholesterol esters, reduction of cholesterol efflux and ultimately in the formation of foam cells, which is an important step in the development of atherosclerosis (Reiss, Patel *et al.* 2004). Furthermore, IFNγ inhibits ApoE production at the post-transcriptional level in a human macrophage cell line (O'Toole and Love 2002).

1.5.2 Cross talk between innate immunity and cholesterol metabolism: Involvement of TLRs and nuclear hormones receptor

1.5.2.1 Innate immune response modulate cholesterol metabolism

Emerging studies indicate that TLR signalling pathways are involved in the regulation of cholesterol metabolism. TLR activation promotes lipid accumulation in endothelial cells (Curtiss and Tobias 2009). The effect of TLRs on cholesterol metabolism seems to have an effect on mainly the cholesterol efflux through the modulation of the ABCs proteins. For example TLR agonists suppress the expression of *ABCA1* and cholesterol efflux in macrophages and LPS treatment reduced the nuclear levels of RAR alpha and decreased *ABCA1* expression and cholesterol efflux via IRAK activation in wild-type mice. TLRs activation has been reported to induce the increase of the oxysterol; 25 hydroxy-cholesterol (Bauman, Bitmansour *et al.* 2009). The reduction of cholesterol efflux by TLRs activation is involved in the development of atherosclerosis as it leads to foam cell formation.

Furthermore, Castrillo and colleagues have demonstrated that TLRs regulate expression of cholesterol efflux associated genes through IRF3 and the nuclear receptors LXRα and RXRs, independently of IFNβ production. In this study, microbial ligand activation of the IRF3 pathway blocked the induction of LXR target genes such as *ABCA1* and inhibited cholesterol efflux from macrophages (Castillo and Kowalik 2004).

Although IFN treatment modulates cholesterol metabolism, little is known of the mechanisms and signalling pathway involved. Recently, Hao and colleagues have shown that the decrease in cholesterol efflux and in *Abca1* expression induced by

IFNγ is LXR and STAT1 dependent in human macrophage-derived foam cells (Wang, Panousis *et al.* 2002; Hao, Cao *et al.* 2009).

1.5.2.2 Cholesterol modulates innate immune response

The cholesterol levels have also been demonstrated to modulate the innate response. For example, Prunet and colleagues have shown that oxysterol, (the oxidised form of cholesterol), initiates an inflammatory response in macrophages (Prunet, Montange *et al.* 2006) .

Moreover, the level of intracellular cholesterol can modify the innate response. Increased cellular free cellular cholesterol enhances TLR signalling (Joseph, Castrillo *et al.* 2003; Naiki, Sorrentino *et al.* 2008) and enhances pro inflammatory cytokine response (Zhu, Lee *et al.* 2008)(Zhu 2008). In addition, *Abca1* -/- mice show a reduction in IFN and ILl signalling in macrophages (Yvan-Charvet, Wang *et al.* 2010), indicating that cholesterol efflux is specifically involved in the regulation of the synthesis of inflammatory cytokines.

1.5.2.3 Limitation of current studies

It is worth noting that the interplay between innate immunity and cholesterol metabolism is generally studied using only two models. The first uses macrophage derived foam cells in the context of atherosclerosis. The second model uses hepatic cell. These 2 cell types are specifically involved in the regulation of lipid metabolism. They have probably a specific response to inflammatory stimuli, which differ from other cell type, such as fibroblasts or macrophages. Strikingly**,** very little is known about the interplay between innate immunity and cholesterol metabolism in other cell types.

1.6 Hypothesis and objectives of the thesis

Today there is a body of evidence that suggests that viral infection depends on cholesterol metabolism and sterol biosynthesis especially. On the other hand, innate immunity cross reacts with the regulation of cholesterol metabolism through inflammatory cytokines and cellular signalling. However, the question remains open as to whether changes in cholesterol metabolism are also part of a protective immune regulation. The effect of mCMV infection on cholesterol metabolism and its consequences for the host and the virus are largely unknown. Nevertheless evidence suggests that cholesterol is involved in the CMV infection.

These studies led us to hypothesise that the innate immune response plays a role in the regulation of cholesterol metabolism following mCMV infection.

To test our hypothesis, 4 objectives were defined:

- To characterise the regulation of cholesterol metabolism by mCMV infection.
- To test whether these changes play a role in modulating the infection.
- To investigate the role of the innate immunity in the changes of cholesterol metabolism induced by the infection.
- To understand which signalling pathways triggered by the immune response are involved in the regulation of cholesterol metabolism induced by the infection.

2 Chapter 2: Materials and methods

2.1 Tissue culture

2.1.1 Cell lines

NIH/3T3 cells and MEFs-P53 -/- cells were obtained from American Type Culture Collection (Manassas, VA.). NIH/3T3 (ATCC CRL1658) cells are spontaneously immortalized fibroblast cells, derived from mouse embryonic origin. MEFs P53 -/- (ATCC CRL2645) was derived from mouse embryos that had null mutations in the p53 gene only.

L929 cells were from Sigma (85011425, Poole, Dorset, UK). Cell line L929 is a subclone of the parental strain L, which was derived from normal subcutaneous areolar and adipose tissue.

NIH-Bam25 cells were derived from NIH/3T3 cells by co-transfecting pBAMB25 (a plasmid containing the mCMV *ie1* and *ie3* genes by Angulo and colleagues (Angulo, Ghazal *et al.* 2000).

All cell lines were cultured at 5% CO_2, 95% humidity and at 37°C.

2.1.2 Cell media and tissue culture consumable

NIH/3T3 culture media: DMEM supplemented with 10% Calf Serum (CS) (v/v), 0.3 mg/ml L-glutamine, 1.3% (w/v) streptomycin and 0.6% (w/v) penicillin.

MEFs-P53 -/- culture media: DMEM supplemented with 10% (v/v) Fetal Calf Serum (FCS), 0.3 mg/ml L-glutamine, 1.3% (w/v) streptomycin and 0.6% (w/v) penicillin.

L929 culture media: DMEM supplemented with 10% (v/v) FCS, 0.3 mg/ml L-glutamine, 1.3% (w/v) streptomycin and 0.6% (w/v) penicillin.

BMDMs culture media: DMEM-F12 supplemented with 10% (v/v) FCS, 10% L929 conditioned media, 0.3 mg/ml L-glutamine, 1.3% (w/v) streptomycin and 0.6% (w/v) penicillin.

Bam25 culture media: DMEM supplemented with 10% (v/v) FCS, 0.3 mg/ml L-glutamine, 1.3% (w/v) streptomycin and 0.6% (w/v) penicillin.

All tissue culture flasks and 96, 48 or 6-well were obtained from Nunc (Copenhagen, Denmark). DMEM was from Lonza (UK), DMEM-F12 was from Gibco (Paisley,

UK), L-glutamine, streptomycin and penicillin were from Invitrogen, trypsin/EDTA was from Lonza.

2.1.3 Passaging cells

Cell lines were expanded by passaging them 1:3 to 1:5 every 3 to 4 days when they reached 70 to 80% confluency. Cells were washed in phosphate-buffered saline (PBS) and incubated with trypsin/EDTA for a few minutes at room temperature (the volume of trypsin/EDTA depending of the size of the flask).
After the cells started to detach from the flask, trypsin/EDTA was discarded and the cells were harvested and resuspended in an appropriate volume of medium.
Cells were then centrifuged at 262 g-force for 5 minutes at room temperature, supernatant was discarded and the pellet was resuspended in medium to an appropriate volume.
Low passage cells were used as much as possible for all experiments.

2.1.4 Cell count with trypan blue exclusion assay

Cells were trypsinised, resuspended in media and diluted (1/2 to 1/10) with trypan blue (0.4%).
Ten µl of the solution was then injected by capillary in a haemocytometer and the viable cells counted in the 16 marked squares. The original numbers of viable cells per ml were estimated by using the following equation:

*Number of viable cells per ml = number of counted cell in the haemocytometer * $1*10^{-4}$ cm^3 * dilution factor.*

2.1.5 Cryopreservation of cells

Cells were trypsinised as described previously, transferred to a plastic Falcon tube and centrifuged at 262 g-force for 5 min at room temperature. The cell pellets were resuspended in freezing medium (DMEM 40%, FCS 50%, dimethyl sulfoxide (DMSO) 10%) and stored into 1.8 ml cryovials (Corning, UK).

Cells were first frozen at –80°C overnight to avoid fast cooling and breaking of the cell membrane. After 24 hours cells were transferred to a liquid nitrogen container (-170°C) for long-term storage.

2.1.6 Thawing cells from liquid nitrogen

Frozen cells from the liquid nitrogen container were transferred on ice and then thawed at 37°C. Cells were then transferred to a Falcon tube and culture medium was added to dilute the DMSO contained in the freezing medium. Cells were then centrifuged at 262 g-force for 5 min at room temperature. The supernatant was discarded and the cell pellet resuspended in normal medium and transferred to a 25 cm^2 tissue culture flask and grown as previously described.

2.1.7 Sterility and cells contamination control

The study of host/pathogens interactions and of the immune system requires strict control over the contamination of the cell lines by pathogens such as bacteria or fungi as these could dramatically affect the results of the experiments.
To avoid cell contamination:

- Cells were grown in media supplemented with antibiotics: penicillin/streptomycin.
- Incubators and tissue culture hoods were weekly disinfected with antimicrobial agents.
- Every 3 months, random samples of cells were grown in antibiotic free media to examine for the presence of mycoplasma and lymphotoxin contamination (SNBTS (Edinburgh, UK)).

New cells brought to the tissue culture from other laboratories were placed in a quarantine incubator for a few days. Cells were then cultured in antibiotic free medium and tested for the presence of mycoplasma before being used.

2.1.8 Seeding cell

For each cell type, optimal seeding density was determined for every plate's format.

Table 2-1: Cell seeding density

	96 well plates	*48 well plates*	*24 well plates*	*6 well plates*
NIH/3T3	*2 10^4*	*4 10^4*	*1 10^5*	*3 10^5*
MEFs-P53 -/-	*NA*	*2 10^4*	*4 10^4*	*3 10^5*
Bam25	*2 10^4*	*4 10^4*	*1 10^5*	*3 10^5*
BMDMs	*4 10^4*	*NA*	*2.5 10^5*	*1 10^6*

NA: Not applicable

2.1.9 Preparation of L929 conditioned media

L929 media was used as a source of Macrophage Colony Stimulating Factor 1 (M-CSF1) to differentiate bone marrow hematopoietic stem cells into macrophages.

M-CSF is a cytokine secreted by mesenchymal cells that stimulates survival, proliferation and differentiation of bone marrow hematopoietic stem cells into mature monocytes. Constant stimulation of monocytes by M-CSF leads ultimately to their differentiation into bone marrow macrophages (BMDMs).

L929 cells were grown in a T175 tissue culture flask until 80% confluence. Cells were then trypsinised, split into 30 T175 flasks and grow for 14 days.

The supernatant was then collected into 50 ml Falcon tubes and centrifuged at 262 g-force to pellet the cell debris. Supernatant was then collected, aliquoted and stored at -70°C until used.

2.1.10 BMDMs culture

2.1.10.1 Bone marrow primary cell isolation

Bone marrow primary cells were extracted from 10-12 week old male BALB/c mice. Mice were sacrificed by cervical dislocation and femurs were gently extracted under sterile conditions. After removing excess tissue of the bones, both ends of each femur were cut off and the marrow cavity of the femur was flushed with DMEM-F12 supplemented with 10% FCS and 10% L929 conditioned media. Cells were recovered in a 50 ml Falcon tube on ice and counted as previously described.

2.1.10.2 Characterisation of BMDMs by FACS analysis

Mature Macrophages, express specific proteins including CD14, CD11b, F4/80 (mice), Lysozyme M, MAC-1/MAC-3 and CD68. The efficiency of the differentiation of the bone marrow cell into macrophages is assessed using fluorescence-activated cell sorting (FACS) analysis to measure the presence of these antigens on the surface of the cell.

Freshly extracted bone marrow stem cells were plated on either 6- or 24- well plates and cultured for 7 days in DMEM-F12 containing 10% FCS and 10% L929 conditioned media. Media was replaced every 3 days for 7 days to maintain constant stimulation with M-CSF1. To ensure that monocytes had developed fully into mature macrophages, the presence of F4/80 and CD11b protein at the surface of the macrophages was tested by flow cytometry analysis. Monocytes were cultured for seven days in the presence of M-CSF1 in order to mature into macrophages. At day seven, cells were washed with PBS twice, scraped off and transferred to a polystyrene FACS tubes (BD Falcon, UK). Tubes were then centrifuged at 1000 g for 5 minutes at 4 °C. After discarding the supernatant, the cells were washed with cold PBS and resuspended in 100 µl of blocking solution (10% mouse serum) for 30 minutes on ice to avoid non-specific antibody binding. Allophycocyanin (APC) conjugated monoclonal rat-anti-mouse F4/80 (IgG2a·, Caltag Laboratories, UK) and fluorescein isothiocyanate (FITC) conjugated rat-anti-mouse CD11b (IgG2b·, eBiosciences, UK) and their isotype controls were then added to the cells (1/100 dilution in blocking solution) and incubated for further 30 minutes in the dark. Cells

were then washed with cold PBS, centrifuged and resuspended in 250 µl of cold PBS.

A FACScan instrument (Becton Dickinson, UK) was used to perform FACS analysis of the samples and the data were analysed using Cell Quest software (Becton Dickinson, UK) or FlowJo software (Treestar, USA). Figure 2-1 shows that 93.1% of the cells were both F4/80 and CD11b antibody positive but not for their isotypes. These results indicate that our protocol for maturation of monocytes with M-CSF1 was efficient and gave a homogenous population of mature macrophages after 7 days treatment.

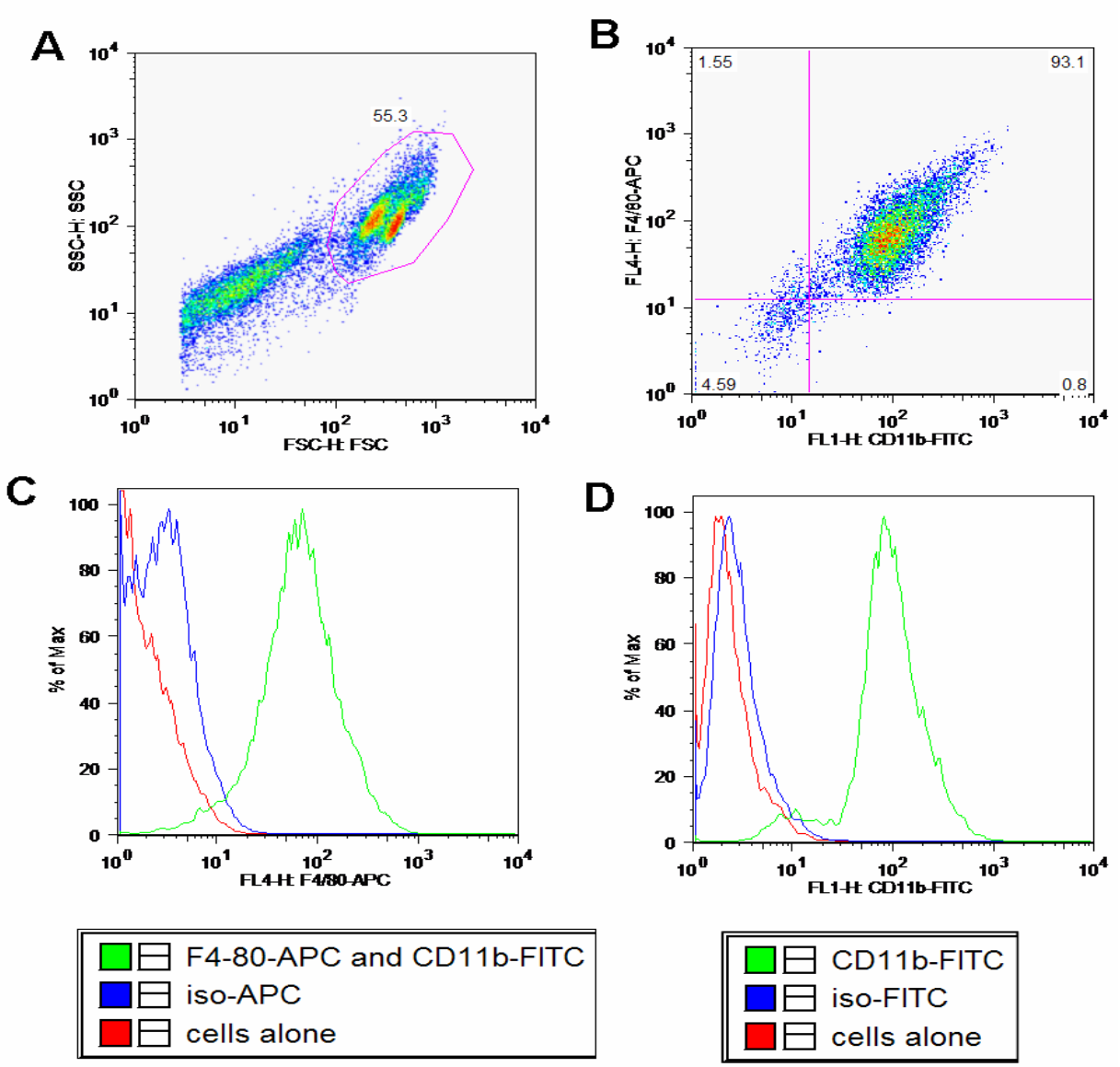


Figure 2-1: Phenotypic characterisation of BMDMs by flow cytometry

The level of expression of surface proteins F4/80 and CD11b and their respective isotypes was analysed by flow cytometry. A. Dot blot showing the gating in forward scatter (FSC) and side scatter (SSC). B. Histograms for C. F4/80 and D. CD11b staining. Experiments were performed in collaboration with Dr Sara Rodriguez Martin from The Division of Pathway Medicine (Edinburgh University).

2.2 General methods for virology

2.2.1 Viruses

C3X strain viral stock was generated from the recombinant bacterial artificial chromosome clone pSM3fr, originally derived from the mCMV strain MW97.01 (Messerle, Hahn *et al.* 2000).

PSM3fr-rev (called mCMV-GFP in this study) viral stock was generated from the recombinant virus originally named pSM3fr (Angulo, Ghazal *et al.* 2000) using homologous recombination in *E. coli*. mCMV-GFP recombinant viruses express the green fluorescent protein (GFP) marker inserted in front of the *ie2* gene.

C3X-IE3 KO virus originally named pSM3frdie3 and was generated by homologous recombination from the pSM3fr BAC(Angulo, Ghazal *et al.* 2000). C3X-IE3 virus is lacking the *ie3* gene.

Details of the genome structure of the viruses are presented in Figure 2-2.

HSV and RSV viral stock were kindly provided by Diwakar S Kumar and Dr Suzanne Stewart (University of Edinburgh) respectively.

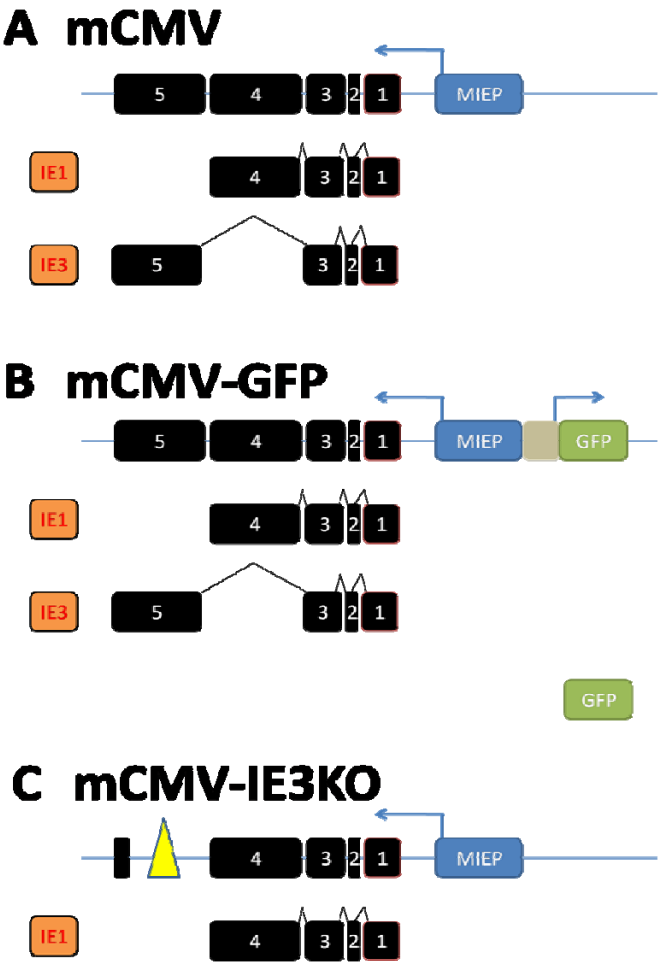


Figure 2-2: Schematic representation of the immediate early gene coding region of mCMV, mCMV-GFP and mCMV-IE3KO viruses.

Coding exons are shown as black rectangles. The blue box represents the mCMV enhancer *ie*1/*ie*3 promoter. The structures of the *ie*1 and *ie*3 transcripts are indicated below the genome representation. Starting with A: mCMV: the *ie*1 transcript of mCMV wild type viruses consists of exons 1 to 4, and the *ie*3 transcript is composed of exons 1 to 3 and 5. B, mCMV-GFP: GFP gene is shown (green box) and C, mCMV-IE3KO, the deletion of the fifth exon of the *ie*1 gene is marked by the delta. All viruses were generated by successive rounds of homologous recombination in *E. coli* (Angulo, Ghazal *et al.* 2000).

2.2.2 Viral stock propagation

70 to 80% confluent cells in a tissue culture flask were infected with an MOI of 0.1 in 3% serum media. The cells and the supernatant were harvested 5-7 days after infection when a nearly complete cytopathic effect (CPE) was observed. The supernatant was collected, spun at 1640 g-force for 15 minutes to remove cell debris and transferred to ice cold in 50 ml Falcon tubes. The concentrated viral stock was then stored at -70°C.

In order to avoid or limit the risk of viral mutations occurring during multiple rounds of infection, viral stocks were generated in two steps. First a master stock was produced in a T25 tissue culture flask from the original viral stock (low passage number) and aliquots stored at -70°C. These aliquots were then used to generate working viral stocks in T175 tissue culture flasks. NIH/3T3 cells were used to grow C3X and CMV-GFP viral stock, while Bam25 cells were used to grow the IE3-KO viral stocks, as Bam25 cells express the *ie3* gene.

2.2.3 Concentrating viral stock

In order to increase viral titre and to purify the viral particles, all the viral stocks were concentrated and resuspended in fresh media or PBS.

Frozen viral stock were thawed in a 37^{o}C water bath and stored on ice at 4^{o}C. Ten ml of a 20% sorbitol - 0.01 M Tris-buffered saline (TBS) pH 7.8 solutions was added to centrifuge tubes (Sorvall) on ice. The viral stock (20 ml) was gently and slowly pipetted down the side of the tube so as not to disturb the sorbitol cushion and to ensure that separate layers form. The tubes were weighed and balanced within 0.5 of a gram and centrifuged at 26 000 x *g* for 70 minutes at 4^{o}C to precipitate the viral particles. The supernatant was removed and pellets were resuspended in a small volume with DMEM, 10% FCS or PBS. Viral preparations were pooled together, aliquoted in a small volume (50 to 200 µl) and stored at -70 ^{o}C for further use.

As a large percentage of viral particles were lost during the purification, concentrating the viral stock concentration will on average increase the viral titre by 10 fold.

2.2.4 Viral infection

All cell types were infected with the mCMV viruses (C3X, mCMV-GFP, mCMV-IE3) at a MOI of 1, unless specified. The viral inoculum was diluted to the appropriate concentration in fresh DMEM 3% CS (FCS for P53-/- and BMDMs), and penicillin/streptomycin. Cells were left for 1 hr adsorption with gentle shaking of the plate every 10 minutes at 37°C. After 1 hr absorption, inoculum was aspirated and cells washed twice with PBS. Fresh DMEM with either 10 % CS or FCS, and penicillin/streptomycin were then added to cells.

2.2.4.1 Multiplicity of infection

The multiplicity of infection or MOI has been defined has the ratio of infectious unit to cell. An infectious unit refers to the viral particles capable of producing an infection in a susceptible cell.

In permissive cells, infection depends on the random collision of cells and virus particles and the distribution of virus particles per cell follows a Poisson distribution:

$$P(K) = \frac{e^{-m}m^{k}}{K!}$$

Where *m* is the multiplicity of infection or MOI, *k* is the number of infectious agents that enter the infection target, and $P(n)$ is the probability that an infection target (a cell) will get infected by *n* infectious agents.

Consequently, when susceptible cells are mixed with a suspension of virus some cells are uninfected and other cells may receive one or more viral particles. Therefore a MOI=1 (1 infectious unit per cell) is not sufficient to infect the total cell population. Increasing the MOI number augment the number of infected cells: for example permissive cells infected at a MOI of 1 will results in the infection of 63.2% of the cell population while infection with a MOI of 5 will results in the infection of 99.3%.

In non or less permissive cells for a given virus (for example macrophages and mCMV), the infection of the cells does not depends only on the random collision of viruses and cells but also in the cellular factors affecting the viral entry and

replication. As a result, infection with a given MOI results in a smaller percentage of infected cells.

2.2.5 Plaque assay and viral stock titration

The Plaque assay is the gold standard technique to quantify the titre of infectious viruses in an infected sample. The principle of a plaque assay is based on the dilution of a solution of the virus, which is applied to a culture dish containing a layer of the host cells. The spreading of the infection is prevented by making the medium very viscous. After incubation the plaque forming units or PFUs (areas in which cells have been lysed), can be counted and the number of infective virus particles in the original suspension estimated. To minimize error, only the wells containing between 10 and 100 plaques are counted. Low passage (less than 10) MEFs P53-/- were grown to reach 70 to 80% confluency, tryspinsed and plated ($5x10^{\wedge 4}$ cells per well) in 48 well plates.

The following day, cells were infected as described before with 100 µl of the viral suspension (10^{-2} to 10^{-8} in DMEM supplemented with 3% FCS). After 1 hr absorption, cells were washed with DMEM and supplemented with 1 ml of DMEM supplemented with 3% FCS at 37 ^{o}C mixed with 2.5% melted agarose.

Plates were placed in an incubator and after 3 to 4 days incubation. Plaques were counted in each well and the viral titres were expressed as plaque forming units per ml (PFU/ml) using the following equation:

$$\text{Viral titre in PFU/ml} = \frac{\text{Number of plaques} * \text{dilution of viral stock}}{\text{Volume of viral inoculums (ml)}}$$

The kinetics growth of each virus was also quantified with a plaque assay for 5 days post infection at least in two independent assays with triplicate for each time point.

2.2.5.1 Growth kinetics of mCMV-C3X in NIH/3T3 fibroblasts and BMDMs

Replication of the wild type virus mCMV-C3X was analysed in NIH/3T3 fibroblasts and BMDMs by performing single and multi step-growth curve characterisation. A single-step growth curve (high MOI) allows the study of the infection of the total population of cells simultaneously and displays information related to the burst and replication of the virus. The multi-step growth curve (low MOI resulting in multiple rounds of infection) displays information related to the budding and the cell to cell spread of the virus.

NIH/3T3 fibroblasts and BMDMs were infected at low (0.1) and high (1) MOI for five days and the number of viral particles in the supernatant was estimated each day using a standard plaque assay technique (2.2.5).

For the single-step growth analysis, fibroblast cultures that were infected with $10^{\wedge 5}$ mCMV-C3X viral particles displayed rapid increases in the viral titres after two days of infection (around $1.5x10^{\wedge 5}$ PFU), followed by a continuous increase at day three and four (up to $4.10^{\wedge 6}$ PFU. At day 5 the number of viral particles levelled off or even decreased ($1\ 10^{\wedge 6}$ PFU) reflecting the saturation and death of the infected cells (Figure 2-3A).

For the multi-step growth analysis NIH/3T3 fibroblasts were infected with $10^{\wedge 4}$ PFU (MOI 0.1) resulting in a continuous increase of viral particles from day 2 ($5.10^{\wedge 3}$ PFU) up to day 5 ($3.10^{\wedge 6}$ PFU), reflecting the increasing numbers of infected cells producing *de novo* viral particles (Figure 2-3A).

Single step growth analysis with BMDMs infected with $10^{\wedge 5}$ PFU showed (Figure 2-3B) a lower number of viral particles produced from day 2 ($5.10^{\wedge 3}$ to day 5 ($3.10^{\wedge 4}$) PFU) of infection compare to infected fibroblasts Multi-step growth analysis of BMDMs infected with $10^{\wedge 4}$ PFU also showed a lower production of viral particles from day 2 to day 5 post infection ($10^{\wedge 3}$ PFU after 5 days of infection) (Figure 2-3B).

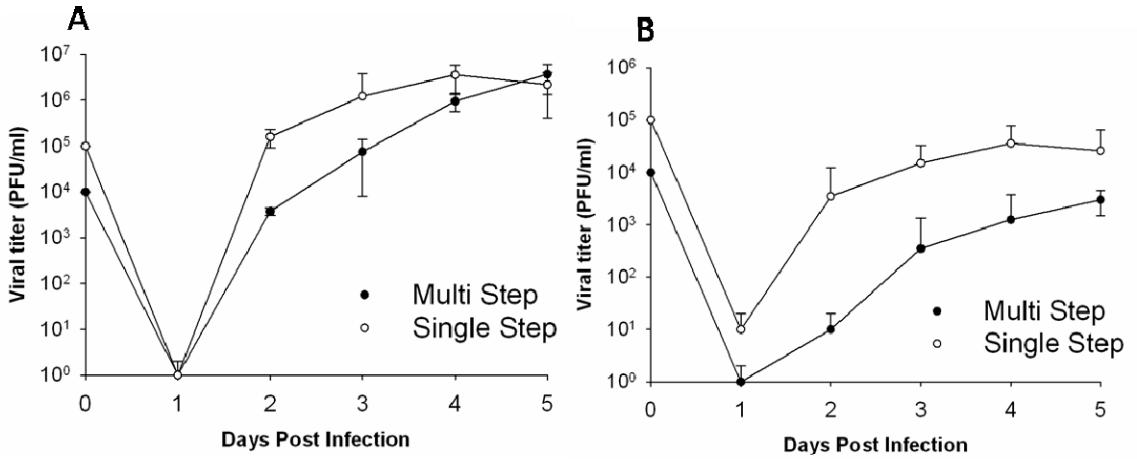


Figure 2-3: Kinetics of mCMV-C3X in murine NIH/3T3 fibroblasts and BMDMs

A: NIH/3T3 fibroblasts were infected at an MOI of 1 (Single step) or 0.1 PFU (Multi step) per cell with wild-type mCMV-C3X viruses. One experiment with three biological replicates was performed (n=3). Each data point represents the average and standard deviation from three independent wells.

B: BMDMs from Balb/c mice were infected at an MOI of 1 (Single step) or 0.1 PFU (Multi step) per cell with wild-type mCMV-C3X viruses.

At the indicated time points after infection supernatants from the infected cultures were harvested, and titres were determined by standard plaque assay on MEFs p53 -/- cells. One experiment with three biological replicates was performed (n=3). Each data point represents the average and standard deviation from three independent wells.

2.2.6 Viral growth kinetics comparison of mCMV-C3X and mCMV-GFP in NIH/3T3 fibroblasts

mCMV-GFP virus was used in this study to measure the replication of the virus upon treatment of NIH/3T3 live cells with siRNA or various drugs. The amount of fluorescence emitted from the infected cells was used to estimate viral replication (2.9). To ensure that mCMV-GFP and the wild type viruses had the same characteristics of replication, growth kinetic analysis of the 2 viruses in NIH/3T3 fibroblastss was performed at low and high MOI. Results (Figure 2-4) show that the growth kinetics of the mCMV-GFP virus and the wild type were comparable at low and high MOI. These results are similar to those of the study done by Angulo and colleagues (Angulo, Ghazal *et al.* 2000).

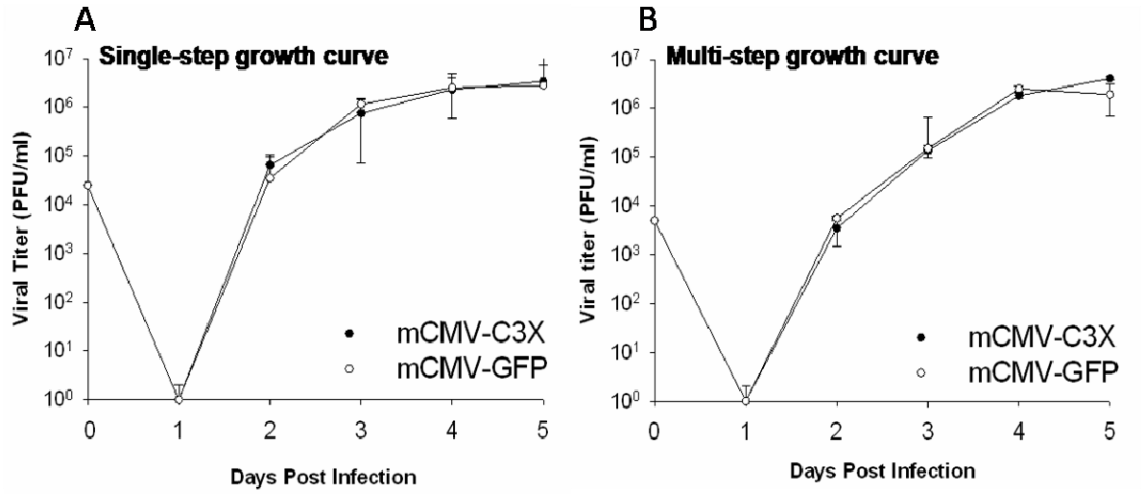


Figure 2-4: Growth kinetics comparison of mCMV-C3X and mCMV-GFP in NIH/3T3 fibroblastss

A: Cells were infected at an MOI of one (Single step growth curve), or B: 0.1 PFU per cell (Multi step growth curve) with wild-type mCMV-C3X or the GFP tagged mCMV-GFP virus. At the indicated time, supernatants from the infected cultures were harvested, and titres were determined by standard plaque assay on MEFs p53 -/- cells. For both graphs, one experiment with three biological replicates was performed (n=3). Each data point represents the average and standard deviation from three independent wells.

2.2.7 Viral growth kinetics comparison of mCMV-C3X and mCMV-IE3KO in BMDMs

In this study, mCMV-IE3KO (2.2.1) was used to investigate the consequence of a non productive infection. To ensure that mCMV-IE3KO was not able to replicate in BMDMs, growth kinetic analysis of mCMV-IE3KO virus compared to mCMV-C3X was performed in BMDMs at low and high MOI. Results (Figure 2-5: Comparison of growth kinetics of mCMV-C3X and mCMV-IE3 in Figure 2-5) show that mCMV-IE3KO infection of BMDMs in low and high MOI does not result in any plaque formation as seen with the wild type virus over 5 days of infection.

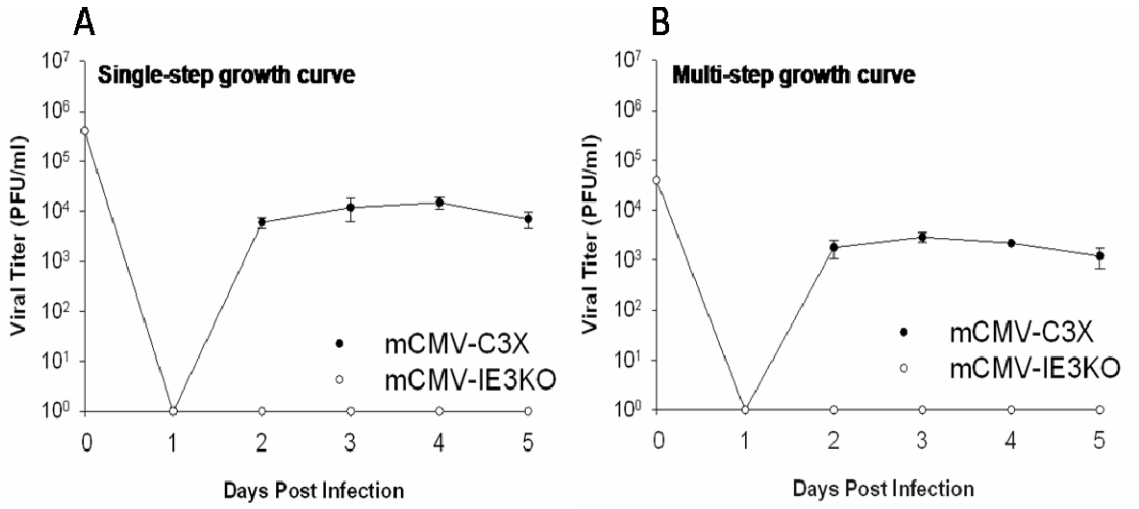


Figure 2-5: Comparison of growth kinetics of mCMV-C3X and mCMV-IE3 in BMDMs

BMDMs from C57bl6 were infected at an MOI of 1 (Single step growth curve) or 0.1 (Multi step growth curve) PFU per cell with wild-type mCMV-C3X virus or the mutant mCMV-IE3KO virus. At the indicated time points after infection (dpi) supernatants from the infected cultures were harvested, and titres were determined by standard plaque assays on MEFs p53 -/- cells. For both graphs, one experiment with three biological replicates was performed (n=3). Each data point represents the average and standard deviation from three independent wells.

2.3 Cytokines and pharmacological treatment

IFNγ was from Boehinger Manheim Corp and IFNβ, IL6, TNF and IL1β were from Biosource International, USA. Each cytokine was dissolved in PBS and 0.1% albumin, aliquoted and stored at – 70^{o}C

Simvastatin (Sigma-Aldrich) was activated by hydrolysis of the lactone ring by adding 1 ml of 0.1 N NaOH, 96 % ethanol to 25 mg of simvastatin powder.
After heating at 50 °C for 2 hrs the solution was neutralized with HCl to a pH of 7.2 and sterilised by filtration through a 0.2 μM filter.
The stock solution was diluted to the appropriate concentration in sterile PBS and the solution was aliquoted and stored at −20°C and used within a month.

Mevalonate (Sigma-Aldrich, Germany) was resuspended in media to the appropriate concentration by vortexing the solution, sterilized by filtration through a 0.2 μm filter and stored at -20^{o}C

Geranylgeraniol, farnesol and squalene (Sigma-Aldrich) were dissolved to the appropriate stock concentration in DMSO, sterilised by filtration through a 0.2 μm filter and stored at -20°C. For tissue culture experiments geraniol and farnesol were diluted in media before use.

Methyl-cyclodextrin was resuspended on the day of the experiment in media (with no serum) to the appropriate concentration by vortexing the solution and sterilised by filtration through a 0.2 μm filter.

Gancyclovir (Cymevene, Hoffman-La Roche, UK) was resuspended in saline solution and sterilised by filtration through a 0.2 μm filter. Gancyclovir was then diluted in media to appropriate concentration.

2.4 In vivo studies

2.4.1 Mice and infection

Mice were housed in the animal facilities at the University of Edinburgh under pathogen free conditions.

All experiments and handling of the animals were carried out under appropriate personal and project licences (Prof P.Ghazal, University of Edinburgh) in accordance with the Home Office and the University of Edinburgh regulations.

Mice (C57/BL6, Charles River, 12 weeks of age) were randomized into 2 groups of six animals a week prior to the experiment.

After treatment with simvastatin (see below) at day 0, at day 1 of the experiment, mice were inoculated with 2 x 10^6 PFU by intraperitoneal injection (i.p) and monitored daily. At day 5, mice were sacrificed by cervical dislocation. This experiment was repeated twice independently.

2.4.2 Simvastatin treatment

Mice were given 50 mg/kg of activated simvastatin (Sigma) resuspended in 150 µl of PBS by gavage by a trained animal technician daily.

The dosage of statins used in the present investigation was chosen according to the literature (Gower and Graham 2001) and was approved by the Home Office. No toxic effect in the mice during the course of the treatment was observed in response to simvastatin treatment

2.4.3 Sample processing

All the following procedures are performed on ice at 4^{o}C in a class I cabinet. Spleen, liver, kidneys, lungs and hearts were removed and placed in 500 μl of PBS. Organs were weighed and resuspended in 10% (w/v) DMEM media under sterile condition.

Tissues were homogenised with a tissue homogeniser and sonicated (1 pulse of 10 s, maximum power (800 W, Fisher Scientific) in order to free the viral particles from the cells. Samples were centrifuged to pellet the cell debris and supernatant was taken and kept at -70^{o}C until further use. Viral titres were determined by standard plaque assay as mentioned above.

2.5 Molecular methods

2.5.1 RNA extraction using Trizol®

Cells were washed twice with PBS, and lysed with the appropriate volume of Trizol® reagent (Invitrogen, CA): 200 µl for 24 well-plates and 500 µl for 6 well-plates. After incubation for 5 minutes at room temperature, samples were transferred to a 1.5 ml microfuge tube and stored at -70 ºC until further use. RNA was extracted after samples defrosted at room temperature by the addition of 1/5 of volume trizol. Samples were mixed gently by hand and incubated at room temperature for 1 minute and centrifuged (12000 g-force, 4 ºC for 15 minutes). The aqueous phase containing the RNA was collected carefully in a fresh tube in order to avoid proteic organic lower contamination. An equal volume of chloroform was added to the tubes to perform a second chloroform extraction as mentioned before. Finally, the upper aqueous layer was placed in a fresh tube and 0.1 volumes 3 M sodium acetate (NaOAc), 2.5 volumes ethanol (EtOH) were added to precipitate the RNA. Samples were incubated (-70 ºC, 1 hr) and then centrifuged (12000 g-force, 4ºC, 30 minutes). Supernatant was removed and the RNA pellets washed in 200 µl 70% (v/v) ethanol and centrifuged as before. This step was repeated twice. Samples were then DNAse (Sigma) treated to avoid any DNA contamination and RNA pellets were then resuspended in 50 µl RNAse-free H_2O.

2.5.2 RNA quantitation

RNA quantity and purity was assayed using a spectrophotometer (Nanodrop ND-1000 (Nanodrop Technologies, Germany). Ratio of the absorption at 260 and 280 nm (A_{260}/A_{280}) was used to assess the protein (optimal absorbance at 280nm) contamination of a DNA sample (optimal absorbance at 260 nm).

Ratio A_{260}/A_{230} was used to assess the organic (phenol, chloroform) contamination, which tends to have an optimal absorbance at 230 nm.

Samples with a ratio $A_{260}/A_{230} \geq 1.8$ and a ratio $A_{260}/A_{280} \geq 2$ were selected for further quantitative PCR analysis.

To assess the RNA integrity, a 2100 Bioanalyzer (Agilent) was used and the samples having a RNA integrity number (RIN number) of ≥ 9.0 were chosen.

2.5.3 Transcript quantification with real time quantitative PCR

2.5.3.1 Principle

Taqman primers probe/set contains 2 set of primers (forward and reverse) which are complementary oligonucleotides to the 5' and 3' area of the region of the cDNA to amplify. Taq polymerase will initiate the formation of the amplicon defined by the 2 primers. TaqMan primers/probe set also contains a probe, which encloses 2 fluorescent reporter dyes, 6 -carboxyfluorescein (FAM) and 6-carboxy-tetramethyl-rhodamine (TAMRA). FAM has its emission quenched due to the spatial proximity of TAMRA.

During the extension phase of the PCR, the Taq polymerase cleaves the TaqMan probe, resulting in the release and accumulation of FAM no longer quenched by TAMRA. The amount of fluorescence will hence determine the initial concentration of RNA allowing quantification and therefore comparison. Because the PCR reaction is exponential until it reaches a plateau due to shortage of resources, the quantification of the fluorescence is done during the exponential phase of the PCR reaction using cycle threshold (Ct) value as a unit. Ct values represent the number of cycles requires for the fluorescence signal to reach a threshold of detection. Ct values are inversely proportional to the number of copies of the RNA targeted in the samples. The lower the Ct level the greater the amount of target RNA in the sample.

2.5.3.2 Methods

Commercially available "ready to use" Taqman primer probes set (Applied Biosystems, CA) was used for all Quantitative PCR experiments (QPCR).

PCR reactions were performed using the Brilliant II QRT-PCR Master MixnKit 1 step from Stratagene. Brilliant Master Mix contains the StrataScript® Reverse Transcriptase (RT), and enough oligonucleotides and magnesium to allow the formation of cDNA and its PCR amplification from the original mRNA in the same sample.

For each sample QPCR was performed in 20 µl final volumes using MicroAmp Optical 96-well reaction plates and MicroAmp Optical Caps (Applied Biosystems).

Per reaction, 2 µl diluted RNA sample (~100 ng of RNA) was added to 10 µl 2 X Brilliant II master mixes, 1 µl Taqman primer/probe set for the gene of interest, 0.25 µl StrataScript (Stratagene) and 6.25 µl double-distilled H_2O.
Data were collected using the Mx3000P instrument (Stratagene).
After an initial incubation at 50 °C for 30 seconds to activate the RNA reverse transcriptase and allow cDNA synthesis, samples were then subject to 40 cycles under Taqman standard conditions: combined annealing and primer extension phase at 60 °C for 1 minute and a short denaturation at 72 °C for 30 s.

2.5.3.3 Analysis of the data

Relative transcript quantification of the genes of interest was performed using the ΔΔct method(Livak and Schmittgen 2001) This method calculates the ratio of Ct value of the gene of interest to the Ct value of a housekeeping gene to give a 'normalised' value. The normalised values are used to determine differential gene expression of the samples treated with various conditions.
Gapdh was used as a housekeeping gene for each experiment. The stability of *Gapdh* expression with or without mCMV infection was checked in several micro array data sets (data not shown).
Efficiency of the PCR reaction for each primers probe set was calculated by comparing the results of a standard curve using serial dilution of RNA samples.
Stratagene MXPro software was then used to analyse the data. Threshold of detection and ratio determinations were automatically performed by the software and the normalised values were exported into Microsoft Excel for further statistical analysis.

2.5.4 Primer list

The following lipid genes were used to examine the effect of mCMV infection as summarised in Table 2-2.

Table 2-2: TaqMan primers/probes

GENE SYMBOL	GENE NAME	UNIGENE ID	ASSAY ID	EXON BOUNDARY	AMPLICON LENGTH (KBP)	PCR EFFICIENCY
Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	Mm.475623	Mm01282500_g1	19 - 20	82	82%
Hmgcs1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	Mm.61526	Mm00524111_m1	2 - 3	62	98%
Sqle	squalene epoxidase	Mm.218665	Mm00436772_m1	2 - 3	72	105%
Ldlr	low density lipoprotein receptor	Mm.3213	Mm00440169_m1	14 - 15	63	95%
Ch25h	cholesterol 25-hydroxylase	Mm.30824	Mm00515486_s1	1 - 1	126	69%
Idi1	isopentenyl-diphosphate delta isomerase	Mm.29847	Mm00836417_g1	2 - 3	82	101%
Abca1	ATP-binding cassette, sub-family A (ABC1), member 1	Mm.277376	Mm01350760_m1	2 - 3	65	74%

2.6 Measurement of free cholesterol concentration by enzymatic method

Intracellular cholesterol concentration was determined enzymatically using the Amplex-Red cholesterol Assay Kit (Molecular Probes, the Netherlands) according to manufacturer protocol (Cullen, Tegelkamp *et al.* 1997; Amundson and Zhou 1999).
Briefly, cells were washed with 1 ml cold PBS and then lysed in 200 µl cold "Lipid buffer" containing 0.5M of potassium phosphate, pH 7.4, 0.25 mM cholic acid, and 0.5% Triton X-100. Cell lysates were sonicated on ice with three 10-second pulses at high intensity (800 W).
To determine free cholesterol concentration, 20 µl of the samples were then mixed in a 96 well plate with 80 µl assay solution containing 300 µM Amplex Red reagent, 2 U per ml of horse radish peroxidise (HRP) and 2 U per ml cholesterol oxidase, 0.1M of potassium phosphate, pH 7.4, 0.05 mM cholic acid, and 0.1% Triton X-100.
After preincubation for 30 min at 37 °C in the dark, fluorescence was measured for 30 min (excitation signal of 530 ± 2.5 nm and fluorescence detection at 590 ± 2.5 nm) using a Polarstar Optima Multifunciton Microplate Reader (BMG Labtech, UK). The values were corrected for the background signal determined by blank samples value.
A standard curve was constructed using the manufacturer's supplied free cholesterol and was used to calculate the amount of free intra cellular cholesterol.

2.7 Analysis of lipids using high performance liquid chromatography/mass spectrometry and Electrospray ionization.

Lipid extraction of samples was performed using Bligh and Dyer method (Bligh and Dyer 1959) with chloroform methanol 2:1 ration. Samples were dried under Argon and re-suspended in chloroform 1:1. An Agilent high performance liquid chromatography (HPLC) system coupled with an Applied Biosystem Triple Quadrupole/Ion Trap mass spectrometer (4000Qtrap) was used for quantification of individual polar lipids (Phospholipids and sphingolipids). Electrospray ionization-based multiple reaction monitoring (MRM) transitions were set up for the quantitative analysis of various polar lipids (Fei, Shui *et al.* 2008). HPLC atmosphere

chemical ionization (APCI)/MS were carried out for analysis of sterols (Huang, Shen *et al.* 2006). Samples preparation and lipid extraction was performed by M BLANC, University of Edinburgh, UK, lipidomic analysis was performed by Dr G SHUI, National University of Singapore, Singapore.

2.8 Transfection and SiRNA knock down assay

To transfect NIH/3T3 cell in 96 well plate's format at a SiRNA concentration of 25 nM, the following protocol was applied.

NIH/3T3 cells were grown to 70-80 % confluency, washed with PBS, trypsynised and resuspended in antibiotic free media on ice until transfection.

SiRNAs (SMARTpools -ON-TARGETplus modification) were from Thermo Fisher Inc, USA. Various SiRNA samples (5 nM) were supplied in powder form, dissolved and aliquoted (2 μM) in SiRNA buffer 1X (Thermo Fisher).

For each transfected well, 1 μl of siRNA SMARTpool was used with 9 μl of Optimem solution (Invitrogen, CA, USA) while 0.4 μl of Dharmafect1 (Dharmacon, Perbio Science, Bonn, Germany) was mixed with 9.6 μl Optimem. Following incubation for 5 minutes, the SiRNA mix was added to the Dharmafect1 mix (0.4%) and incubated for a further 30 minutes.

NIH/3T3 cells ($1.5x10^4$ in 80 μl of DMEM 10% CS medium lacking antibiotics) were added to the SiRNA-Dharmafect1 complexes and mixed gently for few min. Growth medium was then removed from the wells and cells were washed once with PBS before 100 μl of the siRNA:. Dharmafect1-liposomes mixture was added carefully to the well.

Transfection conditions were optimized by using SiGLO red (Dharmacon) as an indicator of transfection efficiency and cell viability was assessed using Cell titer blue assay (Promega) according to manufacturer instruction. SiGLO red contains fluorescent oligonucleotides that localise to the nucleus thus permitting the visual assessment of SiRNA uptake into mammalian cells.

For negative control, SiGLO Risk free, non-targeting (SiRNA having no target sequence in human, mouse and rat genome) and Dharmafect1 only (0.04%) were used.

Transfection efficiency during each experiment was monitored by using eGFP SiRNA to knock-down the GFP expression induced by the viral gene expression.

2.9 Live cell replication assay

To measure the effect of multiple drugs and SiRNA transfection on viral growth, a sensitive live cell infection assay was developed using the properties of the mCM GFP tagged virus. 1.5 x 104 NIH/3T3 cells were infected for 1 hr in black 96 well plates (Costar, UK) at a MOI of 0.2 in 25 µl of fresh DMEM phenol red-free media, 3% FCS, and 100 U of penicillin/streptomycin per ml. After infection, the inoculums was carefully removed by pipetting and replaced by 150 µl of DMEM phenol red-free media with 10% FCS.

Viral growth was measured by recording the GFP signal over time using an OPTIMA Polarstar plate reader (excitation wave length of 485 nm and emission of 520 nm). As an optimisation step we checked the correlation between GFP levels and MOIs. Results showed a correlation between multiplicity of infection and growth kinetics (Figure 2-6). Levels of GFP signal corresponding to different levels of virus was checked by comparing the GFP value and number of viral particles per ml using plaque assay. A strong correlation between differences in levels of GFP expression and differences in number of viral particles assessed by plaque assay: a drop of 20% of GFP signals corresponding to log difference in the number of viral particles established by plaque assay.

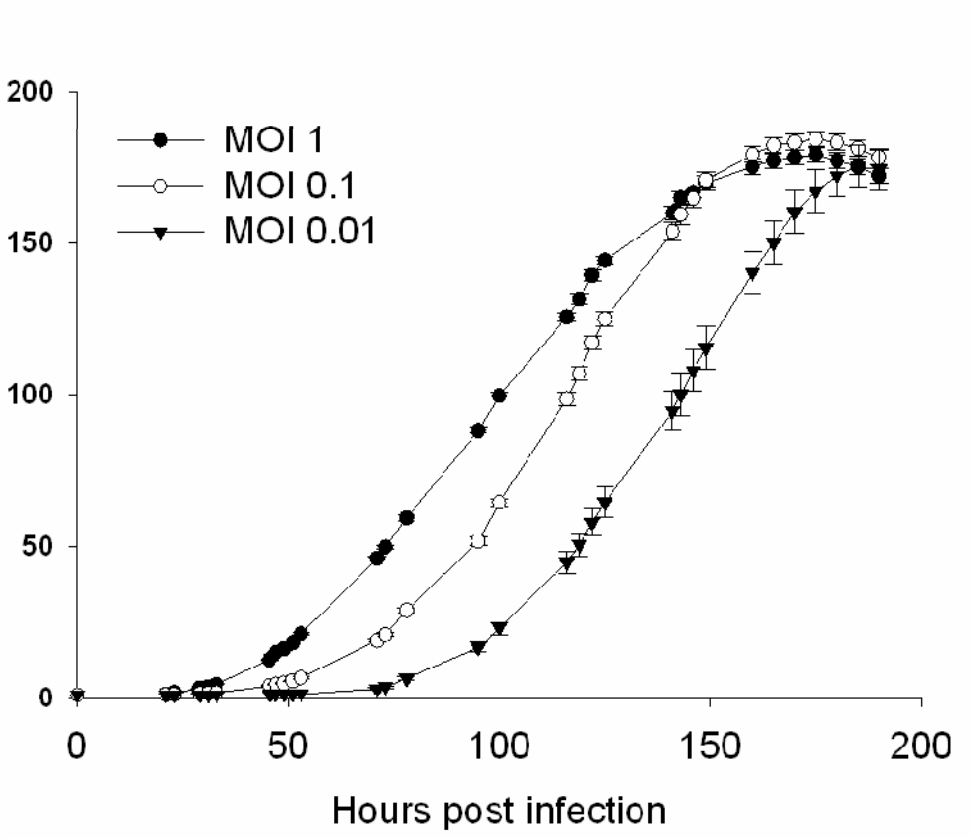


Figure 2-6: Correlation between multiplicity of infection and GFP signal

1.5 x 104 NIH/3T3 cells were infected for 1 hr in black 96 well plates (Costar, UK) at a MOI of 0.0.1, 0.1 or 1 in 25 µl of fresh DMEM phenol red-free media, 3% FCS, and 100 U of penicillin/streptomycin per ml. After infection, the inoculums were carefully removed by pipetting and replaced by 150 µl of DMEM phenol red-free media with 10% FCS.

Viral growth was measured by recording the GFP signal over time using an OPTIMA Polarstar plate reader (excitation wave length of 485 nm and emission of 520 nm). One experiment with six biological replicates for each conditions was performed (n=6).Each data point represents the average and standard deviation from six independent wells.

2.10 Bioinformatics analysis

2.10.1 Micro-array experiment and data analysis

For the research reported in this thesis, three micro-array data sets were used to examine the effect of interferon treatment or viral infection on BMDMs. Experiments were performed by Dr Garwin Sing and Andrew Livingstone, array data processing was performed by Marie Craigon and Alan Ross. Data analysis was performed by Thorsten Forster and Dr Paul Dickinson. All are from the Division of Pathway Medicine (University of Edinburgh). Details of the experiments and data processing and analysis are in appendix 3.

2.10.2 Pathway analysis

Generation of lipid associated genes list

In order to create a database of lipid associated genes (LAGs) list, we used a published database of 1224 proteins associated to lipid or lipid metabolism (http://www.lipidmaps.org). Lipid maps proteome database (LMPD) is an object-relational publicly available database of lipid-associated protein sequences and annotations using UniProt, EntrezGene, ENZYME, GO, KEGG and other public resources. Using web resources: Netaffx (http://www.affymetrix.com), DAVID (http://david.abcc.ncifcrf.gov) and NCBI website, a list of 1080 LAGs was created. Each LAGs is associated with LMPD identification number, gene symbol, uniprot Id, unigen ID, David ID, Affy ID, Agilent ID, lipids categories (appendix 4).

KEGG analysis

KEGG metabolic pathway analysis of the LAGs was performed with DAVID, online pathway annotation software based on scoring and visualization of the metabolic pathways collected in the KEGG database (http://david.abcc.ncifcrf.gov/home.jsp). Fisher's exact test was applied to determine whether or not the proportion of those genes is overrepresented in the KEGG pathway. P-value < 0.05 cut off was chosen to select significantly enriched pathways.

Ingenuity Pathway network analysis (IPA)

To extend the pathway analysis of significantly altered lipid associated genes we also use the *IPA* tool (www.ingenuity.com). IPA analysis generates networks connecting the differentially regulated genes from published interactions. This approach has the advantage of building pathways independently of the traditional canonical pathways database. Therefore, this approach potentially highlights unknown relationships and connections between genes and proteins. The relationship between the genes (nodes in the pathway) is indicated by a line, which corresponds to at least one reference from literature. The significance of a canonical pathway is controlled by p-value (right-tailed Fisher's exact test). The significance threshold of a canonical pathway is set to two which is derived by -log10 [p value], with $p \geq 2$.

Lipid class analysis

Traditionally lipids are grouped in structurally related classes. In order to study the representation of lipids class associated with the regulated LAGs by mCMV infection or IFNγ treatment or both, genes were grouped by lipid classes according to LMPD classification (http://www.lipidmaps.org).

Clustering analysis of selected metabolic pathways

Altered metabolic pathways were clustered by the number of common genes significantly regulated in each pathway. For this purpose, a matrix of distance of the genes shared between each of the significantly altered pathways was created by attributing a score of 1 if a gene belongs to both pathways or 0 if not. From the matrix a dendogramm representing the distance between each pathway was created. The distance between branches represents, how related the metabolic pathways are by number of shared altered genes.

2.11 Statistical analysis

For QPCR and cholesterol metabolism assay, the normal distribution of the data was tested by performing a Kolmogorov-Smirnov (KS) test using the online resource: (http://www.physics.csbsju.edu/stats/KS-test.n.plot_form.html).

The data set of the measurement of the gene expression of *Hmgcs1*: mock vs. mCMV which represented 15 data points in each group was used to control the normal distribution of the QPCR.

For the group1: mock treated samples: 0.680 0.700 0.750 0.780 0.950 0.950 0.960 1.02 1.03 1.20 1.23 1.30 1.35 1.36 1.45.

KS test showed that the data were consistent with a normal distribution: P= 0.95 where the normal distribution has a mean= 1.051 and an sdev= 0.2794.

For the group2: mCMV infected samples: 6.000E-02 0.150 0.210 0.250 0.260 0.290 0.300 0.340 0.350 0.380 0.400 0.410 0.430 0.520 0.600.

KS test that the data were consistent with a normal distribution: P= 0.89 where the normal distribution has a mean= 0.3314 and an sdev= 0.1675.

We also evaluated the distribution of the free cholesterol measurement and showed with a KS test that there were also following a normal distribution (data not shown)

As a result for evaluation of statistical significance of QPCR and free cholesterol measurement, we used a Welch's *t* test assuming that the two groups of samples had possibly an unequal variance.

For *in vivo* experiment, a Mann-Whitney non-parametric test was performed for evaluation of statistical significance.

Statistical analysis was performed with Excel software.

3 Chapter 3: System level analysis of perturbation of host lipid pathways associated with mCMV

3.1 Introduction

Perturbation of lipid metabolism in cells is a hallmark of infection. However there is little published evidence of the lipidomic changes induced by mCMV infection in BMDMs (1.4.2).

The concentrations of lipids are stringently regulated by the cell using a range of proteins acting as enzymes or transport proteins. These proteins control the uptake, efflux, synthesis and degradation of lipid species. Consequently, the study of the expression of the genes coding for these proteins defined as lipid associated genes (LAGs) constitutes a valuable tool to understand the role played by lipid pathways in biological processes.

In this chapter, we have sought to undertake a system level analysis approach using bioinformatics and pathway analysis tools to perform a transcriptional analysis of infected BMDMs in response to mCMV infection. The aim is to characterise the global cellular lipid response to mCMV infection in BMDMs at the transcriptional level.

.

3.2 Results

3.2.1 Identification of altered genes by mCMV infection

The first objective of the study was to identify the genes regulated by mCMV infection in BMDMs. For this purpose a micro array data set of BMDMs infected for 24 hrs with mCMV at an MOI of 1 was analysed (n= 3 biological replicates) (2.10.1).

Using this resource a stringent p-value cut off of $p < 0.05$ and a fold change ≥ 1.25 were chosen to select the significantly regulated genes and to avoid a maximum of false positive. As a result 1076 genes were considered significantly up or down regulated by the viral infection. Four hundred and forty six genes (41.5%) were down regulated and 630 genes (58.5%) were up regulated (Appendix 1 and 2).

3.2.2 Identification of metabolic pathways altered by mCMV infection

We next looked at the overrepresentation of metabolic pathways of these altered genes to identify if any were regulated by mCMV infection. KEGG, a database of genes, metabolic interactions and enzymatic pathways was queried using the web resource DAVID to identify any overrepresented pathways (Kanehisa and Goto 2000; Sherman, Huang da *et al.* 2007) (See section 2.10).

Of the 1028 genes altered by mCMV infection, 24 % (247) were identified as being part of the KEGG metabolic pathways. For the pathway enrichment analysis a p-value < 0.05 was chosen as significant threshold. As a result, 8 metabolic pathways were identified from the 101 up regulated genes and 17 metabolic pathways were identified from the 146 down regulated genes (Table 3-1 and Table 3-2).

Table 3-1: Significant metabolic pathways up regulated by mCMV infection in BMDMs

Metabolic pathway	KEGG ID	Genes present in the pathway	P-value <
Antigen processing and presentation	nmu04612	25	2.58E-12
Type I diabetes	nmu04940	17	1.18E-08
Proteasome	nmu03050	11	1.05E-06
Cell adhesion molecules (CAMs)	nmu04514	21	2.06E-05
Haematopoeitic cell lineage	nmu04640	11	5.81E-03
Parkinson disease	nmu05020	5	1.41E-02
P53 signalling pathway	nmu04115	8	3.66E-02
Fatty acid elongation in mitochondria	nmu00062	3	4.16E-02

Table 3-2: Significant metabolic pathways down regulated by mCMV infection in BMDMs

Metabolic pathway	KEGG ID	Genes present in the pathway	P-value <
Biosynthesis of steroids	nmu00100	15	3.72E-15
Pentose and glucuronate metabolism	nmu00040	8	4.68E-06
Porphyrin and chlorophyll metabolism	nmu00860	8	2.09E-04
Terpenoids biosynthesis	nmu00900	4	4.90E-04
Cell cycle	nmu04110	13	6.77E-04
Focal adhesion	nmu4510	18	7.20E-04
Androgen and estrogen metabolism	nmu00150	8	1.24E-03
Cytochrome P450	nmu00980	9	3.90E-03
Starch and sucrose metabolism	nmu00500	8	6.62E-03
B cell receptor signalling pathway	nmu04662	8	8.64E-03
P53 signalling pathway	nmu04115	8	1.02E-02
ECM-receptor interaction	nmu04512	9	1.27E-02
Pancreatic cancer	nmu05212	7	2.46E-02
Adherents junction	nmu04520	7	2.57E-02
Pentose phosphate pathway	nmu00030	4	2.61E-02
Bladder cancer	nmu5219	5	3.59E-02
Colorectal cancer	nmu5210	7	3.98E-02

3.2.3 Analysis of KEGG pathways altered by mCMV infection

Two pathways identified by our analysis related to lipids metabolism were significantly regulated. The fatty acid elongation in mitochondria (p-value =0.0416, 3 genes) was found to be up regulated and the sterol biosynthesis pathway (p-value =3.72E-15, 15 genes) was the most significantly down-regulated pathway.

The fatty acid elongation pathway allows the synthesis of very long chain fatty acids (VLCFA) from C16 and C18 fatty acids (Cinti, Cook *et al.* 1992). C16 and C18 species represent more than 90% of total fatty acids present in most mammalian tissues. However, many biological processes depend on VLCFA (Poulos 1995). VLCFA are essential for the synthesis of sphingolipids and phospholipids (Lester, Wells *et al.* 1993). Three genes associated with fatty acid elongation were found to be up-regulated: Acca2, +1.52, Hadhb, +1.33 and Hadha2, +1.45).

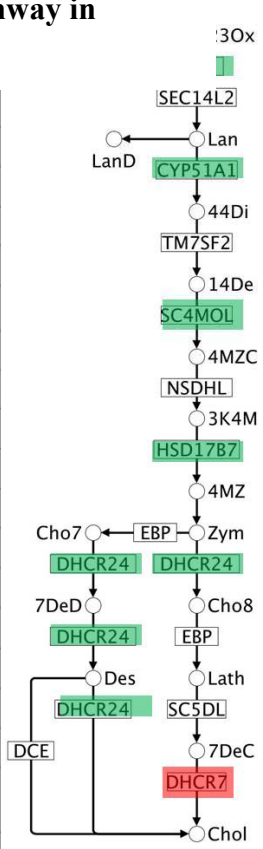
Strikingly, 10 genes of the sterol pathway were down regulated by mCMV infection. These are *Hmgcs1*: -3.25, *Idi1*: -3.22, Fdps: -3.06, *Sqle*: -3.05, Scd2: -2.79, *Sc4mol*: -2.5, *Fdft1*: -2.17, *Mvd*: -1.85, *Hmgcr*: -1.81, *Hsd17b7*: -1.52, *Lss*: -1.45, *Dhcr24*: -1.41. More than 60% of the main genes regulating the sterol pathway are down regulated, which indicate that there might be coordinate down regulation in response to mCMV infection (Figure 3-1).

Furthermore, several of the other identified metabolic pathways have previously been shown to be modulated in response to infection. Indeed, among the eight significantly up regulated pathways, five were linked with the activation of the immune and inflammatory response. These include antigen processing and presentation type I diabetes, P53 signalling, haematopoietic cell lineage. These pathways also have been previously shown to be modulated in response to mCMV infection (Hanson, Slater *et al.* 1999; Chan, Stinski *et al.* 2004; Crane, Hokeness-Antonelli *et al.* 2009). In addition, among the 17 down regulated pathways, six were related to the cell cycle and DNA production. These are cell cycle, P53 signalling pathway, pancreatic bladder and colorectal cancer pathways as well as pentose and glucuronate interconversions and pentose phosphate pathway. This is in agreement with previous studies, that show that mCMV and hCMV induce cell cycle arrest and

FPPD

Figure 3-1: Collective down regulation of the sterol biosynthesis pathway in response to mCMV in BMDMs

Gene Symbol	Gene Name	Fold Change
Hmgcs1	Hmgcs1 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	*-3.63*
Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	*-1.81*
Mvk	mevalonate kinase	*NC*
Pmvk	hosphomevalonate kinase	*NC*
Mvd	mevalonate (diphospho) decarboxylase	*-1.85*
Fdft1	farnesyl-diphosphate farnesyltransferase 1	*-2.17*
Sqle	squalene epoxidase	*-3.05*
Sec14l2	SEC14-like 2	*NC*
Lss	lanosterol synthase	*-1.45*
Cyp51a1	cytochrome P450, subfamily 51	*-2.54*
Tm7msf2	delta14-sterol reductase	*A/NC*
Sc4mol	sterol-C4-methyl oxidase-like	*-2.5*
Nsdhl	NAD(P) dependent steroid dehydrogenase-like	*A/NC*
Hsd17b7	hydroxysteroid (17-beta) dehydrogenase 7	*-1.52*
Ebp	phenylalkylamine Ca2+ antagonist (emopamil) binding protein	*A/NC*
Dhcr24	24-dehydrocholesterol reductase	*-1.41*
Sc5dl	sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, S. cerevisiae)-like	*A/NC*
Dhcr7	7-dehydrocholesterol reductase	*+1.28*



prevent cellular DNA replication (Castillo and Kowalik 2004; Cinatl, Vogel *et al.* 2004; Sanchez and Spector 2008) and confirmed the validity of the dataset.

The pathway is shown in KEGG notation (above and to the right hand side of the table), with abbreviated metabolites. Genes highlighted in green are down regulated and in red are up regulated. The last column of the table shows the fold changes from the microarray experiment of the corresponding genes.

Metabolic abbreviation: ACoA: acetyl – CoA, AaCoA: Acetoacetyl-CoA, HCoA: HMG-CoA, M:mevalonate, M5P: mevalonate-5P, M5PP: mevalonate-5PP, IsPP: isopentyl-PP, FPP: farnesyl-PP, Squa: squalene, 23Ox: 2,3 oxydosqualene, Lan: lanesterol, 44Di: 4,4 dimethyl-cholesta, 8,14,24-trienol, 14De: 14-demethyl-lanosterol, 4MZC: 4-methylzymosterol-carboxylate, 3K4M: 3-keto-4-methyl-zymosterol, Zym: zymosterol, Cho8: cholesta-8,en-3beta-ol, Lath: lathosterol, 7DeC: 7-dehydro-cholesterol, Cho7: cholesta-7,24-dien-3beta-ol, 7DeD: 7-dehydro-desmosterol, Des: desmosterol, Chol: cholesterol.

3.2.4 Identification and expression of altered Lipid Associated Genes in mCMV infected macrophages

KEGG analysis of the genes altered in response to mCMV infection highlighted the importance of sterol and fatty acid pathways in response to mCMV infection. However, the number of significant lipid pathways differentially regulated by the infection was relatively small.

Since regulation of metabolic pathways often involves small coordinate changes, we suspected that many of the changes in the metabolic pathways were below our previous detection limit. As a consequence, we decided to use another strategy and to restrict our analysis of expression data exclusively to lipid-associated genes. A combination of literature and data-mining identified 1089 genes that had published direct and indirect functions relating to cellular lipid metabolism, regulation and synthesis (742.10 and Appendix 3).

Of the 1089 LAGs identified, 789 (72%) were present in the micro array dataset and 643 (59%) were found expressed in BMDMs. Using our previous selection criteria (P-value ≤ 0.05 and cut off threshold of 1.25), 74 LAGs were found significantly up or down regulated (33 down and 41 up) upon infection. These represent 18% of the total LAGs expressed in the array and 8% of all altered genes (Table 3-3and Table 3-4).

These results indicate that there are not general changes in the regulation of lipid associated genes in response to infection after 24 hrs, rather the infection causes specific regulation of them. It is also worth noting that, as mentioned earlier, the observed quantifiable level of reduction in expression of the lipid associated genes at the specific transcript level are relatively modest (ranging from 1.3 to 4).

Table 3-3: Up regulated lipid associated genes in response to mCMV infection

GENE SYMBOL	AFFY ID	FC	P-VALUE
Ptafr	94158_f_at	2.14	2.52E-07
St3gal6	102208_at	1.86	5.02E-08
Pld4	103299_at	1.64	6.56E-08
Crabp2	100127_at	1.62	3.09E-06
Acaa2	95064_at	1.52	2.65E-07
Abhd5	102052_at	1.52	5.25E-06
Crabp1	98108_at	1.51	2.12E-05
Stard3	95607_at	1.47	6.55E-07
Gpd2	98984_f_at	1.46	8.01E-07
Nmt1	102047_at	1.39	7.57E-06
Pla2gl2a	104343_f_at	1.38	3.92E-06
Slcl0a3	103218_at	1.35	1.46E-04
Ugcg	96623_at	1.35	2.03E-05
Pgrmc1	101585_at	1.34	2.19E-05
Pparg	97926_s_at	1.34	1.53E-03
Ch25h	104509_at	1.34	2.67E-05
Decrl	160711_at	1.34	5.03E-04
Mcee	102022_at	1.33	6.14E-05
Hadhb	96913_at	1.33	4.79E-06
Phyh	96608_at	1.33	9.03E-06
Bckdha	96035_at	1.32	3.50E-05
Pik3ca	92452_at	1.31	6.96E-05
Dgka	103596_at	1.30	8.33E-03
Arsa	100931_at	1.28	1.83E-04
Pigx	160444_at	1.28	2.63E-04
Dhrs7	95620_at	1.28	3.90E-05
Lrrc8a	104250_at	1.27	1.23E-04
Sptlc1	100608_at	1.27	1.07E-04
Chpt1	93994_at	1.26	9.40E-04
Cds2	104627_at	1.26	2.68E-05
Vkorc1	95709_at	1.26	5.02E-05
Apobec1	98398_s_at	1.25	5.35E-05

Table 3-4: Down regulated lipid associated genes in response to mCMV infection

GENE SYMBOL	AFFY ID	FC	P-VALUE
Ldlr	160832_at	-3.63	7.51E-10
Hmgcs1	94325_at	-3.25	2.14E-10
Idi1	96269_at	-3.22	1.12E-10
Fdps	160424_f-at	-3.06	4.73E-09
Sqle	94322_at	-3.05	4.57E-10
Scd2	162077_f_at	-2.79	1.18E-09
Fpr1	99387_at	-2.60	3.40E-08
Cyp51	94916_at	-2.54	7.77E-08
Sc4mol	160388_at	-2.50	2.14E-09
Fdft1	97518_at	-2.17	6.34E-08
Fabp5	160544_at	-1.98	3.02E-08
Cav1	160280_at	-1.92	4.44E-07
Mvd	160770_at	-1.85	2.09E-08
Hmgcr	104285_at	-1.81	3.41E-06
Fasn	98575_at	-1.64	3.20E-07
Anxa3	101393_at	-1.64	3.55E-07
Gpx3	101676_at	-1.56	7.91E-07
Akrlb8	100884_at	-1.53	6.23E-06
Hsdl7b7	94177_at	-1.52	4.22E-07
Pcyt2	103914_at	-1.50	9.25E-06
Lss	160737_at	-1.45	8.08E-05
Srebf1	93264_at	-1.43	1.40E-06
Dhcr24	160369_at	-1.41	7.27E-06
Dbi	97248_at	-1.40	1.56E-06
Ptgs1	95597_at	-1.39	5.08E-06
Galc	93131_at	-1.37	9.65E-05
Dhcr7	98989_at	-1.36	1.14E-05
Npc1	98114_at	-1.34	1.04E-05
Rdh11	99114_at	-1.34	1.04E-05
Oxct1	92845_at	-1.33	1.02E-05
Prkcd	104531_at	-1.32	3.00E-05
Abcc1	99329_at	-1.31	1.09E-05
Pitpna	160409_at	-1.31	8.44E-05
Aoah	99838_at	-1.30	9.50E-05
Ugtlal	99580_s_at	-1.30	2.15E-05
Atp6v0al	103275_at	-1.27	3.08E-05
Neu1	101546_at	-1.27	1.67E-04
Nqol	94351_r_at	-1.26	3.50E-03
Impad1	95457_at	-1.26	3.52E-04
B4galt6	102936_at	-1.25	1.70E-03

3.2.5 Lipid class analysis

We then asked if a specific class of lipids was affected by the viral infection. Thus, we used the constructed lipidomic gene list and associated LMPD classification to look at overrepresentation of regulated lipid class by mCMV infection. At the time of the analysis lipids were classified in six major classes: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids (Fahy, Subramaniam *et al.* 2009).

The specific distribution of lipids classes of the altered LAG list in comparison to the normal distribution of lipid class in the 1079 LAGs was analysed. Results show that among the altered LAGs, genes associated with sterol lipid class were largely overrepresented in the group of down regulated genes, 34 % of the altered LAGs against 13% of the total (Table 3-5 and Table 3-6). These results indicated that mCMV infection induced the selective down regulation of the genes associated with sterol and prenol metabolism. Results show also a trend for the overrepresentation of genes that control fatty acid and glycerophospholipids levels in the up-regulated list. This may indicate that these pathways are activated in response to infection.

Table 3-5: Lipid class analysis of 33 significantly up regulated LAGs by mCMV infection

LIPID CLASS	NUMBER OF LAGS	% OF TOTAL REGULATED LAGS	% OF THE GENES OF THE LIPID CLASS
Not classified	13	39	45
Fatty acids/Eicosanois (FA)	6	18	15
Glycerophospholipids (GP)	6	18	12
Sterol lipids (ST)	4	12	13
Prenol lipids (PR)	2	6	3
Glycerolipids (GP)	1	3	5
Sphingolipids (SP)	1	3	6

Table 3-6: Lipid class analysis of 41significantly down regulated LAGs by mCMV infection

LIPID CLASS	NUMBER OF LAGS	% OF TOTAL REGULATED LAGS	% OF THE GENES OF THE LIPID CLASS
Sterol lipids (ST)	14	34	13
Not classified	7	17	45
Fatty acids/Eicosanois (FA)	7	17	15
Glycerophospholipids (GP)	4	10	12
Prenol lipids (PR)	3	7	5
Glycerolipids (GP)	3	7	5
Sphingolipids (SP)	3	7	6

NB: The genes involved in lipid metabolism with missing or incomplete information are marked as “not classified”.

3.2.6 Lipidomic profiling of mCMV infected BMDMs

We next studied the changes induced by mCMV infection in the content of the major lipid class.

Lipid extraction of mock treated and mCMV infected macrophages at 24 and 48 hrs was carried out using chloroform methanol at Edinburgh University and the APCI/MS analysis were performed in a collaborative project by Dr Markus Wenk and Dr Shui Guanghou at the University of Singapore (Methods). Electrospray ionization mass spectrometry was used to quantify the major membrane lipid: phosphatidyl choline (PC); phosphatidyl ethanolamine (PE); phosphatidylserine (PS); phosphatidylglycerol (PG); phosphatidylinositol (PI);sphingomyelin (SM), ceramide (Cer), ganglisoside 3 (GM3), and triacylglycerol (TAG). We found no substantial differences in the overall levels of major glycerophospholipids (phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine) at 24 and 48 hrs post mCMV infection (Figure 3-2). However, significant increases due to the infection includes GM3 and Cer at 24 and 48 hrs post infection, while decreases due to infection includes PG and SM at 24 and 48 hrs post infection. PG levels were found only significantly decreased at 48 hrs post infection.

Using the same technique, we then characterized the individual lipid species level. All SM and PG species and some PC were significantly decreased in response to infection. All PI and Cer species were increased in infected BMDMs.

Interestingly, polyunsaturated fatty acid (PUFA) containing phospholipids were significantly increased in most phospholipids while more saturated FA containing phospholipids were significantly decreased.

These results indicate that there are not global changes of membrane lipid content in response to infection but rather a selective alteration of lipids species by mCMV infection in BMDMs.

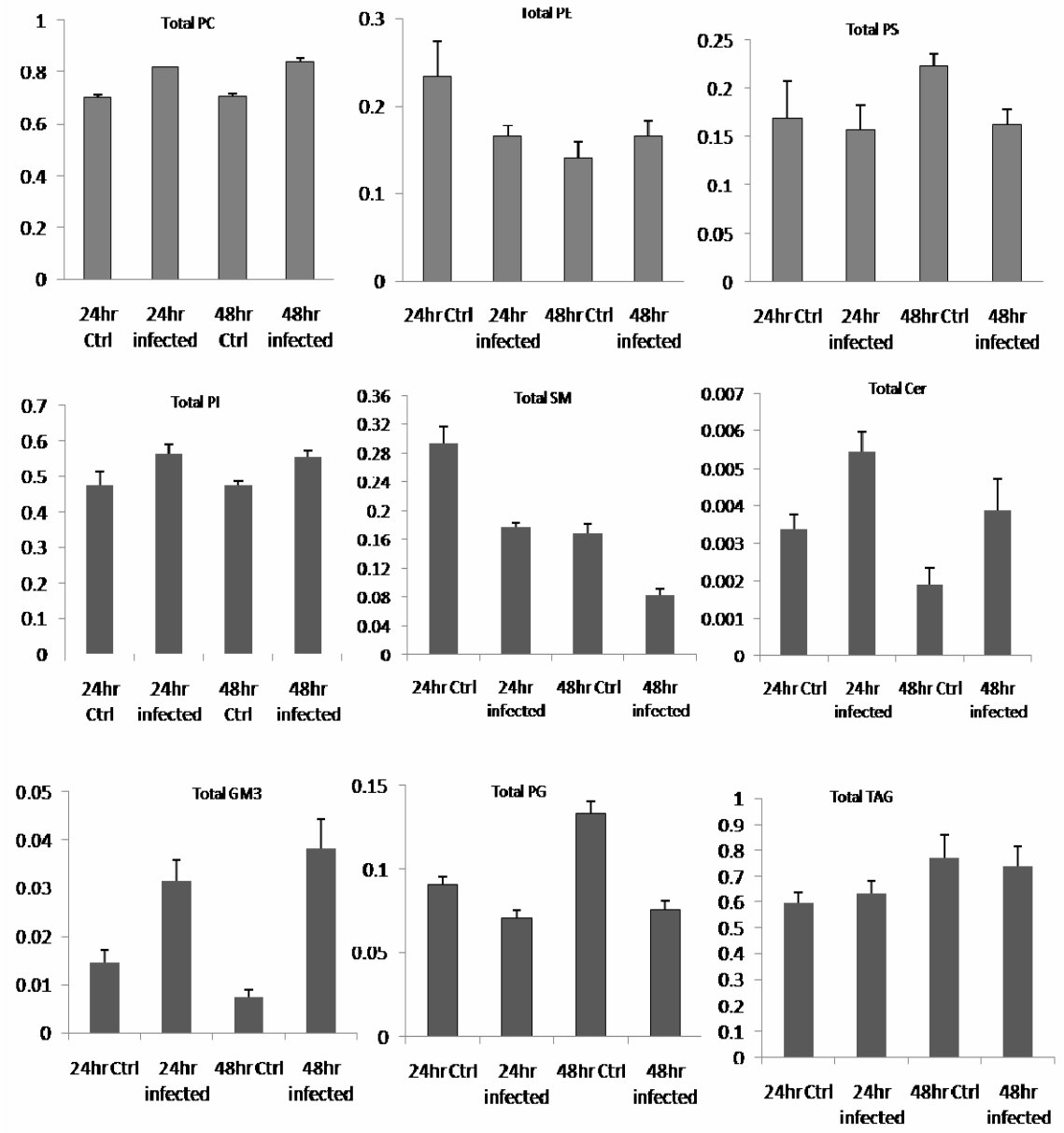
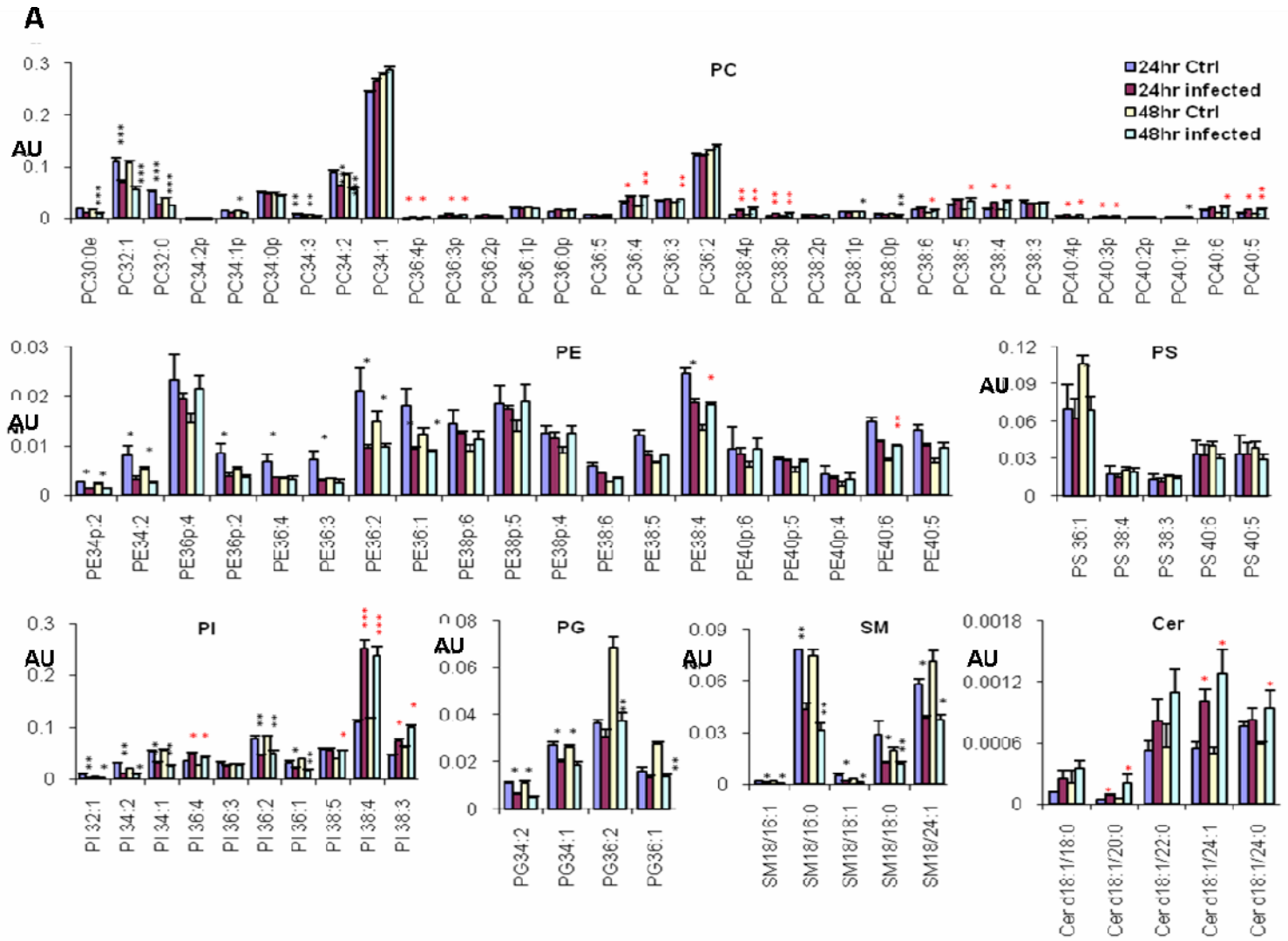


Figure 3-2: Lipidomic analysis of the changes in the major membrane lipid class induced by mCMV infection

Y-axis: normalized intensity (NI). One independent experiment with triplicate assays was performed for this analysis (n=3) and ESI-based MRM analysis was used of the analysis of the samples.


A
PC
24hr Ctrl
24hr infected
48hr Ctrl
48hr infected
AU
PC30:0e
PC32:1
PC32:0
PC34:2p
PC34:1p
PC34:0p
PC34:3
PC34:2
PC34:1
PC36:4p
PC36:3p
PC36:2p
PC36:1p
PC36:0p
PC36:5
PC36:4
PC36:3
PC36:2
PC38:4p
PC38:3p
PC38:2p
PC38:1p
PC38:0p
PC38:6
PC38:5
PC38:4
PC38:3
PC40:4p
PC40:3p
PC40:2p
PC40:1p
PC40:6
PC40:5
PE
PE34p:2
PE34:2
PE36p:4
PE36p:2
PE36:4
PE36:3
PE36:2
PE36:1
PE38p:6
PE38p:5
PE38p:4
PE38:6
PE38:5
PE38:4
PE40p:6
PE40p:5
PE40p:4
PE40:6
PE40:5
PS
PS 36:1
PS 38:4
PS 38:3
PS 40:6
PS 40:5
PI
PI 32:1
PI 34:2
PI 34:1
PI 36:4
PI 36:3
PI 36:2
PI 36:1
PI 38:5
PI 38:4
PI 38:3
PG
PG34:2
PG34:1
PG36:2
PG36:1
SM
SM18/16:1
SM18/16:0
SM18/18:1
SM18/18:0
SM18/24:1
Cer
Cer d18:1/18:0
Cer d18:1/20:0
Cer d18:1/22:0
Cer d18:1/24:1
Cer d18:1/24:0

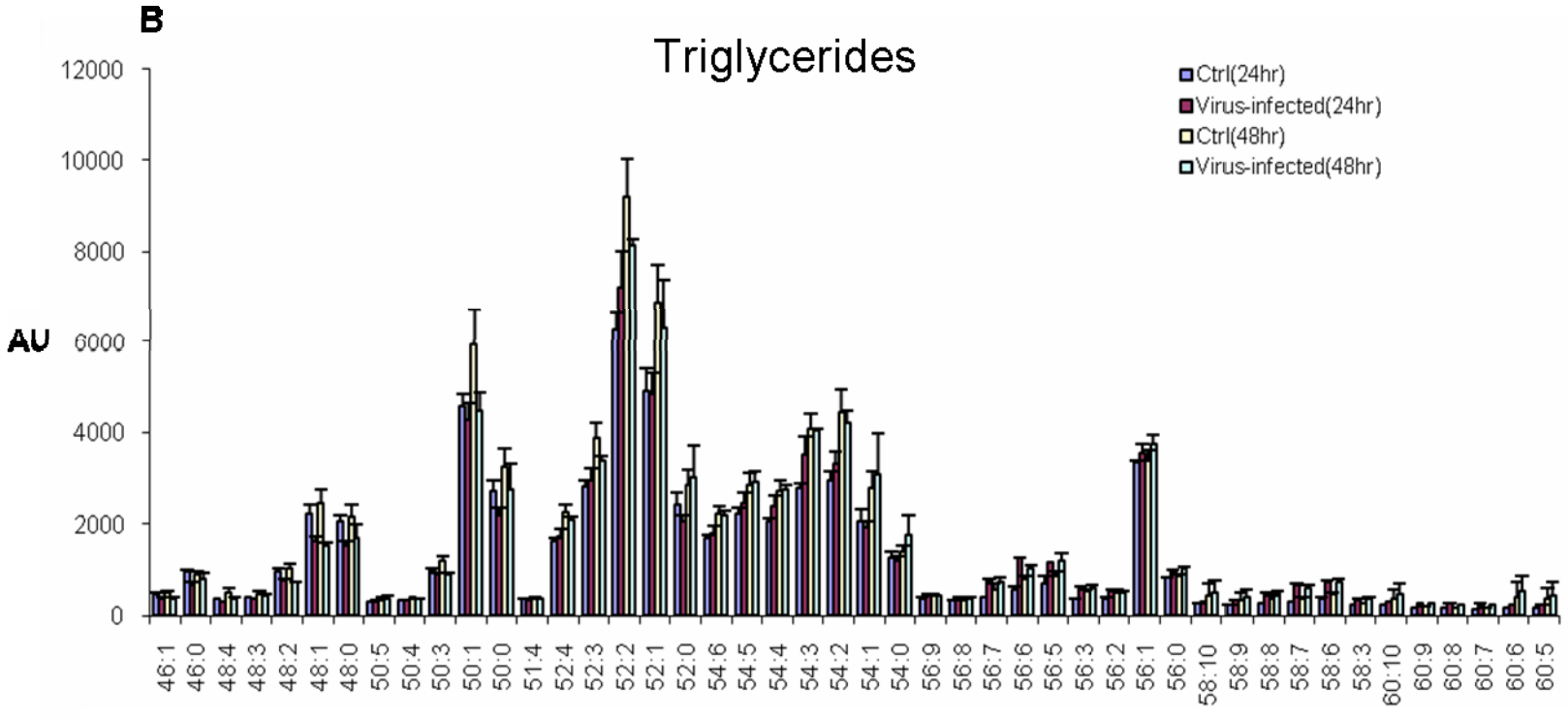


Figure 3-3: Lipidomic analysis at high sensitivity of the changes in the major membrane individual molecular lipid species induced by mCMV infection.

Y-axis: Arbitrary Unit. One independent experiment with triplicate assays was performed for this analysis (n=3) and ESI-based MRM analysis was used of the analysis of the samples. Significance was calculated using a Student T-test: *, $P<0.05$; **, $p<0.01$; ***, $p<0.005$); red and black indicated significant increases and decreases, respectively. Panel A, PC: Phosphatidylcholine, PE: phosphatidylethanolamine, PS: phosphatidylserine, PI: phosphatidylinositol, PG: phosphatidylglycerol, SM: sphingomyelin, Cer: ceramide. Panel B: tryglycerides. The signal intensity of each MRM value was normalized to the sum of MRM intensities of all species.

3.2.7 IPA canonical pathway analysis

The list of significantly altered LAGs was used to perform a canonical pathways analysis using IPA software (2.10.2). IPA canonical pathways analysis identified the most significant known biological pathways for a given set of genes. We used also IPA to independently verify the previous KEGG analysis. The list of significantly altered LAGs was uploaded and over expression of pathways calculated. A stringent p-value ≤ 0.01was chosen (right-tailed Fisher's exact test). Results show that 22 lipid pathways were significantly altered by mCMV infection. As expected the most significant down regulated pathway was the biosynthesis of steroids (-Log (p-value) =14.7, 9 genes). Moreover fatty acid elongation in mitochondria was also present (-Log (p-value) =2.60, 2 genes). Furthermore, we could identify 20 significantly lipids related pathways that were altered by mCMV infection and that had not been identified by our previous analysis (Table 3-7).

Table 3-7: Significantly altered lipid pathways in response to mCMV infection in BMDMs

Ingenuity Canonical Pathways	-Log(P-value)	Ratio	Downregulated	Upregulated
Biosynthesis of Steroids	14.70	7.03E-02	8	1
Valine, Leucine and Isoleucine Degradation	6.17	5.41E-02	2	4
Glycerophospholipid Metabolism	4.74	3.61E-02	1	6
Sphingolipid Metabolism	4.73	4.46E-02	2	3
Synthesis and Degradation of Ketone Bodies	4.68	1.58E-01	2	1
Aminophosphonate Metabolism	3.91	4.62E-02	1	2
Inositol Metabolism	3.70	4.08E-02	3	1
LXR/RXR Activation	3.54	4.65E-02	4	0
TR/RXR Activation	3.21	4.26E-02	3	1
RAR Activation	3.05	2.79E-02	2	3
Fatty Acid Elongation in Mitochondria	2.60	4.44E-02	0	2
Propanoate Metabolism	2.58	2.31E-02	1	2
Butanoate Metabolism	2.56	2.26E-02	2	1
Arachidonic Acid Metabolism	2.52	1.77E-02	3	1
Tryptophan Metabolism	2.48	1.57E-02	2	2
Nitric Oxide Signaling in the Cardiovascular System	2.33	3.26E-02	2	1
Androgen and Estrogen Metabolism	2.31	2.1E-02	2	1
Endothelin-1 Signaling	2.16	2.67E-02	2	3
FXR/RXR Activation	2.13	3E-02	2	1
Virus Entry via Endocytic Pathways	2.13	3.12E-02	2	1
NRF2-mediated Oxidative Stress Response	2.06	2.16E-02	3	1
ERK/MAPK Signaling	2.05	2.08E-02	1	3

NB: Ratios represent the number of LAGs represented in the total number of genes present in the given canonical pathway (Parameter of the analysis: -Log (P-value ≥ 2).

3.2.8 Clustering of metabolic pathways

To make biological sense of the previous results and since lipid pathways are strongly interconnected, we thought of clustering the altered lipid pathways to identify the biological "nodes" which could be at the origin of these regulation. For that purpose, we thought to build a matrix of distance representing the number of shared genes among two canonical pathways. Agglomerative hierarchical clustering, using function hclust() in R statistical programming language was performed by Thorsten Forster from the DPM (University of Edinburgh).The results are presented as a dendogram (Figure 3.4) .

Using this approach we could identify 4 major clusters of pathways altered by the viral infection, which were clearly separated. We used literature-based analysis to understand which biological "node" could be at the origin of the alteration of these pathways (Table 3.8).

Many of the pathways belonging to the cluster one and four were related to G proteins-dependent signalling. Indeed, G-protein phosphorylation has been shown to activate ERK MAPK kinase pathways (Goldsmith and Dhanasekaran 2007), retinoic acid receptor (Kiefer, Lai *et al.* 2005), nitric oxide signalling (Ushio-Fukai 2009), viral entry (Unutmaz, KewalRamani *et al.* 1998) as well as in arachidonic acid, sphingolipids, aminophosphate and inositol mechanism (Naor 2009). G proteins also called "molecular switches" are guanine nucleotide binding proteins involved in the activation of second messengers. These second messengers are mainly lipid compounds. G-protein alternate from a dephosphorylated form (GDP) to an active phosphorylated form (GTP) and are usually associated with G-protein coupled receptors (GPCR) (although soluble G-proteins are also present in the cells). GPCRs represent a large family of extracellular transmembrane receptors. A large number of ligands bind GPCRs and activate the phosphorylation of G-proteins which trigger downstream signal transduction (Gilman 1987; Neves, Ram *et al.* 2002; Vassilatis, Hohmann *et al.* 2003).

We could identify several key genes involved in the activation of these pathways, which were modulated by the viral infection. For example, phosphatidylinositol 3-kinase (PI3K), key in the inositol metabolism is slightly but significantly up

regulated by the virus (+1.32 fold change), while the expression of protein Kinase C (PKC) is down regulated (-1.32).

Furthermore, the expression of phospholipase A_2 (PlA_2), a key enzyme responsible of the control of cellular arachidonic acids (AA), is up regulated (+1.38). AA is the principle substrate for the prostaglandin pathway. This pathway is key in the control of the inflammatory response. Indeed, AA is converted into prostaglandin by PGH_2 synthase in a reaction creating reactive oxygen intermediates (ROI), which will then activate NFκB. Prostaglandin pathway activates the leukotriene pathway that is involved in the control of inflammatory mechanisms. Interestingly in our dataset, prostaglandin-endoperoxide synthase 1 (*Ptgs1*) expression is down regulated (-1.39). Furthermore, two genes involved in the regulation of retinoic acid pathway were found to be up regulated. These two are related, cellular retinoic acid-binding protein 1 and 2 (*Crabp1* and *2*) (+1.51, +1.62). However, it is difficult to predict if these pathways are being activated or blocked, since the change in the level of expression of these genes is small. Furthermore the regulation of these kinases or enzymes does not only depend of their gene expressions levels and other mechanisms of modulation could occur. However, the number of genes significantly regulated belonging to the G-protein downstream signalling pathways are sufficient to indicate that G-proteins are activated at 24 hours in response to mCMV infection.

Almost all the pathways of the second cluster are related to acetyl-CoA. Indeed carbohydrate, butanoate, valine, leucine and isoleucine and ketone bodies can all be converted into acetyl-CoA and induce the production of ATP to provide energy for the cell (Hers and Hue 1983). Acetyl-CoA is a coenzyme which is at the cross road of many metabolic pathways regulating the production of energy, lipogenesis or the synthesis of fatty acids and *de novo* sterol production. Acetyl CoA is also involved in the degradation and synthesis of amino acids. (Barnes and Weitzman 1986). Interestingly, several important genes in the production of acetyl-CoA were up regulated. These are methylmalonyl CoA epimerase (*Mcee*, +1.33), Acetyl-COA carboxylase (*Acca2*, +1.52), which may indicate that this pathway is activated in

response to infection. Fatty acid synthesis and elongation is also directly dependent on the production of acetyl-CoA.

Finally in the third cluster as expected by our previous analysis, the pathways leading to the biosynthesis of steroids are statistically altered by mCMV infection in macrophages. This cluster also linked the biosynthesis of sterols with the expression of proliferator-activated receptors γ (*Pparγ*, +1.34), a nuclear receptor which has been linked with immune and inflammatory response (1.5.1). In addition, cholesterol 25-hydroxylase (*Ch25h*), which transforms cholesterol in 25-hydroxycholesterol, is up regulated (+1.34) in cluster 3. Notably, 25-hydroxycholesterol control cholesterol metabolism and plays a role in inflammatory processes.

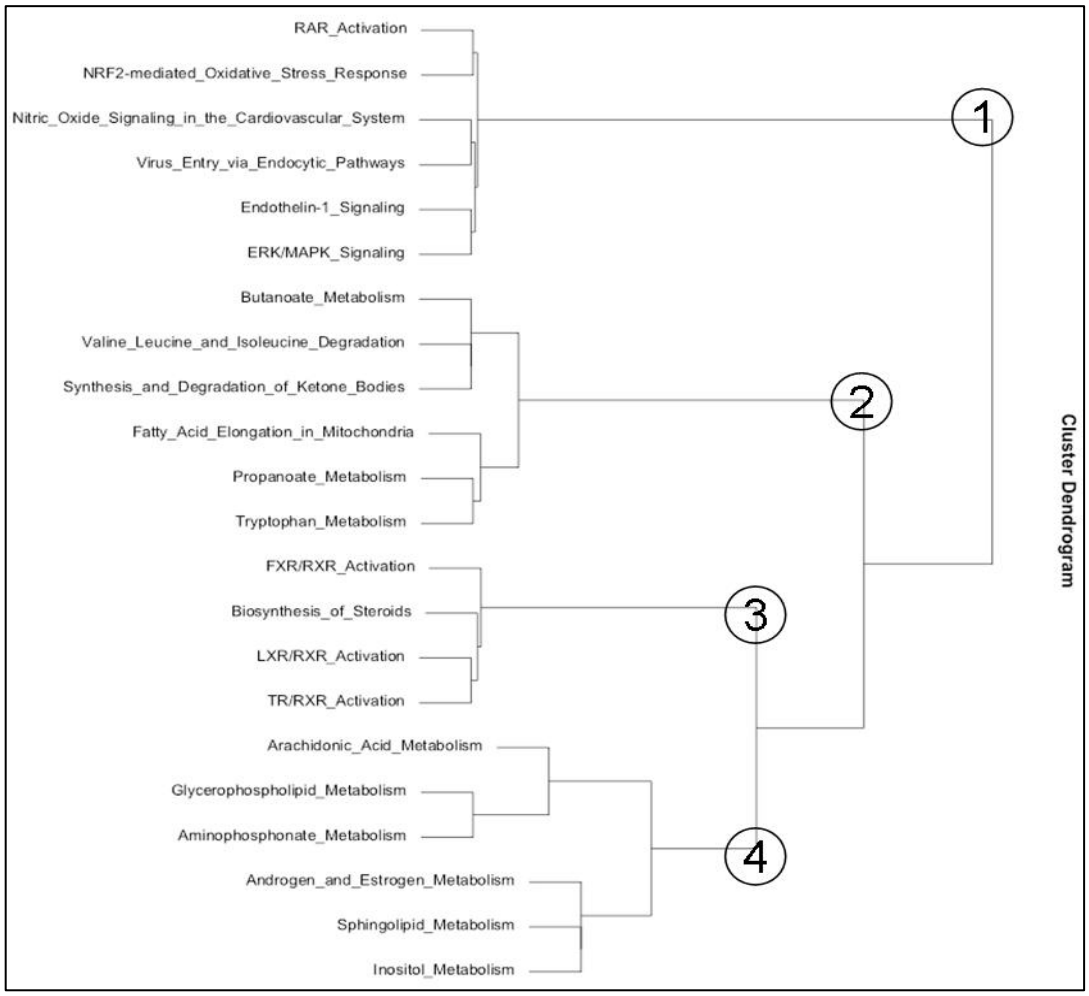


Figure 3-4: Dendogram representing the clustering of altered lipid pathway in mCMV infection

Table 3-8: Clustering of metabolic pathway

CLUSTER	PROPOSED CENTRAL BIOLOGICAL PROCESS	CLUSTERED METABOLIC PATHWAYS	REGULATED LAGS IN THE CLUSTERS
1	G-protein signalling	RAR activation, NRF2 mediated oxidative stress response Nitric oxide signalling in the cardiovascular system, virus entry via endocytic pathways, endotothelin 1 signalling, ERK/MAPK signalling	CRABP1, CRABP2, PRKCD, RDH11, PIK3CA, PIK3CA, ABCC1, NQO, CAV1, PLA2G12A, PTGS1, PLD4, PPARG
2	Actetyl-CoA	Butanoate metabolism Valine leucine and isoleucine degradation, Synthesis and degradation of ketone bodies, fatty acid elongation in mithochondria, propanoate metabolism, tryptophan metabolism	OXCT1, HADHB, *HMGCS1*, MCEE, ACAA2, BCKDHA, *DHCR24*, *CYP51*A1
3	Nuclear receptor and sterol biosynthesis	FXR/RXR activation, Biosynthesis of steroids,LXR,RXR activation, TR/RXR activation	FDPS, GGPS1, *FDFT1*, *SQLE*, CYP24A1, *IDI1*, *DHCR7*, *HMGCR*, VKORC1, *LSS*, NQO1, *MVD SREBF1*, *APOE*, *ABCA1*, NR1H2, *LDLR*, FASN,*CH25H*
4	G-protein signalling	Arachidonic acid metabolism, aminophosphate metabolism, androgen and estrogen metabolism, sphingolipid metabolism, inositol metabolism	*CYP51*A1, PLA2G12A, PTGS1, GPX3, GPD2, PLA2G12A, CDS2, DGKA, PCYT2, CHPT1, PLD4, PCYT2, BCKDHA, CHPT1, *HSD17B7*, ARSA, UGT1A1, SPTLC1, ARSA, UGCG, GALC, NEU1, *DHCR24*, RDH11, *SC4MOL*, DECR1

3.3 Discussion

Increasing evidence suggest that the regulation of lipid metabolism is critical to the outcome of viral infection. However the modulation of lipids by viral infections are still not well characterised. In this chapter we used gene expression and pathway analysis of mCMV infected BMDMs to characterise the lipid pathways altered by mCMV infection.

Genome wide analysis of mCMV infected BMDMs at 24 hrs post infection identified 1028 up or down regulated genes. Pathway analysis using public domain resources (DAVID and KEGG) to study the overrepresentation of these genes revealed that fatty acid elongation is up-regulated and sterol biosynthesis is down regulated in response to mCMV infection. Several studies have shown the modulation of gene regulation and metabolism of fatty acid in HCV infection (Diamond, Syder *et al.* ; Kapadia and Chisari 2005) and HIV (Chan, Qian *et al.* 2007; Ringrose, Jeeninga *et al.* 2008; Chan, Sutton *et al.* 2009). Recently, Munger and colleagues have shown that fatty acid synthesis up regulated upon hCMV infection (Munger, Bennett *et al.* 2008). Moreover, inhibition of the fatty acid pathway by drugs decreases the infectivity of viruses (Kapadia and Chisari 2005; Munger, Bennett *et al.* 2008). Here we provide evidence that among fatty acid synthesis, fatty acid elongation is increased by mCMV infection at the gene expression level after 24 hrs post infection. Further studies are necessary to understand if fatty acid elongation plays a crucial role for CMV infection.

The most pronounced pathway-related changes occurred in sterol biosynthesis. Significantly, it appears that gene expression associated with sterol biosynthesis is co-ordinately down regulated during the infection. To our knowledge, this was the first time that the coordinate down regulation of the entire sterol synthesis pathway has been described in response to a Herpes virus infection. In addition, these results support the observation of Robinson and colleagues who have recently shown that Measles virus induce the down regulation of sterol biosynthesis pathway (Robinzon, Dafa-Berger *et al.* 2009). Furthermore, several viruses such as HIV, HCV, West Nile virus have developed multiple strategies to up regulate the sterol biosynthesis for

their benefit (Zheng, Plemenitas *et al.* 2003; Mackenzie, Khromykh *et al.* 2007; Park, Jun *et al.* 2009). This raises the question whether the down regulation of the sterol pathway induced by mCMV infection was directly triggered by the viral gene expression and if the alteration of the pathway is beneficial or not for viral replication.

Inhibition of the cholesterol synthesis pathway by drug has an antiviral effect for many viruses (See 1.4.2.2). Therefore it is possible that the down regulation of the sterol pathway in response to mCMV infection may be antiviral. This may indicate an activation of an immune mechanism directed against the virus. Recently, several studies have shown that the nuclear receptor LXRα was activated by an innate immune response following an exposure to pathogens. This response regulates cholesterol efflux (Castrillo, Joseph *et al.* 2003). Here we show the potential involvement of another nuclear receptor, PPARγ in the regulation of sterol biosynthesis. Further studies need to be conducted to evaluate the role of PPARγ in mCMV infection and its relation to the regulation of sterol biosynthesis.

SREBP2 proteins have been shown to modulate the expression of most of the genes of the sterol synthesis pathway (See 1.4.1.3). This sterol regulatory element binding protein constitutes therefore a strong candidate to explain the collective down regulation of the entire pathway in mCMV infection. Unfortunately, SREBP2 was not present in the microarray chip and therefore we could not evaluate its regulation by mCMV infection in this analysis.

It is worth noticing that the down regulation of the sterol genes is relatively small and did not exceed 4 fold. Furthermore, *Hmgcr*, the rate-limiting enzyme of the pathway, is very weakly down regulated. We can speculate that the modulation of cholesterol biosynthesis via coordinated small transcriptional changes offers advantages over control by a single enzyme. Biologically, coordinate control can potentially increase the robustness of modulation; the redundant rate limiting interactions downstream of the true rate limiting interaction can protect the pathway from surges in the levels of downstream metabolites. Coordinate control also increases the specificity of the pathway modulation as a small reduction of the enzyme level in an interaction ensures that the level of the interacting metabolite need not drop as far to affect a reduction in flux. This has the advantage of potentially lessening the impact on other

branched pathways that use the same metabolites and thus provides a high degree of pathway specificity.

In regards to the small number of the altered lipid pathways, we decided to restrict our analysis of gene expression data to that of the lipid-associated genes. Using this new approach, we could identify 74 LAGs specifically regulated by mCMV infection.

Lipid class analysis of the 74 altered LAGs has showed that sterol associated genes were specifically down regulated by the virus. Furthermore, lipidomic analysis using electrospray ionization mass spectrometry showed that mCMV infection altered specific membrane lipids in response to infection such as ganglioside-3 and ceramides.

To understand which lipid pathways were affected by mCMV infection, a new pathway analysis of the restricted altered lipid genes using proprietary pathway tools (Ingenuity software IPA) was performed. Results showed that 22 lipid-associated pathways were significantly altered during infection.

Taken together, these results suggest that mCMV infection alter selectively and specifically lipids and lipid regulatory pathways in BMDMs.

Since lipid pathways are strongly interconnected, activation of a specific pathway or metabolite will have an effect on many neighbouring lipid pathways. We thought to use these properties to identify the common biological events or "nodes" leading to the alteration of the downstream pathways. For that reason, we clustered the 22 lipids pathways altered in response to infection by the number of their shared regulatory elements. This analysis revealed 4 major clusters of pathways altered by mCMV infection.

Applying literature searching, two of the clusters were clearly linked by the activation of G-protein signalling. UV inactivated hCMV triggers within the first hr post infection the activation of the G-protein-coupled receptor dependent pathways leading to important cellular metabolic changes (AbuBakar, Boldogh *et al.* 1990; Albrecht, Fons *et al.* 1991; Fortunato, McElroy *et al.* 2000).

The presence of numerous G protein-coupled receptor (GPCR) homologues has been found in several Herpes viral genomes. In addition, hCMV carry a cell-derived phospholipase A2 required for its infectivity (Welch, McGregor *et al.* 1991; Allal,

Buisson-Brenac *et al.* 2004). This suggests an essential role for G-protein in CMV replication.

Furthermore, inhibition of many members of these pathways limit CMV replication (Speir, Shibutani *et al.* 1996; Shibutani, Johnson *et al.* 1997; Speir, Yu *et al.* 1998; Kucic, Mahmutefendic *et al.* 2005). Moreover, G protein activation in response to infection activates phospholipase C (PLC). This resulted in the production of a second messenger inositol (1,4,5)-trisphosphate (PIP3) and diacylglycerol (DG) which will activate the release of Ca^{2+}, many kinases: PKC, PKA, PI3K, MAPK (Kristoffersen, Tasken *et al.* 1994; Keay and Baldwin 1996; Sherrill, Stropes *et al.* 2009). We identified several of the key genes of these pathways such as Pkc, Pi3k and Pla2 as being regulated by the virus.

In addition, lipidomic analysis at high sensitivity of the changes in the major membrane individual molecular lipid species induced by mCMV infection showed that lipid second messenger induced by G-coupled receptor activation such as phosphoinositols, ceramides and gangliosides were increased in response to infection.

To date, the direct mechanisms to explain G-protein-dependent crucial activation for the efficient replication of the CMV virus are still not completely elucidated. One possible explanation is that the secondary messengers induced by the activation of G-protein directly enhance viral gene expression. Indeed, the immediate early promoter of CMV contains binding sites for ligands such as retinoic acid and NFκB (Kowalik, Wing *et al.* 1993; Angulo, Suto *et al.* 1996). On the other hand, the activation of the G-protein pathway activates many other pathways which could play an indirect role in the regulation of the virus. Exemple might be the leukotriene pathway or the MAPK kinases pathway involved in the modulation of inflammatory response.

In addition, activation of the G-protein signalling pathways has been shown to modulate cytokine and chemokines expression. Shingai and colleagues have shown that soluble G protein of respiratory syncytial virus inhibits Toll-like receptor 3/4-mediated IFNβ induction (Shingai, Azuma *et al.* 2008). In view of this evidence, we can speculate that by interacting with the G-proteins derived pathways, the virus limits, the innate immune response. So far the activation of these pathways in response to CMV infection has been an early event post infection. Our results

provide evidence that these pathways are still modulated 24 hrs after infection and may still play a role in viral infection. Further studies will be necessary to validate and understand the role of the G-proteins in mCMV infection at later times post infection.

Another interesting result from our analysis reveals the importance of acetyl-CoA in many lipid pathways that are regulated by mCMV infection. Since acetyl-CoA is at the cross road of various biological processes, such as energy supply, protein synthesis, fatty acid and sterol synthesis, it is not surprising that it is modulated by viral infection.

Diamond and colleagues have shown that HCV infection shifts the production of energy towards the synthesis of cellular metabolites supporting viral life cycle through the modulation of glycolysis and TCA cycle (Diamond, Syder *et al.*)

Munger and colleagues have shown that hCMV induced an increase in the level of acetyl-CoA at early and late stage of infection (Munger, Bajad *et al.* 2006). They speculate that the virus modulates the cellular metabolic pathways to its advantage. Our results complement Munger's study and highlight the important role of glycolysis and the TCA cycle in the viral cycle. Further analyses are required to understand whether mCMV modulates a specific pathway, which requires acetyl-CoA. However, since we have shown an up regulation of fatty acid elongation and a down regulation of sterol synthesis it is possible that the virus targets specifically theses two pathways by regulating the level of Acetyl-CoA. Alternatively, the changes observed reflect a compensatory mechanism. The understanding of the mechanisms involved could help to identity new antiviral targets.

To conclude, in this chapter we have characterised the lipid metabolic pathways regulated in response to mCMV infection in macrophages. Our analysis showed that there is a specific and selective regulation of lipid metabolism upon infection. Some of these pathways such as fatty acid, G-protein activated second messenger or acetyl-CoA associated pathways have previously been linked with physiological processes occurring at early time post mCMV infection. Here we provide evidence that these pathways are still modulated by the virus at 24 hrs post infection. In addition, we identified for the first time that the sterol biosynthesis pathway is co-ordinately down regulated in response to mCMV infection

4 Chapter 4: mCMV infection impaired cholesterol homeostasis at metabolic and transcriptional levels

4.1 Introduction

Several viruses such as HCV, HIV, Western Nile virus, have been shown to alter the intra cellular cholesterol synthesis of their host (Zheng, Plemenitas *et al.* 2003; Mackenzie, Khromykh *et al.* 2007; Waris, Felmlee *et al.* 2007). Furthermore, cholesterol homeostasis and pharmacological inhibition of the sterol biosynthesis pathway have been shown to modulate the infection of many viruses, suggesting an important role for cholesterol homeostasis in viral infection (1.4). However, to date, very little is known about the regulation of intra cellular cholesterol synthesis by mCMV infection. Nevertheless, there is evidence that cholesterol is important for Herpes virus life cycle. For example, Herpes Zoster infection has been shown to induce the level of serum cholesterol in infected patients and cholesterol has been shown to be important for Herpes viruses entry and replication (Bender, Whitbeck *et al.* 2003; Potena, Frascaroli *et al.* 2004; Cohen 2005; Hill, Steiner *et al.* 2005; Hambleton, Steinberg *et al.* 2007; Del Pozo, van de Beek *et al.* 2010). Furthermore, Hajjar and colleagues have shown that Herpes virus altered the transcriptional and lipidomic cholesterol homeostasis in smooth muscle cells (Hajjar, Falcone *et al.* 1985; Hsu, Nicholson *et al.* 1995).

Since we identified the sterol biosynthesis pathway to be co-ordinately down regulated in response to mCMV infection, the aim of this chapter was to validate our previous observations and to characterise the changes in cholesterol synthesis induced by mCMV infection.

In addition, given that the cellular cholesterol homeostasis is also regulated by the cholesterol entry, storage and efflux, we studied the regulation of these other pathways by mCMV infection. Finally we examined the consequences of mCMV infection on the overall cellular cholesterol concentration.

4.2 Results

4.2.1 Characterization of the cholesterol regulating genes expression upon mCMV infection

The expression of cholesterol synthesis, uptake, storage, efflux and regulators marker genes in mCMV infected or mock treated cells were measured by quantitative PCR. For this experiment BMDMs and 3T3-NIH fibroblasts were mock treated or infected at a MOI of 1 and RNA was extracted and purified at 24 hrs post infection (2.5.3).

Hmgcs1, *Hmgcr*, *Idi1* and *Sqle* were chosen as marker genes of the sterol biosynthesis pathway because they were the most down regulated genes in our previous micro-array experiment (See Chapter 3). QPCR analysis with Taqman primer-probes sets confirmed that *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* gene expression were down regulated at 24 hrs post infection in both BMDMs (Figure 4-1A) and 3T3-NIH fibroblasts (Figure 4-1B). The fold changes in gene expression for infected compared to mock treated cells were for BMDMs: *Hmgcs1*:-4.28, *Hmgcr*: -2.64, *Idi1*: -7.14, *Sqle*: -6.83 and for 3T3 NIH fibroblasts: *Hmgcs1*:-7.95, *Hmgcr*: -4.04, *Idi1*: -7.2, *Sqle*: -18.8

The scale of the regulation in BMDMs and NIH/3T3 was comparable although the alteration is more pronounced in the fibroblast cells. Furthermore, these changes are very similar to the ones observed in the previous micro-array analysis and confirm with an independent technique that mCMV infection down regulates the sterol biosynthesis pathway. In addition these results showed that the response is not specific to BMDMs.

We then measured the expression of the genes involved in the regulation of cholesterol uptake (*Ldlr*), storage (*Soat1*) and efflux (*Abcg1* and *Apoe*) in mCMV infected compared to mock treated cells. Results show that *Soat1*, *Abcg1* and *Apoe* are significantly up regulated after 24 hrs infection in BMDMs and NIH/3T3 fibroblasts (fold changes for BMDMs: *Soat1*:+4.39, *Abcg1*: +3.25, *Apoe*: +4.29 and for NIH/3T3 *Soat1*:+7.62, *Abcg1*: +4.70, *Apoe*: +4.29) (Figure 4-1C and Figure 4-1D). However, *Ldlr* expression is significantly down regulated by mCMV

infection at 24hrs post infection in BMDMs and NIH/3T3 fibroblasts (fold changes -7 and -6.37 respectively) (Figure 4-1C and Figure 4-1D). These results validate our previous observations and indicate that storage and efflux of cholesterol are up regulated while uptake of cholesterol into the cell is down regulated at the transcriptional level.

Since we observed a coordinate down regulation of the sterol pathways genes in response to infection, we next wondered if the expression of Srebfs genes, master regulators of the sterol and fatty acid metabolism, was also regulated by the mCMV infection. We were especially interested in the regulation of *Srebf2* since it has been shown to specifically regulate the sterol pathway (1.4.1.103).
Previous micro array analysis showed that *Srebf1* was weakly down regulated; however a *Srebf2* probe was not present in the micro array chip. To investigate whether Srebfs genes were modulated by mCMV infection, gene expression levels of *Srebf1* and *Srebf2* in infected and mock treated BMDMs and NIH/3T3 for 24 hrs were compared.

Results in Figure 4-1E and Figure 4-1F show that *Srebf1* was not significantly down regulated by mCMV infection in BMDMs and NIH/3T3 although there is a trend for the down regulation of the gene. However *Srebf2* was found significantly down regulated at 24 hrs post infection (p-value<0.05) in both cell types. Significant fold changes of *Srebf2* were: -3.27 in BMDMs and-3.70 in NIH/3T3s.
These results indicate the *Srebf2* is down regulated in response to infection, which could explain the co-ordinate down regulation of the sterol biosynthesis pathway in response to mCMV infection.

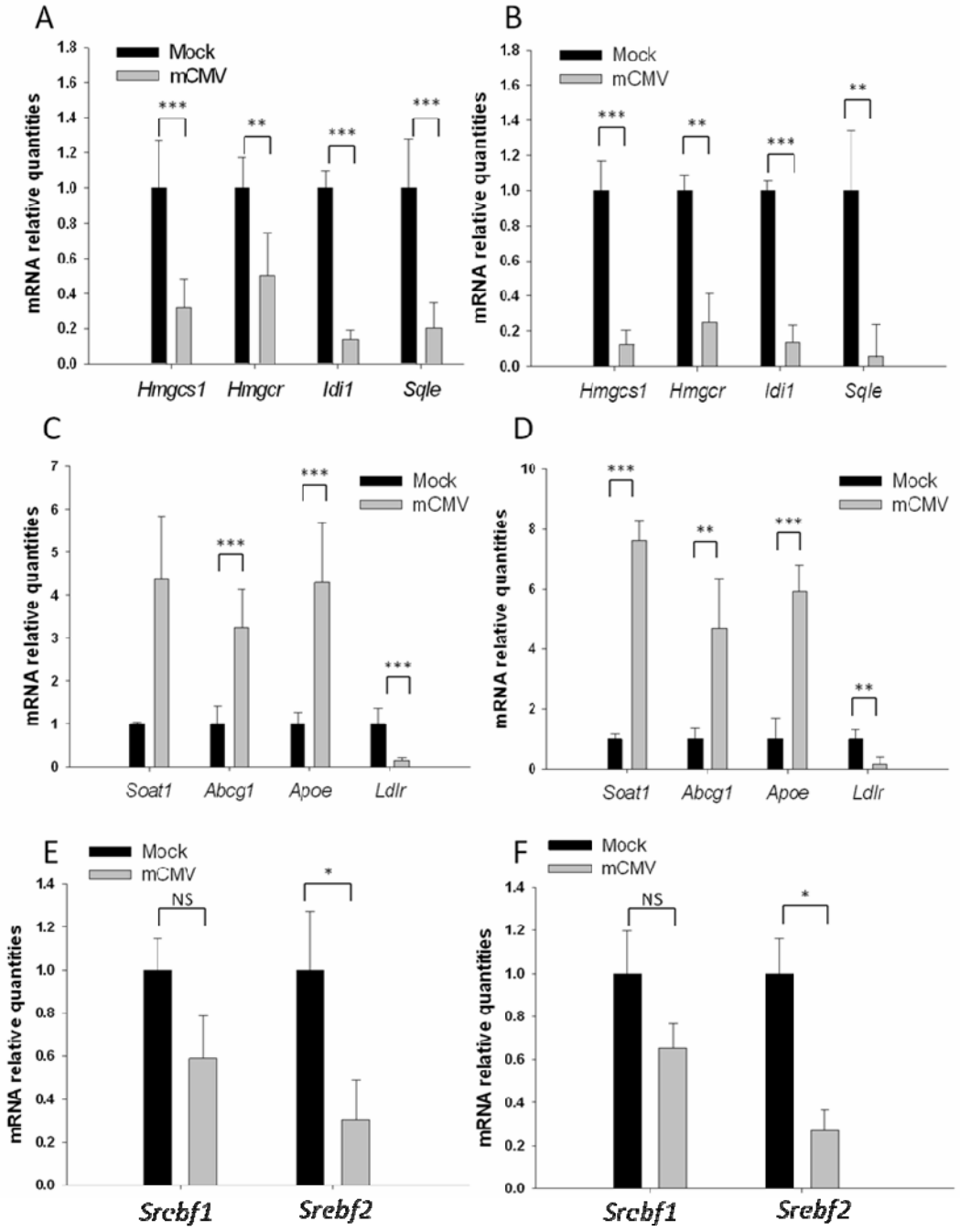

A
Mock
mCMV
mRNA relative quantities
Hmgcs1
Hmgcr
Idi1
Sqle
B
C
Soat1
Abcg1
Apoe
Ldlr
D
E
NS
Srebf1
Srebf2
F

Figure 4-1: Changes in the genes expression of the cholesterol metabolism upon mCMV infection in BMDMs and NIH/3T3 compare to mock treated cells

A –B: Changes in gene expression of Acat1, *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* expression in BMDMs (A) and NIH/3T3 (B) at 24 hrs post infection compared to mock treated cell.

Bars represent means ± S.D. of 5 independent experiments with triplicate measurements for each experiments for BMDMs (n=15) and 2 independent experiments with quadruplicate measurements for NIH/3T3 (n=8).

C-D: Changes in gene expression of *Soat1*, *Abcg1*, *Apoe* and *Ldlr* expression in BMDMs (C) and NIH/3T3 (D) at 24 hrs post infection compared to mock treated cell.

Bars represent means ± S.D. of 3 independent experiments with triplicate measurements for each experiments for BMDMs (n=9) and 2 independent experiments with quadruplicate measurements for NIH/3T3 (n=8).

E-F: Changes in gene expression of *Srebf1* and *Srebf2* expression in BMDMs (E) and NIH/3T3 (F) at 24 hrs post infection compared to mock treated cell.

Bars represent means ± S.D. of 2 independent experiments with triplicate measurements for each experiment for BMDMs (n=6) and 2 independent experiments with quadruplicate measurements for NIH/3T3 (n=8).

A Welsh t-test was used for evaluation of statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

4.2.2 Kinetics of free cholesterol concentration in mCMV infected BMDMs and NIH/3T3 fibroblasts

mCMV infection induces the transcriptional regulation of the uptake synthesis, storage and efflux genes. However, given that the changes are quantitatively modest it was not clear whether such small, coordinate temporal reductions in the level of individual components of cholesterol homeostasis will have any functional consequence on the overall pool of intra cellular cholesterol. Especially since *Hmgcr* the rate-limiting enzyme of the cholesterol synthesis pathway is very weakly regulated by mCMV infection at the transcriptional level.

To measure the dynamic changes of the free cellular cholesterol concentration in BMDMs and NIH/3T3s, we developed a biochemical assay based on an enzymatic assay method (2.6). Changes were measured over a period of 72 hrs after mCMV infection at a MOI of 1 to take into account the delay of the proteins translation and consequent metabolic adjustments.

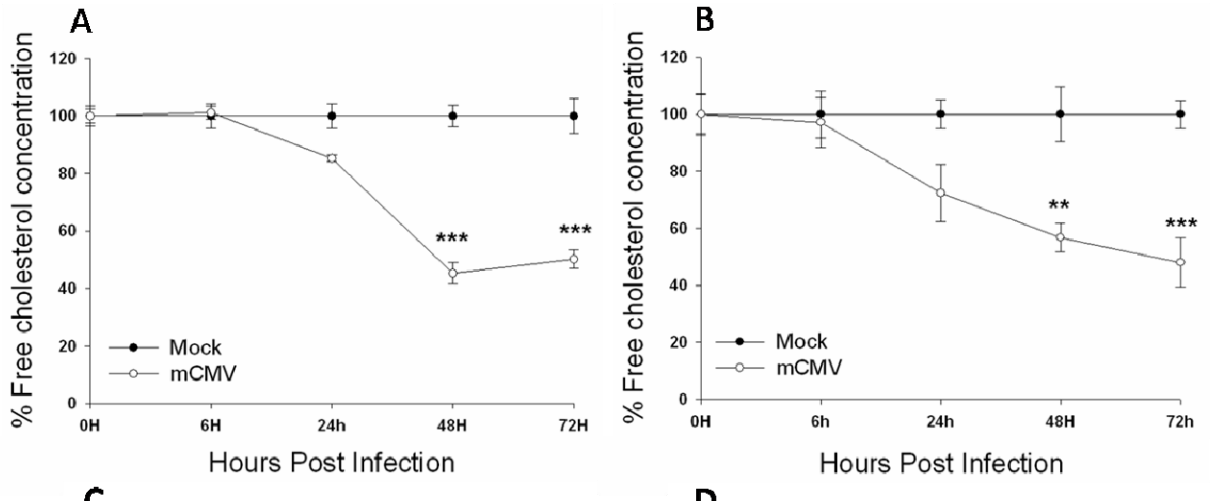
Results (Figure 4-2) show that mCMV infection induced a significant decrease of free intra-cellular cholesterol concentration after 48 hrs of infection up to 72 hrs in both BMDMs and NIH/3T3s. Furthermore, the reduction in both cell types is comparable (approximately 50 to 60% of the initial amount of free cholesterol).
These results demonstrate that mCMV infection induces a drop of free intra cellular cholesterol after 48 hrs infection. However, these results are not sufficient to attribute the drop of free intra cellular cholesterol to the down regulation of the sterol biosynthesis pathway since the cholesterol efflux storage and uptake could also modulate the intra cellular cholesterol concentration.

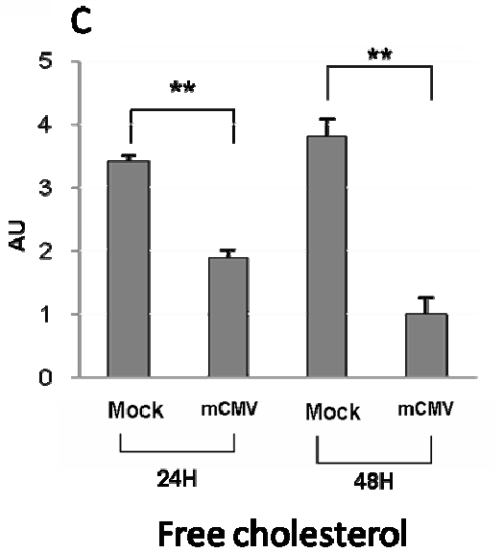
To further validate these results and investigate the role of each component of the cholesterol homeostasis, an independent analytical technique based on APCI/MS was used to examine the level of free cholesterol, of an immediate precursor of cholesterol: 7-dehydrocholesterol and of cholesterol ester, as well as the storage form of cholesterol. Lipid extraction of mock treated and mCMV infected macrophages at 24 and 48 hrs was carried out using chloroform methanol at Edinburgh University

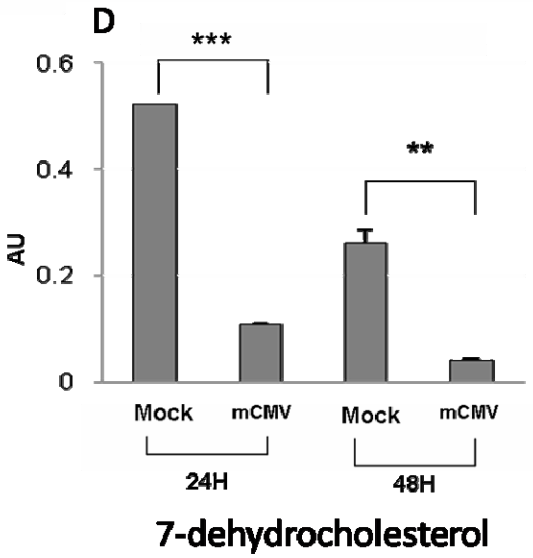
and the APCI/MS analysis were performed in a collaborative project by Dr Markus Wenk and Dr Shui Guanghou at the University of Singapore (See section 2.6).

As expected, the analysis shows a decrease of 50 to 65% of free intra cellular cholesterol level in response to infection at both 24 and 48 hrs post infection confirming our previous analysis (Figure 4-2C). The level of 7-dehydrocholesterol was also dramatically reduced after 24 hrs and 48 hrs post infection (80 and 90%) (Figure 4-2D). Furthermore, the level of cholesterol ester was increased at 24 and 48 hrs post infection (8 and 5 fold respectively) (Figure 4-2E).

These results confirmed with an independent assay that mCMV infection induces a significant decrease of free intra cellular cholesterol in response to infection after 24 and 48 hrs post infections. Furthermore, the synthesis of free cholesterol is also reduced by 80 to 90% as a consequence of the infection. In addition, the storage of cholesterol in its ester form is increased (5 to 8 fold) by mCMV infection in BMDMs.


A
% Free cholesterol concentration
120
100
80
60
40
20
0
0H
6H
24h
48H
72H
***
***
Mock
mCMV
Hours Post Infection
B
% Free cholesterol concentration
120
100
80
60
40
20
0
0H
6h
24h
48H
72h
**
***
Mock
mCMV
Hours Post Infection


C
AU
5
4
3
2
1
0
**
**
Mock
mCMV
Mock
mCMV
24H
48H
Free cholesterol


D
AU
0.6
0.4
0.2
0
***
**
Mock
mCMV
Mock
mCMV
24H
48H
7-dehydrocholesterol

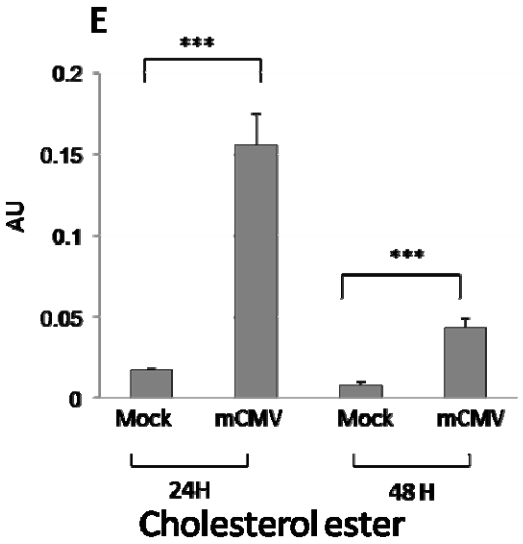

E
AU
0.2
0.15
0.1
0.05
0
***
***
Mock
mCMV
Mock
mCMV
24H
48 H
Cholesterol ester

Figure 4-2: Changes in cholesterol concentration in mCMV infected BMDMs and NIH/3T3 fibroblasts

A-B: Free cholesterol concentration was determined at 0, 6, 24, 48 and 72 hrs post infection in BMDMs (A) and NIH/3T3 cells (B) infected with mCMV. Three independent experiments with quadruplicate assays for each experiment were performed (n=12). Cholesterol content is presented as the percentage of free intracellular cholesterol concentration from infected cells compared to mock treatment. Graphs show means ± S.D

C-E: Free cholesterol and 7-dehydrocholesterol analysis was performed using APCI/MS from mCMV infected BMDMs at 24 and 48 hrs post infection. Dramatic decreases in free cholesterol (p-value = 0.009 at 24 hrs and 0.003 at 48 hrs) and 7-dehydrocholesterol (p-value = 0.00002 at 24 hrs and 0.0074 at 48 hrs) were observed in infected mice. Y-axis, arbitrary unit (AU), relative ratio to deuterated sterol standards. One independent experiment with triplicate assays for each experiment was performed for this analysis (n=3).

Statistical significance in a Welsh t-test: *P < 0.05, **P < 0.01, ***P < 0.001.

4.2.3 Temporal regulation of cholesterol biosynthesis pathway

We next were interested in understanding the dynamic of the regulation of the sterol genes in the first hrs post infection. Indeed, early changes in cellular gene expression are indicators of the event following viral gene entry into the cell (Fortunato, McElroy *et al.* 2000; Kropp, Simon *et al.* 2009).

To analyse the changes in temporal gene expression of the sterol regulating genes, two micro-array time-courses dataset of mCMV infected (MOI of 1) BMDMs and NIH/3T3 respectively were analysed (2.10.1).

For the first experiment, BMDMs were infected for 12 hours; RNA samples were extracted every 30 minutes for the first 12 hrs following the infection and RNA was analysed using a Mouse Agilent V2 chip array. In a separate experiment NIH/3T3 infected RNA samples were taken at 3, 16 and 24 hrs post infection and processed in an Affymetrix U74 chip. Results are presented figure 4.2 as a heat map representing the log fold changes of expression of infected compared to the mock treated samples. (Experiment, processing and statistical analysis were performed by Dr Garwin Sing and Dr Paul Dickinson in the Division of Pathway and Medicine – See section 2.10.1).

Temporal analysis of the changes in expression of the sterol associated genes in response to mCMV infection confirmed our previous observation, indeed, for both BMDMs and NIH/3T3 the expression of the sterol genes was down regulated at 12 and 24 hrs post infection. Furthermore, the down regulation of the biosynthesis pathway occurs in a gradual and temporal manner from an early time (3 hrs) up to at least 12 hrs post infection for BMDMs and 24 hrs for NIH/3T3.

In the BMDMs infected experiment, the sterol-associated genes are at first briefly up regulated in response to infection (up to 300 min) before being down regulated. In addition, there are differences in the timing of the down regulation of some sterol associated genes in BMDMs: While *Hmgcs1*, *Hmgcr*, *Fdft1*, *Sqle*, *Lss*, *Sc4mol*, *Nsdhl*, *Hsd17b7*, *Dhcr24* and *Dhcr7* are down regulated at 300 minutes, *Mvk*, *Pmvk*, *Mvd*, and *Cyp51*and *Ebp* begins to be down regulated at a later stage of the infection (around 600 minutes). Moreover, in BMDMs but not NIH/3T3s, the expression of

Cyp51 is increased until 600 minutes post infection before being down regulated in comparison to mock treated cells.

Taken together these results indicate that the co-ordinate down regulation of the sterol genes consecutively to mCMV infection occurs both in BMDMs and NIH/3T3 at an early time post infection and follow an initial up regulation stage.

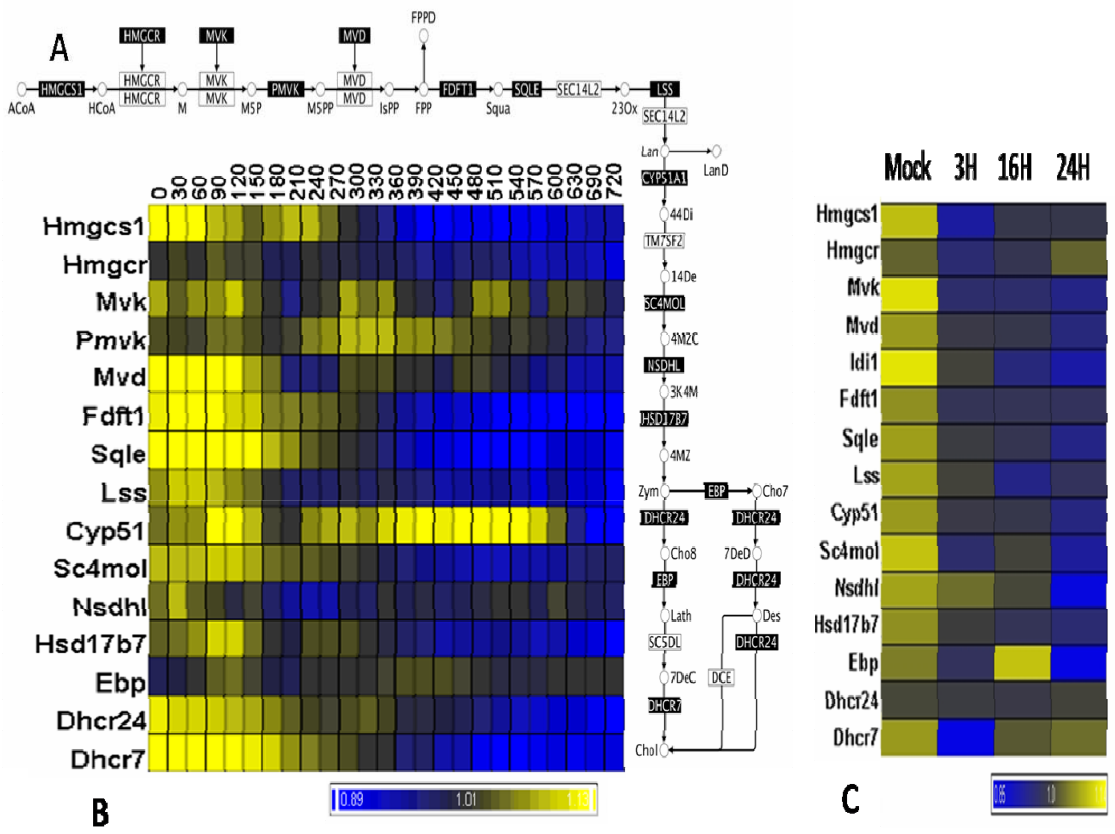


Figure 4-3: Down regulation of the cholesterol biosynthesis pathway upon mCMV infection in BMDMs and NIH/3T3

A. The Sterol biosynthesis pathway, shown in KEGG notation,

B. Heat map of the temporal expression levels of 15 genes of the cholesterol biosynthesis pathway during the first 12 hrs of mCMV infection in BMDMs. Columns indicate time in minutes. Scale: (from, in Log scale, -0.83 to +1.13)

C. Heat map representing the temporal expression levels of 15 genes of the cholesterol biosynthesis pathway at 3, 16 and 24 hrs of mCMV infection in NIH/3T3. Scale: (from, in Log scale, -0.85 to +1.14)

Yellow represents an increase in expression from the median while blue represents a decrease.

4.2.4 Regulation of the sterol genes expression by mCMV IE3Ko infection

The early regulation of the sterol associated genes in response to mCMV infection led us to wonder if this alteration of the pathway was due to the binding and entry of the virion into the cell or was driven by viral gene expression. Indeed virion binding and tegument proteins trigger a large transcriptional host response (Fortunato, McElroy *et al.* 2000), which could be at the origin of the down regulation of the sterol biosynthesis pathway. In the other hand, immediate early gene expression during mCMV infection occurs as early as 30 minutes post infection (Kropp, Simon *et al.* 2009). Since our results show that sterol genes are initially up regulated in response to infection before being gradually down regulated, it was possible that the down regulation of the cholesterol associated genes is driven by viral gene expression or by the cellular host response.

To understand whether the down regulation of the sterol pathway was related to an early host response to infection or induced by viral gene expression, we used a replication and early/late gene defective mCMV virus (mCMV-IE3KO) (See section 2.2.1). mCMV-IE3KO is capable of infecting cells at levels equivalent to wild-type virus but is incapable of expressing its genome downstream of a rather restricted immediate-early set of genes (Angulo, Ghazal et al. 2000; Lacaze 2010).

For these experiments, BMDMs were infected with mCMV wild type or mCMV-IE3KO viruses or mock treated for 24 hrs in BMDMs and RNA extracted to perform QPCR analysis of *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* gene expression.

Results in Figure 4-3show that the mCMV and mCMV-IE3Ko induce the down regulation of the sterol genes. Additionally, the scale of the down regulation induced by mCMV-IE3 infection is even greater than the wild type. Fold changes for *Hmgcs1* (mCMV: -2.5 and mCMV-IE3Ko: -5.2), for *Hmgcr* (mCMV: Not significant and mCMV-IE3Ko: -1.72), for *Idi1* (mCMV: -2.9 and mCMV-IE3Ko: -18.65) and for *Sqle* (mCMV: -3.3 and mCMV-IE3Ko: -11.6).

These results clearly indicate that the alteration of the sterol biosynthesis pathway in response to mCMV infection is not driven by late gene expression and suggest that viral gene expression limits the down regulation of the sterol pathway in response to infection.

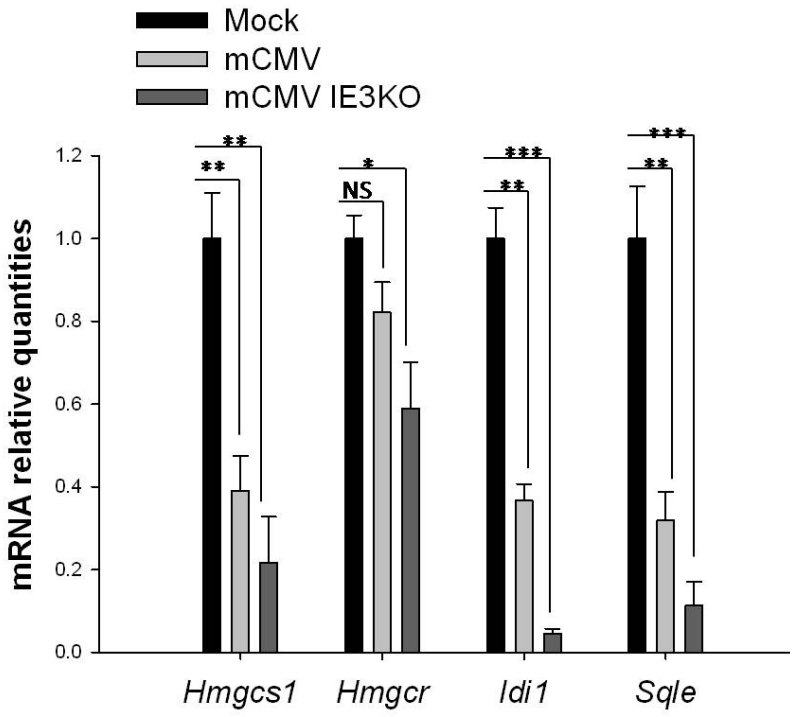


Figure 4-4: Regulation of sterol gene expression by non-replicative mCMV-IE3 KO virus in BMDMs

Changes in gene expression of Acat1, *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* expression in mock treated, mCMV or mCMV-IE3KO infected BMDMs at 24 hrs post infection. Bars represent means ± S.D. of 2 independent experiments with triplicate measurements for each experiment (n=6). A Welsh t-test was used for evaluation of statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

4.2.5 The specificity of the viral infective agent

Previous results showed that the down regulation of the sterol biosynthesis associated genes by mCMV infection were not dependent on late viral gene expression. We next asked if the alteration of the sterol pathway was specific to mCMV infection or rather a general cellular response to viral infection.

For that purpose we exploited an Affymterix MOE430v2 chip micro array dataset of BMDMs mock treated or infected with Herpes Simplex virus 1 (HSV1), Semliki Forest Virus (SFV), Vaccinia virus (VV) or adenovirus (AD) for 24 hrs post infection (See section 2.2.1).

This experiment presented four families of viruses having very different characteristics and therefore, it is a good representation of a broad viral infection. HSV1 is an enveloped DNA alpha Herpes virus replicating in the nucleus of the cell, VV is an enveloped DNA virus that replicates only in the cytoplasm of the infected cells, AD is a non-enveloped DNA virus and finally SFV is a positive-stranded RNA virus which replicates from its negative strands in the cytoplasm of the cell.

Mock treatment, HSV1 (MOI=1), SFV (MOI=10), VV (MOI=1) infection of BMDMs for 24 hrs were performed in 3 independent replicates while only 2 independent replicates where used for Ad (MOI=100) infection of BMDMs. RNA was hybridised and processed in an Affymetrix MOE 430-2 chip array (Experiment, processing and statistical analysis were performed by Dr Garwin Sing and Dr Paul Dickinson in the Division of Pathway and Medicine –See section 2.10.1)

To verify that the infection was successful for every viral species, activation of IFN response and of the MHC class II presentation genes was checked and the results showed that while there is almost no activation of the IFN signalling or MHC class II in the mock treated macrophages, the infection of the 4 viruses induced a strong immune response (Figure 4-5).

We then looked at the regulation of the sterol biosynthesis genes and of the genes responsible for uptake, storage, efflux and regulation of intra cellular cholesterol concentration (*Ldlr*, *Soat1*, *Apoe*, *Abca1* and *Abcg1*).

Results in Figure 4-5, showed that after 24 hrs infection in BMDMs the sterol biosynthesis regulating genes were globally down regulated by HSV1, SFV, VV and

Ad compared to the mock treated samples. These results are similar to the one observed in response to mCMV infection. Moreover, *Ldlr* implicated in the cellular entry of cholesterol was down regulated, and the genes implicated in storage and effluxes of cellular cholesterol (respectively *Soat1*, *Apoe*, *Abca1 and Abcg1*) were globally up regulated in response to infection.

Srebf2 was significantly down regulated by all four viruses while *Srebf1* is not altered by the infection of the four viruses.

These results are similar to the effect of mCMV infection in BMDMs and NIH/3T3s sterols associated genes previously observed and strongly argue that the down regulation of sterol pathway synthesis is a general host response towards viral infection.

To further validate these results the gene expression of *Hmgcs1*. *Hmgcr*, *Idi1* and *Sqle* of HSV1 or mCMV infected BMDMs for 24 hrs was measured by QPCR.

Even if a formal statistical analysis was not performed on these samples, as only a few replicates could be analysed, nevertheless these results confirmed that HSV1 and mCMV infection down regulate *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* gene expression after 24 hrs infection in BMDMs.

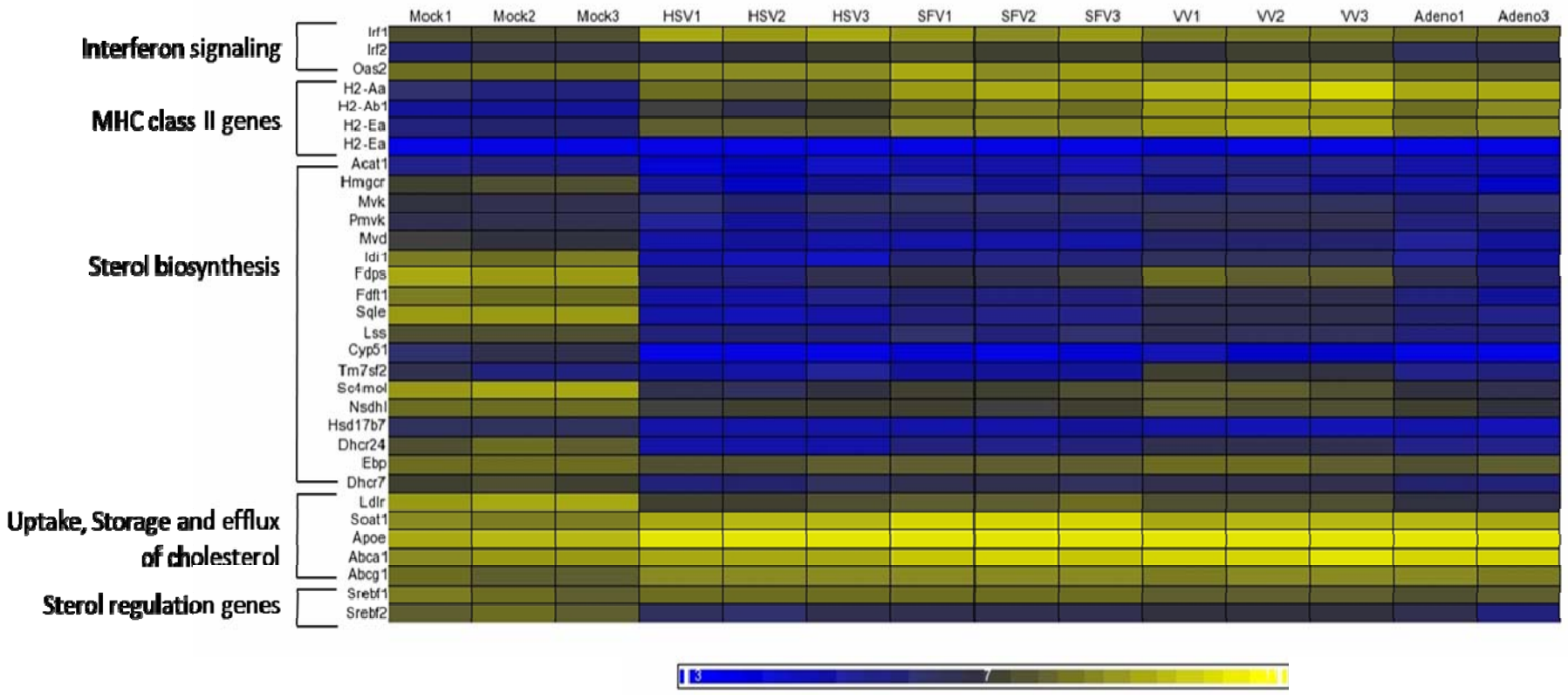


Figure 4-5: Alteration of cholesterol regulating genes by HSV1, SVF, VV and adenovirus infection after 24 hrs infection in BMDMs

Hit map representing the fold changes of gene expression of the cholesterol regulating genes HSV1, SFV and VV infected BMDMs compared to mock treated samples.

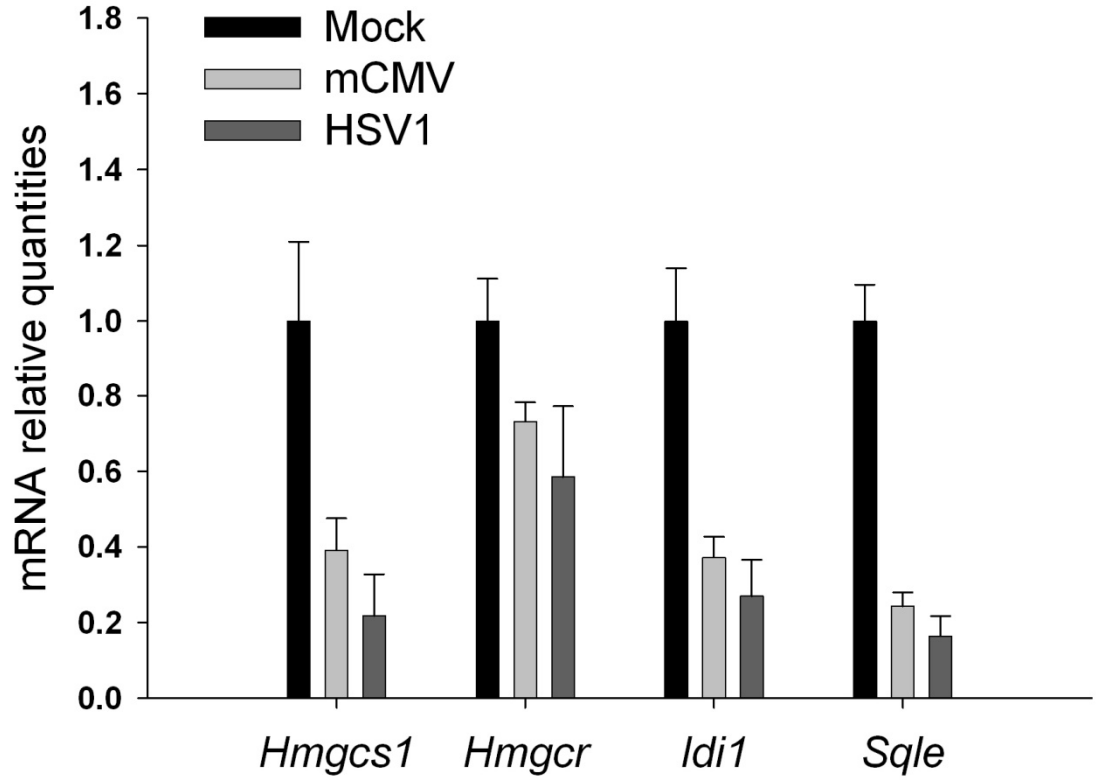


Figure 4-6: Quantitative PCR of Hmgcs1, Hmgcr, and Idi1 and Sqle gene expression in mock treated or HSV1, SVF and VV infected BMDMs at 24 hrs post infection

Bars represent means ± S.D. of one independent experiment with quadruplicates measurements (n=4).

4.3 Conclusion

The aim of this chapter was to characterise the genetic and metabolic regulation of the cholesterol homeostasis in response to mCMV infection.

We first look at the transcriptional regulation of the genes responsible for the control of uptake, synthesis, efflux and storage representing the main pathways regulating cholesterol homeostasis: First of all, measurement of gene expression of infected macrophages by RT-QPCR confirmed the down regulation of the sterol biosynthesis in BMDMs at 24 hrs post mCMV infection. We have also shown that this response was similar in NIH/3T3 fibroblasts cells. In addition, this study provides evidence that mCMV infection induced the transcriptional down regulation of cholesterol uptake (*Ldlr*) and the up regulation of the storage (*Soat1*) and of the efflux of cholesterol (Abaca1, *Abcg1*, *Apoe*) in BMDMs and NIH/3T3s.

In addition, we have shown that *Srebf2*, a key gene involved in the coordinate regulation of the entire sterol synthesis pathway is down regulated upon infection. Further studies need to be done to assess whether *Srebf2* is directly responsible for the down regulation of the cholesterol pathway in response to infection.

Since the regulation of genes associated with cholesterol homeostasis is complex and is normally driven by feedback mechanisms (Simons and Ikonen 2000), we then wonder if the changes induced by mCMV infection in the regulation of cholesterol homeostasis at the transcriptional level resulted in an alteration of the concentration of intra cellular cholesterol. Free intracellular measurement using two independent analytical methods shows that mCMV infection results in a drop of free intra cellular cholesterol of 50% after 48 and 72 hrs experiments. Additionally, we have shown that the biosynthesis of de novo cholesterol was directly reduced since the concentration of a sterol intermediary of the sterol synthesis pathway 7-hydroxycholesterol was decreased by 70 to 80% in response to infection. In addition cholesterol ester concentration was increased by 5 to 8 fold as a result of infection.

Taken together these results indicate that, mCMV infection induces a complete reprogramming of the regulation of cholesterol homeostasis at the transcriptional and metabolic level. Indeed mCMV infection decreases the gene expression of the synthesis and uptake of cholesterol, and increases the one of the storage and efflux.

Conversely, mCMV infections decrease the level of production of de novo cholesterol as well as the overall pool of free intra-cellular cholesterol and increase the cholesterol ester concentration. However, these results are not sufficient to conclude that transcriptional changes in the regulation of the cholesterol associated genes directly induce changes at the metabolic levels since other regulatory mechanisms could also playing a role. Measurements of the protein levels by Western Blot technique and use of radioactive probe such as [^{3}H] mevalonate, [^{3}H] LDL and [^{3}H] HDL to measure qualitative synthesis, uptake and efflux of cholesterol (Xu, Zhou *et al.* 2009) are required to understand completely the dynamic of the changes in intra cellular cholesterol in response to mCMV infection. Never the less these study characterise for the first time the modulation of cholesterol homeostasis in response to mCMV infection in BMDMs and NIH/3T3.

In addition, these results designate that drop in cholesterol concentration is an early marker of mCMV infection in vitro. Further work will be required to evaluate if the level of cholesterol could constitute a marker of acute infection *in vivo*.

Previously, Herpes viruses and especially MDV has been shown to increase free cholesterol and cholesterol ester level in vitro in smooth cell muscles and in the arterial wall(Hajjar, Falcone *et al.* 1985). Furthermore, *Ldlr* and *Hmgcr* expression has been shown to be increased in response to hCMV and MDV infection (Hsu, Nicholson *et al.* 1995; Zhou, Guetta *et al.* 1996).

Our results differ from previous published studies since both *Ldlr* and *Hmgcr* gene expression is decreased by mCMV infection and HSV1 in BMDMs. Furthermore we have shown that total cholesterol level was decreased in response to mCMV infection in BMDMs and NIH/3T3s while Hajjar and colleagues have shown that there is an increase of total cholesterol. Here, we can speculate that smooth cell muscle may have a different cellular response in the regulation of sterol associated genes in response to infection or that experimental conditions were different. In any case further study will be required to understand the differences in these results.

Nevertheless, our results show that cholesterol ester is increased in response to mCMV infection and are in agreement with Hajjar and colleagues and others. This points at a possible mechanism for macrophage foam cell formation in response to

mCMV infection and could provide new mechanism in the understanding of the initiation of the atherosclerosis disease.

To understand the mechanisms at the origin of the transcriptional modulation of the cholesterol homeostasis, we next considered the temporal regulation of the sterol associated genes in response to infection. Temporal gene expression analysis of two independent micro array data sets showed that the modulation of the genes associated with cholesterol concentration was occurring at a very early time post infection (around 3hrs pi). Since both, viral binding and expression of early viral gene could have been at the origin of the regulation, we wondered if the regulation of these genes was the consequence of viral gene expression or a cellular response to viral entry. Infection of BMDMs with a mutant virus mCMV-IE3KO unable to express any viral transcript showed that the modulation of the cholesterol associated genes was maintained and even greater than the one observed with the wild type virus infection. These results strongly support the view that the modulation of the cholesterol regulating genes in response to viral infection is triggered by viral binding or entry into the cell and not by viral gene expression. However, mCMV-Ie3-KO have been shown to have very low expression of immediate early and early genes compare to the wild type virus (Lacaze 2010), therefore we cannot exclude that this very weak expression regulates sterol associated genes.

We then looked if the regulation of the expression the sterol biosynthesis pathway was specific to mCMV infection or was a general response to infection. Analysis of micro array data set from BMDMs infected with 4 structurally very different viruses (HSV1, SFV, VV and Ad) showed that all four infections induced the down regulation of the sterol associated genes.

Taking together, these results indicate that the regulation of cholesterol synthesis in response viral infection is a general cellular response.

To date, several viruses such as HIV, HCV have been shown to manipulate intra cellular cholesterol location for their own benefit, for example HCV hijacks the lipid droplets pathway for efficient replication and budding (Boulant, Douglas *et al.* 2008), HIV redistributes cellular cholesterol to specific membrane compartment to increase its replication. HCV viruses have been shown to hijack and redistribute the intra-cellular cholesterol pool (Mackenzie, Khromykh *et al.* 2007).

Furthermore, HIV, HCV and measles viruses have been shown to code for viral proteins which increase the sterol biosynthesis gene expression. NEF for HIV and NS4B for HCV, have been shown to directly up regulate the expression of SR*EBP*s (Zheng, Plemenitas *et al.* 2003; Mackenzie, Khromykh *et al.* 2007; Waris, Felmlee *et al.* 2007).

Recently, measles viruses have been shown to induce the down regulation of the sterol biosynthesis pathway in persistent infection but not in acute infection. The authors of this study conclude that alteration of cholesterol synthesis may limit the budding of the virus, which is a characteristic of measles persistent infection (Robinzon, Dafa-Berger *et al.* 2009).

In this study we are showing that down regulation of the sterol biosynthesis is a general cellular response to viral infection occurring independently of viral gene expression. Since viruses have evolved to take advantage of the host cholesterol homeostasis, it is possible that cells have developed innate immune mechanisms to counteract viral infection by influencing the cholesterol homeostasis. Our results raise also the question whether the down regulation of the sterol biosynthesis pathways and the decrease in intracellular cholesterol concentration in response to viral infection have an effect on the output of the infection.

Since pharmacological inhibition of *Hmgcr* the first step of the sterol synthesis pathway has been shown to be antiviral for many viruses *in vitro* and *in vivo* (See 1.4.2.2), it is possible that the down regulation of the cholesterol synthesis pathway by the cell in response to viral infection is antiviral. Antiviral effects of statins have been attributed to the drop of cholesterol but most often of the prenylation of proteins. Drop in free cholesterol concentration might results in alteration of viral virion structure, intra cellular movement and budding and therefore be beneficial for the infected organism. Alternatively, reduction of sterol biosynthesis flux may alter the prenylation of proteins require for efficient viral growth such as FBL2 for HCV(Wang, Gale *et al.* 2005).

In addition, alteration of intra cellular cholesterol may alter the stability and number of the lipid rafts. However, it is difficult to predict what effects the alteration of lipid rafts will have on viral infection. Indeed, alteration of rafts may perturb the viral replication cycle but may also decrease antiviral pathways such as MHC class I

presentation (Mackenzie, Khromykh *et al.* 2007). Interestingly since lipid raft have been shown to be essential for the cellular entry of many viruses (See section 1.4.2.2), we can speculate that drop of cholesterol in response to infection may also affect further viruses entry. Further experiments will be required to assess the effect of the drop of cholesterol synthesis on mCMV replication.

To conclude, in this chapter we have shown for the first time that mCMV infection induces the impairment of cholesterol homeostasis as both transcriptional and metabolic level in BMDMs and NIH/3T3. In addition, we have shown that the down regulation of the sterol associated genes is occurring at a very early time post infection (three hrs) and is not dependent of the viral genes. Finally, we provide evidence that the down regulation of sterol associated genes is a general cellular response towards viral infection.

5 Chapter 5: Alteration of the sterol biosynthesis pathway is antiviral for mCMV infection

5.1 Introduction

Previously, we have shown that the coordinate down regulation of the sterol biosynthesis pathway at the transcriptional and metabolic level is induced by mCMV infection in BMDMs and NIH/3T3s (See chapter 3 and 4).

Recently, sterol biosynthesis has been identified as an important modulator of viral infection (See section 1.4.2.2). This raises the question whether cellular down regulation of the sterol synthesis pathway in response to mCMV infection affects the outcome of mCMV infection.

Inhibition of sterol synthesis by statins has been shown to limit hCMV infection *in vitro* (Potena, Frascaroli *et al.* 2004). However to date, the contribution of sterol synthesis to mCMV infection has not been studied *in vitro* or *in vivo*.

To address this question, we used a combined approach using pharmacological inhibition and selective gene expression knock downs to perturb the sterol biosynthesis pathway and monitor the effects on mCMV infection (Figure 5-1).

Furthermore, several studies have shown that the alteration of protein prenylation, a branch of the mevalonate pathway, was implicated in the modulation of viral replication (Bordier, Ohkanda *et al.* 2003; Einav and Glenn 2003; Glenn 2006). For that reason, we performed a supplementation assay with a mevalonate intermediate in Statin-treated cells to understand the role of prenylation in the modulation of mCMV infection.

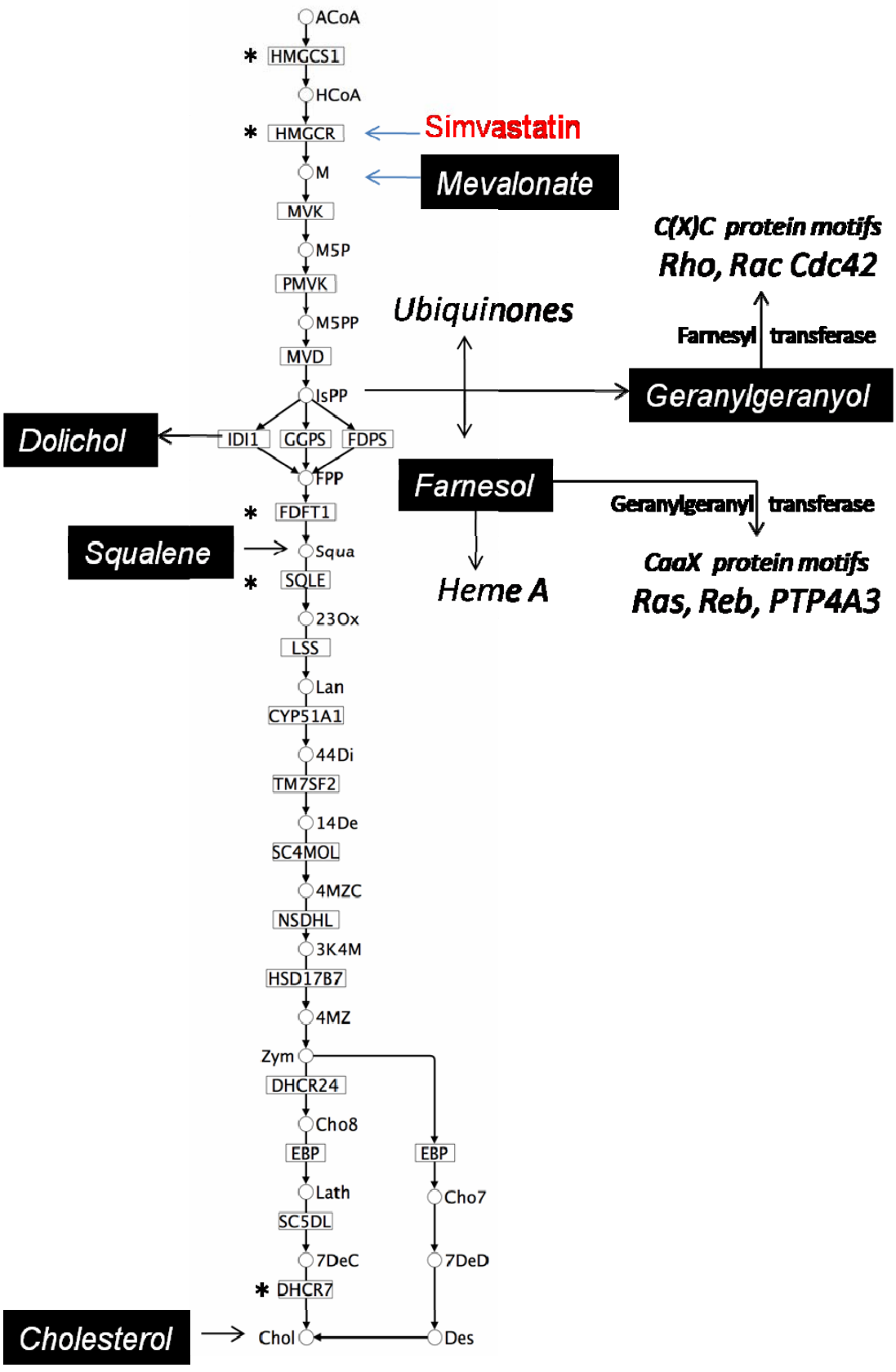

ACoA
HMGCS1
HCoA
HMGCR
Simvastatin
M
Mevalonate
MVK
M5P
PMVK
M5PP
MVD
IsPP
Ubiquinones
C(X)C protein motifs
Rho, Rac Cdc42
Farnesyl transferase
Geranylgeranyol
Dolichol
IDI1
GGPS
FDPS
FPP
Farnesol
Geranylgeranyl transferase
FDFT1
Squalene
Squa
SQLE
Heme A
CaaX protein motifs
Ras, Reb, PTP4A3
23Ox
LSS
Lan
CYP51A1
44Di
TM7SF2
14De
SC4MOL
4MZC
NSDHL
3K4M
HSD17B7
4MZ
Zym
DHCR24
Cho8
EBP
EBP
Lath
Cho7
SC5DL
7DeC
7DeD
DHCR7
Cholesterol
Chol
Des

Figure 5-1: Pharmacological inhibition and supplementation with an intermediary of the mevalonate pathway helps to decipher the involvement of different branches of the pathway in mCMV replication

Statins inhibit *Hmgcr*, the rate-limiting enzyme of the sterol pathway. Activated statins bind approximately 1,000 times more effectively to *HMGCR* than its natural substrate HMG-CoA. Statin treatment inhibits the formation of both mevalonate and its downstream product, isopentyl pyrophosphate (IPP). This inhibition can be reversed completely by replenishment with mevalonate. Additions of farnesol restore farnesylation but not geranylgeranylation, as IPP is not available to convert farnesyl pyrophosphate (FPP) into geranylgeranyl pyrophosphate (GGPP). Addition of geranylgeranyol will only restore geranylgeranylation while addition of squalene and cholesterol will restore sterol biosynthesis (Liao 2002; Mo and Elson 2004).

Proteins containing a carboxy terminal CaaX motif (C is a cysteine, a is an aliphatic amino acid and X is an amino acid) such as Ras, Rheb and PTP4A3 families are farnesylated by farnesyl transferase while proteins containing a CC or C(X)C sequence such as Rho, Rac and Cdc42 families are geranylgeranylated by geranylgeranyl transferase. A few G-proteins such as RHOB and NRAS can either be farnesylated or geranylgeranylated (Takai, Sasaki *et al.* 2001). In addition, other isoprenoid side-branches are also altered by Statin inhibition: Dolichol pyrophosphate which plays a role in the synthesis of oligosaccharide chains of glycoproteins and is found in many membranes of cells, Ubiquinone, (Coenzyme Q), is involved in the electron transfer chain and finally Heme A, a constituent of respiratory chain complexes, which contain a farnesyl side-chain.

Stars represent the genes targeted by SiRNA knock down during this study: *Hmgcs1*, *Hmgcr*, *Fdft1*, *Sqle* and *Dhcr7*.

5.2 Results

5.2.1 Effect of simvastatin treatment on NIH/3T3 fibroblast cell viability

Pharmacological inhibition of the sterol pathway using statins, inhibitors of the rate limiting enzyme of the cholesterol synthesis pathway have been used extensively to study the involvement of cholesterol synthesis for the viral replication of many viruses (See section 1.4.2.2). Since simvastatin treatment has been shown to induce cellular toxicity and to trigger apoptosis (Demierre, Higgins *et al.* 2005), we first determined the range of simvastatin concentration which was non-toxic for the cells.
NIH/3T3 fibroblast cells (2.5×10^4) were seeded in 96-well plates and treated with increasing concentrations (0.25 – 25 μM) of simvastatin for 120 hrs. Cell viability was measured using the cell titre blue assay (Promega). As seen in Figure 5-2A, increasing concentrations of simvastatin resulted in no significant difference in the cell viability of the NIH/3T3 fibroblasts after 72 hrs of treatment with 0.25 to 10 μM. However, a significant decrease in cell viability is observed when the cells are treated with more than 15 μM of simvastatin, and when treated with 25 μM, cells were no longer viable. BMDMs however were more susceptible to simvastatin toxicity than NIH/3T3. Indeed, macrophage viability was affected with concentrations as low as 1 μM after 72 hrs (Figure 5-2B).
To avoid any results which could have been biased by the toxicity of the simvastatin per se, we decided to restrict our analysis to the NIH/3T3 cells as the down regulation of the sterol pathway regulation in response to infection was observed in both cell types.

5.2.2 Consequences of simvastatin treatment on mCMV infection *in vitro*

We next evaluated whether inhibition of the biosynthesis pathway by simvastatin will affect mCMV infection. For this purpose, we performed a one-step growth analysis of NIH/3T3 fibroblasts infected with mCMV at a MOI of one in the presence and absence of a non-toxic dose of 10 μM of simvastatin for 5 days (Figure 5-2C). At different times after infection, the presence of extracellular virus in the cultures was determined by standard plaque assay (See section 2.2.5). As shown in Figure 5-3, the rates of growth of mCMV decreased markedly in the presence of

Simvatstatin, where there is at least one log difference in the amount of virus measured between the treated and untreated cells after three days and up to five days post-infection.
These results indicate that simvastatin has an antiviral effect on the viral replication of mCMV in NIH/3T3 cells.

5.2.3 Dose effect of simvastatin treatment on mCMV infection

We next looked at the dose dependency of the inhibition of mCMV growth by simvastatin treatment. For that purpose, we used a live cell assay using mCMV-GFP tagged virus to monitor the effect of increasing doses of simvastatin on mCMV replication. In this assay, the amount of GFP signal emitted in an infected cell is directly proportional to the amount of viral replication (See section 2.9).
For this experiment, NIH/3T3 were infected with mCMV-GFP at a MOI of 0.2 and then treated immediately after with an increasing amount of simvastatin (0.001 to 10 μM). As a positive control, NIH/3T3 cells were also treated in a similar manner with an increasing amount of gancyclovir (0.001 to 10 μM), a drug known to alter mCMV infection (Matthews and Boehme 1988). The GFP-fluorescence was recorded over 96 hrs to monitor the growth of the virus.
Results (Figure 5-4D) demonstrate that simvastatin inhibits mCMV-GFP viral growth in a dose dependent manner with a half maximal inhibitory concentration (IC_{50}) equal to 2μM. Gancyclovir, as expected, also inhibited mCMV-GFP viral growth with an IC_{50} of 1 μM (Gschwentner, Susanna *et al.* 1995).

5.2.4 Specificity of the simvastatin antiviral effect

Statins have been shown to have other effects than inhibiting the mevalonate pathway (Demierre, Higgins *et al.* 2005; Shaw, Fildes *et al.* 2009). For that reason, we investigated if the antiviral effect of simvastatin in mCMV viral growth was specific to the mevalonate pathways.
Since mevalonate concentrations are decreased by simvastatin treatment (Goldstein and Brown 1990), NIH/3T3 fibroblasts were infected with mCMV-GFP and treated with 2.5μM of simvastatin supplemented with or without 300μM of mevalonate. Results (Figure 5-2E) show that while simvastatin treatment altered mCMV growth,

this effect is abolished by the addition of mevalonate. Moreover, the level of infection was restored to a level comparable to the untreated cells. Altogether, these results indicate that inhibition of viral growth by simvastatin is specific to the inhibition of the mevalonate pathway.

5.2.5 Effect of simvastatin treatment on mCMV infection *in vivo*

We next evaluated the antiviral effect of simvastatin towards mCMV infection *in vivo*. In two separate experiments representing a total of 2 groups (control and treated) of 12 animals each, Balb/C mice received simvastatin (50mg/kg/mice) daily for five days by gavage. The non-toxic dose of the drug administered was chosen according to previous studies (Gower and Graham 2001).

At day 1 post-treatment, mice were then challenged with 2 x 10^6 PFU of mCMV by intraperitoneal injection, and sacrificed at 4 days post-infection (See section 2.4).

Spleen, liver, kidney, heart and lung were harvested and the viral titre of each organ was measured by plaque assay (See section 2.2.5). Each of the organs treated with simvastatin over 5 days had a significant lower amount of virus (approximately a log less) that those treated with vehicle (Figure 5-2F). These results indicate that simvastatin treatment has an antiviral effect on mCMV replication *in vivo* in Balb/c mice.

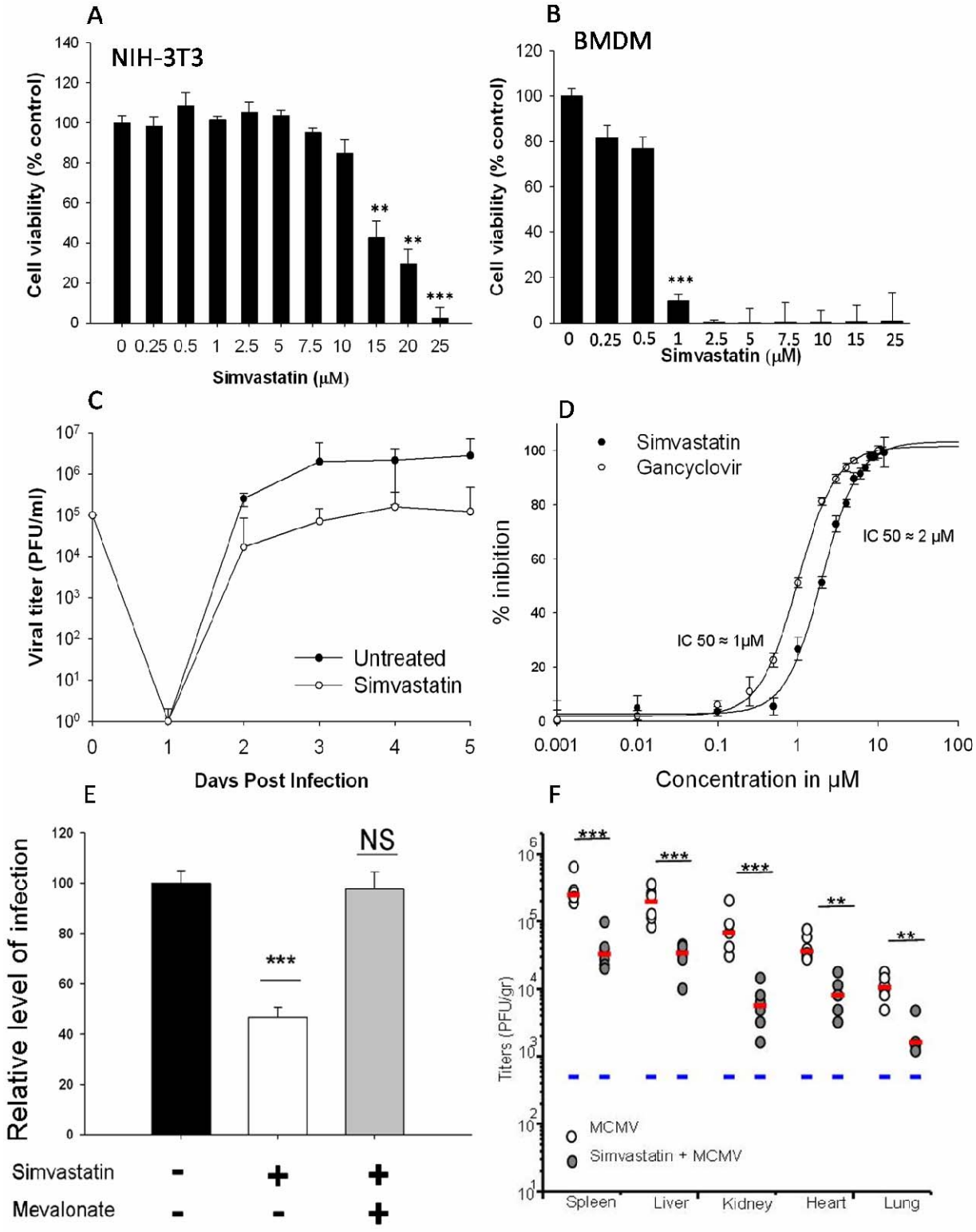

A
NIH-3T3
Cell viability (% control)
Simvastatin (μM)
B
BMDM
Cell viability (% control)
Simvastatin (μM)
C
Viral titer (PFU/ml)
Untreated
Simvastatin
Days Post Infection
D
Simvastatin
Gancyclovir
% inibition
IC 50 ≈ 1μM
IC 50 ≈ 2 μM
Concentration in μM
E
Relative level of infection
NS
Simvastatin
Mevalonate
F
Titers (PFU/gr)
MCMV
Simvastatin + MCMV
Spleen
Liver
Kidney
Heart
Lung

Figure 5-2: Pharmacological inhibition of mCMV viral infection with simvastatin

A-B: Dose effect of simvastatin on NIH/3T3and BMDM cell viability.

NIH/3T3 fibroblasts (A) or BMDMs (B) were treated with an increasing range of simvastatin concentration (0 to 25μM) or with vehicle for 72 hrs. Cell viability was determined using the Cell titre blue assay and is expressed as the percent of fluorescence signal from treated cells in comparison to untreated cells. Graphs represent the average values (± S.D.) of two independent experiments in triplicate (n=6). A Welsh t-test was used for the evaluation of statistical significance.

C: Effect of simvastatin on mCMV replication measured by plaque assay.

NIH/3T3 cells were infected with mCMV at a MOI of 1 PFU/cell. After one hr incubation, fresh media containing 10 μM simvastatin or vehicle was added to the culture for 5 days. At the different times indicated in the graph, aliquots of the culture were taken and the amount of viable viral particles present in the media was determined by standard plaque assay (see methods). Each data point represents the average values (± S.D.) of two independent experiments in triplicate (n=6).

D: Dose dependent inhibition of mCMV replication by simvastatin.

NIH/3T3 cells were infected with mCMV-GFP at a MOI of 0.2 for one hr and then treated with increasing concentrations of simvastatin or gancyclovir immediately after infection (0 to 10 μM). GFP expression was measured to establish the level of infection (Materials and methods). The graph represents the percentage of viral inhibition as a function of drug treatment where 100% inhibition represents the maximum level of inhibition of the simvastatin or gancyclovir treatment. Data points represent average values (± S.D.) of two independent experiments with six replicates for each experiment (n=12).

E: Mevalonate rescues infectivity of NIH/3T3 infected mCMV-GFP.

NIH/3T3 cells were infected for one hr at a MOI of 0.2 and then immediately treated with vehicle or 2.5 μM of simvastatin with or without 300 μM of mevalonate. The level of infection was determined by measuring the level of GFP fluorescence at 72 hrs (Materials and methods). The bars represent average values (± S.D.) of 3 independent experiments with 5 replicates for each experiment (n=15). A Welsh t-test was used for the evaluation of statistical significance.

F: Effect of simvastatin treatment on mCMV replication in vivo.

Two groups (control and treated) of 12 animals were given either simvastatin (50mg/kg/mice) or PBS daily for five days by gavage. After one day of drug treatment, the mice were then challenged with 2 x 10^6 PFU of mCMV by intraperitoneal injection, and sacrificed at 4 days post-infection. Spleen, liver, kidney, heart and lung were harvested and the viral titres were measured by plaque assay. Individual viral titres for each mouse (white circle or dark circle) are expressed per gram of tissue. The mean value of 4 to 6 mice is represented by a red horizontal bar, the blue bar represents the limit of detection of the assay, and the values were subjected to a Mann-Whitney U test for determination of statistical significance.

Statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

5.2.6 Gene-by-gene knock down of the sterol pathway

To corroborate our previous results and study in more detail the role of sterol biosynthesis in mCMV infection, we used a mCMV-GFP-based replication assay (See section 2.8) to study the effect of knocking-down the expression of *Hmgcs1*, *Hmgcr*, *Sqle*, *Fdft1* and *Dhcr7* on mCMV viral growth.

Once SiRNA are transfected into the cell, the amount of the related mRNA decreases within 24–48 hrs and the targeted protein is knocked down 48 to72 hrs later for approximately 5 days (Elbashir, Harborth *et al.* 2001). Consequently, NIH/3T3 cells were transfected for 48 hrs to allow gene and protein knock down before being infected. The fluorescence signal was monitored at 76 hrs post infection to compare the level of viral replication.

Transfection conditions and SiRNA concentration were optimised to obtain the best knock down, using the lowest amount of SiRNA and transfection reagent possible. Indeed high concentrations of SiRNA or transfection reagent have been reported to trigger off-target effects such as unspecific knock down and activation of the immune response (Lacaze, Raza *et al.* 2009). Non-target SiRNA was used as a negative control to test whether the transfection conditions alone may affect viral replication because it contains unspecific sequence to the mouse genome. We also included as a control the treatment of the cells with the transfection reagent only: Dharmafect-1 called “lipid only” in our figures.

The efficiency of the transfection for each assay was monitored by transfecting the cells with EGFP SiRNA which knocked down the viral GFP gene expression. However, since mCMV-GFP virus express the WtGFP and the SiRNA targets the mutated version of the gene: EGFP, the knock down of the signal was incomplete. Nevertheless previous experiments have demonstrated that a reduction of 30 to 40% of the GFP signal using EGFP SiRNA corresponded to efficient transfection conditions (data not shown).

A combination of a concentration of 25 nM of SiRNA and 0.4% of Dharmafect-1 was found to be the optimal conditions to ensure the best knock down efficiency for the minimal amount of SiRNA and transfection reagent possible (data not shown). With these conditions, *Hmgcs1*, *Hmgcr*, and *Sqle* expressions were decreased

dramatically by SiRNA transfection (81%, 70% and 82% respectively) (Figure 5-3A).

Because of time constraint and costs, the efficiency of *Fdft1* and *Dhcr7* transfection could not be tested. We therefore assumed in view of the previous results that the knock down of these genes would be comparable. Given that differences in cell number affects the level of GFP signal regardless of the treatment, which could lead to the mis-interpretation of the results, we checked the effect of knocking down the five genes on the cell viability of NIH/3T3 cells after 48 hrs post-transfection. Results (Figure 5-3B) showed that transfection with each of the five SiRNAs used to target the sterol synthesis pathway did not affect the viability of the NIH/3T3 fibroblasts after 48 hrs. This assay was also repeated after 100 hrs post-transfection and none of the SiRNAs used affected the long term viability of the cells (data not shown).

Once confident in our experimental setup, the effect of knocking down *Hmgcs1*, *Hmgcr*, *Sqle*, *Fdft1* and *Dhcr7* on mCMV replication was measured with the live cell replication assay.

Figure 5F shows that knocking down *Hmgcs1* and *Hmgcr* significantly decreases (25% and 27%) the level of infection of mCMV compared to the control non-targeted SiRNA. In contrast, knocking down *Sqle*, *Fdft1*and *Dhcr7* significantly increased the level of infection by 32, 41 and 20 % respectively.

These results indicate that decreases in gene expression of the first step of sterol biosynthesis results in an inhibition of viral replication. Moreover, since decreasing expression of the genes that specifically regulate the last steps of sterol synthesis results in an increase of viral replication, this strongly suggest that one or several side isoprenoid pathways are responsible for the modulation of the viral replication rather than cholesterol synthesis itself.

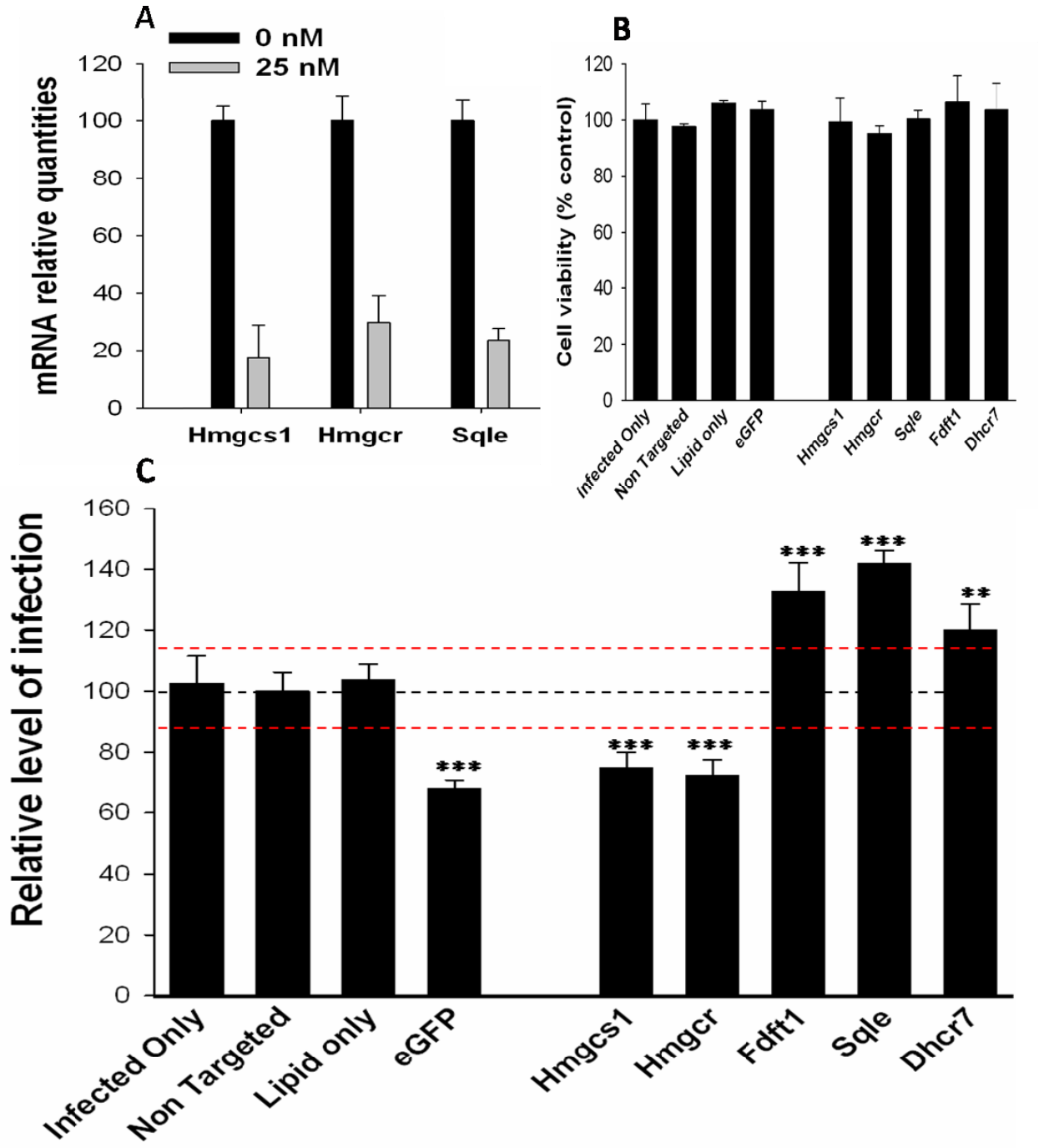

A
0 nM
25 nM
mRNA relative quantities
120
100
80
60
40
20
0
Hmgcs1
Hmgcr
Sqle
B
Cell viability (% control)
120
100
80
60
40
20
0
Infected Only
Non Targeted
Lipid only
eGFP
Hmgcs1
Hmgcr
Sqle
Fdft1
Dhcr7
C
Relative level of infection
160
140
120
100
80
60
40
20
0
***
***
***
***
**
Infected Only
Non Targeted
Lipid only
eGFP
Hmgcs1
Hmgcr
Fdft1
Sqle
Dhcr7

Figure 5-3: Knock down of Hmgcs1, Hmgcr, Sqle, Fdft1 and Dhcr7 and its effect on mCMV replication.

A: Knock down efficiency of *Hmgcs1*, *Hmgcr* and *Sqle* SiRNA:
NIH/3T3 cells were transfected with *Hmgcs1*, *Hmgcr* or *Idi1* On target plus SiRNA smart pool (Dharmacon). After 48 hrs, RNA was collected and QPCR was performed to measure the gene expression of *Hmgcs1*, *Hmgcr* and *Idi1*. Results represent the % of gene expression in the transfected cells (*Hmgcs1*, *Hmgcr* or *Sqle* SiRNA) compared to the level of expression in the control cells (non-targeting SiRNA). Bars represent average values ± S.D of one experiment with 3 biological replicates (n=3).
B: Effect of SiRNA transfection on the cell viability of NIH/3T3 fibroblasts:
NIH/3T3 cells were transfected with lipid only, None-targeted, eGFP, *Hmgcs1*, *Hmgcr*, *Sqle*, *Fdft1* and *Dhcr7* On-target plus SiRNA smart pool (Dharmacon). After 48 hrs, cell viability was determined using the Cell titre blue assay. Cell viability is expressed as the percent of fluorescence signal from treated cells compared to controls, Bars represent the average values ± S.D. of 3 independent experiments with 5 biological replicates for each experiment (n=15).
C: Effect of inhibition of *Hmgcs1*, *Hmgcr*, *Sqle*, *Fdft1* and *Dhcr7* mCMV replication. After 48 hrs, media was removed and cells infected for 1 hr with mCMV-GFP virus at a MOI of 0.2 and the GFP signal was recorded for 100 hrs. At 76 hrs, the level of non-targeting siRNA treated cells was used as a baseline estimate for the cut off point (using two standard deviations and a p-value < 0.001). Bars represent average values ± S.D. of 3 independent experiments with 5 biological replicates for each experiment (n=15).
A Welsh t-test was used for the evaluation of statistical significance: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

5.2.7 Contribution of prenylation in the Simvastatin antiviral effect

Isoprenoids are at the branch point of the synthesis of several downstream metabolites, such as cholesterol and prenylpyrophosphate (farnsesyl pyorphospahte and geranygeranylpyrophosphate), as well as dolichol, a precursor of Heme A and ubiquinone (Figure 5-1).

In order to identify which one of the branches of the mevalonate pathway was responsible for the antiviral effect induced by simvastatin on mCMV growth, supplementation experiments were performed with mevalonate pathway intermediates. 300 μM mevalonate, 15μM of geranylgeraniol, 15 μM of farnesol, 15 μM of squalene or 5 μg/ml of water soluble cholesterol were added to the culture media of mCMV-GFP-infected and simvastatin treated cells for 72 hrs (Figure 5-3C). The metabolite doses were chosen by taking the highest concentration of each metabolite which had no significant effect on the cell viability of the NIH/3T3 fibroblasts over a period of 72 hrs (data not shown).

Furthermore, addition of mevalonate and geranylgeraniol completely rescued the decrease of infectivity induced by simvastatin treatment, while addition of farnesol, squalene and water-soluble cholesterol did not rescue the mCMV infectivity (Figure 5-4).

Taken together these results indicate that the alteration of geranylgeranilation is responsible for the antiviral properties of simvastatin in mCMV infection.

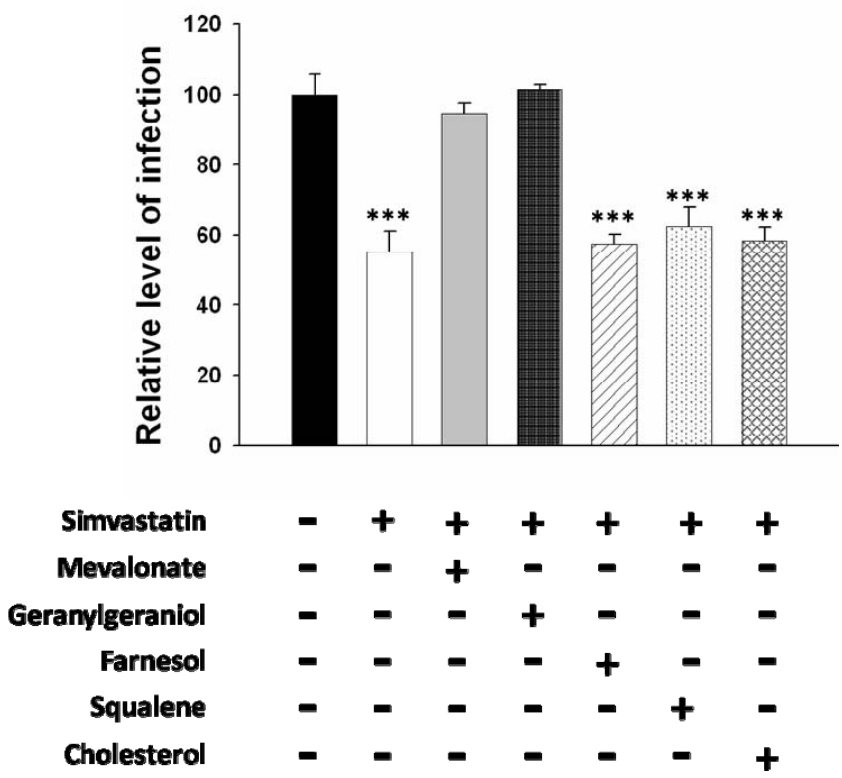


Figure 5-4: Effects of mevalonate pathway intermediates on the level of infection of mCMV in

Simvastatin treated NIH/3T3 fibroblast cells.

300 µM mevalonate, 15µM of geranylgeraniol, 15 µM of farnesol, 15 µM of squalene or 5 µg/ml of water soluble cholesterol (See section 2.3) were added to the culture media of mCMV-GFP infected and simvastatin treated NIH/3T3 fibroblast cells for 72 hrs and GFP fluorescence was recorded with a spectrophotometer. Results represent the relative level of infection compared to the untreated cells. The level of infection was determined by measuring GFP fluorescence at 72 hrs (See section 2.8). Graphs represent average values ± S.D. of 3 independent experiments with 5 replicates for each experiment (n=15). Statistical significance was used for the evaluation by a Welsh t-test: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

5.3 Discussion

The aim of this chapter was to investigate whether the cellular modulation of the sterol synthesis pathway altered mCMV infection. To answer that question, we applied a combined approach using pharmacological inhibition and gene expression knock-down to modulate the sterol pathway and monitor effects on mCMV infection.

Pharmacological inhibition of the sterol biosynthesis pathway with simvastatin decreased the amount of mCMV replication in a dose dependent manner (IC_{50} = 2μM). This effect was not due to an alteration of cell growth due to simvastatin treatment, since cell viability was not affected at the dose having an antiviral effect. Furthermore, we have shown that inhibition of mCMV replication by simvastatin was specifically due to the alteration of the mevalonate pathway since addition of mevalonate in simvastatin treated cells restored the infection to a level comparable to the non-treated cells. This is the first time that the involvement of sterol biosynthesis in the regulation of mCMV infection has been demonstrated. These results are comparable to the inhibition of hCMV replication by fluvastatin in human endothelial cells, as demonstrated by Potena and colleagues (Potena, Frascaroli *et al.* 2004). In this study, the expression of immediate early genes is reduced by fluvastatin treatment, suggesting that modulation of sterol synthesis alters viral gene expression. We can speculate that the mechanisms of inhibition of mCMV replication by simvastatin are similar. Nevertheless, further studies will be require to understand the mechanisms driving the antiviral effect of simvastatin on mCMV replication and especially whether simvastatin alters the gene expression of mCMV or perturbs the assembly and budding of the viral particle. Since simvastatin treatment induced cell death of BMDMs even at a very low concentration, combined with the difficulty of transfecting primary macrophages, we could not study the effect of the inhibition of the sterol pathway on mCMV infection in the macrophage model.

As a result we cannot exclude that the inhibition of sterol synthesis in BMDMs will not have the same effect on mCMV infection as that in 3T3 cells.

In this chapter we have also demonstrated that simvastatin treatment of Balb/C mice *in vivo* significantly reduced (one log scale) the level of mCMV infection in the liver, spleen, lung, kidney and heart after 4 days of infection compared to untreated mice. To our knowledge, this is the first time that the antiviral effect of statins on herpes virus replication has been demonstrated *in vivo*. However, these results do not prove a direct correlation between down regulation of the sterol biosynthesis pathway and the reduction of mCMV infection. Indeed, simvastatin has been shown to have various effects not linked to the inhibition of cholesterol synthesis. In particular, statins have been shown to modulate the adaptive immune response which is crucial in the control of mCMV infection. In order to directly correlate the antiviral effect of statins *in vivo* to the inhibition of the sterol biosynthesis pathway, the antiviral effects of statin treatment supplemented with mevalonate or the use of drugs targeting downstream proteins of the mevalonate pathway will be required *in vivo*. Although it is still controversial, several studies have shown that statin treatment could be used efficiently as an antiviral agent against HCV and HIV in patients. Our results suggest that statins could also be potentially used as a therapeutic treatment against acute cytomegalovirus or herpes virus infection in patients. However, the dose of simvastatin used in the *in vivo* experiment (50 mg per day per kilo) is 50 to 100 fold higher than the doses prescribed to patients. Since there are more than nine different statins available, each with different properties and inhibitory effects, it will be of interest to study the antiviral effect of each statin (IC_{50} and specificity) on mCMV replication to reduce the dose required for an antiviral effect.

We also confirmed with SiRNA gene knock down that reduction of the gene expression of *Hmgcr* and *Hmgcs1* reduced viral replication. To our knowledge this is the first time that transcriptional down regulation of sterol synthesis genes have been shown to have an antiviral effect.

In order to understand if sterol biosynthesis or the isoprenoid alternative pathway (prenylation, dolichol, heme A) were responsible for the antiviral effect we also knocked down the expression of *Fdft1*, *Sqle* and *Dhcr7*, three genes positioned downstream of the isporenoid branch points of the pathway and specifically involved in cholesterol synthesis. Surprisingly, the knock down of these three genes

significantly increased viral replication. Pharmacological inhibition of squalene synthase (*Fdft1*) with zaragocid acid has been shown to induce the accumulation of upstream intermediates and enzyme gene expression (Bergstrom, Kurtz *et al.* 1993; Vaidya, Bostedor *et al.* 1998), therefore we can speculate that the knock down of *Fdft1* may be beneficial for the virus by increasing upstream metabolites or gene expression involved in mCMV replication. In addition, we have demonstrated that addition of geranylgeranyol, but not farnesol or squalene and cholesterol, could restore the infectivity of mCMV infection in simvastatin treated cells. Further experiments are required to confirm the direct involvement of geranylgeranylation in mCMV replication. For example, knock down of the gene expression of the enzymes responsible for prenylation or the use of chemical inhibitors of prenylation (FTIs and GGTIs) will confirm that the inhibition of geranylgeranylation is responsible for the inhibition of mCMV replication. Nevertheless, taken together, these results provide evidence for the first time that inhibition of geranylgeranylation, rather than the synthesis of cholesterol itself, is responsible for the inhibition of mCMV replication in NIH/3T3 cells. Since the use of statins as an antiviral agent may be problematic because of their pleitotropic effects and the high dose required, our results suggest that the use of chemical inhibitors of geranylgeranylation may be more appropriate as an antiviral agent. Further investigation should look at the antiviral effect of geranylgeranyl transferase inhibitors (GGTIs) in herpes virus infection, *in vitro* and *in vivo*.

The mechanisms involved in the modulation of viral infection by the prenylation of proteins remains unclear. Geranylgeranylation has been previously shown to regulate the replication of other viruses such as HCV, HDV, EBV (Bordier, Ohkanda *et al.* 2003; Einav and Glenn 2003; Glenn 2006). Some viruses have been shown to require viral protein prenylation (HDV) (Glenn 2006), while other viruses require a host protein geranylgeranylation (HCV) (Wang, Gale *et al.* 2005). Further work is required to understand the mechanisms involved in the modulation of mCMV infection by geranylgeranylation and especially if a specific host protein from the Rho, Rac or CD32 small G-protein family requires geranylgeranylation for efficient replication of the virus. We can also not exclude that a viral protein requires direct geranylgeranylation for efficient replication of the virus.

In conclusion, we have shown that inhibition of the sterol pathway at the transcriptional and protein level reduces viral replication. These results suggest that regulation of sterol biosynthesis upon mCMV infection contribute to the establishment of an antiviral state.

6 Chapter 6: Type I interferon receptor regulates the sterol biosynthesis pathway

6.1 Introduction

In the previous chapters, we provided evidence that the down regulation of the sterol biosynthesis pathway is a general cellular response to viral infection, and is independent of viral gene expression (see chapters 3 and 4). We then demonstrated that transcriptional and protein inhibition of the sterol biosynthesis pathway inhibits viral replication (see chapter 5).

Altogether, these observations led us to hypothesise that the modulation of the sterol synthesis pathway in response to infection is part of a host innate immune response.

Upon infection, the innate immune response takes place in two distinct phases. The first is initiated by the detection of viral pathogen associated molecular patterns (PAMPs) by host pattern recognition receptors (PRRs), such as TLRs. Activation of these receptors lead to activation of transcription factors that activate the primary antiviral response. Some of the transcription factors activated, such as IRF3 or IRF7, will then rapidly induce the secretion of inflammatory cytokines and chemokines. When secreted, these molecules bind to specific cellular receptors, and via an autocrine loop, induce a cascade of signalling events leading to the secondary immune response (see the introduction chapter). In macrophages and NIH/3T3 cells, Interleukins (IL-1β, Il6), IFNα and β, as well as Tumour necrosis factor alpha (TNFα), are secreted rapidly in response to infection (see the introduction chapter).

The aim of this chapter is to understand whether the down regulation of sterol biosynthesis is part of the innate immune response triggered by infection, and to identify the mediators involved in this response.

6.2 Results

6.2.1 Effect of conditioned media on the regulation of the sterol biosynthesis pathway

To test whether immune factors secreted in response to infection were capable of regulating the sterol biosynthesis pathway, we thought of using the media of infected cells that contain inflammatory cytokines, but no viral particles, to treat uninfected BMDMs.

Conditioned media from 8 hrs infected BMDMs at a MOI of 1, or fresh media, were immediately added to uninfected BMDMs. The absence of any viral particle in the conditioned media was tested by plaque assay (Figure 6-1A). After 24 hrs incubation, macrophage RNA was extracted and the expression of *Hmgcs1*, *Hmgcr* and *Sqle* was measured by QPCR. Results (Figure 6-1B) show that each of the three genes are down regulated significantly by treatment with conditioned media compared to the mock treated macrophages. Fold change for *Hmgcs1*: -2.56, *Hmgcr*: -1.72 and *Sqle* : -2.32.

These results indicate that after viral infection, one or several factors secreted in response to infection are responsible for the down regulation of genes associated with cholesterol synthesis.

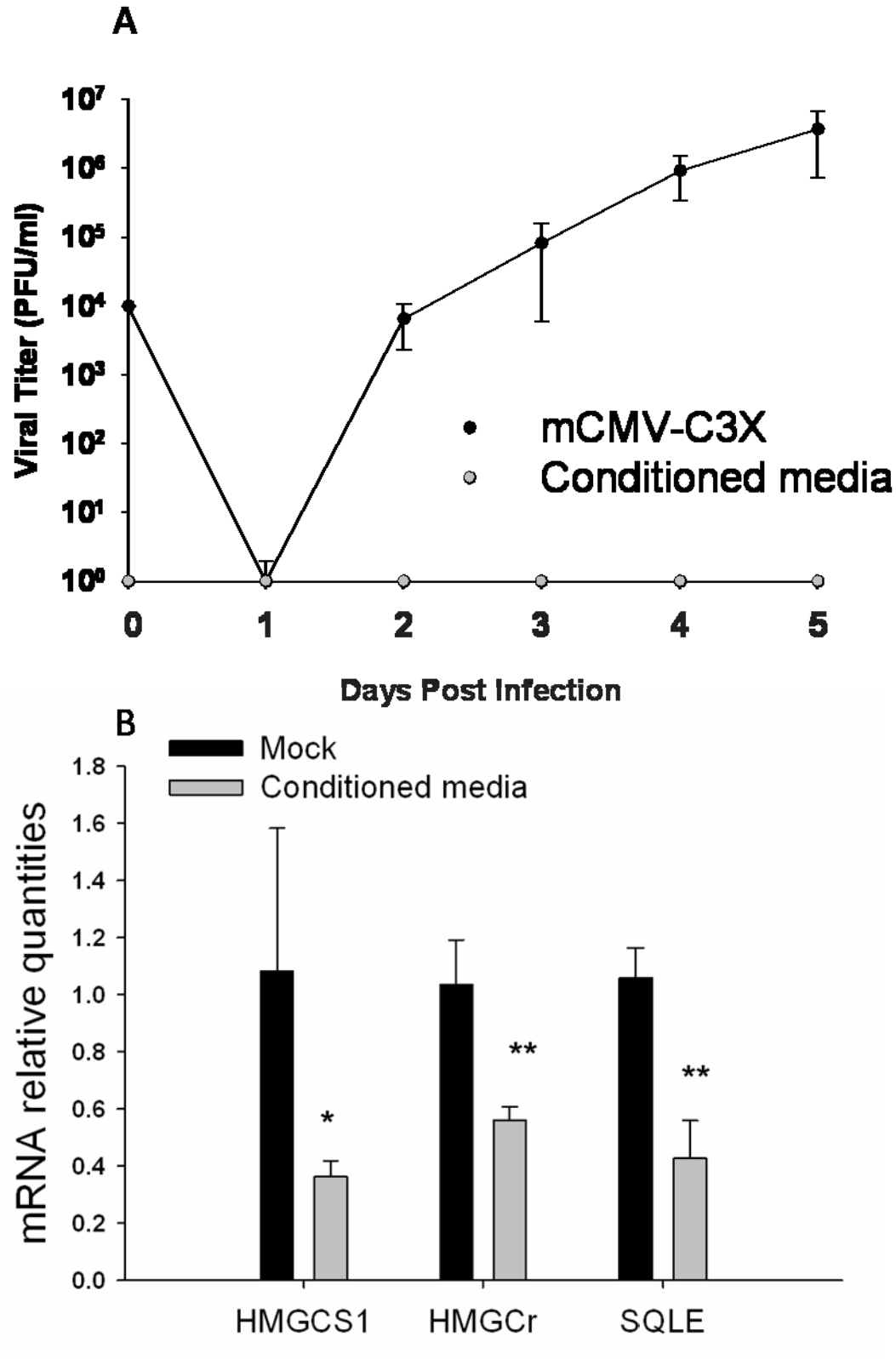

A
Viral Titer (PFU/ml)
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
1
2
3
4
5
Days Post Infection
mCMV-C3X
Conditioned media
B
Mock
Conditioned media
mRNA relative quantities
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
**
**
HMGCS1
HMGCr
SQLE

Figure 6-1: Effect of conditioned media on the regulation of the sterol biosynthesis pathway

BMDMs were infected with mCMV at a MOI of 1, or mock treated. Supernatant was collected after 8 hrs and added to fresh BMDMs.

A: NIH/3T3 cells were treated with conditioned media, or as a control, infected at a MOI of 0.1 with mCMV-C3X. At the indicated time points after infection (dpi) supernatants from the infected cultures were harvested, and titres were determined by standard plaque assays on MEF p53 -/- cells. Each data point represents the average and standard deviation from three independent wells (n=3).

B: After 24 hrs, RNA was collected and *Hmgcs1*, *Hmgcr* and *Sqle* expression was measured by QPCR. The bars represent the relative amount of RNA compared to the untreated samples. Results represent average ± S.D. of two experiments with triplicate assays (n=6).

Statistical significance was tested using a Welsh t-test: *P < 0.05, **P < 0.01, ***P < 0.001).

6.2.2 Effect of IFNγ, IFNβ, IL1β, IL6 and TNFα treatment on the regulation of the sterol biosynthesis pathway and on free cellular cholesterol levels

Next, we thought of testing if antiviral cytokines could modulate sterol biosynthesis. For that reason BMDMs were treated with fresh media or fresh media containing 10 and 100 units of TNFα and IFNγ, or 10 and 25 units of IFNβ, IL1β, and IL6. BMDMs were also infected with mCMV at a MOI of 1 as a positive control. After 24 hrs incubation, macrophage RNA was extracted and the level of *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* expression was measured by QPCR. Results (Figure 6-2A-E) demonstrate that during mCMV infection, IFNγ and IFNβ treatments significantly down regulated *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* gene expression, and treatment with IL1β, IL6 or TNFα did not affect the expression of these genes. Furthermore, the amplitudes of the decrease in gene expression induced by IFNβ and IFNγ are comparable to those induced by mCMV infection. In addition, the down regulation of the markers of sterol biosynthesis is more pronounced with increasing doses of IFNβ and IFNγ, which might suggest that there is a dose effect in its regulation.

We then measured the effect of cytokine treatment on the levels of free intracellular cholesterol concentration. BMDMs were treated with the same concentration of IFNγ, IFNβ, IL1β, IL6 or TNFα as in the previous experiment for 48 hrs, and the free cholesterol concentration was measured as previously described (see section 2.6).

Results (**Figure 6-3**) show that treatment with IFNβ decreases the amount of free intracellular cholesterol by 40 to 50% after 48 hrs. Inversely, IL1β, IL6 or TNFα treatment did not induce any changes in the free cholesterol concentration of BMDMs. Taken together, these results indicate that among the inflammatory cytokines inhibiting mCMV infection, IFNβ and IFNγ are key players in the regulation of sterol gene expression.

Since IFNβ is secreted in response to infection, these results, and the fact that IFN treatment and the cell response to infection are equally capable of causing a down-regulation of the sterol metabolic pathway, raises the question of whether infection mediated regulation might result from an IFN regulatory loop.

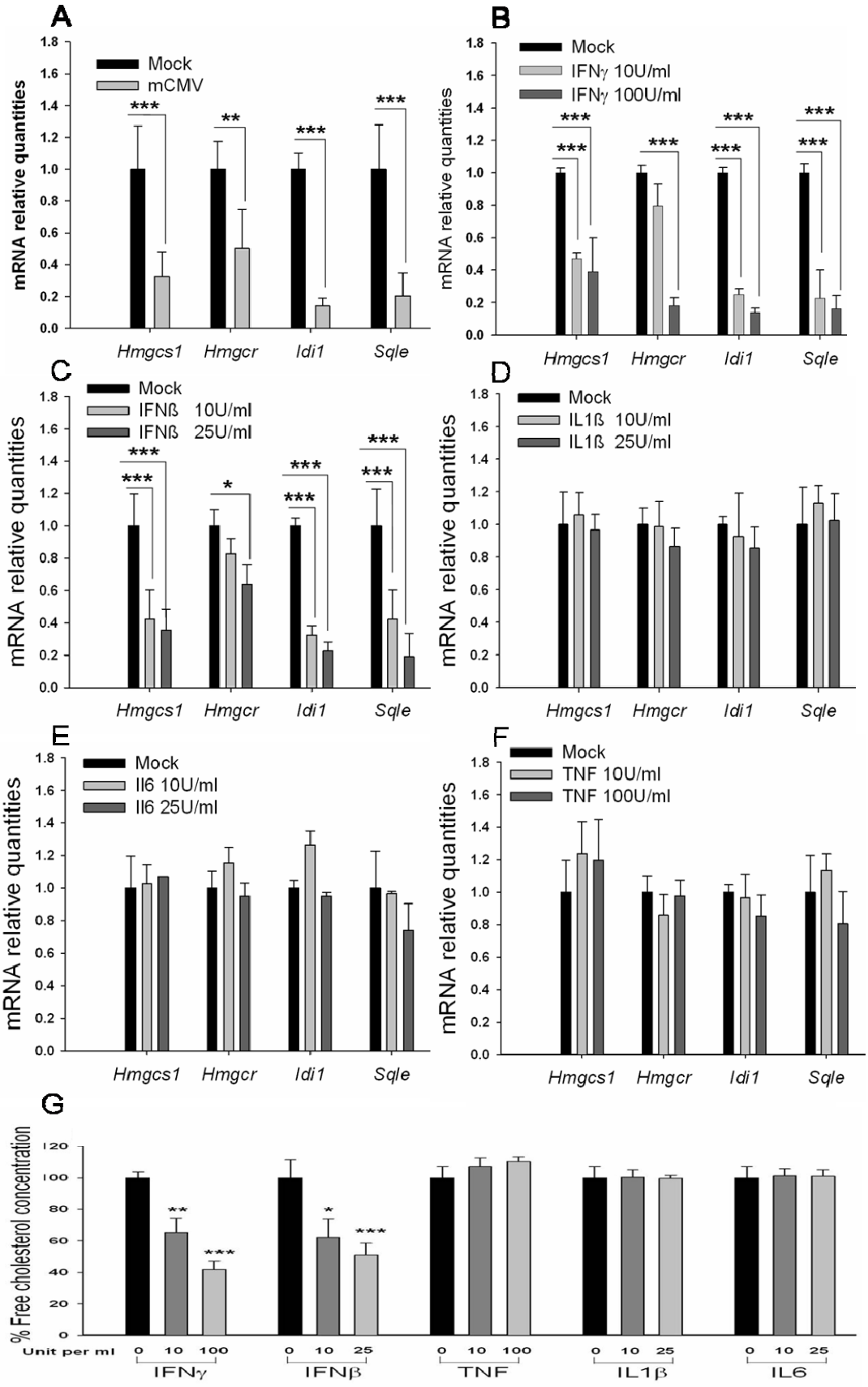

A
Mock
mCMV
mRNA relative quantities
Hmgcs1
Hmgcr
Idi1
Sqle
B
Mock
IFNγ 10U/ml
IFNγ 100U/ml
C
Mock
IFNß 10U/ml
IFNß 25U/ml
D
Mock
IL1ß 10U/ml
IL1ß 25U/ml
E
Mock
Il6 10U/ml
Il6 25U/ml
F
Mock
TNF 10U/ml
TNF 100U/ml
G
% Free cholesterol concentration
Unit per ml
IFNγ
IFNβ
TNF
IL1β
IL6

Figure 6-2: Effect of inflammatory cytokines on BMDM sterol biosynthesis gene expression and cellular free cholesterol levels

A- D: BMDMs were treated with fresh media or fresh media containing 10 and 100 units of TNFα and IFNγ, or 10 and 25 units of IFNβ, IL1β, and IL6. BMDMs were also infected with mCMV at a MOI of 1 as a positive control. After 24 hrs incubation, macrophage RNA was extracted and the level of *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* gene expression was measured by QPCR. Bars represent average values ± S.D. of five independent experiments with triplicate measurements for each experiment (n=15).

E: BMDMs were treated with the same concentration of IFNγ, IFNβ, IL1β, IL6 or TNFα as in the previous experiment for 48 hrs, and the free cholesterol concentration was measured as previously described (see section 2.6). Results represent average ± S.D. of two independent experiments with quadruplicate assays for each experiment (n=8).

Statistical significance in a Welsh t-test: *P < 0.05, **P < 0.01, ***P < 0.001.

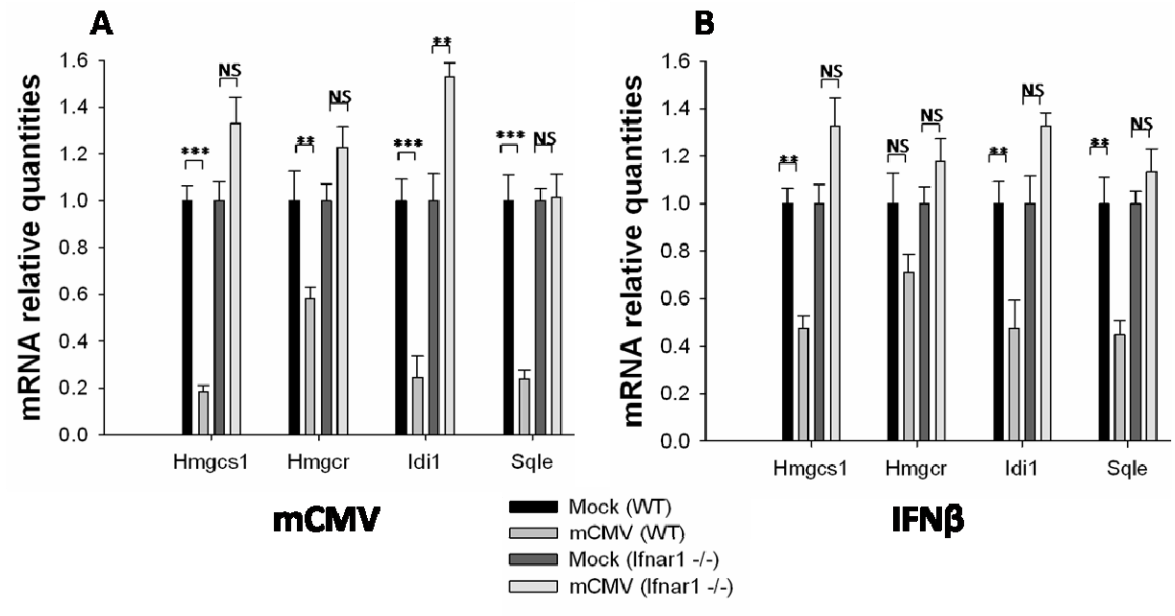
6.2.3 Type I receptor knockout mice abolish transcriptional and metabolic regulation of cellular free cholesterol by mCMV infection and IFNβ.

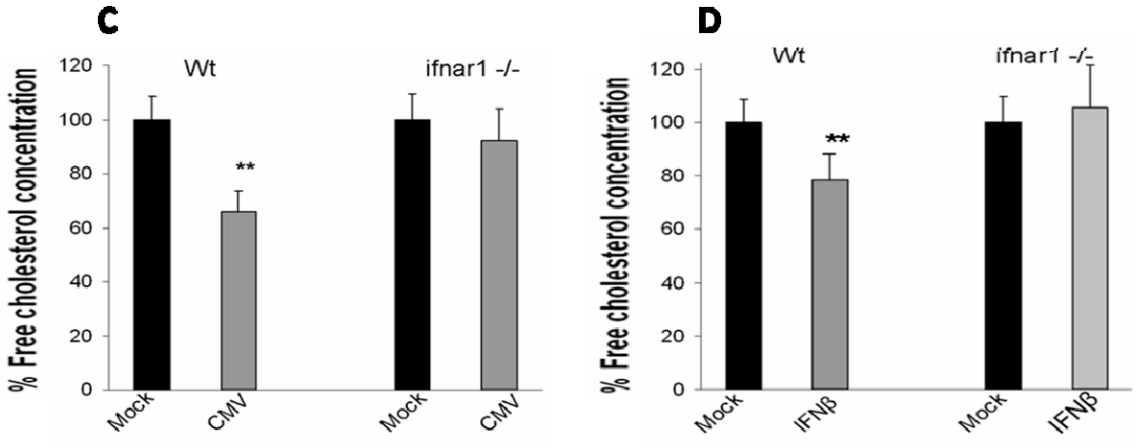
To directly test the hypothesis that down regulation of the sterol synthesis pathway is occurring through a type I interferon autocrine loop, BMDMs cultures from IFNAR1-/- mice lacking sub-unit 1 of the type I receptor were infected with mCMV, or treated with 10 Units per ml of IFNβ. After 24 hrs, the gene expression of *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle*, as well as the level of free cellular cholesterol, were measured. As shown in **Figure 6-3A**-B, in the WT backgrounds, mCMV infection and IFNβ treatment down regulates *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* expression after 24 hrs, as observed previously. Fold changes for mCMV infection: *Hmgcs1*: -5.2, *Hmgcr*: -1.64, *Idi1*: - 4.2 and *Sqle*: -4.54, and for IFNβ treatment: *Hmgcs1*: -2.28, *Hmgcr*: not significant, *Idi1*: - 2.39 and *Sqle*: -2.63. However, the lack of an IFN type I receptor abolished the ability of BMDMs to reduce sterol biosynthesis gene expression upon either infection with mCMV or treatment with IFNβ. Additionally, we measured the level of free cellular cholesterol in WT and IFNAR1 -/- cells treated with the same conditions as the previous experiment. As a result, mCMV infection and IFNβ treatment significantly decreased the level of free cellular cholesterol in WT BMDMs (35 % and 22 % respectively), but not in IFNAR1-/- BMDMs (**Figure 6-3C**-D).

We concluded from these experiments that a type I IFN-dependent innate immune response stringently regulates the transcriptional and metabolic alterations of the sterol biosynthesis pathway and overall free cellular cholesterol levels.

We then wondered if the abolition of the reduction in gene expression of the sterol biosynthesis pathway was specific to IFNβ secretion, since IFNα has also been shown to be produced upon infection (Dalod, Hamilton *et al.* 2003). BMDMs derived from IFNβ gene knockout mice were challenged with mCMV infection for 24 hrs and the sterol biosynthesis gene expression analyzed. As shown in **Figure 6-3E**, the deletion of IFNβ genes only partially, but significantly, abolished the down regulation of the sterol pathway induced by mCMV infection. However, due to time constraints, this experiment could only been done once, limiting our confidence in the validity of these results. Nevertheless, if these results are confirmed, it will

indicate that IFNβ is not the only factor responsible for the down regulation of the sterol pathway through a type I response.


A
B
mRNA relative quantities
Hmgcs1
Hmgcr
Idi1
Sqle
mCMV
IFNβ
Mock (WT)
mCMV (WT)
Mock (Ifnar1 -/-)
mCMV (Ifnar1 -/-)


C
D
% Free cholesterol concentration
Wt
ifnar1 -/-
Mock
CMV
IFNβ

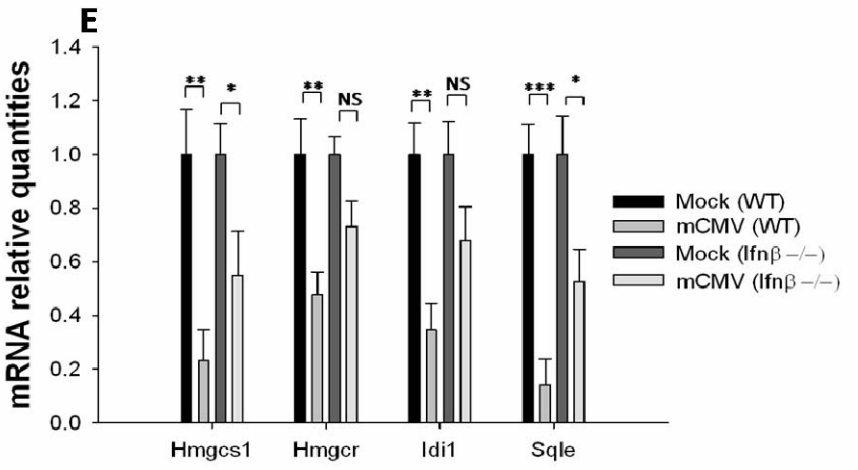

E
mRNA relative quantities
Hmgcs1
Hmgcr
Idi1
Sqle
Mock (WT)
mCMV (WT)
Mock (Ifnβ −/−)
mCMV (Ifnβ −/−)

Figure 6-3: Type I receptor knockout mice abolish transcriptional and metabolic regulation of cellular free cholesterol by mCMV infection and IFNβ

A-B: Wild type BMDMs or BMDMs from IFNAR1-/- mice were mock treated, or infected with mCMV at a MOI of 1 or treated with 10 units of IFNβ. After 24 hrs, RNA was collected and the gene expression of *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* measured by QPCR. Results represent the average values ± S.D. of 2 independent experiments with three biological replicates for each experiment (n=6).

C-D: Wild type or IFNAR1-/- BMDMs were infected with mCMV at a MOI of 1 (left panel) or treated with 10U/ml of IFNβ (right panel). After 48 hrs, free cholesterol concentration was measured (see section 2.6). Results represent the average values ± S.D of 2 independent experiments with three biological replicates for each one (n=6).

E: Wild type or IFNβ -/- BMDMs were mock treated or infected with mCMV at a MOI of 1.

After 24 hrs, RNA was collected and the gene expression of *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* was measured by QPCR. Results represent the average values ± S.D. of one experiment with a total n of four.

Statistical significance in a Welsh t-test: *P < 0.05, **P < 0.01, ***P < 0.001).

6.2.4 IFNγ regulation of the sterol biosynthesis pathway

As demonstrated in section 6.1.1, IFNγ treatment also induces the down regulation of sterol pathway-related gene expression. This raised the question of the involvement of type II interferon in the regulation of sterol biosynthesis. Mechanistically, one possibility is that IFNγ binds to a type II receptor and consequently triggers the initiation of a signalling cascade leading to the down regulation of the sterol pathway. Alternatively, IFNγ could lead to the secretion of IFNβ, which will then bind to its receptor and induce the down regulation of sterol biosynthesis.

To test whether IFNγ treatment of BMDMs induces a type–I independent regulation of the sterol pathway, IFNAR1 knock out or wild type macrophages were treated with 10 U per ml of IFNγ for 24 hrs, and the gene expression of *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* were compared. Results (Figure 6-4) demonstrate that IFNγ treatment of BMDMs results in the down regulation of four gene markers of sterol biosynthesis in WT and IFNAR1 -/- KO BMDMs. Fold changes in WT BMDMs: *Hmgcs1*: -4.4, *Hmgcr*: - 1.7, *Idi1*: -5.2 and *Sqle*:-7.7. Fold changes in IFNAR1 -/- BMDMs: *Hmgcs1*: -5.3, *Hmgcr*:-1.9, *Idi1*: -5.7 and *Sqle*: -4.8. These results clearly show that IFNγ down regulation of sterol synthesis is independent of IFNAR activation

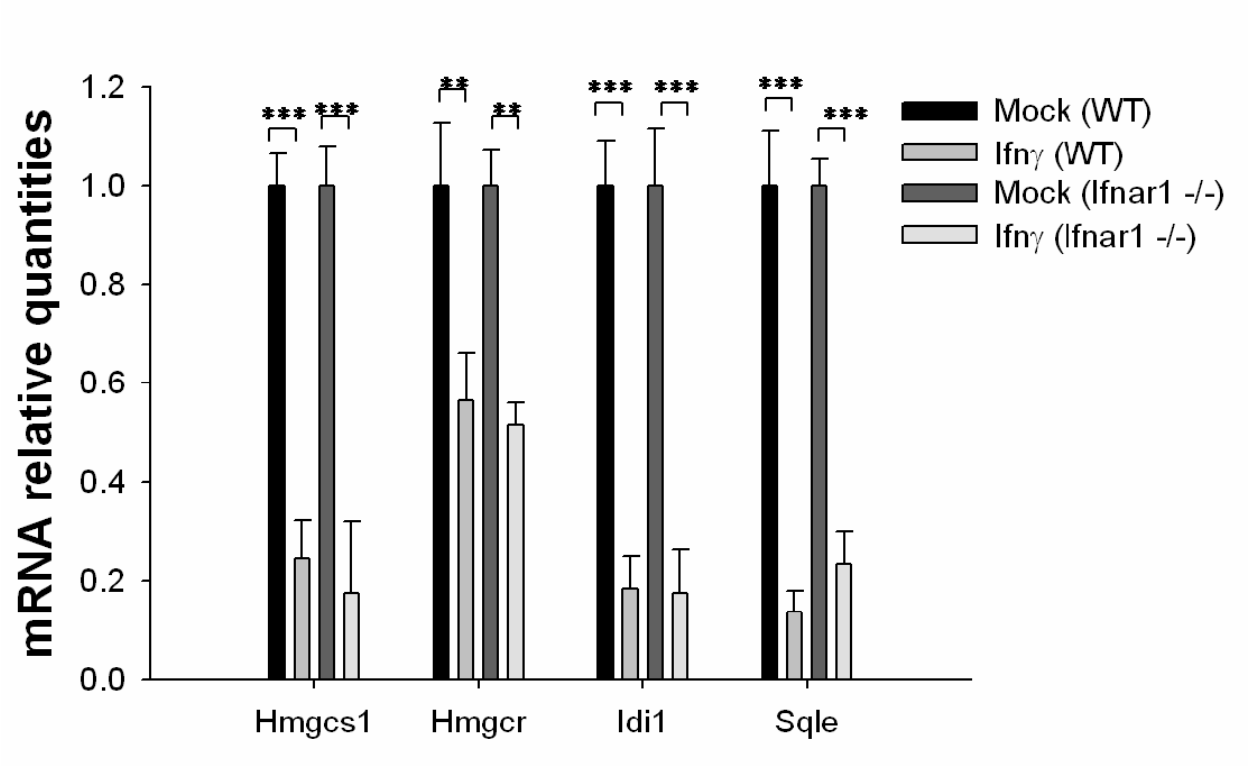


Figure 6-4: Regulation of sterol biosynthesis by IFNγ treatment

Wild type BMDMs or BMDMs from IFNAR1-/- mice were mock treated, or infected with mCMV at a MOI of 1 or treated with 10 units of IFNγ. After 24 hrs, RNA was collected and the gene expression of *Hmgcs1*, *Hmgcr*, *Idi1* and *Sqle* measured by QPCR. Results represent the average values ± S.D. of 2 independent experiments with three biological replicates for each experiments (n=6). Statistical significance in a Welsh t-test: *P < 0.05, **P < 0.01, ***P < 0.001).

6.3 Discussion

In the previous chapters, we have shown that down regulation of sterol biosynthesis is a general response to infection. We also provided evidence that this regulation occurs rapidly after the first contact with the virus, and does not require viral gene expression. In addition, we have demonstrated that transcriptional or protein inhibition of sterol biosynthesis reduces mCMV replication.

This led us to hypothesise that the regulation of the sterol pathway in response to infection is triggered by an innate immune mechanism.

In this study we have shown that the regulation of the sterol cholesterol pathway was dependent on factors secreted in response to infection. This evidence strongly suggests that the regulation of the sterol pathway in response to infection is part of a second autocrine signalling cascade. However, we could not exclude that the primary activation had a direct effect on the regulation of sterol synthesis. Consequently, we aimed to identify the effectors secreted in response to infection which were responsible for the regulation of the sterol pathway. Transcriptional and metabolic screening of the effect of five inflammatory cytokines and chemokines known to induce an antiviral state showed that IFNβ and IFNγ treatment, but not IL6, IL1β or TNFα, down regulated sterol biosynthesis gene expression, and decreased levels of free cellular cholesterol. These results raised the hypothesis for an IFN regulatory loop mechanism that is responsible for modulating sterol biosynthesis. However, we cannot exclude that other secreted factors in response to infection may also modulate the sterol biosynthesis pathway, or that the doses of cytokines and chemokines used for this experiment were not sufficient. Future experiments should characterise and test the effect of other inflammatory cytokines and chemokines. In addition, the use of neutralising antibodies against IFNs in the media of the treated cells should indicate if IFNs are the only effectors of the down regulation of the sterol pathway.

We next investigated whether the sterol response to infection is dependent on the type I IFN receptor and the expression of IFNβ using genetic deficient mice. The lack of IFN type I receptor completely abolished the ability of BMDMs to reduce both sterol biosynthesis gene expression and cholesterol yield upon either infection with mCMV or treatment with IFNβ. However, the reduction of sterol gene expression was only partial in mice lacking the IFNβ gene.

We conclude from these experiments that a type I IFN-dependent innate immune response stringently regulates the metabolic alteration of the sterol biosynthesis network observed upon infection. However, since IFNβ is not the only secreted factor responsible for this regulation, these results suggest that other type I IFN members may compensate for the lack of IFNβ. Further studies will be required to understand the role of type I IFN in the regulation of sterol biosynthesis.

Nevertheless, to our knowledge, this is the first time that the regulation of sterol biosynthesis in response to infection, or direct IFN treatment, has been shown to be type I signalling dependent.

In this chapter, we also demonstrated that type II interferon treatment of BMDMs induced the down regulation of sterol synthesis independently of the type I regulatory response. Since we could not obtain type II receptor knockout mice we could not formally prove that IFNγ regulation of sterol synthesis is the consequence of a direct type II signalling cascade.

To date, cellular metabolic, signalling and regulatory pathways causally linked with immunity have been argued to play a critical role in modulating infection (Diamond, Syder *et al.*). In this regard, toll-like receptors, the central pathogen recognition receptor systems of innate immune signalling, have recently been shown upon bacterial infection to regulate the expression of key lipid-associated genes through IRF3 and the nuclear receptor LXRα. In this study, microbial ligand activation of the IRF3 pathway blocked the induction of LXR target genes, such as *ABCA1*, and inhibited cholesterol efflux from macrophages (Castrillo, Joseph *et al.* 2003). Additionally, knockout mice deficient for LXRα are more susceptible to bacterial infection (Joseph, Bradley *et al.* 2004), suggesting the importance of this pathway in the innate immune response. However, Castrillo and colleagues demonstrated that the regulation of the *ABCA1* gene by the innate immune response through the IRF3-LXRα pathway is independent of the type I signalling response.

Our results complement these studies by revealing an unknown mechanism of cross talk between innate immunity and lipid regulation through a type I response. The involvement of TLR and IRF signalling in the regulation of sterol biosynthesis is still undefined. Since IRF3 has been involved in the transcription of type I interferon genes, we can speculate that it is its activation which leads to sterol synthesis through

a type I signalling cascade. Use of IRF3 deficient macrophages will be required to test this hypothesis.

In addition, the mechanisms of regulation of sterol biosynthesis by the activation of interferon signalling are not yet fully understood. In particular, the involvement of JAK1, TYK2, STAT1 and STAT2 in the regulation of sterol synthesis is still to be addressed. Since both IFNβ and IFNγ induce the down regulation of sterol biosynthesis, we can speculate that STAT1 is involved in the regulation, as activation of both signalling cascades induces the activation of STAT1. However, this hypothesis raises the question of the direct or indirect action of STATs on the regulation of sterol biosynthesis gene expression. Indeed, when activated the STAT proteins migrate to the nucleus, where they bind to promoter elements on the DNA molecule, leading to the expression of target genes. This indicates that IFNAR/STAT regulation of sterol gene synthesis occurs through the active expression of a repressor of sterol biosynthesis. Since we have shown that the free cellular cholesterol levels are decreased in response to infection, the involvement of a negative feedback mechanism in consequence of high cholesterol concentration have to be excluded. Several hypotheses can be proposed to explain the mechanism of this regulation. Firstly, IFNAR activation may induce the regulation of nuclear factors such as PPARs, LXRs FXR. These nuclear factors may in turn down regulate sterol biosynthesis. However, the direct down regulation of *Srebf2* and the sterol biosynthesis genes by activation of nuclear factors has been shown, yet also there is some evidence linking the regulation of *Srebf1* by PPARγ and LXRα in the literature (Schultz, Tu *et al.* 2000; Davidson 2006; Kadegowda, Bionaz *et al.* 2009; Kim, Kim *et al.* 2009).

Another possible mechanism of the regulation of sterol biosynthesis by a IFN/IFNAR dependent pathway, is the involvement of oxysterols as a repressor of the sterol biosynthesis genes. In particular, 25-hydroxycholesterol has been shown to directly interact with SCAP and INSIG proteins to block the cleavage of SREBP2, leading to the down regulation of the sterol biosynthesis genes (Adams, Reitz *et al.* 2004). Given that mCMV infection and IFNβ and IFNγ treatment results in the coordinate transcriptional regulation of sterol biosynthesis, we can speculate that *Srebf2* is involved in this process, as well as being IFNAR dependent, although

further experiments are required to test this hypothesis. Recently, 25-hydroxycholesterol secretion by macrophages has been shown to be dependent on TLR activation (Bauman, Bitmansour *et al.* 2009; Diczfalusy, Olofsson *et al.* 2009). The expression of cholesterol 25-hydroxylase (*CH25H*) catalyses the production of *25-hydroxycholesterol* from cholesterol, and we have seen that mCMV infection and IFNγ treatment increases the expression of *CH25H* at a very early time post-treatment and infection. Furthermore, this up regulation is abolished in IFNAR -/- macrophage cells infected with mCMV or treated with IFNβ and IFNγ (data not shown- unpublished data). We can speculate that the activation of a type I IFN signalling cascade results in the activation of the expression of *CH25H* and consequently regulates sterol biosynthesis gene expression through the inhibition of SREBP2 cleavage. On the other hand, a few studies have shown that STAT proteins are capable of directly inhibiting gene transcription (Foley, Ofori-Acquah *et al.* 2002; Ma, Chang *et al.* 2005), and we cannot exclude a direct negative regulation by STATs on sterol-associated genes and *Srebf2* gene expression.

Moreover, type I interferon activation also interacts with other signalling pathways which may also be involved in the regulation of sterol synthesis. JAK/STAT phosphorylation leads to the activation of the Ras receptor tyrosine kinase (RTK) and mitogen-activated protein kinase (MAPK) pathways, which result in the activation of the phosphoinositide 3-kinase (PI3K) pathway. Recently, activation of PI3K/AKT/mTORc1/AMPK pathway has been shown to regulate the expression of *Srebf1* (Porstmann, Santos *et al.* 2008). This mechanism could also explain the regulation of sterol synthesis by IFN signalling activation.

Finally, a plethora of studies has reported an interplay between the inflammatory response and cholesterol metabolism. For example, it is well documented that the acute phase response induces a rapid decrease of total cholesterol serum levels (Khovidhunkit, Kim *et al.* 2004), and IFNβ treatment has also been shown to decrease cholesterol plasma levels in multiple sclerosis patients (Morra, Coppola *et al.* 2004). However, the mechanisms involved in this process are not fully understood. The results of our study may provide mechanisms to explain this clinical observation. It will be of interest to study the correlation between the type I interferon response and the rapid drop of cholesterol following an acute event.

However, this study did not address certain important questions; firstly, we did not define whether the same regulatory mechanisms occur in other cell types and species. Nevertheless, in view of the fact that the IFN signalling pathway is ubiquitously conserved among vertebrates (Rawlings, Rosler *et al.* 2004), we can speculate that it is the case, but further experiments need to be conducted to test this hypothesis. Secondly, we did not study the dependency to type I signalling of the regulation of the mechanisms regulating cholesterol homeostasis, such as efflux, uptake and storage. Castrillo and colleagues have demonstrated that cholesterol efflux is dependent on IRF3/LXRα activation in an IFNAR independent manner. These results need to be validated in our model and the role of IFN signalling in the regulation of cholesterol efflux, uptake and storage needs to be addressed (Castrillo, Joseph *et al.* 2003).

7 Chapter 7: Discussion

7.1 Rational of the study

Cholesterol metabolism has been shown to be involved in every step of the virus life cycle (see section 1.4.2.2). Indeed, cholesterol has been shown to be structurally important for viral entry, assembly and budding. Additionally, pharmacological inhibition of the *de novo* cholesterol synthesis pathway using statins has also been shown to result in a reduction of viral infection *in vitro* and *in vivo*, highlighting the importance of this process for viral replication. Although the mechanisms are still unclear, it appears that the synthesis of specific isoprenyl lipids, but not of *de novo* cholesterol, is involved in this process. For example, HCV infection requires the geranylgeranylation of a cellular protein FBL2 for efficient replication (Wang, Gale *et al.* 2005).

Since the sterol synthesis pathway is important for viral replication, it is not surprising that some viruses have developed mechanisms to exploit the pathway regulating cholesterol metabolism to increase their replication efficiency. For example, the HIV protein NEF and the HCV protein NS4B have been shown to directly increase the expression of the genes regulating the sterol synthesis pathway (Zheng, Plemenitas *et al.* 2003).

However, the involvement of the sterol biosynthesis pathway in the herpes virus life cycle has not yet been fully characterised. Nonetheless, Potena and colleagues have shown that treatment with statins inhibited hCMV early gene expression, suggesting that sterol biosynthesis is also important for herpes viral replication (Potena, Frascaroli *et al.* 2004). However, the mechanisms of this inhibition are still unknown.

On the other hand, recent studies suggest that the innate immune response modulates several aspects of cholesterol metabolism. Indeed, TLR activation through IRF3 and the nuclear receptor LXRα have been shown to reduce the efflux of cellular cholesterol (Castrillo, Joseph *et al.* 2003). Furthermore, LXRα KO mice have been demonstrated to be more susceptible to viral infection. In addition, TLR activation has been demonstrated to increase oxysterol levels and consequently negatively regulate the activation of B cells (Bauman, Bitmansour *et al.* 2009). However, the

question remains open as to whether innate immunity modulates the sterol biosynthesis pathway and if cholesterol modulation is part of a protective immune response against viral infection.

Moreover, it is well documented that cytokines and chemokines treatment modulates cholesterol metabolism in patients. For example, an acute phase response induces a decrease of total serum cholesterol levels (Khovidhunkit, Kim *et al.* 2004). IFNβ treatment has also been shown to decrease cholesterol plasma levels in multiple sclerosis patients (Morra, Coppola *et al.* 2004). However, the mechanisms involved in this regulation are still unknown.

Taken together, these observations led us to hypothesise that the innate immune response interacts with the regulation of cholesterol to modulate viral infection.

To understand the host-pathogen interaction with cholesterol metabolism, we used mCMV, a large double strand DNA virus which represents one of the few models of a natural infection of its host, and which has both biological and clinical relevance to human CMV diseases. Since the macrophage is one of the central players of the regulation of the innate immune response and of the CMV infection, as well as being implicated in the development of diseases such as atherosclerosis, which results in abnormal cellular cholesterol regulation, we used BMDMs as a model in our study.

At the start of this project, three major questions were defined to validate our hypothesis:

1. Does mCMV infection regulate cholesterol homeostasis?
2. How do those changes modulate viral replication?
3. Does the innate immune response play a role in the modulation of cholesterol metabolism in mCMV infections, and what are the mechanisms involved?

7.2 Summary of the results

The sterol biosynthesis pathway is down regulated at the transcriptional and metabolite level by mCMV infection in BMDMs and NIH/3T3 fibroblasts.

As a first step, we transcriptionally and metabolically characterised the regulation of cholesterol metabolism upon mCMV infection. We first applied a global non-biased transcriptional analysis coupled to the analysis of expression data exclusively restricted to lipogenic associated genes of mCMV-infected BMDMs to characterise the regulation of lipid pathways by mCMV infection. In addition, we performed a lipidomic quantification of the major membrane lipid classes as well as individual molecular lipid species at high sensitivity.

This approach revealed a selective and specific regulation of the lipid pathway by viral infection. Moreover, we provided evidence that fatty acid elongation, acetyl-COA-associated pathways and G-protein signalling are modulated by mCMV infection. Furthermore, this analysis demonstrated that sterol synthesis-regulating genes were co-ordinately down regulated by the mCMV infection.

However, the observed quantifiable level of reduction in expression of the sterol-associated genes was relatively modest (ranging from 1.3 to 3-fold). Hence, to independently validate the microarray data and extend our analysis, we then performed a more focused analysis of the gene expression of the cholesterol homeostasis-associated genes using QPCR and of the free cellular cholesterol changes induced by the mCMV infection in BMDMs. Our results showed that there is a complete reprogramming of the pathways regulating cholesterol homeostasis in response to infection. Indeed, sterol synthesis and uptake were down-regulated and cholesterol storage and efflux was up-regulated at the transcriptional level. Consistent with the transcriptional changes, the free cellular cholesterol levels, as well as its immediate precursor, 7-dehydrocholesterol, were strongly reduced (approximately 50%) by mCMV infection in BMDMs and NIH/3T3 fibroblasts, while the cholesteryl ester levels was increased in BMDMs. Moreover, we have shown that the down regulation of the sterol biosynthesis pathway does not require viral gene expression and is generalised to other viral infections since HSV1, SFV,

VV and Ad infections also down-regulate the expression of the sterol biosynthesis pathway.

Pharmacologic inhibition and siRNA knock-down of the sterol biosynthesis pathway has an antiviral effect.

Since we identified the sterol synthesis pathway as being regulated by mCMV infection, we then wondered if the alteration of the sterol pathway had any consequences on the mCMV replication. We used pharmacological inhibition with simvastatin and gene expressions knock-down to modulate the sterol pathway and monitor its effect on mCMV infection. This combined approach showed that inhibition at the protein and transcriptional level of the sterol biosynthesis pathway induced a decrease in mCMV replication. We also showed that simvastatin treatment has an antiviral effect against acute mCMV infection *in vivo*. These results are in agreement with a previous study (Potena, Frascaroli *et al.* 2004) and indicate that the sterol synthesis pathway is important for cytomegalovirus replication. Furthermore, by performing a selective knock-down assay and sterol intermediary supplementation of simvastatin treated cells, we could identify the prenylation branch and not the synthesis of cholesterol itself as being responsible for the modulation of the mCMV infection. This is the first time that the prenylation pathway has been shown to play a role in herpes virus replication. However, we did not identify the molecular mechanisms of the modulation of mCMV replication by the prenylation pathway. Taken together, these results support a role for the mevalonate-prenylation arm of the sterol pathway for optimal mCMV replication, and highlight its potential in protecting the host from viral infection.

An interferon regulatory loop mechanism induces the transcriptional down-regulation of the sterol biosynthesis pathway in response to infection.

The demonstration that down-regulation of the sterol pathway is a general early response to infection and does not require viral gene expression added to the observation that the alteration of the sterol synthesis inhibits viral replication. This led us to hypothesise that the regulation of sterol biosynthesis is part of an innate

immune response. On the basis that secreted factors in response to infection also down-regulate the sterol synthesis pathway, a screen for a cytokine effect on sterol synthesis gene expression demonstrated that IFNβ and IFNγ, but not IL1β, IL6 or TNFα treatment, were able to alter sterol synthesis pathway. These results raised the hypothesis for a potential IFN regulatory loop mechanism that is responsible for modulating sterol biosynthesis. Consequently, we investigated whether type I IFN receptor was required for the regulation of sterol biosynthesis in response to infection. Our results demonstrated that the lack of IFN type I receptor abolished the ability of BMDMs to reduce both sterol biosynthesis gene expression and cholesterol yield upon either infection with mCMV or treatment with IFNβ, but not IFNγ. We conclude from these experiments that a type I IFN-dependent innate immune response stringently regulates the metabolic alteration of the sterol biosynthesis network observed upon infection. Moreover, we demonstrated that genetic ablation of IFNβ results in a partial loss but still statistically reduced sterol gene expression indicating that it is not absolutely necessary and strongly suggesting that other type I interferons may also play a role in the regulation of the sterol pathway in response to infection.

Additionally we also demonstrated that type II interferon treatment of BMDMs induced the down regulation of sterol synthesis independently of the type I regulatory response suggesting that an alternative type II interferon signalling pathway also modulates the sterol synthesis pathway.

7.3 Conclusion

In this study, we confirmed the central hypothesis of this thesis, that the innate immune response interacts with the regulation of cholesterol to modulate viral infection. Indeed, by using a strategy integrating genomic, lipidomic and biochemical approaches, we identified that the prenylation arm of the sterol pathway as playing a pivotal role in antiviral functions coupled to the type I IFN response. A definitive link to sterol metabolism that is independent of cholesterol was established by the observation that the anti-viral effect of down-regulating the sterol pathway upon infection is completely blocked if cells are provided with an excess of mevalonate but not cholesterol. Furthermore, on the basis of genetic elimination studies, we document for the first time a molecular dependency between sterol biosynthesis and IFN signalling upon infection, leading to small coordinate changes in gene expression.

Our results are consistent with a model involving a canonical two-step IFN response for modulating endogenous sterol pathway activity upon infection.

Figure 7-1 illustrates the two signalling cascades; a virus-induced IFN-producing signal and an IFN receptor-mediated secondary signal. The first is initiated by the detection of viral structural proteins and nucleic acids by host recognition receptors, in the case of mCMV this has been extensively investigated and shown to involve TLR2 and -9 recognition receptors (Compton, Kurt-Jones *et al.* 2003; Tabeta, Georgel *et al.* 2004), and this has been seen to lead to the activation of the transcription factors, NFkB, ATF2/c-Jun, and IRF3 that directly activate IFNAα and β genes. Interestingly, previous studies have shown that microbial activation of TLR3 or TLR4 inhibits LXR target genes such as *ABCA1* by an unknown mechanism, resulting in the inhibition of cholesterol efflux from macrophages, which is reported to occur in a type I interferon-independent manner (Castrillo, Joseph *et al.* 2003). Similar to the microbial-mediated TLR activation of IRF3, many viruses including mCMV potently induce IRF3 and may also have the potential to inhibit LXR functions. Despite the recent progress in finding a link between intracellular cholesterol homeostasis and innate immunity, little is known regarding the impact of an IFN-regulated loop signalling, as the second step, through interactions with IFN receptors that activate the JAK-STAT pathway. We demonstrate here that

transcriptional regulation of the cellular sterol biosynthesis pathway upon infection impacts viral replication and mechanistically requires an IFN-regulated loop dependent on type I interferon signalling. We also showed that viral infection or direct IFN stimulation is accompanied by the down-regulation of sterol biosynthesis genes, and in the context of ligand activation, of IFNAR1. The IFN-dependent coupling of the mevalonate-sterol metabolic network and anti-viral activity represents a previously unrecognised mechanism for the regulation of protective immunity.

Several viruses including human CMV have been reported to be sensitive to statin administration (Gower and Graham 2001; del Real, Jimenez-Baranda *et al.* 2004; Potena, Frascaroli *et al.* 2004; Cohen 2005; Bader, Fazili *et al.* 2008; Mohan, Muller *et al.* 2008), and although the mechanism of action of most is not known, it has been correlated with a lower abundance of cholesterol in lipid rafts of cell membranes. A potential complicating factor of using statins is that suppression of the mevalonate pathway also perturbs synthesis of non-sterol mevalonate derivatives such as geranylgeraniol and farnesol involved in protein farnesylation and prenylation pathways, although in the case of HCV, the mechanisms of the inhibitory effects of the statins have been examined and are related to the prenylation of essential proteins. In our current study we uncoupled the cholesterol synthetic pathway from the non-steroidal modifications through targeted metabolic rescue and siRNA knock-down studies of mCMV and revealed an absolute requirement for the prenylation branch of the sterol pathway for mediating anti-viral effects.

HMGCR is likely to have broad range of non-specific effects on various efferent branch points of the pathway and thus may well not be ideal for an anti-infective therapy. In addition, statins are also known to have a range of immune modulatory activities by mechanisms yet to be fully characterised. In this connection, it is worth noting that type I IFN, especially IFNβ, have considerable overlap with many of the immune related activities of statins (Neuhaus, Stuve *et al.* 2005). Moreover, it is especially noteworthy that IFNβ treatment in patients has also been reported to decrease cholesterol plasma levels. Since our studies uncover a molecular dependency of type 1 signalling this may provide an entirely new therapeutic pathway for lowering cholesterol. Moreover, our findings may have important

implications for the development of new adjuvant strategies to existing anti-infective therapies based on the principal of using metabolic modifiers (drugs that target metabolic pathways) of protective innate immunity.

7.4 Future work

The different findings of this investigation also have raised new questions which should be addressed in future studies to fully understand the mechanisms involved in the regulation of sterol synthesis as an antiviral response by innate immunity.

1. **What are the mechanisms and signalling cascade involved in the down-regulation of the sterol biosynthesis pathway by the activation of the type I interferon receptor?**

    - Are nuclear receptors such as LXRs or PPAR involved in the regulation of the sterol synthesis by interferon signalling?
    - Is TYK2 and STAT1 activation involved in the regulation of sterol biosynthesis, and if so, is it by a direct or indirect mechanism?
    - Is SREBP2 regulated at the protein and/or the expression level by type I interferon signalling?
    - Are oxysterols, especially 25-hydroxycholesterol, involved in this regulation?

2. **What are the mechanisms involving the prenylation branch of the sterol biosynthesis pathway which regulates mCMV replication?**

    - Does a viral protein require prenylation for efficient mCMV replication?
    - Is it due to an alteration of the cell cycle?
    - Is it due to an induction of apoptosis/necrosis?
    - Does it involve the prenylation of a G-protein?

3. **Are other components involved in cholesterol homeostasis (cholesterol efflux, uptake and storage) also regulated by the interferon response to infection?**

4. **What is the signalling cascade involved in the regulation of the sterol biosynthesis pathway by type II interferon?**

7.5 Proposed Model

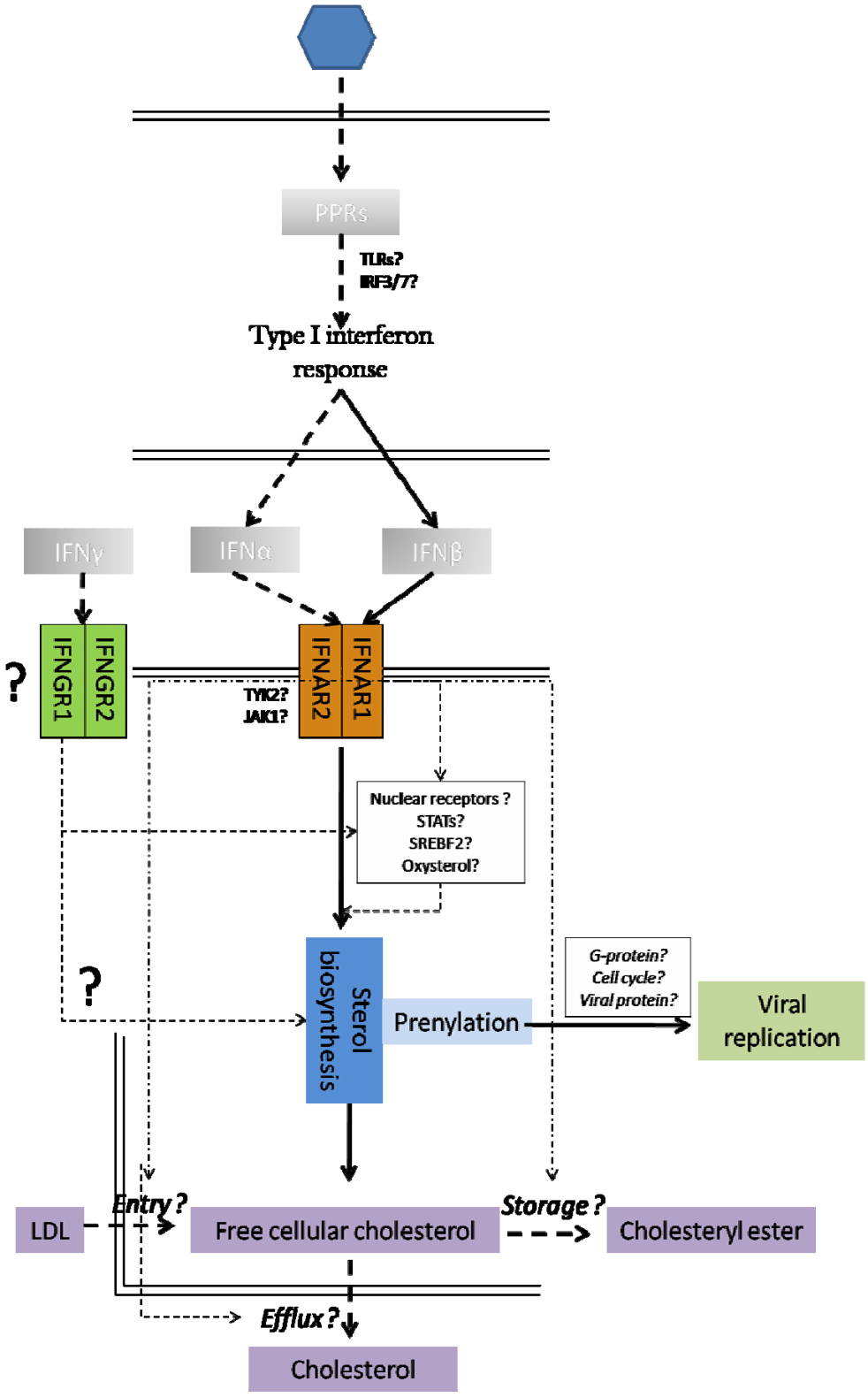


Figure 7-1: Proposed model for the regulation of sterol biosynthesis by IFN in response to infection.

References

Abrahamsen, L. H., M. J. Clay, et al. (1996). "The effects of cytomegalovirus infection on polar lipids and neutral lipids in cultured human cells." Intervirology **39**(4): 223-9.

AbuBakar, S., I. Boldogh, et al. (1990). "Human cytomegalovirus stimulates arachidonic acid metabolism through pathways that are affected by inhibitors of phospholipase A2 and protein kinase C." Biochem Biophys Res Commun **166**(2): 953-9.

Adam, E., J. L. Melnick, et al. (1987). "High levels of cytomegalovirus antibody in patients requiring vascular surgery for atherosclerosis." Lancet **2**(8554): 291-3.

Adams, C. M., J. Reitz, et al. (2004). "Cholesterol and 25-hydroxycholesterol inhibit activation of SREBPs by different mechanisms, both involving SCAP and Insigs." J Biol Chem **279**(50): 52772-80.

Ahn, A., D. L. Gibbons, et al. (2002). "The fusion peptide of Semliki Forest virus associates with sterol-rich membrane domains." J Virol **76**(7): 3267-75.

Akira, S. (2009). "Pathogen recognition by innate immunity and its signaling." Proc Jpn Acad Ser B Phys Biol Sci **85**(4): 143-56.

Akira, S. and K. Takeda (2004). "Toll-like receptor signalling." Nat Rev Immunol **4**(7): 499-511.

Alber, D. G., K. L. Powell, et al. (2000). "Herpesvirus infection accelerates atherosclerosis in the apolipoprotein E-deficient mouse." Circulation **102**(7): 779-85.

Albrecht, T., M. P. Fons, et al. (1991). "Metabolic and cellular effects of human cytomegalovirus infection." Transplant Proc **23**(3 Suppl 3): 48-54, discussion 54-5.

Alcami, A. and U. H. Koszinowski (2000). "Viral mechanisms of immune evasion." Immunol Today **21**(9): 447-55.

Allal, C., C. Buisson-Brenac, et al. (2004). "Human cytomegalovirus carries a cell-derived phospholipase A2 required for infectivity." J Virol **78**(14): 7717-26.

Amundson, D. M. and M. Zhou (1999). "Fluorometric method for the enzymatic determination of cholesterol." J Biochem Biophys Methods **38**(1): 43-52.

Andrews, D. M., C. E. Andoniou, et al. (2001). "Infection of dendritic cells by murine cytomegalovirus induces functional paralysis." Nat Immunol **2**(11): 1077-84.

Andrews, D. M., A. A. Scalzo, et al. (2003). "Functional interactions between dendritic cells and NK cells during viral infection." Nat Immunol **4**(2): 175-81.

Angulo, A., P. Ghazal, et al. (2000). "The major immediate-early gene ie3 of mouse cytomegalovirus is essential for viral growth." J Virol **74**(23): 11129-36.

Angulo, A., M. Messerle, et al. (1998). "Enhancer requirement for murine cytomegalovirus growth and genetic complementation by the human cytomegalovirus enhancer." J Virol **72**(11): 8502-9.

Angulo, A., C. Suto, et al. (1996). "Characterization of the sequences of the human cytomegalovirus enhancer that mediate differential regulation by natural and synthetic retinoids." Mol Endocrinol **10**(7): 781-93.

Arase, H., E. S. Mocarski, et al. (2002). "Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors." Science **296**(5571): 1323-6.
Atalay, R., A. Zimmermann, et al. (2002). "Identification and expression of human cytomegalovirus transcription units coding for two distinct Fcgamma receptor homologs." J Virol **76**(17): 8596-608.
Babic, M., M. Pyzik, et al. (2010). "Cytomegalovirus immunoevasin reveals the physiological role of "missing self" recognition in natural killer cell dependent virus control in vivo." J Exp Med **207**(12): 2663-73.
Bader, T., J. Fazili, et al. (2008). "Fluvastatin inhibits hepatitis C replication in humans." Am J Gastroenterol **103**(6): 1383-9.
Bale, J. F., Jr., J. A. Blackman, et al. (1990). "Outcome in children with symptomatic congenital cytomegalovirus infection." J Child Neurol **5**(2): 131-6.
Banks, T. A., S. Rickert, et al. (2005). "A lymphotoxin-IFN-beta axis essential for lymphocyte survival revealed during cytomegalovirus infection." J Immunol **174**(11): 7217-25.
Barnes, S. J. and P. D. Weitzman (1986). "Organization of citric acid cycle enzymes into a multienzyme cluster." FEBS Lett **201**(2): 267-70.
Basak, S., H. Kim, et al. (2007). "A fourth IkappaB protein within the NF-kappaB signaling module." Cell **128**(2): 369-81.
Bauman, D. R., A. D. Bitmansour, et al. (2009). "25-Hydroxycholesterol secreted by macrophages in response to Toll-like receptor activation suppresses immunoglobulin A production." Proc Natl Acad Sci U S A **106**(39): 16764-9.
Bavari, S., C. M. Bosio, et al. (2002). "Lipid raft microdomains: a gateway for compartmentalized trafficking of Ebola and Marburg viruses." J Exp Med **195**(5): 593-602.
Bender, F. C., J. C. Whitbeck, et al. (2003). "Specific association of glycoprotein B with lipid rafts during herpes simplex virus entry." J Virol **77**(17): 9542-52.
Benedict, C. A., T. A. Banks, et al. (2001). "Lymphotoxins and cytomegalovirus cooperatively induce interferon-beta, establishing host-virus detente." Immunity **15**(4): 617-26.
Bentz, M. H. and J. Magnette (1998). "[Hypocholesterolemia during the acute phase of an inflammatory reaction of infectious origin. 120 cases]." Rev Med Interne **19**(3): 168-72.
Bergstrom, J. D., M. M. Kurtz, et al. (1993). "Zaragozic acids: a family of fungal metabolites that are picomolar competitive inhibitors of squalene synthase." Proc Natl Acad Sci U S A **90**(1): 80-4.
Beutler, B. A. (2009). "TLRs and innate immunity." Blood **113**(7): 1399-407.
Biron, C. A. (2001). "Interferons alpha and beta as immune regulators--a new look." Immunity **14**(6): 661-4.
Biron, C. A., K. S. Byron, et al. (1989). "Severe herpesvirus infections in an adolescent without natural killer cells." N Engl J Med **320**(26): 1731-5.
Bligh, E. G. and W. J. Dyer (1959). "A rapid method of total lipid extraction and purification." Can J Biochem Physiol **37**(8): 911-7.
Boeckh, M. and R. Bowden (1995). "Cytomegalovirus infection in marrow transplantation." Cancer Treat Res **76**: 97-136.
Bordier, B. B., J. Ohkanda, et al. (2003). "In vivo antiviral efficacy of prenylation inhibitors against hepatitis delta virus." J Clin Invest **112**(3): 407-14.

Boulant, S., M. W. Douglas, et al. (2008). "Hepatitis C virus core protein induces lipid droplet redistribution in a microtubule- and dynein-dependent manner." Traffic **9**(8): 1268-82.
Brown, M. S. and J. L. Goldstein (1997). "The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor." Cell **89**(3): 331-40.
Browne, E. P., B. Wing, et al. (2001). "Altered cellular mRNA levels in human cytomegalovirus-infected fibroblasts: viral block to the accumulation of antiviral mRNAs." J Virol **75**(24): 12319-30.
Campbell, A. E., V. J. Cavanaugh, et al. (2008). "The salivary glands as a privileged site of cytomegalovirus immune evasion and persistence." Med Microbiol Immunol **197**(2): 205-13.
Caraglia, M., A. Budillon, et al. (2005). "Isoprenylation of intracellular proteins as a new target for the therapy of human neoplasms: preclinical and clinical implications." Curr Drug Targets **6**(3): 301-23.
Carter, C. J. (2008). "Interactions between the products of the Herpes simplex genome and Alzheimer's disease susceptibility genes: relevance to pathological-signalling cascades." Neurochem Int **52**(6): 920-34.
Castillo, J. P. and T. F. Kowalik (2004). "HCMV infection: modulating the cell cycle and cell death." Int Rev Immunol **23**(1-2): 113-39.
Castrillo, A., S. B. Joseph, et al. (2003). "Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism." Mol Cell **12**(4): 805-16.
Chakravarty, E. F. (2008). "Viral infection and reactivation in autoimmune disease." Arthritis Rheum **58**(10): 2949-57.
Chan, E. Y., W. J. Qian, et al. (2007). "Quantitative analysis of human immunodeficiency virus type 1-infected CD4+ cell proteome: dysregulated cell cycle progression and nuclear transport coincide with robust virus production." J Virol **81**(14): 7571-83.
Chan, E. Y., J. N. Sutton, et al. (2009). "Dynamic host energetics and cytoskeletal proteomes in human immunodeficiency virus type 1-infected human primary CD4 cells: analysis by multiplexed label-free mass spectrometry." J Virol **83**(18): 9283-95.
Chan, G., M. F. Stinski, et al. (2004). "Human cytomegalovirus-induced upregulation of intercellular cell adhesion molecule-1 on villous syncytiotrophoblasts." Biol Reprod **71**(3): 797-803.
Chang, K. O. (2009). "Role of cholesterol pathways in norovirus replication." J Virol **83**(17): 8587-95.
Chang, W. L., N. Baumgarth, et al. (2004). "Human cytomegalovirus-encoded interleukin-10 homolog inhibits maturation of dendritic cells and alters their functionality." J Virol **78**(16): 8720-31.
Chawla, A., W. A. Boisvert, et al. (2001). "A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis." Mol Cell **7**(1): 161-71.
Chazal, N. and D. Gerlier (2003). "Virus entry, assembly, budding, and membrane rafts." Microbiol Mol Biol Rev **67**(2): 226-37.

Chee, M. S., A. T. Bankier, et al. (1990). "Analysis of the protein-coding content of the sequence of human cytomegalovirus strain AD169." Curr Top Microbiol Immunol **154**: 125-69.
Chiarla, C., I. Giovannini, et al. (2004). "The relationship between plasma cholesterol, amino acids and acute phase proteins in sepsis." Amino Acids **27**(1): 97-100.
Chiu, Y. H., J. B. Macmillan, et al. (2009). "RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway." Cell **138**(3): 576-91.
Cinatl, J., Jr., J. U. Vogel, et al. (2004). "Oncomodulatory signals by regulatory proteins encoded by human cytomegalovirus: a novel role for viral infection in tumor progression." FEMS Microbiol Rev **28**(1): 59-77.
Cinti, D. L., L. Cook, et al. (1992). "The fatty acid chain elongation system of mammalian endoplasmic reticulum." Prog Lipid Res **31**(1): 1-51.
Cohen, J. I. (2005). "HMG CoA reductase inhibitors (statins) to treat Epstein-Barr virus-driven lymphoma." Br J Cancer **92**(9): 1593-8.
Colonna, M., A. Krug, et al. (2002). "Interferon-producing cells: on the front line in immune responses against pathogens." Curr Opin Immunol **14**(3): 373-9.
Compton, T., E. A. Kurt-Jones, et al. (2003). "Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2." J Virol **77**(8): 4588-96.
Cosman, D., J. Mullberg, et al. (2001). "ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor." Immunity **14**(2): 123-33.
Cousens, L. P., J. S. Orange, et al. (1997). "Interferon-alpha/beta inhibition of interleukin 12 and interferon-gamma production in vitro and endogenously during viral infection." Proc Natl Acad Sci U S A **94**(2): 634-9.
Crane, M. J., K. L. Hokeness-Antonelli, et al. (2009). "Regulation of inflammatory monocyte/macrophage recruitment from the bone marrow during murine cytomegalovirus infection: role for type I interferons in localized induction of CCR2 ligands." J Immunol **183**(4): 2810-7.
Cull, V. S., E. J. Bartlett, et al. (2002). "Type I interferon gene therapy protects against cytomegalovirus-induced myocarditis." Immunology **106**(3): 428-37.
Cullen, P., K. Tegelkamp, et al. (1997). "Measuring cholesterol in macrophages: comparison of high-performance liquid chromatography and gas-liquid chromatography with enzymatic fluorometry." Anal Biochem **251**(1): 39-44.
Curtiss, L. K. and P. S. Tobias (2009). "Emerging role of Toll-like receptors in atherosclerosis." J Lipid Res **50 Suppl**: S340-5.
Dalod, M., T. Hamilton, et al. (2003). "Dendritic cell responses to early murine cytomegalovirus infection: subset functional specialization and differential regulation by interferon alpha/beta." J Exp Med **197**(7): 885-98.
Dalod, M., T. P. Salazar-Mather, et al. (2002). "Interferon alpha/beta and interleukin 12 responses to viral infections: pathways regulating dendritic cell cytokine expression in vivo." J Exp Med **195**(4): 517-28.
Danesh, J. and P. Appleby (1998). "Persistent infection and vascular disease: a systematic review." Expert Opin Investig Drugs **7**(5): 691-713.
Danesh, J., R. Collins, et al. (1997). "Chronic infections and coronary heart disease: is there a link?" Lancet **350**(9075): 430-6.

Dankner, W. M., J. A. McCutchan, et al. (1990). "Localization of human cytomegalovirus in peripheral blood leukocytes by in situ hybridization." J Infect Dis **161**(1): 31-6.
Davidson, M. H. (2006). "Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids." Am J Cardiol **98**(4A): 27i-33i.
Davignon, J. L., P. Castanie, et al. (1996). "Anti-human cytomegalovirus activity of cytokines produced by CD4+ T-cell clones specifically activated by IE1 peptides in vitro." J Virol **70**(4): 2162-9.
Davison, A. J., R. Eberle, et al. (2009). "The order Herpesvirales." Arch Virol **154**(1): 171-7.
De Fabiani, E., N. Mitro, et al. (2001). "The negative effects of bile acids and tumor necrosis factor-alpha on the transcription of cholesterol 7alpha-hydroxylase gene (CYP7A1) converge to hepatic nuclear factor-4: a novel mechanism of feedback regulation of bile acid synthesis mediated by nuclear receptors." J Biol Chem **276**(33): 30708-16.
DeFilippis, V. R., D. Alvarado, et al. "Human cytomegalovirus induces the interferon response via the DNA sensor ZBP1." J Virol **84**(1): 585-98.
Deigendesch, N., F. Koch-Nolte, et al. (2006). "ZBP1 subcellular localization and association with stress granules is controlled by its Z-DNA binding domains." Nucleic Acids Res **34**(18): 5007-20.
Del Pozo, J. L., D. van de Beek, et al. (2010). "High serum cholesterol levels are associated with herpes zoster infection after heart transplantation." Clin Infect Dis **50**(1): 121-2.
del Real, G., S. Jimenez-Baranda, et al. (2004). "Statins inhibit HIV-1 infection by down-regulating Rho activity." J Exp Med **200**(4): 541-7.
Delale, T., A. Paquin, et al. (2005). "MyD88-dependent and -independent murine cytomegalovirus sensing for IFN-alpha release and initiation of immune responses in vivo." J Immunol **175**(10): 6723-32.
Demierre, M. F., P. D. Higgins, et al. (2005). "Statins and cancer prevention." Nat Rev Cancer **5**(12): 930-42.
Demmler, G. J. (1991). "Infectious Diseases Society of America and Centers for Disease Control. Summary of a workshop on surveillance for congenital cytomegalovirus disease." Rev Infect Dis **13**(2): 315-29.
Desplanques, A. S., H. J. Nauwynck, et al. (2008). "Plasma membrane cholesterol is required for efficient pseudorabies virus entry." Virology **376**(2): 339-45.
Diamond, D. L., A. J. Syder, et al. "Temporal proteome and lipidome profiles reveal hepatitis C virus-associated reprogramming of hepatocellular metabolism and bioenergetics." PLoS Pathog **6**(1): e1000719.
Diczfalusy, U., K. E. Olofsson, et al. (2009). "Marked upregulation of cholesterol 25-hydroxylase expression by lipopolysaccharide." J Lipid Res **50**(11): 2258-64.
Diebold, S. S., M. Montoya, et al. (2003). "Viral infection switches non-plasmacytoid dendritic cells into high interferon producers." Nature **424**(6946): 324-8.
Einav, S. and J. S. Glenn (2003). "Prenylation inhibitors: a novel class of antiviral agents." J Antimicrob Chemother **52**(6): 883-6.
Elbashir, S. M., J. Harborth, et al. (2001). "Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells." Nature **411**(6836): 494-8.

Ettinger, W. H., V. K. Varma, et al. (1994). "Cytokines decrease apolipoprotein accumulation in medium from Hep G2 cells." Arterioscler Thromb **14**(1): 8-13.
Fabricant, C. G. and J. Fabricant (1999). "Atherosclerosis induced by infection with Marek's disease herpesvirus in chickens." Am Heart J **138**(5 Pt 2): S465-8.
Fahy, E., S. Subramaniam, et al. (2009). "Update of the LIPID MAPS comprehensive classification system for lipids." J Lipid Res **50 Suppl**: S9-14.
Farrell, H. E., H. Vally, et al. (1997). "Inhibition of natural killer cells by a cytomegalovirus MHC class I homologue in vivo." Nature **386**(6624): 510-4.
Fei, W., G. Shui, et al. (2008). "Fld1p, a functional homologue of human seipin, regulates the size of lipid droplets in yeast." J Cell Biol **180**(3): 473-82.
Fennie, E. H., Y. S. Lie, et al. (1988). "Reduced mortality in murine cytomegalovirus infected mice following prophylactic murine interferon-gamma treatment." Antiviral Res **10**(1-3): 27-39.
Feramisco, J. D., J. L. Goldstein, et al. (2004). "Membrane topology of human insig-1, a protein regulator of lipid synthesis." J Biol Chem **279**(9): 8487-96.
Fodil-Cornu, N. and S. M. Vidal (2008). "Type I interferon response to cytomegalovirus infection: the kick-start." Cell Host Microbe **3**(2): 59-61.
Foley, H. A., S. F. Ofori-Acquah, et al. (2002). "Stat3 beta inhibits gamma-globin gene expression in erythroid cells." J Biol Chem **277**(18): 16211-9.
Forde, K. A., C. Law, et al. (2009). "Do statins reduce hepatitis C RNA titers during routine clinical use?" World J Gastroenterol **15**(40): 5020-7.
Fortunato, E. A., A. K. McElroy, et al. (2000). "Exploitation of cellular signaling and regulatory pathways by human cytomegalovirus." Trends Microbiol **8**(3): 111-9.
Gamadia, L. E., E. B. Remmerswaal, et al. (2003). "Primary immune responses to human CMV: a critical role for IFN-gamma-producing CD4+ T cells in protection against CMV disease." Blood **101**(7): 2686-92.
Gazit, R., R. Gruda, et al. (2006). "Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1." Nat Immunol **7**(5): 517-23.
Geelen, M. J., D. M. Gibson, et al. (1986). "Hydroxymethylglutaryl-CoA reductase--the rate-limiting enzyme of cholesterol biosynthesis. A report of a meeting held at Nijenrode Castle, Breukelen, The Netherlands, August 24, 1985." FEBS Lett **201**(2): 183-6.
Georges, J. L., H. J. Rupprecht, et al. (2003). "Impact of pathogen burden in patients with coronary artery disease in relation to systemic inflammation and variation in genes encoding cytokines." Am J Cardiol **92**(5): 515-21.
Gerna, G., E. Percivalle, et al. (2000). "Human cytomegalovirus replicates abortively in polymorphonuclear leukocytes after transfer from infected endothelial cells via transient microfusion events." J Virol **74**(12): 5629-38.
Ghazal, P., M. Messerle, et al. (2003). "An essential role of the enhancer for murine cytomegalovirus in vivo growth and pathogenesis." J Virol **77**(5): 3217-28.
Gilman, A. G. (1987). "G proteins: transducers of receptor-generated signals." Annu Rev Biochem **56**: 615-49.
Glenn, J. S. (2006). "Prenylation of HDAg and antiviral drug development." Curr Top Microbiol Immunol **307**: 133-49.
Goldsmith, Z. G. and D. N. Dhanasekaran (2007). "G protein regulation of MAPK networks." Oncogene **26**(22): 3122-42.

Goldstein, J. L. and M. S. Brown (1990). "Regulation of the mevalonate pathway." Nature **343**(6257): 425-30.
Gomez, E., A. Laures, et al. (2005). "Cytomegalovirus replication and "herpesvirus burden" as risk factor of cardiovascular events in the first year after renal transplantation." Transplant Proc **37**(9): 3760-3.
Gordon, S. (1998). "The role of the macrophage in immune regulation." Res Immunol **149**(7-8): 685-8.
Gordon, S. (2002). "Pattern recognition receptors: doubling up for the innate immune response." Cell **111**(7): 927-30.
Gower, T. L. and B. S. Graham (2001). "Antiviral activity of lovastatin against respiratory syncytial virus in vivo and in vitro." Antimicrob Agents Chemother **45**(4): 1231-7.
Grahame-Clarke, C., N. N. Chan, et al. (2003). "Human cytomegalovirus seropositivity is associated with impaired vascular function." Circulation **108**(6): 678-83.
Grandvaux, N., M. J. Servant, et al. (2002). "Transcriptional profiling of interferon regulatory factor 3 target genes: direct involvement in the regulation of interferon-stimulated genes." J Virol **76**(11): 5532-9.
Granucci, F., I. Zanoni, et al. (2004). "A contribution of mouse dendritic cell-derived IL-2 for NK cell activation." J Exp Med **200**(3): 287-95.
Gschwentner, M., A. Susanna, et al. (1995). "Antiviral drugs from the nucleoside analog family block volume-activated chloride channels." Mol Med **1**(4): 407-17.
Hahm, B., M. J. Trifilo, et al. (2005). "Viruses evade the immune system through type I interferon-mediated STAT2-dependent, but STAT1-independent, signaling." Immunity **22**(2): 247-57.
Hajjar, D. P., D. J. Falcone, et al. (1985). "Altered cholesteryl ester cycle is associated with lipid accumulation in herpesvirus-infected arterial smooth muscle cells." J Biol Chem **260**(10): 6124-8.
Hambleton, S., S. P. Steinberg, et al. (2007). "Cholesterol dependence of varicella-zoster virion entry into target cells." J Virol **81**(14): 7548-58.
Hamilton, J. D. and B. J. Seaworth (1985). "Transmission of latent cytomegalovirus in a murine kidney tissue transplantation model." Transplantation **39**(3): 290-6.
Hannah, B. P., T. M. Cairns, et al. (2009). "Herpes simplex virus glycoprotein B associates with target membranes via its fusion loops." J Virol **83**(13): 6825-36.
Hanson, L. K., J. S. Slater, et al. (1999). "Replication of murine cytomegalovirus in differentiated macrophages as a determinant of viral pathogenesis." J Virol **73**(7): 5970-80.
Hao, X. R., D. L. Cao, et al. (2009). "IFN-gamma down-regulates ABCA1 expression by inhibiting LXRalpha in a JAK/STAT signaling pathway-dependent manner." Atherosclerosis **203**(2): 417-28.
Hardardottir, I., A. H. Moser, et al. (1994). "Effects of TNF, IL-1, and the combination of both cytokines on cholesterol metabolism in Syrian hamsters." Lymphokine Cytokine Res **13**(3): 161-6.

Hegarty, B. D., A. Bobard, et al. (2005). "Distinct roles of insulin and liver X receptor in the induction and cleavage of sterol regulatory element-binding protein-1c." Proc Natl Acad Sci U S A **102**(3): 791-6.

Heise, M. T., M. Connick, et al. (1998). "Murine cytomegalovirus inhibits interferon gamma-induced antigen presentation to CD4 T cells by macrophages via regulation of expression of major histocompatibility complex class II-associated genes." J Exp Med **187**(7): 1037-46.

Heise, M. T., J. L. Pollock, et al. (1998). "Murine cytomegalovirus infection inhibits IFN gamma-induced MHC class II expression on macrophages: the role of type I interferon." Virology **241**(2): 331-44.

Heise, M. T. and H. W. t. Virgin (1995). "The T-cell-independent role of gamma interferon and tumor necrosis factor alpha in macrophage activation during murine cytomegalovirus and herpes simplex virus infections." J Virol **69**(2): 904-9.

Hemmi, H., O. Takeuchi, et al. (2000). "A Toll-like receptor recognizes bacterial DNA." Nature **408**(6813): 740-5.

Hengel, H., P. Lucin, et al. (1994). "Restoration of cytomegalovirus antigen presentation by gamma interferon combats viral escape." J Virol **68**(1): 289-97.

Hers, H. G. and L. Hue (1983). "Gluconeogenesis and related aspects of glycolysis." Annu Rev Biochem **52**: 617-53.

Hill, J. M., I. Steiner, et al. (2005). "Statins lower the risk of developing Alzheimer's disease by limiting lipid raft endocytosis and decreasing the neuronal spread of Herpes simplex virus type 1." Med Hypotheses **64**(1): 53-8.

Ho (1995). Cytomegalovirus Biology and Infection. 2nd Ed. Plenum Medical Book Company, New York. 353 pp.

Horne, B. D., J. B. Muhlestein, et al. (2003). "Statin therapy interacts with cytomegalovirus seropositivity and high C-reactive protein in reducing mortality among patients with angiographically significant coronary disease." Circulation **107**(2): 258-63.

Hornung, V., S. Rothenfusser, et al. (2002). "Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides." J Immunol **168**(9): 4531-7.

Horton, J. D. (2002). "Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis." Biochem Soc Trans **30**(Pt 6): 1091-5.

Horton, J. D., J. L. Goldstein, et al. (2002). "SREBPs: transcriptional mediators of lipid homeostasis." Cold Spring Harb Symp Quant Biol **67**: 491-8.

Hsich, E., Y. F. Zhou, et al. (2001). "Cytomegalovirus infection increases development of atherosclerosis in Apolipoprotein-E knockout mice." Atherosclerosis **156**(1): 23-8.

Hsu, H. Y., A. C. Nicholson, et al. (1995). "Altered cholesterol trafficking in herpesvirus-infected arterial cells. Evidence for viral protein kinase-mediated cholesterol accumulation." J Biol Chem **270**(33): 19630-7.

Huang, Q., H. M. Shen, et al. (2006). "Emodin inhibits tumor cell adhesion through disruption of the membrane lipid Raft-associated integrin signaling pathway." Cancer Res **66**(11): 5807-15.

Ikeda, M. and N. Kato (2007). "Life style-related diseases of the digestive system: cell culture system for the screening of anti-hepatitis C virus (HCV) reagents: suppression of HCV replication by statins and synergistic action with interferon." J Pharmacol Sci **105**(2): 145-50.
Ikeda, M. and R. Longnecker (2007). "Cholesterol is critical for Epstein-Barr virus latent membrane protein 2A trafficking and protein stability." Virology **360**(2): 461-8.
Imhoff, H., V. von Messling, et al. (2007). "Canine distemper virus infection requires cholesterol in the viral envelope." J Virol **81**(8): 4158-65.
Itabashi, S., H. Arai, et al. (1997). "Herpes simplex virus and risk of Alzheimer's disease." Lancet **349**(9058): 1102.
Itzhaki, R. F. and M. A. Wozniak (2006). "Herpes simplex virus type 1, apolipoprotein E, and cholesterol: a dangerous liaison in Alzheimer's disease and other disorders." Prog Lipid Res **45**(1): 73-90.
Jonjic, S., W. Mutter, et al. (1989). "Site-restricted persistent cytomegalovirus infection after selective long-term depletion of CD4+ T lymphocytes." J Exp Med **169**(4): 1199-212.
Jonjic, S., I. Pavic, et al. (1990). "Efficacious control of cytomegalovirus infection after long-term depletion of CD8+ T lymphocytes." J Virol **64**(11): 5457-64.
Jonjic, S., I. Pavic, et al. (1994). "Antibodies are not essential for the resolution of primary cytomegalovirus infection but limit dissemination of recurrent virus." J Exp Med **179**(5): 1713-7.
Joseph, S. B., M. N. Bradley, et al. (2004). "LXR-dependent gene expression is important for macrophage survival and the innate immune response." Cell **119**(2): 299-309.
Joseph, S. B., A. Castrillo, et al. (2003). "Reciprocal regulation of inflammation and lipid metabolism by liver X receptors." Nat Med **9**(2): 213-9.
Juckem, L. K., K. W. Boehme, et al. (2008). "Differential initiation of innate immune responses induced by human cytomegalovirus entry into fibroblast cells." J Immunol **180**(7): 4965-77.
Kadegowda, A. K., M. Bionaz, et al. (2009). "Peroxisome proliferator-activated receptor-gamma activation and long-chain fatty acids alter lipogenic gene networks in bovine mammary epithelial cells to various extents." J Dairy Sci **92**(9): 4276-89.
Kanehisa, M. and S. Goto (2000). "KEGG: kyoto encyclopedia of genes and genomes." Nucleic Acids Res **28**(1): 27-30.
Kapadia, S. B. and F. V. Chisari (2005). "Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids." Proc Natl Acad Sci U S A **102**(7): 2561-6.
Kavanagh, D. G., M. C. Gold, et al. (2001). "The multiple immune-evasion genes of murine cytomegalovirus are not redundant: m4 and m152 inhibit antigen presentation in a complementary and cooperative fashion." J Exp Med **194**(7): 967-78.
Kawabata, A., H. Tang, et al. (2009). "y Human herpesvirus 6 envelope components enriched in lipid rafts: evidence for virion-associated lipid rafts." Virol J **6**: 127.
Kawai, T. and S. Akira (2006). "Innate immune recognition of viral infection." Nat Immunol **7**(2): 131-7.

Kawai, T. and S. Akira (2006). "TLR signaling." Cell Death Differ **13**(5): 816-25.
Keay, S. and B. R. Baldwin (1996). "Evidence for the role of cell protein phosphorylation in human cytomegalovirus/host cell fusion." J Gen Virol **77 (Pt 10)**: 2597-604.
Keil, G. M., A. Ebeling-Keil, et al. (1984). "Temporal regulation of murine cytomegalovirus transcription and mapping of viral RNA synthesized at immediate early times after infection." J Virol **50**(3): 784-95.
Khovidhunkit, W., M. S. Kim, et al. (2004). "Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host." J Lipid Res **45**(7): 1169-96.
Kiefer, T. L., L. Lai, et al. (2005). "Differential regulation of estrogen receptor alpha, glucocorticoid receptor and retinoic acid receptor alpha transcriptional activity by melatonin is mediated via different G proteins." J Pineal Res **38**(4): 231-9.
Kim, T. H., H. Kim, et al. (2009). "Interrelationship between liver X receptor alpha, sterol regulatory element-binding protein-1c, peroxisome proliferator-activated receptor gamma, and small heterodimer partner in the transcriptional regulation of glucokinase gene expression in liver." J Biol Chem **284**(22): 15071-83.
Kinzler, E. R. and T. Compton (2005). "Characterization of human cytomegalovirus glycoprotein-induced cell-cell fusion." J Virol **79**(12): 7827-37.
Kleijnen, M. F., J. B. Huppa, et al. (1997). "A mouse cytomegalovirus glycoprotein, gp34, forms a complex with folded class I MHC molecules in the ER which is not retained but is transported to the cell surface." Embo J **16**(4): 685-94.
Klenovsek, K., F. Weisel, et al. (2007). "Protection from CMV infection in immunodeficient hosts by adoptive transfer of memory B cells." Blood **110**(9): 3472-9.
Koszinowski, U. H., M. J. Reddehase, et al. (1991). "The role of CD4 and CD8 T cells in viral infections." Curr Opin Immunol **3**(4): 471-5.
Kovacs, A., M. Schluchter, et al. (1999). "Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1-infected women. Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection Study Group." N Engl J Med **341**(2): 77-84.
Kowalik, T. F., B. Wing, et al. (1993). "Multiple mechanisms are implicated in the regulation of NF-kappa B activity during human cytomegalovirus infection." Proc Natl Acad Sci U S A **90**(3): 1107-11.
Kristoffersen, A. K., K. Tasken, et al. (1994). "Expression of protein kinase A and protein kinase C during ongoing human cytomegalovirus infection." Arch Virol **138**(1-2): 85-93.
Krmpotic, A., D. H. Busch, et al. (2002). "MCMV glycoprotein gp40 confers virus resistance to CD8+ T cells and NK cells in vivo." Nat Immunol **3**(6): 529-35.
Kropp, K. A., C. O. Simon, et al. (2009). "Synergism between the components of the bipartite major immediate-early transcriptional enhancer of murine cytomegalovirus does not accelerate virus replication in cell culture and host tissues." J Gen Virol **90**(Pt 10): 2395-401.
Krug, A., A. R. French, et al. (2004). "TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function." Immunity **21**(1): 107-19.

Kucic, N., H. Mahmutefendic, et al. (2005). "Inhibition of protein kinases C prevents murine cytomegalovirus replication." J Gen Virol **86**(Pt 8): 2153-61.
Kumar, H., T. Kawai, et al. (2009). "Toll-like receptors and innate immunity." Biochem Biophys Res Commun **388**(4): 621-5.
Kurzrock, R., M. F. Rohde, et al. (1986). "Recombinant gamma interferon induces hypertriglyceridemia and inhibits post-heparin lipase activity in cancer patients." J Exp Med **164**(4): 1093-101.
Lacaze, P., S. Raza, et al. (2009). "Combined genome-wide expression profiling and targeted RNA interference in primary mouse macrophages reveals perturbation of transcriptional networks associated with interferon signalling." BMC Genomics **10**: 372.
Le, V. T., M. Trilling, et al. (2008). "Mouse cytomegalovirus inhibits beta interferon (IFN-beta) gene expression and controls activation pathways of the IFN-beta enhanceosome." J Gen Virol **89**(Pt 5): 1131-41.
Lee, C. J., H. R. Lin, et al. (2008). "Cholesterol effectively blocks entry of flavivirus." J Virol **82**(13): 6470-80.
Lenac, T., J. Arapovic, et al. (2008). "Murine cytomegalovirus regulation of NKG2D ligands." Med Microbiol Immunol **197**(2): 159-66.
Lester, R. L., G. B. Wells, et al. (1993). "Mutant strains of Saccharomyces cerevisiae lacking sphingolipids synthesize novel inositol glycerophospholipids that mimic sphingolipid structures." J Biol Chem **268**(2): 845-56.
Liao, J. K. (2002). "Isoprenoids as mediators of the biological effects of statins." J Clin Invest **110**(3): 285-8.
Lilley, B. N., H. L. Ploegh, et al. (2001). "Human cytomegalovirus open reading frame TRL11/IRL11 encodes an immunoglobulin G Fc-binding protein." J Virol **75**(22): 11218-21.
Lippmann, J., S. Rothenburg, et al. (2008). "IFNbeta responses induced by intracellular bacteria or cytosolic DNA in different human cells do not require ZBP1 (DLM-1/DAI)." Cell Microbiol **10**(12): 2579-88.
Liu, S., A. V. Rodriguez, et al. (2006). "Role of simvastatin and methyl-beta-cyclodextrin [corrected] on inhibition of poliovirus infection." Biochem Biophys Res Commun **347**(1): 51-9.
Liu, Y. J. (2005). "IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors." Annu Rev Immunol **23**: 275-306.
Livak, K. J. and T. D. Schmittgen (2001). "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method." Methods **25**(4): 402-8.
Lucin, P., S. Jonjic, et al. (1994). "Late phase inhibition of murine cytomegalovirus replication by synergistic action of interferon-gamma and tumour necrosis factor." J Gen Virol **75 (Pt 1)**: 101-10.
Ma, Z., M. J. Chang, et al. (2005). "Interferon-gamma-activated STAT-1alpha suppresses MMP-9 gene transcription by sequestration of the coactivators CBP/p300." J Leukoc Biol **78**(2): 515-23.
Mackenzie, J. M., A. A. Khromykh, et al. (2007). "Cholesterol manipulation by West Nile virus perturbs the cellular immune response." Cell Host Microbe **2**(4): 229-39.
Malmgaard, L., T. P. Salazar-Mather, et al. (2002). "Promotion of alpha/beta interferon induction during in vivo viral infection through alpha/beta

interferon receptor/STAT1 system-dependent and -independent pathways." J Virol **76**(9): 4520-5.
Manie, S. N., S. de Breyne, et al. (2000). "Measles virus structural components are enriched into lipid raft microdomains: a potential cellular location for virus assembly." J Virol **74**(1): 305-11.
Marjomaki, V., V. Pietiainen, et al. (2002). "Internalization of echovirus 1 in caveolae." J Virol **76**(4): 1856-65.
Martin, S. and R. G. Parton (2005). "Caveolin, cholesterol, and lipid bodies." Semin Cell Dev Biol **16**(2): 163-74.
Matthews, T. and R. Boehme (1988). "Antiviral activity and mechanism of action of ganciclovir." Rev Infect Dis **10 Suppl 3**: S490-4.
McCormick, A. L., A. Skaletskaya, et al. (2003). "Differential function and expression of the viral inhibitor of caspase 8-induced apoptosis (vICA) and the viral mitochondria-localized inhibitor of apoptosis (vMIA) cell death suppressors conserved in primate and rodent cytomegaloviruses." Virology **316**(2): 221-33.
McEwan, I. J. (2009). "Nuclear receptors: one big family." Methods Mol Biol **505**: 3-18.
McLauchlan, J. (2009). "Hepatitis C virus: viral proteins on the move." Biochem Soc Trans **37**(Pt 5): 986-90.
McTaggart, S. J. (2006). "Isoprenylated proteins." Cell Mol Life Sci **63**(3): 255-67.
Medzhitov, R. and C. A. Janeway, Jr. (1998). "Innate immune recognition and control of adaptive immune responses." Semin Immunol **10**(5): 351-3.
Melnick, J. L., E. Adam, et al. (1996). "Cytomegalovirus and atherosclerosis." Arch Immunol Ther Exp (Warsz) **44**(5-6): 297-302.
Messerle, M., G. Hahn, et al. (2000). "Cytomegalovirus bacterial artificial chromosomes: a new herpesvirus vector approach." Adv Virus Res **55**: 463-78.
Mettenleiter, T. C. (2002). "Brief overview on cellular virus receptors." Virus Res **82**(1-2): 3-8.
Miller, D. M., B. M. Rahill, et al. (1998). "Human cytomegalovirus inhibits major histocompatibility complex class II expression by disruption of the Jak/Stat pathway." J Exp Med **187**(5): 675-83.
Miller, D. M., Y. Zhang, et al. (1999). "Human cytomegalovirus inhibits IFN-alpha-stimulated antiviral and immunoregulatory responses by blocking multiple levels of IFN-alpha signal transduction." J Immunol **162**(10): 6107-13.
Mintern, J. D., E. J. Klemm, et al. (2006). "Viral interference with B7-1 costimulation: a new role for murine cytomegalovirus fc receptor-1." J Immunol **177**(12): 8422-31.
Mitchell, B. M., A. Leung, et al. (1996). "Murine cytomegalovirus DNA in peripheral blood of latently infected mice is detectable only in monocytes and polymorphonuclear leukocytes." Virology **223**(1): 198-207.
Miyanari, Y., K. Atsuzawa, et al. (2007). "The lipid droplet is an important organelle for hepatitis C virus production." Nat Cell Biol **9**(9): 1089-97.
Mo, H. and C. E. Elson (2004). "Studies of the isoprenoid-mediated inhibition of mevalonate synthesis applied to cancer chemotherapy and chemoprevention." Exp Biol Med (Maywood) **229**(7): 567-85.

Mocarski, E. S., Jr. (2002). "Immunomodulation by cytomegaloviruses: manipulative strategies beyond evasion." Trends Microbiol **10**(7): 332-9.
Mocarski, E. S., Jr. and G. W. Kemble (1996). "Recombinant cytomegaloviruses for study of replication and pathogenesis." Intervirology **39**(5-6): 320-30.
Mohan, K. V., J. Muller, et al. (2008). "Defective rotavirus particle assembly in lovastatin-treated MA104 cells." Arch Virol **153**(12): 2283-90.
Montoya, C. J., F. Jaimes, et al. (2009). "Antiretroviral effect of lovastatin on HIV-1-infected individuals without highly active antiretroviral therapy (The LIVE study): a phase-II randomized clinical trial." Trials **10**: 41.
Morra, V. B., G. Coppola, et al. (2004). "Interferon-beta treatment decreases cholesterol plasma levels in multiple sclerosis patients." Neurology **62**(5): 829-30.
Munger, J., S. U. Bajad, et al. (2006). "Dynamics of the cellular metabolome during human cytomegalovirus infection." PLoS Pathog **2**(12): e132.
Munger, J., B. D. Bennett, et al. (2008). "Systems-level metabolic flux profiling identifies fatty acid synthesis as a target for antiviral therapy." Nat Biotechnol **26**(10): 1179-86.
Murphy, D. J. (2001). "The biogenesis and functions of lipid bodies in animals, plants and microorganisms." Prog Lipid Res **40**(5): 325-438.
Naiki, Y., R. Sorrentino, et al. (2008). "TLR/MyD88 and liver X receptor alpha signaling pathways reciprocally control Chlamydia pneumoniae-induced acceleration of atherosclerosis." J Immunol **181**(10): 7176-85.
Naor, Z. (2009). "Signaling by G-protein-coupled receptor (GPCR): studies on the GnRH receptor." Front Neuroendocrinol **30**(1): 10-29.
Ness, G. C., D. Lopez, et al. (1998). "Effects of 15-oxa-32-vinyl-lanost-8-ene-3 beta,32 diol on the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase and low density lipoprotein receptor in rat liver." Arch Biochem Biophys **357**(2): 259-64.
Neuhaus, O., O. Stuve, et al. (2005). "Putative mechanisms of action of statins in multiple sclerosis--comparison to interferon-beta and glatiramer acetate." J Neurol Sci **233**(1-2): 173-7.
Neves, S. R., P. T. Ram, et al. (2002). "G protein pathways." Science **296**(5573): 1636-9.
Nishi, K. and K. Saigo (2007). "Cellular internalization of green fluorescent protein fused with herpes simplex virus protein VP22 via a lipid raft-mediated endocytic pathway independent of caveolae and Rho family GTPases but dependent on dynamin and Arf6." J Biol Chem **282**(37): 27503-17.
Norkin, L. C., H. A. Anderson, et al. (2002). "Caveolar endocytosis of simian virus 40 is followed by brefeldin A-sensitive transport to the endoplasmic reticulum, where the virus disassembles." J Virol **76**(10): 5156-66.
O'Leary, J. G., J. L. Chan, et al. (2007). "Atorvastatin does not exhibit antiviral activity against HCV at conventional doses: a pilot clinical trial." Hepatology **45**(4): 895-8.
O'Toole, D. and W. C. Love (2002). "Interferon-beta-1b and interferon-gamma have similar inhibitory effects on apolipoprotein-E production in the monocyte/macrophage." Mult Scler **8**(2): 124-9.
Orange, J. S., B. Wang, et al. (1995). "Requirement for natural killer cell-produced interferon gamma in defence against murine cytomegalovirus infection and

enhancement of this defence pathway by interleukin 12 administration." J Exp Med **182**(4): 1045-56.
Osborne, T. F. (2000). "Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action." J Biol Chem **275**(42): 32379-82.
Ostrowski, M. A., D. C. Krakauer, et al. (1998). "Effect of immune activation on the dynamics of human immunodeficiency virus replication and on the distribution of viral quasispecies." J Virol **72**(10): 7772-84.
Park, C. Y., H. J. Jun, et al. (2009). "Hepatitis C virus nonstructural 4B protein modulates sterol regulatory element-binding protein signaling via the AKT pathway." J Biol Chem **284**(14): 9237-46.
Paulus, C., S. Krauss, et al. (2006). "A human cytomegalovirus antagonist of type I IFN-dependent signal transducer and activator of transcription signaling." Proc Natl Acad Sci U S A **103**(10): 3840-5.
Peters, K. L., H. L. Smith, et al. (2002). "IRF-3-dependent, NFkappa B- and JNK-independent activation of the 561 and IFN-beta genes in response to double-stranded RNA." Proc Natl Acad Sci U S A **99**(9): 6322-7.
Pfeffer, L. M., B. C. Kwok, et al. (1985). "Interferon stimulates cholesterol and phosphatidylcholine synthesis but inhibits cholesterol ester synthesis in HeLa-S3 cells." Proc Natl Acad Sci U S A **82**(8): 2417-21.
Pinto, A. K. and A. B. Hill (2005). "Viral interference with antigen presentation to CD8+ T cells: lessons from cytomegalovirus." Viral Immunol **18**(3): 434-44.
Platanias, L. C. (2005). "Mechanisms of type-I- and type-II-interferon-mediated signalling." Nat Rev Immunol **5**(5): 375-86.
Popa, C., M. G. Netea, et al. (2007). "The role of TNF-alpha in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk." J Lipid Res **48**(4): 751-62.
Porstmann, T., C. R. Santos, et al. (2008). "SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth." Cell Metab **8**(3): 224-36.
Potena, L., G. Frascaroli, et al. (2004). "Hydroxymethyl-glutaryl coenzyme a reductase inhibition limits cytomegalovirus infection in human endothelial cells." Circulation **109**(4): 532-6.
Poulos, A. (1995). "Very long chain fatty acids in higher animals--a review." Lipids **30**(1): 1-14.
Presti, R. M., J. L. Pollock, et al. (1998). "Interferon gamma regulates acute and latent murine cytomegalovirus infection and chronic disease of the great vessels." J Exp Med **188**(3): 577-88.
Price, P., J. G. Winter, et al. (1987). "Genetically determined resistance to murine cytomegalovirus: a role for lymphocytostatic macrophages." J Gen Virol **68 (Pt 12)**: 2997-3008.
Prunet, C., T. Montange, et al. (2006). "Multiplexed flow cytometric analyses of pro- and anti-inflammatory cytokines in the culture media of oxysterol-treated human monocytic cells and in the sera of atherosclerotic patients." Cytometry A **69**(5): 359-73.
Radhakrishnan, A., Y. Ikeda, et al. (2007). "Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: oxysterols block transport by binding to Insig." Proc Natl Acad Sci U S A **104**(16): 6511-8.

Raghow, R., C. Yellaturu, et al. (2008). "SREBPs: the crossroads of physiological and pathological lipid homeostasis." Trends Endocrinol Metab **19**(2): 65-73.
Raghu, H., N. Sharma-Walia, et al. (2007). "Lipid rafts of primary endothelial cells are essential for Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8-induced phosphatidylinositol 3-kinase and RhoA-GTPases critical for microtubule dynamics and nuclear delivery of viral DNA but dispensable for binding and entry." J Virol **81**(15): 7941-59.
Randolph-Habecker, J., M. Iwata, et al. (2002). "Interleukin-1-mediated inhibition of cytomegalovirus replication is due to increased IFN-beta production." J Interferon Cytokine Res **22**(7): 765-72.
Rawlings, J. S., K. M. Rosler, et al. (2004). "The JAK/STAT signaling pathway." J Cell Sci **117**(Pt 8): 1281-3.
Rawlinson, W. D., H. E. Farrell, et al. (1996). "Analysis of the complete DNA sequence of murine cytomegalovirus." J Virol **70**(12): 8833-49.
Reddehase, M. J. (2002). "Antigens and immunoevasins: opponents in cytomegalovirus immune surveillance." Nat Rev Immunol **2**(11): 831-44.
Reddehase, M. J., M. Balthesen, et al. (1994). "The conditions of primary infection define the load of latent viral genome in organs and the risk of recurrent cytomegalovirus disease." J Exp Med **179**(1): 185-93.
Reddehase, M. J., W. Mutter, et al. (1987). "CD8-positive T lymphocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity." J Virol **61**(10): 3102-8.
Reddehase, M. J., J. Podlech, et al. (2002). "Mouse models of cytomegalovirus latency: overview." J Clin Virol **25 Suppl 2**: S23-36.
Reddehase, M. J., C. O. Simon, et al. (2008). "Murine model of cytomegalovirus latency and reactivation." Curr Top Microbiol Immunol **325**: 315-31.
Reddehase, M. J., F. Weiland, et al. (1985). "Interstitial murine cytomegalovirus pneumonia after irradiation: characterization of cells that limit viral replication during established infection of the lungs." J Virol **55**(2): 264-73.
Redpath, S., A. Angulo, et al. (1999). "Murine cytomegalovirus infection down-regulates MHC class II expression on macrophages by induction of IL-10." J Immunol **162**(11): 6701-7.
Reiss, A. B., C. A. Patel, et al. (2004). "Interferon-gamma impedes reverse cholesterol transport and promotes foam cell transformation in THP-1 human monocytes/macrophages." Med Sci Monit **10**(11): BR420-5.
Reusch, U., W. Muranyi, et al. (1999). "A cytomegalovirus glycoprotein re-routes MHC class I complexes to lysosomes for degradation." Embo J **18**(4): 1081-91.
Reyburn, H. T., O. Mandelboim, et al. (1997). "The class I MHC homologue of human cytomegalovirus inhibits attack by natural killer cells." Nature **386**(6624): 514-7.
Reynolds, C. P., B. J. Maurer, et al. (2004). "Ceramide synthesis and metabolism as a target for cancer therapy." Cancer Lett **206**(2): 169-80.
Ringrose, J. H., R. E. Jeeninga, et al. (2008). "Proteomic studies reveal coordinated changes in T-cell expression patterns upon infection with human immunodeficiency virus type 1." J Virol **82**(9): 4320-30.

Robinzon, S., A. Dafa-Berger, et al. (2009). "Impaired cholesterol biosynthesis in a neuronal cell line persistently infected with measles virus." J Virol **83**(11): 5495-504.
Rothwell, C., A. Lebreton, et al. (2009). "Cholesterol biosynthesis modulation regulates dengue viral replication." Virology **389**(1-2): 8-19.
Ruzek, M. C., A. H. Miller, et al. (1997). "Characterization of early cytokine responses and an interleukin (IL)-6-dependent pathway of endogenous glucocorticoid induction during murine cytomegalovirus infection." J Exp Med **185**(7): 1185-92.
Sagedal, S., A. Hartmann, et al. (2005). "The impact of early cytomegalovirus infection and disease in renal transplant recipients." Clin Microbiol Infect **11**(7): 518-30.
Sainz, B., Jr., H. L. LaMarca, et al. (2005). "Synergistic inhibition of human cytomegalovirus replication by interferon-alpha/beta and interferon-gamma." Virol J **2**: 14.
Samsa, M. M., J. A. Mondotte, et al. (2009). "Dengue virus capsid protein usurps lipid droplets for viral particle formation." PLoS Pathog **5**(10): e1000632.
Samuel, C. E. (2001). "Antiviral actions of interferons." Clin Microbiol Rev **14**(4): 778-809.
Sanchez, V. and D. H. Spector (2008). "Subversion of cell cycle regulatory pathways." Curr Top Microbiol Immunol **325**: 243-62.
Sato, M., H. Suemori, et al. (2000). "Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction." Immunity **13**(4): 539-48.
Scalzo, A. A., N. A. Fitzgerald, et al. (1990). "Cmv-1, a genetic locus that controls murine cytomegalovirus replication in the spleen." J Exp Med **171**(5): 1469-83.
Schleiss, M. R. and T. C. Heineman (2005). "Progress toward an elusive goal: current status of cytomegalovirus vaccines." Expert Rev Vaccines **4**(3): 381-406.
Schneider, K., A. Loewendorf, et al. (2008). "Lymphotoxin-mediated crosstalk between B cells and splenic stroma promotes the initial type I interferon response to cytomegalovirus." Cell Host Microbe **3**(2): 67-76.
Schneider, R. J. and I. Mohr (2003). "Translation initiation and viral tricks." Trends Biochem Sci **28**(3): 130-6.
Schultz, J. R., H. Tu, et al. (2000). "Role of LXRs in control of lipogenesis." Genes Dev **14**(22): 2831-8.
Scott, I. (2009). "Degradation of RIG-I following cytomegalovirus infection is independent of apoptosis." Microbes Infect **11**(12): 973-9.
Sears, A. E. and B. Roizman (1990). "Amplification by host cell factors of a sequence contained within the herpes simplex virus 1 genome." Proc Natl Acad Sci U S A **87**(23): 9441-4.
Shaw, S. M., J. E. Fildes, et al. (2009). "Pleiotropic effects and cholesterol-lowering therapy." Cardiology **112**(1): 4-12.
Sherman, B. T., W. Huang da, et al. (2007). "DAVID Knowledgebase: a gene-centered database integrating heterogeneous gene annotation resources to facilitate high-throughput gene functional analysis." BMC Bioinformatics **8**: 426.

Sherrill, J. D., M. P. Stropes, et al. (2009). "Activation of intracellular signaling pathways by the murine cytomegalovirus G protein-coupled receptor M33 occurs via PLC-{beta}/PKC-dependent and -independent mechanisms." J Virol **83**(16): 8141-52.
Shibutani, T., T. M. Johnson, et al. (1997). "Pertussis toxin-sensitive G proteins as mediators of the signal transduction pathways activated by cytomegalovirus infection of smooth muscle cells." J Clin Invest **100**(8): 2054-61.
Shingai, M., M. Azuma, et al. (2008). "Soluble G protein of respiratory syncytial virus inhibits Toll-like receptor 3/4-mediated IFN-beta induction." Int Immunol **20**(9): 1169-80.
Simon, C. O., R. Holtappels, et al. (2006). "CD8 T cells control cytomegalovirus latency by epitope-specific sensing of transcriptional reactivation." J Virol **80**(21): 10436-56.
Simons, K. and E. Ikonen (2000). "How cells handle cholesterol." Science **290**(5497): 1721-6.
Sinzger, C. and G. Jahn (1996). "Human cytomegalovirus cell tropism and pathogenesis." Intervirology **39**(5-6): 302-19.
Speir, E., T. Shibutani, et al. (1996). "Role of reactive oxygen intermediates in cytomegalovirus gene expression and in the response of human smooth muscle cells to viral infection." Circ Res **79**(6): 1143-52.
Speir, E., Z. X. Yu, et al. (1998). "Aspirin attenuates cytomegalovirus infectivity and gene expression mediated by cyclooxygenase-2 in coronary artery smooth muscle cells." Circ Res **83**(2): 210-6.
Spencer, J. V., K. M. Lockridge, et al. (2002). "Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10." J Virol **76**(3): 1285-92.
Stinski, M. F. and H. Isomura (2008). "Role of the cytomegalovirus major immediate early enhancer in acute infection and reactivation from latency." Med Microbiol Immunol **197**(2): 223-31.
Strobl, B., I. Bubic, et al. (2005). "Novel functions of tyrosine kinase 2 in the antiviral defence against murine cytomegalovirus." J Immunol **175**(6): 4000-8.
Sun, L. P., J. Seemann, et al. (2007). "Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: Insig renders sorting signal in Scap inaccessible to COPII proteins." Proc Natl Acad Sci U S A **104**(16): 6519-26.
Swiecki, M., S. Gilfillan, et al. (2010). "Plasmacytoid dendritic cell ablation impacts early interferon responses and antiviral NK and CD8(+) T cell accrual." Immunity **33**(6): 955-66.
Tabeta, K., P. Georgel, et al. (2004). "Toll-like receptors 9 and 3 as essential components of innate immune defence against mouse cytomegalovirus infection." Proc Natl Acad Sci U S A **101**(10): 3516-21.
Tailor, P., T. Tamura, et al. (2007). "The feedback phase of type I interferon induction in dendritic cells requires interferon regulatory factor 8." Immunity **27**(2): 228-39.
Takai, Y., T. Sasaki, et al. (2001). "Small GTP-binding proteins." Physiol Rev **81**(1): 153-208.
Tang-Feldman, Y. J., A. Wojtowicz, et al. (2006). "Use of quantitative real-time PCR (qRT-PCR) to measure cytokine transcription and viral load in murine cytomegalovirus infection." J Virol Methods **131**(2): 122-9.

Tang, H., A. Kawabata, et al. (2008). "Human herpesvirus-6 infection induces the reorganization of membrane microdomains in target cells, which are required for virus entry." Virology **378**(2): 265-71.
Unutmaz, D., V. N. KewalRamani, et al. (1998). "G protein-coupled receptors in HIV and SIV entry: new perspectives on lentivirus-host interactions and on the utility of animal models." Semin Immunol **10**(3): 225-36.
Ushio-Fukai, M. (2009). "Vascular signaling through G protein-coupled receptors: new concepts." Curr Opin Nephrol Hypertens **18**(2): 153-9.
Vaidya, S., R. Bostedor, et al. (1998). "Massive production of farnesol-derived dicarboxylic acids in mice treated with the squalene synthase inhibitor zaragozic acid A." Arch Biochem Biophys **355**(1): 84-92.
Varani, S., M. Cederarv, et al. (2007). "Human cytomegalovirus differentially controls B cell and T cell responses through effects on plasmacytoid dendritic cells." J Immunol **179**(11): 7767-76.
Vassilatis, D. K., J. G. Hohmann, et al. (2003). "The G protein-coupled receptor repertoires of human and mouse." Proc Natl Acad Sci U S A **100**(8): 4903-8.
Vilkuna-Rautiainen, T., P. J. Pussinen, et al. (2006). "Serum antibody response to periodontal pathogens and herpes simplex virus in relation to classic risk factors of cardiovascular disease." Int J Epidemiol **35**(6): 1486-94.
Vliegen, I., A. Duijvestijn, et al. (2004). "Cytomegalovirus infection aggravates atherogenesis in apoE knockout mice by both local and systemic immune activation." Microbes Infect **6**(1): 17-24.
Wang, C., M. Gale, Jr., et al. (2005). "Identification of FBL2 as a geranylgeranylated cellular protein required for hepatitis C virus RNA replication." Mol Cell **18**(4): 425-34.
Wang, G., S. J. Jones, et al. (2006). "Identification of genes targeted by the androgen and PKA signaling pathways in prostate cancer cells." Oncogene **25**(55): 7311-23.
Wang, X., S. M. Huong, et al. (2003). "Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus." Nature **424**(6947): 456-61.
Wang, X. Q., C. G. Panousis, et al. (2002). "Interferon-gamma-mediated downregulation of cholesterol efflux and ABC1 expression is by the Stat1 pathway." Arterioscler Thromb Vasc Biol **22**(5): e5-9.
Ware, C. F. (2005). "Network communications: lymphotoxins, LIGHT, and TNF." Annu Rev Immunol **23**: 787-819.
Waris, G., D. J. Felmlee, et al. (2007). "Hepatitis C virus induces proteolytic cleavage of sterol regulatory element binding proteins and stimulates their phosphorylation via oxidative stress." J Virol **81**(15): 8122-30.
Welch, A. R., L. M. McGregor, et al. (1991). "Cytomegalovirus homologs of cellular G protein-coupled receptor genes are transcribed." J Virol **65**(7): 3915-8.
Welsh, R. M., J. O. Brubaker, et al. (1991). "Natural killer (NK) cell response to virus infections in mice with severe combined immunodeficiency. The stimulation of NK cells and the NK cell-dependent control of virus infections occur independently of T and B cell function." J Exp Med **173**(5): 1053-63.
Wenk, M. R. (2005). "The emerging field of lipidomics." Nat Rev Drug Discov **4**(7): 594-610.
Wenk, M. R. (2006). "Lipidomics of host-pathogen interactions." FEBS Lett **580**(23): 5541-51.

Wesoly, J., Z. Szweykowska-Kulinska, et al. (2007). "STAT activation and differential complex formation dictate selectivity of interferon responses." Acta Biochim Pol **54**(1): 27-38.
Wild, P., M. Engels, et al. (2005). "Impairment of nuclear pores in bovine herpesvirus 1-infected MDBK cells." J Virol **79**(2): 1071-83.
Wilson, N. S. and J. A. Villadangos (2005). "Regulation of antigen presentation and cross-presentation in the dendritic cell network: facts, hypothesis, and immunological implications." Adv Immunol **86**: 241-305.
Wong, A., K. H. Tan, et al. (2000). "Seroprevalence of cytomegalovirus, toxoplasma and parvovirus in pregnancy." Singapore Med J **41**(4): 151-5.
Wynn, K. K., Z. Fulton, et al. (2008). "Impact of clonal competition for peptide-MHC complexes on the CD8+ T-cell repertoire selection in a persistent viral infection." Blood **111**(8): 4283-92.
Xu, M., H. Zhou, et al. (2009). "ABCG1 mediated oxidized LDL-derived oxysterol efflux from macrophages." Biochem Biophys Res Commun **390**(4): 1349-54.
Ye, J., C. Wang, et al. (2003). "Disruption of hepatitis C virus RNA replication through inhibition of host protein geranylgeranylation." Proc Natl Acad Sci U S A **100**(26): 15865-70.
Yoneyama, M., W. Suhara, et al. (1998). "Direct triggering of the type I interferon system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300." EMBO J **17**(4): 1087-95.
Yvan-Charvet, L., N. Wang, et al. (2010). "Role of HDL, ABCA1, and ABCG1 transporters in cholesterol efflux and immune responses." Arterioscler Thromb Vasc Biol **30**(2): 139-43.
Zheng, Y. H., A. Plemenitas, et al. (2003). "Nef increases the synthesis of and transports cholesterol to lipid rafts and HIV-1 progeny virions." Proc Natl Acad Sci U S A **100**(14): 8460-5.
Zhou, Y. F., E. Guetta, et al. (1996). "Human cytomegalovirus increases modified low density lipoprotein uptake and scavenger receptor mRNA expression in vascular smooth muscle cells." J Clin Invest **98**(9): 2129-38.
Zhu, X., J. Y. Lee, et al. (2008). "Increased cellular free cholesterol in macrophage-specific Abca1 knock-out mice enhances pro-inflammatory response of macrophages." J Biol Chem **283**(34): 22930-41.
Zucchini, N., G. Bessou, et al. (2008). "Individual plasmacytoid dendritic cells are major contributors to the production of multiple innate cytokines in an organ-specific manner during viral infection." Int Immunol **20**(1): 45-56.
Zucchini, N., G. Bessou, et al. (2008). "Cutting edge: Overlapping functions of TLR7 and TLR9 for innate defence against a herpesvirus infection." J Immunol **180**(9): 5799-803.

Appendix 1. - List of the 446 down regulated genes by mCMV in BMDMs after 24 hrs infection

Probe Set ID	Gene Symbol	Gene Title	FC	P-Value
94305_at	Col1a1	collagen, type I, alpha 1	-4.48	4.30E-09
160406_at	Ctsk	cathepsin K	-3.73	3.66E-11
160832_at	*Ldlr*	low density lipoprotein receptor	-3.63	7.51E-10
93100_at	Acta2	actin, alpha 2, smooth muscle, aorta	-3.32	1.80E-09
92567_at	Col5a2	collagen, type V, alpha 2	-3.31	1.62E-09
94325_at	*Hmgcs1*	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	-3.25	2.14E-10
96269_at	*Idi1*	isopentenyl-diphosphate delta isomerase	-3.22	1.12E-10
99098_at	Fdps	farnesyl diphosphate synthetase	-3.06	4.73E-09
94322_at	*Sqle*	squalene epoxidase	-3.05	4.57E-10
96049_at	Bgn	biglycan	-2.94	2.48E-09
95758_at	Scd2	stearoyl-Coenzyme A desaturase 2	-2.79	1.18E-09
103957_at	Tfrc	transferrin receptor	-2.71	5.08E-10
94769_at	Mmp8	matrix metallopeptidase 8	-2.67	1.31E-08
101160_at	Cxcl2	chemokine (C-X-C motif) ligand 2	-2.61	6.84E-08
101130_at	Col1a2	collagen, type I, alpha 2	-2.60	6.14E-09
99387_at	Fpr1	formyl peptide receptor 1	-2.60	3.40E-08
94916_at	*Cyp51*	cytochrome P450, family 51	-2.54	7.77E-08
160388_at	*Sc4mol*	sterol-C4-methyl oxidase-like	-2.50	2.14E-09
93294_at	Ctgf	connective tissue growth factor	-2.50	4.32E-07
94294_at	Ccnb2	cyclin B2	-2.41	2.32E-09
97203_at	Marcksl1	MARCKS-like 1	-2.39	2.42E-08
103958_g_at	Tfrc	transferrin receptor	-2.39	1.05E-09
103226_at	Mrc1	mannose receptor, C type 1	-2.37	1.75E-07
160118_at	Mmp14	matrix metallopeptidase 14 (membrane-inserted)	-2.36	2.47E-08
160424_f_at	Fdps	farnesyl diphosphate synthetase	-2.31	2.83E-09
99457_at	Mki67	antigen identified by monoclonal antibody Ki 67	-2.29	2.14E-09
94817_at	Serpinh1	serine (or cysteine) peptidase inhibitor, clade H, member 1	-2.23	3.71E-07
92833_at	Hal	histidine ammonia lyase	-2.20	2.38E-08
96784_at	Anln	anillin, actin binding protein	-2.19	1.55E-08
94784_at	Iqgap3	IQ motif containing GTPase activating protein 3	-2.17	3.84E-09
95348_at	Cxcl1	chemokine (C-X-C motif) ligand 1	-2.17	1.10E-06
97518_at	*Fdft1*	farnesyl diphosphate farnesyl transferase 1	-2.17	6.34E-08
97282_at	Mela	melanoma antigen	-2.13	5.98E-08
102629_at	Tnf	tumor necrosis factor	-2.12	1.36E-08
97320_at	Slc39a4	solute carrier family 39 (zinc transporter),	-2.11	3.88E-09

		member 4		
104333_at	D17H6S56E-5	DNA segment, Chr 17, human D6S56E 5	-2.03	6.23E-08
99979_at	Cyp1b1	cytochrome P450, family 1, subfamily b, polypeptide 1	-2.02	3.15E-07
160544_at	Fabp5	fatty acid binding protein 5, epidermal	-1.98	3.02E-08
96886_at	Stab1	stabilin 1	-1.96	3.39E-08
101073_at	Lrp1	low density lipoprotein receptor-related protein 1	-1.92	1.80E-07
160280_at	Cav1	caveolin 1, caveolae protein	-1.92	4.44E-07
104149_at	Nfkbia	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	-1.92	1.33E-08
160501_at	Kif20a	kinesin family member 20A	-1.91	3.29E-07
104451_at	Slc11a2	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	-1.91	2.47E-08
95032_at	Prc1	protein regulator of cytokinesis 1	-1.90	5.52E-08
93573_at	Mt1	metallothionein 1	-1.89	1.00E-08
99578_at	Top2a	topoisomerase (DNA) II alpha	-1.87	9.50E-09
103448_at	S100a8	S100 calcium binding protein A8 (calgranulin A)	-1.85	1.29E-07
160770_at	*Mvd*	mevalonate (diphospho) decarboxylase	-1.85	2.09E-08
160159_at	Ccnb1	cyclin B1	-1.85	2.44E-07
97238_at	Tacc3	transforming, acidic coiled-coil containing protein 3	-1.85	4.26E-08
160755_at	Kif2c /// LOC631653	kinesin family member 2C /// similar to Kinesin-like protein KIF2C (Mitotic centromere-associated kinesin) (MCAK)	-1.82	2.42E-08
94288_at	Hist1h1c	histone cluster 1, H1c	-1.82	6.84E-08
93273_at	Snca	synuclein, alpha	-1.82	2.34E-07
93777_at	Golph3l	golgi phosphoprotein 3-like	-1.82	1.34E-08
160901_at	Fos	FBJ osteosarcoma oncogene	-1.81	6.66E-07
99425_at	*Hmgcr*	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	-1.81	3.41E-06
92639_at	Aurka	aurora kinase A	-1.81	1.55E-08
101561_at	Mt2	metallothionein 2	-1.81	4.92E-08
162077_f_at	Scd2	stearoyl-Coenzyme A desaturase 2	-1.80	2.89E-07
101521_at	Birc5	baculoviral IAP repeat-containing 5	-1.80	4.82E-08
98773_s_at	Irg1	immunoresponsive gene 1	-1.80	3.41E-07
92593_at	Postn	periostin, osteoblast specific factor	-1.80	3.33E-07
103667_at	Atp13a3	ATPase type 13A3	-1.78	2.76E-07
161000_i_at	Nusap1	nucleolar and spindle associated protein 1	-1.78	1.03E-06
160973_at	C330027C09Rik	RIKEN cDNA C330027C09 gene	-1.78	3.15E-07
101571_g_at	Igfbp4	insulin-like growth factor binding protein 4	-1.78	1.60E-07
93574_at	Serpinf1	serine (or cysteine) peptidase inhibitor, clade F, member 1	-1.77	9.99E-07
96319_at	Cdc20	cell division cycle 20 homolog (S. cerevisiae)	-1.75	3.06E-08
99186_at	Ccna2	cyclin A2	-1.74	3.85E-07
104314_r_at	1110032A03Rik	RIKEN cDNA 1110032A03 gene	-1.74	1.04E-04

94211_at	6720460F02Rik	RIKEN cDNA 6720460F02 gene	-1.73	4.26E-08
97160_at	Sparc	secreted acidic cysteine rich glycoprotein	-1.73	1.48E-06
100616_at	Cenpa	centromere protein A	-1.71	7.10E-07
100128_at	Cdc2a	cell division cycle 2 homolog A (S. pombe)	-1.71	3.37E-08
93541_at	Tagln	transgelin	-1.70	7.21E-08
93099_f_at	Plk1	polo-like kinase 1 (Drosophila)	-1.68	1.91E-06
94910_at	Nde1	nuclear distribution gene E homolog 1 (A nidulans)	-1.68	3.15E-07
104056_at	Ccdc50	coiled-coil domain containing 50	-1.67	1.28E-07
100116_at	2810417H13Rik	RIKEN cDNA 2810417H13 gene	-1.65	5.68E-07
104285_at	*Hmgcr*	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	-1.65	3.21E-07
160095_at	Lox	lysyl oxidase	-1.65	1.18E-07
161946_r_at	Sorbs1	sorbin and SH3 domain containing 1	-1.65	3.51E-03
92790_at	Kpna2	karyopherin (importin) alpha 2	-1.65	1.29E-06
98631_g_at	*Nsdhl*	NAD(P) dependent steroid dehydrogenase-like	-1.64	6.84E-08
98575_at	Fasn	fatty acid synthase	-1.64	3.20E-07
93868_at	*Nsdhl*	NAD(P) dependent steroid dehydrogenase-like	-1.64	2.67E-07
101393_at	Anxa3	annexin A3	-1.64	3.55E-07
160655_at	Cpd	carboxypeptidase D	-1.63	1.27E-06
98059_s_at	Lmna	lamin A	-1.63	8.78E-07
95062_at	Cast	calpastatin	-1.63	1.51E-06
101560_at	Emb	embigin	-1.63	6.84E-08
97105_at	Zc3h12c	zinc finger CCCH type containing 12C	-1.62	1.23E-07
98305_at	Foxm1	forkhead box M1	-1.62	3.46E-07
94971_at	Cdkn3	cyclin-dependent kinase inhibitor 3	-1.62	7.86E-08
104097_at	Bub1	budding uninhibited by benzimidazoles 1 homolog (S. cerevisiae)	-1.62	1.74E-07
100885_at	Nek2	NIMA (never in mitosis gene a)-related expressed kinase 2	-1.62	3.84E-07
94953_at	Racgap1	Rac GTPase-activating protein 1	-1.62	3.71E-07
102632_at	Aspm	asp (abnormal spindle)-like, microcephaly associated (Drosophila)	-1.61	3.85E-07
98982_at	Tmpo	thymopoietin	-1.61	2.46E-07
96551_at	Clec4e	C-type lectin domain family 4, member e	-1.61	1.18E-06
96168_at	Kif23	kinesin family member 23	-1.60	2.76E-07
99149_at	Trim59	tripartite motif-containing 59	-1.60	3.83E-07
100348_at	---	---	-1.60	3.59E-07
93833_s_at	Hist1h2bc	histone cluster 1, H2bc	-1.59	6.82E-07
94243_at	Ckap5	cytoskeleton associated protein 5	-1.58	8.74E-07
104522_at	Itsn1	intersectin 1 (SH3 domain protein 1A)	-1.58	4.48E-07
94805_f_at	Hist1h2ab /// Hist1h2ac /// Hist1h2ad /// Hist1h2ae /// Hist1h2af /// Hist1h2ag ///	histone cluster 1, H2ab /// histone cluster 1, H2ac /// histone cluster 1, H2ad /// histone cluster 1, H2ae /// histone cluster 1, H2af /// histone cluster 1, H2ag /// histone cluster 1,	-1.57	3.02E-07

	Hist1h2ah /// Hist1h2ai /// Hist1h2ak /// Hist1h2an /// Hist1h2ao /// Hist3h2a /// RP23-480B19.10	H2ah /// histone cluster 1, H2ai /// histone cluster 1, H2ak /// histone cluster 1, H2an /// histone cluster 1, H2ao /// histone cluster 3, H2a /// similar to histone 2a		
103978_at	Zc3h11a	zinc finger CCCH type containing 11A	-1.57	5.28E-06
99040_at	Slc6a4	solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	-1.57	1.57E-05
97295_at	Cdca8	cell division cycle associated 8	-1.57	3.76E-07
99541_at	Kif11	kinesin family member 11	-1.56	7.14E-07
93019_at	H2afx	H2A histone family, member X	-1.56	1.95E-06
95721_at	Mapkapk2	MAP kinase-activated protein kinase 2	-1.56	3.49E-07
101676_at	Gpx3	glutathione peroxidase 3	-1.56	7.91E-07
96081_at	Tk1	thymidine kinase 1	-1.56	3.14E-07
103994_at	Eif2c2	eukaryotic translation initiation factor 2C, 2	-1.55	2.19E-05
103845_at	Slc31a1	solute carrier family 31, member 1	-1.55	4.89E-07
93990_at	Hnrnph1	heterogeneous nuclear ribonucleoprotein H1	-1.55	5.00E-07
92782_at	Tmpo	thymopoietin	-1.55	4.49E-07
92978_s_at	Serpinb2	serine (or cysteine) peptidase inhibitor, clade B, member 2	-1.54	3.84E-07
104322_at	Ckap2	cytoskeleton associated protein 2	-1.54	1.37E-06
98968_at	Myo5a	myosin VA	-1.54	2.51E-05
92350_at	Mapre1	microtubule-associated protein, RP/EB family, member 1	-1.54	3.25E-06
104320_at	Pdxk	pyridoxal (pyridoxine, vitamin B6) kinase	-1.54	1.00E-05
160479_at	Cat	catalase	-1.54	9.04E-07
93414_at	Abcb1b	ATP-binding cassette, sub-family B (MDR/TAP), member 1B	-1.53	3.56E-07
100884_at	Akr1b8	aldo-keto reductase family 1, member B8	-1.53	6.23E-06
100923_at	Myo10	myosin X	-1.53	3.56E-07
103709_at	Col1a1	collagen, type I, alpha 1	-1.52	5.68E-07
161530_r_at	Sema4a	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A	-1.52	4.66E-06
160546_at	Aldoc	aldolase C, fructose-bisphosphate	-1.52	5.26E-07
94177_at	*Hsd17b7*	hydroxysteroid (17-beta) dehydrogenase 7	-1.52	4.22E-07
102768_i_at	Sc5d	sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog (S. cerevisae)	-1.52	6.29E-05
160894_at	C*Ebp*d	CCAAT/enhancer binding protein (C/*EBP*), delta	-1.52	1.72E-06
104214_at	Slc7a8	solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	-1.51	9.42E-07
104059_at	Chd8	chromodomain helicase DNA binding protein 8	-1.51	3.29E-06
95292_at	Itga4	integrin alpha 4	-1.51	3.41E-06
104467_at	Cpd	carboxypeptidase D	-1.51	9.13E-07
160461_f_at	Tubb6	tubulin, beta 6	-1.51	1.10E-06
102655_at	Itga4	integrin alpha 4	-1.51	1.39E-05
162379_r_at	---	---	-1.51	3.77E-06

104712_at	Myc	myelocytomatosis oncogene	-1.51	7.74E-05
100050_at	Id1	inhibitor of DNA binding 1	-1.50	1.86E-04
103914_at	Pcyt2	phosphate cytidylyltransferase 2, ethanolamine	-1.50	9.25E-06
97364_at	Asf1b	ASF1 anti-silencing function 1 homolog B (S. cerevisiae)	-1.50	6.66E-07
104644_at	Kif4	kinesin family member 4	-1.50	3.79E-07
95010_at	Traf3	TNF receptor-associated factor 3	-1.50	4.27E-07
104579_r_at	Actn1	actinin, alpha 1	-1.50	1.75E-05
92925_at	C*Ebp*b	CCAAT/enhancer binding protein (C/*EBP*), beta	-1.49	5.60E-07
92984_g_at	Ptprj	protein tyrosine phosphatase, receptor type, J	-1.49	1.25E-04
99823_r_at	D18Ertd232e	DNA segment, Chr 18, ERATO Doi 232, expressed	-1.49	3.44E-06
161796_r_at	Kcnq1	potassium voltage-gated channel, subfamily Q, member 1	-1.48	2.48E-05
97095_at	Bub1	budding uninhibited by benzimidazoles 1 homolog (S. cerevisiae)	-1.48	1.15E-05
92280_at	Actn1	actinin, alpha 1	-1.48	1.01E-05
97421_at	Smc2	structural maintenance of chromosomes 2	-1.48	7.18E-06
161314_r_at	Ubr4	ubiquitin protein ligase E3 component n-recognin 4	-1.47	6.32E-04
101350_g_at	Plk1	polo-like kinase 1 (Drosophila)	-1.47	4.07E-06
160489_at	Tnfaip2	tumor necrosis factor, alpha-induced protein 2	-1.47	7.26E-06
95542_at	Tpm4	tropomyosin 4	-1.47	1.10E-06
92412_s_at	Spag5	sperm associated antigen 5	-1.47	6.79E-05
92852_at	Fn1	fibronectin 1	-1.47	7.42E-05
98306_g_at	Foxm1	forkhead box M1	-1.47	3.34E-06
104578_f_at	Actn1	actinin, alpha 1	-1.46	1.49E-06
97527_at	Cks2	CDC28 protein kinase regulatory subunit 2	-1.45	5.17E-06
99392_at	Tnfaip3	tumor necrosis factor, alpha-induced protein 3	-1.45	8.23E-07
95731_at	LOC100047324 /// Sesn1	similar to Sesn1 protein /// sestrin 1	-1.45	7.57E-06
160737_at	*Lss*	lanosterol synthase	-1.45	8.08E-05
103201_at	Ttk	Ttk protein kinase	-1.45	1.85E-05
99621_s_at	Sfpq	splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	-1.45	6.89E-06
102812_i_at	Uba5	ubiquitin-like modifier activating enzyme 5	-1.45	1.67E-05
98774_at	Irg1	immunoresponsive gene 1	-1.45	2.48E-05
98988_at	Nfkbiz	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	-1.45	1.22E-03
93471_at	Slc4a7	solute carrier family 4, sodium bicarbonate cotransporter, member 7	-1.45	1.11E-05
96930_at	Ehd1	EH-domain containing 1	-1.45	4.76E-06
100095_at	Scarb1	scavenger receptor class B, member 1	-1.45	1.99E-06
160762_at	Abr	active BCR-related gene	-1.44	1.25E-06
92301_at	Ikbke	inhibitor of kappaB kinase epsilon	-1.44	5.99E-07

100928_at	Fbln2	fibulin 2	-1.44	1.80E-06
93758_at	Incenp	inner centromere protein	-1.44	8.69E-07
93111_at	Kpnb1	karyopherin (importin) beta 1	-1.43	1.74E-06
93264_at	*Srebf1*	sterol regulatory element binding transcription factor 1	-1.43	1.40E-06
103736_at	Sash1	SAM and SH3 domain containing 1	-1.43	3.42E-06
160255_at	Ahnak	AHNAK nucleoprotein (desmoyokin)	-1.43	9.72E-06
99073_at	Ccnf	cyclin F	-1.43	4.66E-06
161137_r_at	Ube2c	ubiquitin-conjugating enzyme E2C	-1.43	5.66E-06
94712_at	Vegfc	vascular endothelial growth factor C	-1.43	1.28E-04
95693_at	Idh2	isocitrate dehydrogenase 2 (NADP+), mitochondrial	-1.43	1.06E-06
101902_at	Rbpj	recombination signal binding protein for immunoglobulin kappa J region	-1.42	1.31E-05
160387_at	Cd302	CD302 antigen	-1.42	2.69E-06
95625_at	Slc6a8	solute carrier family 6 (neurotransmitter transporter, creatine), member 8	-1.42	1.77E-06
160101_at	Hmox1	heme oxygenase (decycling) 1	-1.42	3.78E-06
162402_r_at	Hoxa4	homeo box A4	-1.42	3.10E-06
161856_f_at	Kif20a	kinesin family member 20A	-1.42	4.10E-05
95543_at	EG665825 /// Tpm4	predicted gene, EG665825 /// tropomyosin 4	-1.42	7.11E-05
160369_at	*Dhcr24*	24-dehydrocholesterol reductase	-1.41	7.27E-06
160335_at	Gclm	glutamate-cysteine ligase, modifier subunit	-1.41	8.13E-06
103720_at	Rest	RE1-silencing transcription factor	-1.41	1.33E-03
101554_at	Nfkbia	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	-1.41	1.16E-06
100530_at	Ralgds	ral guanine nucleotide dissociation stimulator	-1.41	1.38E-05
160885_at	Nucks1	nuclear casein kinase and cyclin-dependent kinase substrate 1	-1.41	4.38E-05
100089_at	Ppic	peptidylprolyl isomerase C	-1.41	4.21E-06
160638_at	Cdkn2c	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	-1.40	1.49E-06
103614_at	Nfkb2	nuclear factor of kappa light polypeptide gene enhancer in B-cells 2, p49/p100	-1.40	5.45E-06
102642_at	Slc11a2	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	-1.40	8.09E-06
102934_s_at	Cdc25c	cell division cycle 25 homolog C (S. pombe)	-1.40	1.99E-05
97411_at	Ect2	ect2 oncogene	-1.40	2.91E-05
95665_at	Sec14l1	SEC14-like 1 (S. cerevisiae)	-1.40	1.39E-05
94362_at	Nras	neuroblastoma ras oncogene	-1.40	6.43E-06
97383_at	Slc6a6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	-1.40	1.04E-05
98766_at	Sh3bp5	SH3-domain binding protein 5 (BTK-associated)	-1.40	8.75E-06
97248_at	Dbi	diazepam binding inhibitor	-1.40	1.56E-06
160296_at	Wsb2	WD repeat and SOCS box-containing 2	-1.39	4.11E-05
95597_at	Ptgs1	prostaglandin-endoperoxide synthase 1	-1.39	5.08E-06
97148_at	Kifc1	Kinesin family member C1 (Kifc1), mRNA	-1.39	2.49E-05

95466_at	Cotl1	coactosin-like 1 (Dictyostelium)	-1.39	1.57E-03
103886_at	Reep5	receptor accessory protein 5	-1.39	3.60E-05
96042_at	Sod2	superoxide dismutase 2, mitochondrial	-1.39	5.77E-05
94697_at	Fgr	Gardner-Rasheed feline sarcoma viral (Fgr) oncogene homolog	-1.39	2.18E-05
99009_at	Nnt	nicotinamide nucleotide transhydrogenase	-1.39	4.93E-06
98586_at	Nap1l1	nucleosome assembly protein 1-like 1	-1.39	1.77E-04
97556_at	Anp32e	acidic (leucine-rich) nuclear phosphoprotein 32 family, member E	-1.38	3.40E-05
94217_f_at	Cdca3	cell division cycle associated 3	-1.38	3.46E-06
95349_g_at	Cxcl1	chemokine (C-X-C motif) ligand 1	-1.38	4.73E-05
162124_r_at	Rusc1	RUN and SH3 domain containing 1	-1.38	7.34E-05
160571_at	Idh1	isocitrate dehydrogenase 1 (NADP+), soluble	-1.38	1.24E-04
161524_r_at	Gpi1	glucose phosphate isomerase 1	-1.38	2.74E-05
102767_at	Gng12	guanine nucleotide binding protein (G protein), gamma 12	-1.38	4.17E-05
101877_at	Slc31a1	solute carrier family 31, member 1	-1.38	6.77E-05
101982_at	Vasp	vasodilator-stimulated phosphoprotein	-1.38	6.69E-06
102337_s_at	Fcgr2b	Fc receptor, IgG, low affinity IIb	-1.38	2.80E-03
96885_at	Nt5dc2	5'-nucleotidase domain containing 2	-1.37	3.77E-06
93015_at	Gsta3	glutathione S-transferase, alpha 3	-1.37	6.68E-05
98828_at	Itgam	integrin alpha M	-1.37	3.57E-04
160580_at	Man1a	mannosidase 1, alpha	-1.37	4.30E-05
95117_at	Igf2r	insulin-like growth factor 2 receptor	-1.37	3.69E-06
95753_at	Ncaph	non-SMC condensin I complex, subunit H	-1.37	4.80E-06
92858_at	Slpi	secretory leukocyte peptidase inhibitor	-1.37	1.33E-05
93131_at	Galc	galactosylceramidase	-1.37	9.65E-05
160648_at	Fign11	fidgetin-like 1	-1.37	6.80E-06
98053_at	Ywhab	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide	-1.37	8.95E-05
160469_at	LOC640441 /// Thbs1	similar to thrombospondin 1 /// thrombospondin 1	-1.37	2.56E-03
93250_r_at	Hmgb2 /// OTTMUSG00000011058	high mobility group box 2 /// predicted gene, OTTMUSG00000011058	-1.36	1.04E-04
100949_at	Fam20c	family with sequence similarity 20, member C	-1.36	3.92E-06
98989_at	*Dhcr7*	7-dehydrocholesterol reductase	-1.36	1.14E-05
103619_at	Cyb5b	cytochrome b5 type B	-1.36	2.59E-05
102334_at	Dok2	docking protein 2	-1.36	6.38E-06
93306_at	Mapre1	microtubule-associated protein, RP/EB family, member 1	-1.36	9.02E-06
98060_at	Lmna	lamin A	-1.36	1.25E-05
101964_at	Tkt	transketolase	-1.36	4.80E-05
94361_at	Ddx21	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21	-1.36	4.66E-05
103440_at	Gabpa	GA repeat binding protein, alpha	-1.36	2.57E-06

103091_at	Relb	avian reticuloendotheliosis viral (v-rel) oncogene related B	-1.36	3.37E-06
97273_at	Srrt	serrate RNA effector molecule homolog (Arabidopsis)	-1.36	4.57E-06
104735_at	Kctd12	potassium channel tetramerisation domain containing 12	-1.36	2.59E-05
97468_at	Cks1b	CDC28 protein kinase 1b	-1.36	4.22E-06
95288_i_at	Ccdc88a	coiled coil domain containing 88A	-1.36	1.21E-03
160378_at	Tmem109	transmembrane protein 109	-1.35	3.67E-05
102209_at	Nfatc1	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	-1.35	6.43E-06
104755_at	Tnip1	TNFAIP3 interacting protein 1	-1.35	2.30E-05
97197_r_at	AI506816	expressed sequence AI506816	-1.35	1.01E-03
93298_at	Papss1	3'-phosphoadenosine 5'-phosphosulfate synthase 1	-1.35	2.63E-05
162264_s_at	Bub1	budding uninhibited by benzimidazoles 1 homolog (S. cerevisiae)	-1.35	8.36E-06
96215_f_at	---	---	-1.35	7.59E-06
98630_at	*Nsdhl*	NAD(P) dependent steroid dehydrogenase-like	-1.35	8.74E-06
94963_at	Vcl	vinculin	-1.35	2.63E-05
101464_at	Timp1	tissue inhibitor of metalloproteinase 1	-1.35	1.15E-05
160111_at	Eif1ay	eukaryotic translation initiation factor 1A, Y-linked	-1.35	3.60E-05
95596_at	Foxk2	forkhead box K2	-1.35	2.36E-05
99800_at	L1cam	L1 cell adhesion molecule	-1.34	7.76E-06
96677_at	Setd8	SET domain containing (lysine methyltransferase) 8	-1.34	8.78E-05
98114_at	Npc1	Niemann Pick type C1	-1.34	1.04E-05
93676_at	Rad51ap1	RAD51 associated protein 1	-1.34	3.41E-05
102101_f_at	---	---	-1.34	1.05E-03
101029_f_at	Actc1 /// LOC100048431	actin, alpha, cardiac muscle 1 /// similar to alpha-actin (AA 27-375)	-1.34	3.94E-05
161684_r_at	Lcn2	lipocalin 2	-1.34	1.44E-04
160246_at	Tnfaip8	tumor necrosis factor, alpha-induced protein 8	-1.34	1.01E-05
102142_r_at	OTTMUSG00000017864	predicted gene, OTTMUSG00000017864	-1.34	1.40E-04
93860_i_at	EG622147	predicted gene, EG622147	-1.34	2.99E-05
99592_f_at	Rdh11	retinol dehydrogenase 11	-1.34	1.04E-05
162417_at	1500001M20Rik	RIKEN cDNA 1500001M20 gene	-1.33	4.77E-04
160234_at	Usp1	ubiquitin specific peptidase 1	-1.33	3.48E-05
97967_at	Plxnd1	plexin D1	-1.33	2.94E-05
103499_at	Vwf	Von Willebrand factor homolog	-1.33	2.15E-05
92580_at	Hars	histidyl-tRNA synthetase	-1.33	3.00E-05
92845_at	Oxct1	3-oxoacid CoA transferase 1	-1.33	1.02E-05
103001_at	Vegfb	vascular endothelial growth factor B	-1.33	3.43E-05
161390_r_at	Pou6f1	POU domain, class 6, transcription factor 1	-1.33	9.15E-04
98489_at	Dlgap5	discs, large (Drosophila) homolog-associated protein 5	-1.33	8.74E-06

160517_at	Lmnb1	lamin B1	-1.33	7.76E-06
102111_f_at	Rpp30	Ribonuclease P/MRP 30 subunit (human), mRNA (cDNA clone IMAGE:5010429)	-1.33	7.91E-05
103204_r_at	E2f8	E2F transcription factor 8	-1.32	2.41E-04
96674_at	Tnpo3	transportin 3	-1.32	4.54E-05
94504_at	Slc48a1	solute carrier family 48 (heme transporter), member 1	-1.32	7.50E-06
101918_at	Tgfb1	transforming growth factor, beta 1	-1.32	7.18E-05
92354_at	Maf	avian musculoaponeurotic fibrosarcoma (v-maf) AS42 oncogene homolog	-1.32	9.25E-05
103847_at	LOC100039753 /// LOC100039914 /// LOC100039935	similar to putative /// hypothetical protein LOC100039914 /// similar to putative	-1.32	1.95E-03
98525_f_at	Erdr1	erythroid differentiation regulator 1	-1.32	7.37E-05
160916_at	Eepd1	endonuclease/exonuclease/phosphatase family domain containing 1	-1.32	9.72E-05
93852_at	Mef2a	myocyte enhancer factor 2A	-1.32	3.59E-05
101458_at	Wee1	WEE 1 homolog 1 (S. pombe)	-1.32	1.89E-04
104221_at	LOC100047619 /// Slc7a5	similar to solute carrier family 7 (cationic amino acid transporter, y+ system), member 5 /// solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	-1.32	1.33E-05
104725_at	Rhoq	ras homolog gene family, member Q	-1.32	3.24E-05
160698_s_at	Prkcd	protein kinase C, delta	-1.32	3.00E-05
96725_at	Cic	capicua homolog (Drosophila)	-1.32	4.14E-04
160338_at	Ankrd13a	ankyrin repeat domain 13a	-1.32	3.55E-04
93943_f_at	Zfp36l2	zinc finger protein 36, C3H type-like 2	-1.32	2.33E-05
104671_at	Ampd3	adenosine monophosphate deaminase 3	-1.31	2.12E-04
93939_at	LOC100047863 /// Sh2b3	similar to lymphocyte-specific adaptor protein Lnk /// SH2B adaptor protein 3	-1.31	1.71E-04
99045_at	Eno2	enolase 2, gamma neuronal	-1.31	1.21E-03
102944_at	G3bp1	Ras-GTPase-activating protein SH3-domain binding protein 1	-1.31	2.84E-05
100569_at	Anxa2	annexin A2	-1.31	1.07E-05
97425_at	Slc44a1	solute carrier family 44, member 1	-1.31	3.09E-05
160377_at	Tardbp	TAR DNA binding protein	-1.31	1.77E-05
93507_at	Timp2	tissue inhibitor of metalloproteinase 2	-1.31	1.16E-05
160739_at	Wnk1	WNK lysine deficient protein kinase 1	-1.31	5.31E-03
100535_at	Eif4g2	eukaryotic translation initiation factor 4, gamma 2	-1.31	3.47E-05
101589_at	Hmgn2	high mobility group nucleosomal binding domain 2	-1.31	1.03E-03
98469_at	Aurkb	aurora kinase B	-1.31	4.19E-05
92762_at	Clec4a2	C-type lectin domain family 4, member a2	-1.31	1.31E-05
99329_at	Abcc1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	-1.31	1.09E-05
92193_r_at	Pcsk5	proprotein convertase subtilisin/kexin type 5	-1.31	6.00E-05
160409_at	Pitpna	phosphatidylinositol transfer protein, alpha	-1.31	8.44E-05
96526_at	D030029J20Rik	RIKEN cDNA D030029J20 gene	-1.31	1.14E-05

161062_r_at	Rufy3	RUN and FYVE domain containing 3	-1.31	4.10E-04
92540_f_at	Srm	spermidine synthase	-1.31	7.86E-06
95435_at	Arl8b	ADP-ribosylation factor-like 8B	-1.31	1.23E-04
97885_at	Tmem176b	transmembrane protein 176B	-1.31	7.90E-05
92603_at	Atp6v0d1	ATPase, H+ transporting, lysosomal V0 subunit D1	-1.31	6.79E-05
99597_at	Gnai2	guanine nucleotide binding protein (G protein), alpha inhibiting 2	-1.31	7.11E-05
103562_f_at	LOC67527	murine leukemia retrovirus	-1.31	3.91E-04
95468_at	Egln1	EGL nine homolog 1 (C. elegans)	-1.30	3.84E-05
99838_at	Aoah	acyloxyacyl hydrolase	-1.30	9.50E-05
99580_s_at	Ugt1a1 /// Ugt1a10 /// Ugt1a2 /// Ugt1a5 /// Ugt1a6a /// Ugt1a6b /// Ugt1a7c /// Ugt1a9	UDP glucuronosyltransferase 1 family, polypeptide A1 /// UDP glycosyltransferase 1 family, polypeptide A10 /// UDP glucuronosyltransferase 1 family, polypeptide A2 /// UDP glucuronosyltransferase 1 family, polypeptide A5 /// UDP glucuronosyltransferase 1 family, polypeptide A6A /// UDP glucuronosyltransferase 1 family, polypeptide A6B /// UDP glucuronosyltransferase 1 family, polypeptide A7C /// UDP glucuronosyltransferase 1 family, polypeptide A9	-1.30	2.15E-05
95156_g_at	Znrf1	zinc and ring finger 1	-1.30	5.68E-05
103065_at	Slc20a1	solute carrier family 20, member 1	-1.30	1.12E-05
99985_at	Txnrd1	thioredoxin reductase 1	-1.30	1.52E-05
160820_at	Igsf8	immunoglobulin superfamily, member 8	-1.30	1.12E-04
103370_at	Lin7c	lin-7 homolog C (C. elegans)	-1.30	1.25E-04
103642_at	G3bp1	Ras-GTPase-activating protein SH3-domain binding protein 1	-1.30	3.04E-05
103270_at	Gtse1	G two S phase expressed protein 1	-1.30	3.43E-05
99448_at	Tln1	talin 1	-1.30	1.11E-05
93829_at	Rod1	ROD1 regulator of differentiation 1 (S. pombe)	-1.30	6.06E-04
101030_at	Rhob	ras homolog gene family, member B	-1.30	1.72E-04
101632_at	Tnfrsf11a	tumor necrosis factor receptor superfamily, member 11a	-1.30	4.80E-05
93889_f_at	Hist1h2ba	histone cluster 1, H2ba	-1.30	6.17E-05
161348_r_at	EG545743 /// LOC100048338 /// Pdlim1	predicted gene, EG545743 /// similar to Pdlim1 protein /// PDZ and LIM domain 1 (elfin)	-1.30	2.39E-04
97906_at	Siah2	seven in absentia 2	-1.30	2.28E-04
100915_at	Myh9	myosin, heavy polypeptide 9, non-muscle	-1.30	9.68E-05
101882_s_at	Col18a1	collagen, type XVIII, alpha 1	-1.30	7.06E-05
94174_at	Ctnnal1	catenin (cadherin associated protein), alpha-like 1	-1.29	2.00E-04
98996_at	Plk4	polo-like kinase 4 (Drosophila)	-1.29	1.24E-04

95493_at	Col6a1	collagen, type VI, alpha 1	-1.29	2.22E-05
100595_at	Ptp4a2	protein tyrosine phosphatase 4a2	-1.29	6.68E-05
98151_s_at	Ctnnd1	catenin (cadherin associated protein), delta 1	-1.29	1.43E-04
160167_at	Nup62	nucleoporin 62	-1.29	1.86E-05
98427_s_at	Nfkb1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105	-1.29	3.92E-05
94255_g_at	Clic4	chloride intracellular channel 4 (mitochondrial)	-1.29	8.65E-05
95791_s_at	Sfrs2	splicing factor, arginine/serine-rich 2 (SC-35)	-1.29	6.76E-05
93861_f_at	EG622147	predicted gene, EG622147	-1.29	4.35E-05
93193_at	Adrb2	adrenergic receptor, beta 2	-1.29	1.20E-04
94405_at	Slc6a6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	-1.28	2.78E-04
94964_at	Vcl	vinculin	-1.28	1.82E-04
160579_at	Man1a	mannosidase 1, alpha	-1.28	1.98E-04
104501_at	Vapb	vesicle-associated membrane protein, associated protein B and C	-1.28	1.74E-05
99632_at	Mad2l1	MAD2 mitotic arrest deficient-like 1 (yeast)	-1.28	4.72E-05
99120_f_at	Chd4	chromodomain helicase DNA binding protein 4	-1.28	1.07E-04
162360_f_at	Mtmr14	myotubularin related protein 14	-1.28	4.34E-04
94004_at	Cnn2	calponin 2	-1.28	2.95E-03
93749_at	Maoa	monoamine oxidase A	-1.28	1.16E-04
102144_f_at	---	---	-1.28	4.94E-04
92256_at	Ctsb	cathepsin B	-1.28	3.59E-04
160353_i_at	Mapkapk2	MAP kinase-activated protein kinase 2	-1.28	6.91E-05
99638_at	Col18a1	collagen, type XVIII, alpha 1	-1.28	3.95E-05
102280_at	Pcdh7	protocadherin 7	-1.28	8.50E-05
94238_at	Prss23	protease, serine, 23	-1.28	2.75E-04
99138_at	Rcc1	regulator of chromosome condensation 1	-1.28	4.54E-05
160430_at	Ctnnb1	catenin (cadherin associated protein), beta 1	-1.28	5.79E-05
103713_at	Usp9x	ubiquitin specific peptidase 9, X chromosome	-1.27	3.40E-05
160785_at	Arhgap21	Rho GTPase activating protein 21	-1.27	2.09E-04
160988_r_at	Fubp1	far upstream element (FUSE) binding protein 1	-1.27	1.67E-04
161931_r_at	---	---	-1.27	3.04E-04
103275_at	Atp6v0a1	ATPase, H+ transporting, lysosomal V0 subunit A1	-1.27	3.08E-05
100425_at	Syk	spleen tyrosine kinase	-1.27	1.00E-04
99439_at	LOC100048871 /// Mas1	similar to MAS1 oncogene /// MAS1 oncogene	-1.27	6.75E-05
160682_at	6430706D22Rik	RIKEN cDNA 6430706D22 gene	-1.27	4.07E-05
100020_at	Slc4a2	solute carrier family 4 (anion exchanger), member 2	-1.27	9.45E-05
100548_at	Pea15a	phosphoprotein enriched in astrocytes 15A	-1.27	3.69E-05
102193_at	Exod1	exonuclease domain containing 1	-1.27	2.33E-05

101349_at	Plk1	polo-like kinase 1 (Drosophila)	-1.27	3.13E-04
96811_at	Rab31	RAB31, member RAS oncogene family	-1.27	6.95E-05
101040_at	Capn2	calpain 2	-1.27	3.97E-05
94548_at	Mfsd1	major facilitator superfamily domain containing 1	-1.27	2.13E-05
95134_at	Mid1ip1	Mid1 interacting protein 1 (gastrulation specific G12-like (zebrafish))	-1.27	1.09E-03
93604_f_at	Cadm1	cell adhesion molecule 1	-1.27	3.00E-05
102221_at	Syngr1	synaptogyrin 1	-1.27	2.46E-05
101546_at	Neu1	neuraminidase 1	-1.27	1.67E-04
103308_at	C79407	expressed sequence C79407	-1.27	4.17E-05
103855_at	Plec1	plectin 1	-1.27	3.82E-03
98552_at	Pmf1	polyamine-modulated factor 1	-1.27	7.12E-05
160091_at	Pgam1	phosphoglycerate mutase 1	-1.27	3.68E-04
98044_at	Dab2	disabled homolog 2 (Drosophila)	-1.27	8.39E-05
96519_at	Pdxk	pyridoxal (pyridoxine, vitamin B6) kinase	-1.27	2.31E-04
94985_at	Syncrip	synaptotagmin binding, cytoplasmic RNA interacting protein	-1.27	3.20E-04
92983_at	OTTMUSG00000014243 /// OTTMUSG00000014245 /// Ptprj	predicted gene, OTTMUSG00000014243 /// predicted gene, OTTMUSG00000014245 /// protein tyrosine phosphatase, receptor type, J	-1.27	8.25E-05
92773_at	Nrp1	neuropilin 1	-1.27	1.36E-04
94063_at	Sema4a	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A	-1.27	5.89E-05
103529_at	Tnfaip8l1	tumor necrosis factor, alpha-induced protein 8-like 1	-1.27	3.59E-05
93803_at	Psme3	proteaseome (prosome, macropain) 28 subunit, 3	-1.27	3.35E-04
160568_at	Eno1	enolase 1, alpha non-neuron	-1.26	1.00E-04
96285_at	Myadm	myeloid-associated differentiation marker	-1.26	3.95E-05
94768_at	Rad21	RAD21 homolog (S. pombe)	-1.26	2.81E-04
94351_r_at	Nqo1	NAD(P)H dehydrogenase, quinone 1	-1.26	3.50E-03
93276_at	Hn1	hematological and neurological expressed sequence 1	-1.26	1.00E-04
160462_f_at	Tubb3	tubulin, beta 3	-1.26	2.96E-05
94147_at	Serpine1	serine (or cysteine) peptidase inhibitor, clade E, member 1	-1.26	2.08E-04
161172_f_at	Ncaph	non-SMC condensin I complex, subunit H	-1.26	8.69E-03
160676_at	Lrrc58	leucine rich repeat containing 58	-1.26	1.18E-04
96862_at	1110002B05Rik	RIKEN cDNA 1110002B05 gene	-1.26	3.75E-04
94435_at	LOC100047601 /// Nus1	similar to DNA segment, Chr 10, ERATO Doi 438, expressed /// nuclear undecaprenyl pyrophosphate synthase 1 homolog (S. cerevisiae)	-1.26	7.48E-03
160970_at	LOC100047199 /// Odf2	similar to outer dense fiber of sperm tails 2 /// outer dense fiber of sperm tails 2	-1.26	8.48E-05
101913_at	Clcn5	chloride channel 5	-1.26	5.55E-05

101954_at	H2afz	H2A histone family, member Z	-1.26	4.09E-04
161161_r_at	LOC100046344 /// Nme1	similar to Nucleoside diphosphate kinase A (NDK A) (NDP kinase A) (Tumor metastatic process-associated protein) (Metastasis inhibition factor NM23) (NDPK-A) (nm23-M1) /// non-metastatic cells 1, protein (NM23A) expressed in	-1.26	1.05E-03
98602_at	Rangap1	RAN GTPase activating protein 1	-1.26	5.46E-05
104531_at	Prkcd	protein kinase C, delta	-1.26	7.59E-05
102001_at	Rrm2	ribonucleotide reductase M2	-1.26	8.13E-05
93102_f_at	Actg2	actin, gamma 2, smooth muscle, enteric	-1.26	3.44E-05
103553_at	Mcm10	minichromosome maintenance deficient 10 (S. cerevisiae)	-1.26	8.78E-05
94511_at	LOC636537 /// Ssr1	similar to signal sequence receptor, alpha /// signal sequence receptor, alpha	-1.26	1.43E-04
160371_at	Arl6ip1	ADP-ribosylation factor-like 6 interacting protein 1	-1.26	3.50E-05
94384_at	Ier3	immediate early response 3	-1.26	1.36E-04
95457_at	Impad1	inositol monophosphatase domain containing 1	-1.26	3.52E-04
160726_at	Qk	quaking	-1.26	3.63E-03
104036_at	Dpp7	dipeptidylpeptidase 7	-1.25	7.79E-05
97706_at	---	---	-1.25	1.56E-04
93850_at	Iqgap1	IQ motif containing GTPase activating protein 1	-1.25	1.59E-02
103039_at	Itga5	integrin alpha 5 (fibronectin receptor alpha)	-1.25	2.22E-04
162097_r_at	Cdh3	cadherin 3	-1.25	1.91E-04
98587_at	Nap1l1	nucleosome assembly protein 1-like 1	-1.25	2.41E-04
160326_at	Cdv3	carnitine deficiency-associated gene expressed in ventricle 3	-1.25	2.57E-04
95137_at	Tmem97	transmembrane protein 97	-1.25	2.92E-04
96211_at	Dpp8	dipeptidylpeptidase 8	-1.25	2.59E-04
92637_at	Pfkl	phosphofructokinase, liver, B-type	-1.25	3.07E-04
102936_at	B4galt6	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 6, mRNA (cDNA clone MGC:19296 IMAGE:4037384)	-1.25	1.70E-03

Appendix 2. - List of the 630 up regulated genes by mCMV in BMDMs after 24 hrs infection

Probe Set ID	Gene Symbol	Gene Title	FC	P-Value
98406_at	Ccl5	chemokine (C-C motif) ligand 5	10.61	1.97E-12
100944_at	AW112010	expressed sequence AW112010	7.04	3.29E-12
98373_at	Ms4a4c	membrane-spanning 4-domains, subfamily A, member 4C	6.00	1.67E-11

92315_at	Slfn4	schlafen 4	4.84	5.15E-10
102906_at	Tgtp /// Tgtp2	T-cell specific GTPase /// T-cell specific GTPase 2	4.51	3.11E-11
102264_at	Slfn1	schlafen 1	4.08	8.47E-10
98299_s_at	Slfn3 /// Slfn4	schlafen 3 /// schlafen 4	3.69	4.91E-09
161968_f_at	Ccr5	chemokine (C-C motif) receptor 5	3.68	8.02E-11
103432_at	Isg20	interferon-stimulated protein	3.36	5.09E-10
96764_at	Iigp1	interferon inducible GTPase 1	3.30	9.79E-11
95303_at	Ifitm6	interferon induced transmembrane protein 6	3.28	1.52E-09
101793_at	Fcgr1	Fc receptor, IgG, high affinity I	3.06	5.28E-10
104669_at	Irf7	interferon regulatory factor 7	3.02	8.02E-11
93078_at	Ly6a	lymphocyte antigen 6 complex, locus A	2.99	5.15E-10
103639_at	Ifit2	interferon-induced protein with tetratricopeptide repeats 2	2.95	9.89E-10
95471_at	Cdkn1c	cyclin-dependent kinase inhibitor 1C (P57)	2.91	6.34E-08
100484_at	Mmp13	matrix metallopeptidase 13	2.90	8.36E-10
92689_at	Il18bp	interleukin 18 binding protein	2.88	4.57E-10
93397_at	Ccr2	chemokine (C-C motif) receptor 2	2.84	9.57E-08
93717_at	Ccl12	chemokine (C-C motif) ligand 12	2.81	4.02E-09
95508_at	Nckap1	NCK-associated protein 1	2.74	2.63E-09
102879_s_at	Fcgr1	Fc receptor, IgG, high affinity I	2.73	4.57E-10
94247_at	Dsp	desmoplakin	2.60	8.47E-10
102718_at	Ccr5	chemokine (C-C motif) receptor 5	2.56	1.26E-09
161511_f_at	677168 /// Isg15	predicted gene, 677168 /// ISG15 ubiquitin-like modifier	2.49	1.10E-08
101845_s_at	Csprs /// EG665317 /// EG665378 /// LOC100041022 /// LOC100041903	component of Sp100-rs /// predicted gene, EG665317 /// predicted gene, EG665378 /// similar to HSR /// similar to putative G-protein coupled receptor	2.48	8.27E-08
95743_at	Paip2	polyadenylate-binding protein-interacting protein 2	2.45	1.00E-08
98417_at	Mx1	myxovirus (influenza virus) resistance 1	2.44	6.00E-09
93956_at	Ifit3	interferon-induced protein with tetratricopeptide repeats 3	2.42	2.32E-09
102712_at	Saa3	serum amyloid A 3	2.41	4.86E-08
92406_at	Cd7	CD7 antigen	2.41	3.11E-08
93511_at	Itm2a	integral membrane protein 2A	2.41	8.59E-09
101878_at	Cd72	CD72 antigen	2.37	2.36E-09
104606_at	Cd52	CD52 antigen	2.36	8.65E-10
93865_s_at	H2-T10 /// H2-T17 /// H2-T22 /// H2-T9	histocompatibility 2, T region locus 10 /// histocompatibility 2, T region locus 17 /// histocompatibility 2, T region locus 22 /// histocompatibility 2, T region locus 9	2.36	1.23E-09
97322_at	Ms4a6b	membrane-spanning 4-domains, subfamily A, member 6B	2.30	1.09E-08
95338_s_at	Mmp12	matrix metallopeptidase 12	2.29	3.88E-09
162202_f_at	Irf7	interferon regulatory factor 7	2.27	1.78E-08

102254_f_at	LOC625360	similar to 2-cell-stage, variable group, member 3	2.26	1.29E-07
101876_s_at	H2-T10 /// H2-T17 /// H2-T22 /// H2-T9	histocompatibility 2, T region locus 10 /// histocompatibility 2, T region locus 17 /// histocompatibility 2, T region locus 22 /// histocompatibility 2, T region locus 9	2.26	2.32E-09
93563_s_at	Nid2	nidogen 2	2.26	7.82E-08
99583_at	Gstp1	glutathione S-transferase, pi 1	2.22	2.36E-09
97844_at	Rgs2	regulator of G-protein signaling 2	2.17	1.51E-08
98092_at	Plac8	placenta-specific 8	2.16	3.96E-08
101658_f_at	H2-Q8	histocompatibility 2, Q region locus 8	2.14	1.46E-08
94158_f_at	Ptafr	platelet-activating factor receptor	2.14	2.52E-07
93779_at	100039742	predicted gene, 100039742	2.14	4.66E-09
103963_f_at	Iigp1	interferon inducible GTPase 1	2.11	4.25E-08
93451_at	Lmo7	LIM domain only 7	2.11	1.41E-07
98472_at	C920025E04Rik /// H2-T23	RIKEN cDNA C920025E04 gene /// histocompatibility 2, T region locus 23	2.10	5.07E-09
101436_at	Cxcl9	chemokine (C-X-C motif) ligand 9	2.10	7.08E-08
98822_at	Isg15	ISG15 ubiquitin-like modifier	2.08	7.40E-09
95024_at	LOC100048346 /// Usp18	similar to ubiquitin specific protease UBP43 /// ubiquitin specific peptidase 18	2.07	4.66E-09
94425_at	Ly86	lymphocyte antigen 86	2.07	8.59E-09
92642_at	Car2	carbonic anhydrase 2	2.06	1.55E-08
92989_f_at	Cadps	Ca2+-dependent secretion activator	2.05	3.46E-07
160150_f_at	Cnn3 /// LOC100047856	calponin 3, acidic /// similar to calponin 3, acidic	2.04	2.47E-08
101429_at	Ddit3	DNA-damage inducible transcript 3	2.02	3.21E-07
95339_r_at	Mmp12	matrix metallopeptidase 12	2.00	1.95E-08
104464_s_at	Kdelr3	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3	2.00	1.08E-06
160934_s_at	Sgip1	SH3-domain GRB2-like (endophilin) interacting protein 1	1.98	6.84E-08
103029_at	Pdcd4	programmed cell death 4	1.97	1.55E-08
96766_s_at	LOC100048488 /// Tyro3	similar to Rse /// TYRO3 protein tyrosine kinase 3	1.96	1.59E-06
160933_at	Igtp	interferon gamma induced GTPase	1.95	7.31E-08
103517_at	A530040E14Rik	RIKEN cDNA A530040E14 gene	1.95	3.07E-08
93497_at	C3	complement component 3	1.95	3.15E-07
94224_s_at	100040462 /// Ifi203 /// Ifi204 /// Ifi205 /// LOC192690 /// LOC640890 /// Mnda	predicted gene, 100040462 /// interferon activated gene 203 /// interferon activated gene 204 /// interferon activated gene 205 /// similar to interferon activated gene 205 /// similar to Interferon-activatable protein 205 (IFI-205) (D3 protein) /// myeloid cell nuclear differentiation antigen	1.94	3.96E-08
102330_at	Aif1	allograft inflammatory factor 1	1.94	3.09E-08
103446_at	Ifih1	interferon induced with helicase C domain 1	1.93	2.42E-08
104597_at	Gbp2	guanylate binding protein 2	1.93	7.11E-09

92786_at	Efhd1	EF hand domain containing 1	1.91	1.36E-07
103035_at	Tap1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	1.90	1.34E-08
93389_at	Prom1	prominin 1	1.90	2.15E-06
93268_at	Glo1	glyoxalase 1	1.89	1.81E-07
99378_f_at	H2-gs10	MHC class I like protein GS10	1.87	8.36E-09
102161_f_at	EG630499 /// H2-D1 /// H2-K1 /// H2-L /// H2-Q2 /// H2-T17 /// H2-T22 /// H2-T23 /// H2-t9 /// LOC547349 /// LOC676708	predicted gene, EG630499 /// histocompatibility 2, D region locus 1 /// histocompatibility 2, K1, K region /// histocompatibility 2, D region /// histocompatibility 2, Q region locus 2 /// histocompatibility 2, T region locus 17 /// histocompatibility 2, T region locus 22 /// histocompatibility 2, T region locus 23 /// MHC class Ib T9 /// similar to MHC class I antigen precursor /// similar to H-2 class I histocompatibility antigen, L-D alpha chain precursor	1.87	8.29E-09
104750_at	Ifi47	interferon gamma inducible protein 47	1.87	2.42E-08
92988_i_at	Cadps	Ca2+-dependent secretion activator	1.87	4.83E-05
99379_f_at	LOC676689	similar to H-2 class I histocompatibility antigen, L-D alpha chain precursor	1.86	5.31E-08
97173_f_at	LOC676708	similar to H-2 class I histocompatibility antigen, L-D alpha chain precursor	1.86	2.60E-08
93445_at	Cd5l	CD5 antigen-like	1.86	2.13E-08
102208_at	St3gal6	ST3 beta-galactoside alpha-2,3-sialyltransferase 6	1.86	5.02E-08
93269_at	Glo1	glyoxalase 1	1.85	4.37E-08
103571_at	Lst1	leukocyte specific transcript 1	1.85	7.38E-08
103080_at	Samhd1	SAM domain and HD domain, 1	1.85	1.24E-07
97541_f_at	H2-D1 /// H2-K1 /// LOC100044874	histocompatibility 2, D region locus 1 /// histocompatibility 2, K1, K region /// similar to H-2K(d) antigen	1.84	1.55E-08
96088_at	Ndrg2	N-myc downstream regulated gene 2	1.83	8.01E-07
94774_at	Ifi202b	interferon activated gene 202B	1.81	2.48E-07
92644_s_at	Myb	myeloblastosis oncogene	1.81	3.69E-06
94159_at	Ptafr	platelet-activating factor receptor	1.81	2.37E-07
93898_at	Sgcb	sarcoglycan, beta (dystrophin-associated glycoprotein)	1.80	6.15E-08
103202_at	Gbp3	guanylate binding protein 3	1.79	6.33E-08
94746_at	H2-T24	histocompatibility 2, T region locus 24	1.78	3.93E-07
97507_at	Lgals3bp	lectin, galactoside-binding, soluble, 3 binding protein	1.77	1.55E-08
100706_f_at	Sfmbt2	Scm-like with four mbt domains 2	1.77	4.27E-07
95523_at	2900062L11Rik /// 6530401D17Rik	RIKEN cDNA 2900062L11 gene /// RIKEN cDNA 6530401D17 gene	1.77	1.56E-05
96055_at	Cck	cholecystokinin	1.77	7.07E-07
98423_at	Gjb2	gap junction protein, beta 2	1.77	2.10E-06
96900_at	Chchd10	coiled-coil-helix-coiled-coil-helix domain containing 10	1.77	1.55E-06

99956_at	Kit	kit oncogene	1.76	3.37E-08
99607_at	Skp1a	S-phase kinase-associated protein 1A	1.75	2.20E-05
160264_s_at	Pcp4l1	Purkinje cell protein 4-like 1	1.74	1.37E-06
92866_at	H2-Aa	histocompatibility 2, class II antigen A, alpha	1.72	3.02E-08
103235_at	Npy	neuropeptide Y	1.71	3.70E-08
103066_at	Cmpk2	cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial	1.70	1.32E-06
98465_f_at	Ifi204	interferon activated gene 204	1.69	1.24E-07
101510_at	Psme1	proteasome (prosome, macropain) 28 subunit, alpha	1.69	5.80E-08
97949_at	Fgl2	fibrinogen-like protein 2	1.69	2.76E-05
97125_f_at	H2-K1	histocompatibility 2, K1, K region	1.69	8.23E-08
93085_at	Psmb9	proteasome (prosome, macropain) subunit, beta type 9 (large multifunctional peptidase 2)	1.68	3.37E-08
97772_at	Plau	plasminogen activator, urokinase	1.68	1.97E-04
94085_at	Srgn	serglycin	1.68	1.31E-06
161504_i_at	Cisd1	CDGSH iron sulfur domain 1	1.68	2.36E-05
93077_s_at	LOC100045833 /// Ly6c1 /// Ly6c2	similar to Lymphocyte antigen 6C precursor (Ly-6C) /// lymphocyte antigen 6 complex, locus C1 /// lymphocyte antigen 6 complex, locus C2	1.68	5.90E-08
100981_at	Ifit1	interferon-induced protein with tetratricopeptide repeats 1	1.68	7.86E-08
101054_at	Cd74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	1.67	9.42E-07
100154_at	Tapbp	TAP binding protein	1.67	6.33E-08
95974_at	Gbp1	guanylate binding protein 1	1.66	2.78E-07
101424_at	Nmi	N-myc (and STAT) interactor	1.66	1.41E-07
93871_at	Il1rn	interleukin 1 receptor antagonist	1.66	1.09E-06
98801_at	Klra2	killer cell lectin-like receptor, subfamily A, member 2	1.66	6.66E-07
102904_at	H2-Ea	histocompatibility 2, class II antigen E alpha	1.66	1.19E-06
160108_at	Nupr1	nuclear protein 1	1.66	1.60E-07
102689_at	Tapbp	TAP binding protein	1.66	3.46E-07
98438_f_at	H2-Q7	histocompatibility 2, Q region locus 7	1.66	5.68E-07
104696_at	Ctse	cathepsin E	1.65	3.29E-06
103422_at	Cd1d1	CD1d1 antigen	1.65	3.87E-05
99816_at	Hspa2	heat shock protein 2	1.64	7.09E-07
103299_at	Pld4	phospholipase D family, member 4	1.64	6.56E-08
102237_at	Cd28	CD28 antigen	1.64	6.86E-06
96920_at	Htra1	HtrA serine peptidase 1	1.63	1.98E-06
93560_at	Acyp1	acylphosphatase 1, erythrocyte (common) type	1.63	1.24E-07
99030_at	Il7r	interleukin 7 receptor	1.63	3.20E-07
102104_f_at	Ms4a6c	membrane-spanning 4-domains, subfamily	1.63	2.28E-07

		A, member 6C		
97409_at	Irgm1	immunity-related GTPase family M member 1	1.63	1.36E-07
102360_at	Mthfr	5,10-methylenetetrahydrofolate reductase	1.63	9.13E-07
103816_at	F11r	F11 receptor	1.63	3.29E-07
96956_at	Prdx5	peroxiredoxin 5	1.62	2.61E-06
100127_at	Crabp2	cellular retinoic acid binding protein II	1.62	3.09E-06
100134_at	Eng	endoglin	1.62	2.49E-07
92310_at	Plk2	polo-like kinase 2 (Drosophila)	1.61	2.88E-05
92472_f_at	Slfn2	schlafen 2	1.61	3.74E-06
92356_at	Ptpn22	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	1.61	2.50E-07
101886_f_at	H2-D1	histocompatibility 2, D region locus 1	1.60	1.60E-07
160921_at	Acss1	acyl-CoA synthetase short-chain family member 1	1.59	2.17E-06
104417_at	Flt4	FMS-like tyrosine kinase 4	1.59	2.28E-04
92459_at	Ccl8	chemokine (C-C motif) ligand 8	1.59	6.23E-07
97477_at	Timm8b	translocase of inner mitochondrial membrane 8 homolog b (yeast)	1.59	1.60E-06
93909_f_at	100043775 /// 100043821 /// BC094435 /// Ccrn4l /// LOC280487 /// Sgip1	predicted gene, 100043775 /// predicted gene, 100043821 /// cDNA sequence BC094435 /// CCR4 carbon catabolite repression 4-like (S. cerevisiae) /// pol polyprotein /// SH3-domain GRB2-like (endophilin) interacting protein 1	1.58	7.31E-07
102279_at	Ube1l	ubiquitin-activating enzyme E1-like	1.58	3.46E-07
97504_at	Ccnd2	cyclin D2	1.58	2.04E-05
92471_i_at	Slfn2	schlafen 2	1.57	7.14E-07
93908_f_at	100043775 /// 100043821 /// BC094435 /// Ccrn4l /// LOC280487 /// Sgip1	predicted gene, 100043775 /// predicted gene, 100043821 /// cDNA sequence BC094435 /// CCR4 carbon catabolite repression 4-like (S. cerevisiae) /// pol polyprotein /// SH3-domain GRB2-like (endophilin) interacting protein 1	1.57	3.53E-06
97297_at	Pcp4l1	Purkinje cell protein 4-like 1	1.57	4.31E-05
103043_at	Mtcp1	mature T-cell proliferation 1	1.57	2.64E-06
98410_at	Irgm2	immunity-related GTPase family M member 2	1.57	3.96E-07
101486_at	Psmb10	proteasome (prosome, macropain) subunit, beta type 10	1.57	1.22E-07
93893_f_at	Klra3	killer cell lectin-like receptor, subfamily A, member 3	1.57	5.79E-05
100554_at	EG545743 /// LOC100048338 /// Pdlim1	predicted gene, EG545743 /// similar to Pdlim1 protein /// PDZ and LIM domain 1 (elfin)	1.57	8.23E-07
102873_at	Tap2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	1.56	2.18E-06
98758_at	Alox15	arachidonate 15-lipoxygenase	1.56	4.54E-06
101653_f_at	H2-D4	histocompatibility 2, D region locus 4	1.56	1.10E-05
96912_s_at	Ctla2a /// Ctla2b	cytotoxic T lymphocyte-associated protein 2 alpha /// cytotoxic T lymphocyte-	1.55	6.74E-07

		associated protein 2 beta		
104155_f_at	Atf3	activating transcription factor 3	1.55	3.56E-07
97825_at	Perp	PERP, TP53 apoptosis effector	1.55	1.39E-05
102831_s_at	Cd86	CD86 antigen	1.55	5.03E-07
98405_at	Serpinb9	serine (or cysteine) peptidase inhibitor, clade B, member 9	1.55	7.25E-07
161173_f_at	Ifi202b	interferon activated gene 202B	1.54	8.01E-07
160617_at	Klf13	Kruppel-like factor 13	1.54	1.01E-06
93894_f_at	Klra3	killer cell lectin-like receptor, subfamily A, member 3	1.53	9.08E-06
94269_at	Rabac1	Rab acceptor 1 (prenylated)	1.53	3.12E-07
160774_at	Entpd1	ectonucleoside triphosphate diphosphohydrolase 1	1.53	9.74E-06
100013_at	Ifi35	interferon-induced protein 35	1.53	1.05E-06
100998_at	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	1.53	3.41E-07
104063_at	Tom1l1	target of myb1-like 1 (chicken)	1.53	1.04E-05
95064_at	Acaa2	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	1.52	2.65E-07
93324_at	Zfp36l1	zinc finger protein 36, C3H type-like 1	1.52	1.07E-05
98498_at	Casp7	caspase 7	1.52	9.80E-07
93838_at	2700038C09Rik	RIKEN cDNA 2700038C09 gene	1.52	1.08E-06
95019_at	Gstt1	glutathione S-transferase, theta 1	1.52	2.28E-05
97206_at	Spint1	serine protease inhibitor, Kunitz type 1	1.52	1.59E-06
102052_at	Abhd5	abhydrolase domain containing 5	1.52	5.25E-06
93321_at	Ifi203	interferon activated gene 203	1.51	4.98E-07
162172_f_at	Nedd4	neural precursor cell expressed, developmentally down-regulated 4	1.51	2.27E-06
98108_at	Crabp1	cellular retinoic acid binding protein I	1.51	2.12E-05
160230_at	Cox17	cytochrome c oxidase, subunit XVII assembly protein homolog (yeast)	1.51	2.57E-06
95662_at	Amz2	archaelysin family metallopeptidase 2	1.51	5.68E-07
101972_at	Napsa	napsin A aspartic peptidase	1.51	4.07E-07
95796_g_at	100041294 /// Supt4h1	predicted gene, 100041294 /// suppressor of Ty 4 homolog 1 (S. cerevisiae)	1.51	1.36E-06
97336_at	Ctsf	cathepsin F	1.50	1.60E-06
103025_at	Mov10	Moloney leukemia virus 10	1.50	3.93E-07
162198_f_at	---	---	1.50	3.62E-05
104172_at	Folr2	folate receptor 2 (fetal)	1.50	3.55E-07
104713_at	Prpf38a	PRP38 pre-mRNA processing factor 38 (yeast) domain containing A	1.50	6.92E-07
103330_at	Strbp	spermatid perinuclear RNA binding protein	1.50	3.83E-05
102791_at	Psmb8	proteasome (prosome, macropain) subunit, beta type 8 (large multifunctional peptidase 7)	1.49	5.47E-07
93907_f_at	100043775 /// 100043821 /// BC094435	predicted gene, 100043775 /// predicted gene, 100043821 /// cDNA sequence	1.49	8.87E-07

	/// Ccrn4l /// Cog6 /// Sgip1	BC094435 /// CCR4 carbon catabolite repression 4-like (S. cerevisiae) /// component of oligomeric golgi complex 6 /// SH3-domain GRB2-like (endophilin) interacting protein 1		
98478_at	Ccng2	cyclin G2	1.49	1.40E-06
101973_at	Cited2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	1.48	1.69E-06
161666_f_at	Gadd45b	growth arrest and DNA-damage-inducible 45 beta	1.48	5.52E-05
161610_at	---	---	1.48	8.87E-07
100564_at	Ddt	D-dopachrome tautomerase	1.48	7.52E-06
93120_f_at	H2-K1	histocompatibility 2, K1, K region	1.48	8.86E-07
97181_f_at	100043775 /// 100043821 /// BC094435 /// Ccrn4l /// Cog6 /// ENSMUSG00000075496 /// Sgip1	predicted gene, 100043775 /// predicted gene, 100043821 /// cDNA sequence BC094435 /// CCR4 carbon catabolite repression 4-like (S. cerevisiae) /// component of oligomeric golgi complex 6 /// predicted gene, ENSMUSG00000075496 /// SH3-domain GRB2-like (endophilin) interacting protein 1	1.48	8.01E-07
101846_r_at	EG665317	predicted gene, EG665317	1.47	2.59E-05
93714_f_at	EG630499	predicted gene, EG630499	1.47	3.84E-07
94643_at	Pvr	poliovirus receptor	1.47	1.25E-04
101461_f_at	Pja1	praja1, RING-H2 motif containing	1.47	2.24E-06
101897_g_at	Cd1d1 /// Cd1d2	CD1d1 antigen /// CD1d2 antigen	1.47	9.46E-06
104412_at	Gnai1	guanine nucleotide binding protein (G protein), alpha inhibiting 1	1.47	2.13E-05
93194_at	Ly9	lymphocyte antigen 9	1.47	2.37E-06
104158_at	Snw1	SNW domain containing 1	1.47	6.54E-06
97540_f_at	H2-D1	histocompatibility 2, D region locus 1	1.47	2.96E-06
96653_at	Rnaset2a /// Rnaset2b	ribonuclease T2A /// ribonuclease T2B	1.47	7.08E-06
95607_at	Stard3	START domain containing 3	1.47	6.55E-07
100609_at	Cfb	complement factor B	1.46	2.36E-05
100465_i_at	Gm1673	gene model 1673, (NCBI)	1.46	4.48E-07
96791_at	Fam101b	family with sequence similarity 101, member B	1.46	2.28E-05
103875_at	Ngrn	neugrin, neurite outgrowth associated	1.46	3.05E-06
98984_f_at	Gpd2	glycerol phosphate dehydrogenase 2, mitochondrial	1.46	8.01E-07
160612_at	*Abcg1*	ATP-binding cassette, sub-family G (WHITE), member 1	1.46	3.95E-05
160344_at	Npc2	Niemann Pick type C2	1.46	1.64E-06
99140_at	Mrpl16	mitochondrial ribosomal protein L16	1.46	1.08E-06
100629_at	Gstm5	glutathione S-transferase, mu 5	1.46	1.27E-06
98597_at	Ube2l6	ubiquitin-conjugating enzyme E2L 6	1.46	1.49E-06
100064_f_at	Gja1	gap junction protein, alpha 1	1.46	1.23E-05
95120_at	Tspan13	tetraspanin 13	1.45	6.09E-05

92384_at	Xpa	xeroderma pigmentosum, complementation group A	1.45	8.36E-06
102859_at	Reep1	receptor accessory protein 1	1.45	9.73E-07
93697_at	Cbx4	chromobox homolog 4 (Drosophila Pc class)	1.45	3.45E-06
97925_at	Csnk1e	casein kinase 1, epsilon	1.45	1.40E-06
100880_at	Gbp6	guanylate binding protein 6	1.45	6.24E-07
99058_at	Hmga2	high mobility group AT-hook 2	1.45	1.04E-05
100407_at	Gal	galanin	1.45	1.34E-06
96104_at	Rnf145	ring finger protein 145	1.45	1.48E-05
100571_at	Laptm4b	lysosomal-associated protein transmembrane 4B	1.45	1.60E-06
100486_at	Ezh1	enhancer of zeste homolog 1 (Drosophila)	1.44	2.19E-06
99085_at	Usp3	ubiquitin specific peptidase 3	1.44	5.99E-07
97972_at	Rnf103	ring finger protein 103	1.44	1.05E-06
160253_at	Ifitm3	interferon induced transmembrane protein 3	1.44	7.58E-07
160666_at	Actr6	ARP6 actin-related protein 6 homolog (yeast)	1.44	1.49E-05
92718_at	Ifi27l2a	interferon, alpha-inducible protein 27 like 2A	1.44	1.59E-06
102896_at	Dok1	docking protein 1	1.44	1.51E-06
101930_at	Nfix	nuclear factor I/X	1.44	7.03E-05
95795_at	100041294	predicted gene, 100041294	1.44	1.01E-05
104030_at	Ptch1	patched homolog 1	1.43	2.28E-06
103615_at	BC094916 /// LOC100048304 /// LOC637605 /// Pyhin1	cDNA sequence BC094916 /// hypothetical protein LOC100048304 /// similar to Gamma-interferon-inducible protein Ifi-16 (Interferon-inducible myeloid differentiation transcriptional activator) (IFI 16) /// pyrin and HIN domain family, member 1	1.43	2.18E-05
96146_at	Btg3 /// EG654432	B-cell translocation gene 3 /// predicted gene, EG654432	1.43	8.44E-05
94761_at	Ccl7	chemokine (C-C motif) ligand 7	1.43	5.80E-05
96670_at	Gstk1	glutathione S-transferase kappa 1	1.43	1.52E-05
98499_s_at	Casp7	caspase 7	1.43	2.12E-06
94952_at	Igf2bp2	insulin-like growth factor 2 mRNA binding protein 2	1.43	2.99E-05
96899_at	Ndufs3	NADH dehydrogenase (ubiquinone) Fe-S protein 3	1.43	6.54E-06
97890_at	Sgk1	serum/glucocorticoid regulated kinase 1	1.43	7.86E-07
96894_at	Tmed4	transmembrane emp24 protein transport domain containing 4	1.42	5.45E-06
103518_at	Ctla2b	cytotoxic T lymphocyte-associated protein 2 beta	1.42	1.08E-06
95675_at	Map4k3	mitogen-activated protein kinase kinase kinase kinase 3	1.42	1.19E-06
160627_at	Ddx52	DEAD (Asp-Glu-Ala-Asp) box polypeptide 52	1.42	4.33E-06
93678_s_at	Klrk1	killer cell lectin-like receptor subfamily K,	1.42	4.39E-06

		member 1		
160958_at	9430016H08Rik	RIKEN cDNA 9430016H08 gene	1.42	5.73E-06
95105_at	Higd2a	HIG1 domain family, member 2A	1.42	1.28E-06
95477_at	Tmem59	transmembrane protein 59	1.41	4.54E-05
96747_at	Rhou	ras homolog gene family, member U	1.41	2.75E-04
95518_at	Fam134b	family with sequence similarity 134, member B	1.41	8.20E-06
94365_at	Hint2	histidine triad nucleotide binding protein 2	1.41	3.05E-06
102699_at	Mx2	myxovirus (influenza virus) resistance 2	1.40	3.62E-05
99579_at	Atp1b3	ATPase, Na+/K+ transporting, beta 3 polypeptide	1.40	2.27E-06
162262_f_at	Gyg	glycogenin	1.40	8.88E-05
93312_at	Ube2g1	ubiquitin-conjugating enzyme E2G 1 (UBC7 homolog, C. elegans)	1.40	1.12E-05
104177_at	Rsad2	radical S-adenosyl methionine domain containing 2	1.40	3.41E-06
93869_s_at	Bcl2a1a /// Bcl2a1c /// Bcl2a1d	B-cell leukemia/lymphoma 2 related protein A1a /// B-cell leukemia/lymphoma 2 related protein A1c /// B-cell leukemia/lymphoma 2 related protein A1d	1.40	7.26E-06
103508_at	LOC100048247 /// Pcgf5	similar to polycomb group ring finger 5 /// polycomb group ring finger 5	1.40	4.07E-05
96711_at	Znrd1	zinc ribbon domain containing, 1	1.40	3.84E-06
101922_at	Kdelr2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2	1.40	4.54E-05
98937_at	Tbrg1	transforming growth factor beta regulated gene 1	1.40	1.04E-05
99413_at	Ccr1	chemokine (C-C motif) receptor 1	1.40	1.14E-05
101525_at	Ndufb10	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 10	1.39	2.03E-05
102342_at	Nsf	N-ethylmaleimide sensitive fusion protein	1.39	1.79E-05
93309_at	Ddx3x	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 3, X-linked	1.39	4.73E-05
102047_at	LOC100045684 /// Nmt1	similar to N-myristoyltransferase 1 /// N-myristoyltransferase 1	1.39	7.57E-06
94841_at	Psma5	proteasome (prosome, macropain) subunit, alpha type 5	1.39	3.16E-06
98335_at	Rfc1	replication factor C (activator 1) 1	1.39	1.20E-05
94995_at	Ggct	gamma-glutamyl cyclotransferase	1.39	1.12E-05
104343_f_at	Pla2g12a	phospholipase A2, group XIIA	1.38	3.92E-06
96881_at	Commd6	COMM domain containing 6	1.38	1.99E-06
101933_at	Rab10	RAB10, member RAS oncogene family	1.38	2.60E-05
104680_at	Ramp1	receptor (calcitonin) activity modifying protein 1	1.38	5.73E-06
95058_f_at	Brp44	brain protein 44	1.38	1.80E-05
102819_at	Nap1l2	nucleosome assembly protein 1-like 2	1.38	1.10E-05
94834_at	Ctsh	cathepsin H	1.38	2.72E-05
97487_at	Serpine2	serine (or cysteine) peptidase inhibitor, clade E, member 2	1.38	5.37E-06
160486_at	Ccdc28b	coiled coil domain containing 28B	1.38	1.10E-05

92243_at	Vprbp	Vpr (HIV-1) binding protein	1.38	3.09E-05
101475_at	Bmi1	Bmi1 polycomb ring finger oncogene	1.37	4.69E-06
101487_f_at	Ly6e	lymphocyte antigen 6 complex, locus E	1.37	5.84E-06
94263_f_at	Psmb7	proteasome (prosome, macropain) subunit, beta type 7	1.37	7.00E-06
98590_at	Sdc4	syndecan 4	1.37	9.07E-06
100398_at	Kif3a	kinesin family member 3A	1.37	4.59E-06
93728_at	Tsc22d1	TSC22 domain family, member 1	1.37	3.33E-05
94339_at	Slc46a1	solute carrier family 46, member 1	1.37	4.09E-05
99086_g_at	Usp3	ubiquitin specific peptidase 3	1.37	5.83E-06
97211_at	Armcx2	armadillo repeat containing, X-linked 2	1.37	2.29E-04
98254_f_at	100043775 /// 100043821 /// BC094435 /// Ccrn4l /// Cog6 /// ENSMUSG00000075496 /// Sgip1	predicted gene, 100043775 /// predicted gene, 100043821 /// cDNA sequence BC094435 /// CCR4 carbon catabolite repression 4-like (S. cerevisiae) /// component of oligomeric golgi complex 6 /// predicted gene, ENSMUSG00000075496 /// SH3-domain GRB2-like (endophilin) interacting protein 1	1.37	8.93E-06
96481_at	Nhedc2	Na+/H+ exchanger domain containing 2	1.37	4.07E-05
95448_at	Psmc2	proteasome (prosome, macropain) 26S subunit, ATPase 2	1.37	5.36E-05
96771_at	Erbb3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	1.37	9.46E-05
160589_at	Ppig	peptidyl-prolyl isomerase G (cyclophilin G)	1.37	1.40E-04
92191_at	Mettl5	methyltransferase like 5	1.36	3.85E-05
93579_at	Jagn1	jagunal homolog 1 (Drosophila)	1.36	1.51E-05
103922_f_at	Cyb5r1	cytochrome b5 reductase 1	1.36	3.67E-05
96657_at	Sat1	spermidine/spermine N1-acetyl transferase 1	1.36	3.46E-06
99629_at	Ei24	etoposide induced 2.4 mRNA	1.36	2.09E-05
99327_at	Klk8	kallikrein related-peptidase 8	1.36	3.47E-05
161359_s_at	Apoa1bp	apolipoprotein A-I binding protein	1.36	5.51E-06
102925_at	Dusp9	dual specificity phosphatase 9	1.36	5.97E-05
160237_at	Ndufa6	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6 (B14)	1.36	2.99E-05
100030_at	Upp1	uridine phosphorylase 1	1.36	2.67E-06
101502_at	Tgif1	TGFB-induced factor homeobox 1	1.36	3.14E-06
101102_at	Igbp1	immunoglobulin (CD79A) binding protein 1	1.36	2.77E-05
99053_at	Icam2	intercellular adhesion molecule 2	1.36	2.36E-05
93522_at	Rad9	RAD9 homolog (S. pombe)	1.36	2.51E-05
100946_at	Hspa1b	heat shock protein 1B	1.36	1.31E-05
100074_at	Tmed9	transmembrane emp24 protein transport domain containing 9	1.36	2.06E-05
101787_f_at	100043775 /// 100043821 /// 2700079J08Rik ///	predicted gene, 100043775 /// predicted gene, 100043821 /// RIKEN cDNA 2700079J08 gene /// CCR4 carbon	1.36	2.30E-05

	Ccrn4l /// EG668525 /// ENSMUSG00000075496 /// LOC674800 /// Sgip1	catabolite repression 4-like (S. cerevisiae) /// predicted gene, EG668525 /// predicted gene, ENSMUSG00000075496 /// similar to IgE-binding protein /// SH3-domain GRB2-like (endophilin) interacting protein 1		
104301_at	Fam128b	family with sequence similarity 128, member B	1.36	1.39E-05
103254_at	Trafd1	TRAF type zinc finger domain containing 1	1.36	4.84E-06
102860_at	Serpina3g	serine (or cysteine) peptidase inhibitor, clade A, member 3G	1.36	8.45E-06
160621_at	Mrps22	mitochondrial ribosomal protein S22	1.36	3.73E-05
103218_at	Slc10a3	solute carrier family 10 (sodium/bile acid cotransporter family), member 3	1.35	1.46E-04
96623_at	Ugcg	UDP-glucose ceramide glucosyltransferase	1.35	2.03E-05
100592_at	Ghitm	growth hormone inducible transmembrane protein	1.35	4.56E-06
99465_at	Mecp2	methyl CpG binding protein 2	1.35	1.87E-04
93261_at	Lgmn	legumain	1.35	5.98E-05
97220_at	Psmg1	proteasome (prosome, macropain) assembly chaperone 1	1.35	1.01E-05
98855_r_at	A930001N09Rik	RIKEN cDNA A930001N09 gene	1.35	3.31E-05
98901_at	Fcf1 /// LOC633406	FCF1 small subunit (SSU) processome component homolog (S. cerevisiae) /// similar to Protein C14orf111 homolog	1.35	1.39E-05
95586_at	P2rx4	purinergic receptor P2X, ligand-gated ion channel 4	1.35	7.57E-06
96708_at	Tmed3	transmembrane emp24 domain containing 3	1.35	1.41E-05
161046_at	Crlf1	cytokine receptor-like factor 1	1.35	7.96E-04
160618_at	Lgals8	lectin, galactose binding, soluble 8	1.35	2.36E-04
92468_at	Ankrd49	ankyrin repeat domain 49	1.35	1.83E-04
100988_at	Bcl2l11	BCL2-like 11 (apoptosis facilitator)	1.35	6.07E-06
101585_at	Pgrmc1	progesterone receptor membrane component 1	1.34	2.19E-05
102731_g_at	H2-M3	histocompatibility 2, M region locus 3	1.34	1.11E-05
95517_i_at	BC004004	cDNA sequence BC004004	1.34	2.54E-05
97869_at	Etfdh	electron transferring flavoprotein, dehydrogenase	1.34	1.40E-04
96785_at	Kank3	KN motif and ankyrin repeat domains 3	1.34	1.97E-04
100957_at	Ssbp1	single-stranded DNA binding protein 1	1.34	3.83E-05
102255_at	Osmr	oncostatin M receptor	1.34	7.33E-04
96688_at	Tmem77	transmembrane protein 77	1.34	4.20E-06
100878_at	---	---	1.34	7.76E-06
93101_s_at	Nedd4	neural precursor cell expressed, developmentally down-regulated 4	1.34	2.59E-05
93025_at	LOC100046168 /// Ndfip1	similar to Nedd4 WW domain-binding protein 5 /// Nedd4 family interacting protein 1	1.34	4.07E-05
160290_at	Ide	insulin degrading enzyme	1.34	3.80E-05

97926_s_at	Pparg	peroxisome proliferator activated receptor gamma	1.34	1.53E-03
104225_at	Snx5	sorting nexin 5	1.34	8.38E-04
102870_at	Dynlt1 /// Dynlt1d /// Tmem181	dynein light chain Tctex-type 1 /// dynein light chain Tctex-type 1D /// transmembrane protein 181	1.34	8.93E-06
98030_at	Trim30	tripartite motif-containing 30	1.34	1.61E-05
103804_at	Reck	reversion-inducing-cysteine-rich protein with kazal motifs	1.34	3.09E-05
104398_at	Tspan33	tetraspanin 33	1.34	9.09E-04
96125_at	Daxx	Fas death domain-associated protein	1.34	5.94E-06
95146_at	Tmem66	transmembrane protein 66	1.34	6.44E-06
104509_at	*Ch25h*	cholesterol 25-hydroxylase	1.34	2.67E-05
99015_at	Pml	promyelocytic leukemia	1.34	6.54E-06
95442_at	Tmem205	transmembrane protein 205	1.34	1.86E-05
92282_at	Rprd1b	regulation of nuclear pre-mRNA domain containing 1B	1.34	3.78E-05
160711_at	Decr1	2,4-dienoyl CoA reductase 1, mitochondrial	1.34	5.03E-04
95103_at	2310065K24Rik	RIKEN cDNA 2310065K24 gene	1.34	8.66E-06
102754_at	Birc6	baculoviral IAP repeat-containing 6	1.34	1.25E-05
102798_at	Adm	adrenomedullin	1.33	2.21E-05
102022_at	Mcee	methylmalonyl CoA epimerase	1.33	6.14E-05
99532_at	Tob1	transducer of ErbB-2.1	1.33	9.16E-06
100979_at	Rnf138	ring finger protein 138	1.33	2.73E-03
94526_at	Cisd1	CDGSH iron sulfur domain 1	1.33	3.57E-05
95139_at	Wipi2	WD repeat domain, phosphoinositide interacting 2	1.33	2.90E-05
104156_r_at	Atf3	activating transcription factor 3	1.33	2.76E-05
94469_at	Mat2b	methionine adenosyltransferase II, beta	1.33	7.54E-06
103654_at	Nsbp1	nucleosome binding protein 1	1.33	1.13E-04
94109_at	Zfp281	zinc finger protein 281	1.33	7.57E-06
96913_at	Hadhb	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit	1.33	4.79E-06
104616_g_at	Galt	galactose-1-phosphate uridyl transferase	1.33	1.71E-04
96341_at	Syf2	SYF2 homolog, RNA splicing factor (S. cerevisiae)	1.33	1.47E-05
99994_at	Cidea	cell death-inducing DNA fragmentation factor, alpha subunit-like effector A	1.33	5.45E-05
95531_at	Amot	angiomotin	1.33	2.62E-05
101255_at	Ubb	ubiquitin B	1.33	4.87E-05
96608_at	Phyh	phytanoyl-CoA hydroxylase	1.33	9.03E-06
98124_at	0610011F06Rik	RIKEN cDNA 0610011F06 gene	1.33	2.13E-05
93988_at	Psma7	proteasome (prosome, macropain) subunit, alpha type 7	1.33	2.43E-04
92222_f_at	H2-Q1	histocompatibility 2, Q region locus 1	1.33	4.71E-04

93815_at	Chchd3	coiled-coil-helix-coiled-coil-helix domain containing 3	1.33	2.97E-05
103020_s_at	Map3k1	mitogen-activated protein kinase kinase kinase 1	1.33	6.47E-06
103861_s_at	Ubfd1	ubiquitin family domain containing 1	1.33	7.79E-06
101507_at	Scnm1	sodium channel modifier 1	1.33	2.98E-05
104399_at	Cstf2	cleavage stimulation factor, 3' pre-RNA subunit 2	1.33	8.02E-06
103344_at	Dnajc1	DnaJ (Hsp40) homolog, subfamily C, member 1	1.33	1.29E-04
103222_at	Eps8 /// LOC632638	epidermal growth factor receptor pathway substrate 8 /// similar to Epidermal growth factor receptor kinase substrate 8	1.33	5.71E-04
96743_at	1810035L17Rik	RIKEN cDNA 1810035L17 gene	1.33	3.42E-05
95460_at	Cops5	COP9 (constitutive photomorphogenic) homolog, subunit 5 (Arabidopsis thaliana)	1.33	1.16E-05
97324_at	5133401N09Rik	RIKEN cDNA 5133401N09 gene	1.33	2.68E-05
160397_at	Ik	IK cytokine	1.33	6.04E-05
102965_at	Znfx1	zinc finger, NFX1-type containing 1	1.32	6.59E-05
96035_at	Bckdha	branched chain ketoacid dehydrogenase E1, alpha polypeptide	1.32	3.50E-05
99419_g_at	Bcl2l11	BCL2-like 11 (apoptosis facilitator)	1.32	3.69E-05
160905_s_at	A030009H04Rik	RIKEN cDNA A030009H04 gene	1.32	1.97E-05
96020_at	C1qb	complement component 1, q subcomponent, beta polypeptide	1.32	1.78E-03
103314_at	Parp8	poly (ADP-ribose) polymerase family, member 8	1.32	5.71E-04
99188_at	Use1	unconventional SNARE in the ER 1 homolog (S. cerevisiae)	1.32	1.42E-05
160772_i_at	Slu7	SLU7 splicing factor homolog (S. cerevisiae)	1.32	2.99E-04
93531_at	Ndufa8	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 8	1.32	1.01E-05
93042_at	Tspo	translocator protein	1.32	8.74E-06
98067_at	Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	1.32	1.58E-05
95380_at	Cd244	CD244 natural killer cell receptor 2B4	1.32	1.02E-03
102914_s_at	Bcl2a1a /// Bcl2a1b /// Bcl2a1c /// Bcl2a1d	B-cell leukemia/lymphoma 2 related protein A1a /// B-cell leukemia/lymphoma 2 related protein A1b /// B-cell leukemia/lymphoma 2 related protein A1c /// B-cell leukemia/lymphoma 2 related protein A1d	1.32	8.97E-05
93088_at	B2m	beta-2 microglobulin	1.32	4.31E-05
96649_at	Txndc14	thioredoxin domain containing 14	1.32	5.79E-05
92375_at	Ascc1	activating signal cointegrator 1 complex subunit 1	1.32	7.44E-05
96921_at	Ttc1	tetratricopeptide repeat domain 1	1.32	1.32E-05
101883_s_at	LOC630164 /// Xlr3a /// Xlr3b /// Xlr3c /// Xlr3d-ps	similar to X-LINKED LYMPHOCYTE-REGULATED PROTEIN 3A (XLR RELATED PROTEIN A12) /// X-linked lymphocyte-regulated 3A /// X-linked lymphocyte-regulated 3B /// X-linked	1.32	3.02E-05

		lymphocyte-regulated 3C /// X-linked lymphocyte-regulated 3D, pseudogene		
96283_at	Itm2c	integral membrane protein 2C	1.32	8.85E-06
98369_f_at	100043775 /// 100043821 /// 2610028J07Rik /// BC094435 /// Ccrn4l /// Cog6 /// ENSMUSG00000075496 /// Sgip1	predicted gene, 100043775 /// predicted gene, 100043821 /// RIKEN cDNA 2610028J07 gene /// cDNA sequence BC094435 /// CCR4 carbon catabolite repression 4-like (S. cerevisiae) /// component of oligomeric golgi complex 6 /// predicted gene, ENSMUSG00000075496 /// SH3-domain GRB2-like (endophilin) interacting protein 1	1.32	3.31E-05
162399_f_at	Atxn2 /// LOC100047323	ataxin 2 /// similar to ataxin 2	1.32	4.48E-04
103501_at	Pura	purine rich element binding protein A	1.32	1.77E-05
160383_at	Cox7a2l	cytochrome c oxidase subunit VIIa polypeptide 2-like	1.32	7.98E-05
102370_at	Hsd17b11	hydroxysteroid (17-beta) dehydrogenase 11	1.32	3.28E-04
95416_at	Usp15	ubiquitin specific peptidase 15	1.32	2.41E-05
160966_at	Zfp187	zinc finger protein 187	1.31	1.43E-04
97318_at	Dtd1 /// LOC100048650	D-tyrosyl-tRNA deacylase 1 homolog (S. cerevisiae) /// similar to D-tyrosyl-tRNA deacylase 1	1.31	7.26E-05
99577_at	Kitl	kit ligand	1.31	3.69E-04
95490_at	Kdelr1	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1	1.31	1.53E-05
92452_at	Pik3ca	phosphatidylinositol 3-kinase, catalytic, alpha polypeptide	1.31	6.96E-05
94327_at	Mrps18a	mitochondrial ribosomal protein S18A	1.31	4.00E-05
98849_at	---	---	1.31	3.74E-05
160667_at	Evl /// LOC100047333	Ena-vasodilator stimulated phosphoprotein /// similar to Ena-VASP-like	1.31	7.06E-05
93672_at	Eif2ak2	eukaryotic translation initiation factor 2-alpha kinase 2	1.31	1.20E-05
94270_at	Krt18	keratin 18	1.31	3.50E-05
101971_at	Rnf181	ring finger protein 181	1.31	1.00E-05
100072_at	Ciao1	cytosolic iron-sulfur protein assembly 1 homolog (S. cerevisiae)	1.31	2.22E-05
102960_at	Rag1ap1	recombination activating gene 1 activating protein 1	1.31	3.91E-05
104257_g_at	Cytip	cytohesin 1 interacting protein	1.31	1.32E-05
101979_at	Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	1.31	3.47E-05
99501_at	Sec62	SEC62 homolog (S. cerevisiae)	1.31	2.90E-04
104419_at	Fndc3a	fibronectin type III domain containing 3A	1.31	1.69E-04
160240_at	1110003E01Rik	RIKEN cDNA 1110003E01 gene	1.31	1.47E-05
94929_at	Ptpn1	protein tyrosine phosphatase, non-receptor type 1	1.31	4.17E-05
98113_at	Psmb1	proteasome (prosome, macropain) subunit, beta type 1	1.31	7.62E-06

97496_f_at	Prkcdbp	protein kinase C, delta binding protein	1.31	2.26E-04
103394_at	Fxyd5	FXYD domain-containing ion transport regulator 5	1.31	1.77E-05
94275_at	Urod	uroporphyrinogen decarboxylase	1.31	1.05E-04
95491_at	Park7	Parkinson disease (autosomal recessive, early onset) 7	1.30	3.05E-05
103044_g_at	Mtcp1	mature T-cell proliferation 1	1.30	1.23E-05
97254_at	Rbm8a	RNA binding motif protein 8a	1.30	1.25E-05
94025_at	Psmb3	proteasome (prosome, macropain) subunit, beta type 3	1.30	8.45E-06
101019_at	Ctsc	cathepsin C	1.30	2.48E-05
103596_at	Dgka	diacylglycerol kinase, alpha	1.30	8.33E-03
94939_at	Cd53	CD53 antigen	1.30	4.54E-05
160361_at	Trappc4	trafficking protein particle complex 4	1.30	5.17E-05
101516_at	Cd59a	CD59a antigen	1.30	2.62E-05
99667_at	Cox6a2	cytochrome c oxidase, subunit VI a, polypeptide 2	1.30	1.67E-04
160705_at	Cited1	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1	1.30	1.03E-04
102197_at	Nucb2	nucleobindin 2	1.30	6.01E-04
104116_at	Stbd1	starch binding domain 1	1.30	1.39E-04
99475_at	Socs2	suppressor of cytokine signaling 2	1.30	1.60E-04
92202_g_at	Zbtb16	zinc finger and BTB domain containing 16	1.30	4.75E-04
96298_f_at	Dynll1	dynein light chain LC8-type 1	1.30	2.20E-04
101484_at	Nbr1	neighbor of Brca1 gene 1	1.30	2.76E-05
95465_s_at	Tmem37	transmembrane protein 37	1.30	4.56E-05
104714_at	Dock9	dedicator of cytokinesis 9	1.30	3.57E-05
160740_at	Gm561	gene model 561, (NCBI)	1.30	4.80E-05
102652_at	LOC100045707 /// Pou3f1	similar to long overlapping ORF; NH2 terminus uncertain /// POU domain, class 3, transcription factor 1	1.30	7.34E-05
99624_at	9530068E07Rik	RIKEN cDNA 9530068E07 gene	1.30	3.83E-05
102009_at	Cyfip2	cytoplasmic FMR1 interacting protein 2	1.29	1.32E-04
99187_f_at	Use1	unconventional SNARE in the ER 1 homolog (S. cerevisiae)	1.29	1.57E-05
102678_at	Trim21	tripartite motif-containing 21	1.29	8.23E-05
95884_at	TLR7	toll-like receptor 7	1.29	5.79E-05
160345_at	Mrpl34	mitochondrial ribosomal protein L34	1.29	1.80E-04
98511_at	Raly	hnRNP-associated with lethal yellow	1.29	6.59E-04
102292_at	Gadd45a	growth arrest and DNA-damage-inducible 45 alpha	1.29	1.12E-04
102318_at	St8sia4	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4	1.29	1.16E-04
102710_at	Apbb1ip	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein	1.29	2.62E-05
93219_at	Acp1	acid phosphatase 1, soluble	1.29	3.54E-05
95067_at	Mrpl2	mitochondrial ribosomal protein L2	1.29	2.32E-05

93538_at	Ttrap	TRAF and TNF receptor associated protein	1.29	3.22E-05
102064_at	Casp1	caspase 1	1.29	3.62E-05
98980_at	Cd37	CD37 antigen	1.29	1.44E-05
98452_at	Flt1	FMS-like tyrosine kinase 1	1.29	2.66E-04
97551_at	Hip1r	huntingtin interacting protein 1 related	1.29	5.85E-05
97460_at	Ube2r2	ubiquitin-conjugating enzyme E2R 2	1.29	3.24E-05
96629_at	Nudt19	nudix (nucleoside diphosphate linked moiety X)-type motif 19	1.28	7.08E-05
104312_at	Abhd14a	abhydrolase domain containing 14A	1.28	4.74E-05
104189_at	Traf6	TNF receptor-associated factor 6	1.28	2.66E-05
94982_f_at	Nme3	non-metastatic cells 3, protein expressed in	1.28	4.00E-05
95480_at	Vps25	vacuolar protein sorting 25 (yeast)	1.28	3.44E-05
99160_s_at	Grina	glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1 (glutamate binding)	1.28	4.07E-05
100931_at	Arsa	arylsulfatase A	1.28	1.83E-04
93011_at	Gabarapl1	gamma-aminobutyric acid (GABA) A receptor-associated protein-like 1	1.28	5.74E-05
98018_at	Procr	protein C receptor, endothelial	1.28	1.17E-04
96774_at	Fermt2	fermitin family homolog 2 (Drosophila)	1.28	1.77E-04
94219_at	Psmb2	proteasome (prosome, macropain) subunit, beta type 2	1.28	4.07E-05
92574_at	Sdhd	succinate dehydrogenase complex, subunit D, integral membrane protein	1.28	1.88E-04
95590_at	Alg5	asparagine-linked glycosylation 5 homolog (yeast, dolichyl-phosphate beta-glucosyltransferase)	1.28	4.54E-05
101481_at	Rnf34	ring finger protein 34	1.28	4.39E-03
160444_at	Pigx	phosphatidylinositol glycan anchor biosynthesis, class X	1.28	2.63E-04
94241_at	Coasy	Coenzyme A synthase	1.28	7.81E-05
97893_at	Tbpl1	TATA box binding protein-like 1	1.28	4.88E-05
95102_at	Shisa5	shisa homolog 5 (Xenopus laevis)	1.28	2.13E-05
104603_at	Gstt2	glutathione S-transferase, theta 2	1.28	3.76E-05
102421_at	Lmf1	lipase maturation factor 1	1.28	2.98E-05
100892_at	Ndufaf1	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 1	1.28	1.39E-04
95699_f_at	Dnajc8	DnaJ (Hsp40) homolog, subfamily C, member 8	1.28	4.99E-05
161104_at	Sep-10	septin 10	1.28	1.46E-03
97933_at	2010111I01Rik	RIKEN cDNA 2010111I01 gene	1.28	1.93E-05
100405_at	Cbx3	chromobox homolog 3 (Drosophila HP1 gamma)	1.28	1.11E-03
95096_at	LOC100046895 /// Qk	similar to Quaking protein /// quaking	1.28	1.04E-04
99586_at	Cst3	cystatin C	1.28	3.62E-05
95620_at	Dhrs7	dehydrogenase/reductase (SDR family) member 7	1.28	3.90E-05
94453_at	1810046J19Rik	RIKEN cDNA 1810046J19 gene	1.28	1.36E-04

98936_at	Sars	seryl-aminoacyl-tRNA synthetase	1.28	3.54E-05
103331_at	C030006K11Rik	RIKEN cDNA C030006K11 gene	1.28	1.04E-04
100633_at	Mosc2	MOCO sulphurase C-terminal domain containing 2	1.28	1.77E-05
104115_at	Psme4	proteasome (prosome, macropain) activator subunit 4	1.28	1.20E-04
99147_at	Znhit1	zinc finger, HIT domain containing 1	1.28	6.35E-05
96728_at	Wdr45	WD repeat domain 45	1.28	3.49E-05
96822_at	Eif2b5	eukaryotic translation initiation factor 2B, subunit 5 epsilon	1.28	2.15E-05
94438_at	Pfkm	phosphofructokinase, muscle	1.28	1.70E-04
102252_at	Pfdn2	prefoldin 2	1.27	4.43E-05
99856_r_at	Ctnnd2 /// LOC100045979	catenin (cadherin associated protein), delta 2 /// similar to arm-repeat protein NPRAP/neurojungin	1.27	1.25E-04
103231_at	Rhoh	ras homolog gene family, member H	1.27	2.12E-04
101483_at	Ccndbp1	cyclin D-type binding-protein 1	1.27	8.85E-05
104188_at	Notch2	Notch gene homolog 2 (Drosophila)	1.27	2.16E-03
94530_at	Eif2b2	eukaryotic translation initiation factor 2B, subunit 2 beta	1.27	7.34E-05
94843_at	Pold4	polymerase (DNA-directed), delta 4	1.27	8.96E-05
92206_at	A430107D22Rik	RIKEN cDNA A430107D22 gene	1.27	1.24E-04
104328_at	Aqp9	aquaporin 9	1.27	2.33E-04
101976_at	Cops4	COP9 (constitutive photomorphogenic) homolog, subunit 4 (Arabidopsis thaliana)	1.27	2.41E-04
160366_at	BC031181	cDNA sequence BC031181	1.27	1.60E-04
160305_at	Psmd11	proteasome (prosome, macropain) 26S subunit, non-ATPase, 11	1.27	7.54E-05
101298_g_at	Ptprc	protein tyrosine phosphatase, receptor type, C	1.27	2.20E-04
102401_at	Irf1	interferon regulatory factor 1	1.27	3.02E-05
94078_at	1110020P15Rik	RIKEN cDNA 1110020P15 gene	1.27	7.86E-05
101045_at	Hsd17b10	hydroxysteroid (17-beta) dehydrogenase 10	1.27	3.67E-05
95730_at	Mrps34	mitochondrial ribosomal protein S34	1.27	1.03E-04
160668_at	Ogfr	opioid growth factor receptor	1.27	3.80E-05
94924_at	Prrg2	proline-rich Gla (G-carboxyglutamic acid) polypeptide 2	1.27	1.01E-04
101106_at	G3bp2	GTPase activating protein (SH3 domain) binding protein 2	1.27	5.38E-05
96577_i_at	Ubap2l	ubiquitin associated protein 2-like	1.27	5.49E-05
96658_at	2900010J23Rik	RIKEN cDNA 2900010J23 gene	1.27	2.31E-04
94860_at	Timm17a	translocase of inner mitochondrial membrane 17a	1.27	1.18E-04
160320_at	Sorbs1	sorbin and SH3 domain containing 1	1.27	1.60E-04
103207_at	Pola1	polymerase (DNA directed), alpha 1	1.27	8.11E-04
160696_at	Tia1	cytotoxic granule-associated RNA binding protein 1	1.27	1.64E-04
94032_at	Apoa1bp	apolipoprotein A-I binding protein	1.27	3.33E-05

104250_at	Lrrc8a	leucine rich repeat containing 8A	1.27	1.23E-04
103547_at	Slc41a1	solute carrier family 41, member 1	1.27	4.44E-03
160236_at	Slain1	SLAIN motif family, member 1	1.27	3.30E-04
98129_at	Tmsb10	thymosin, beta 10	1.27	8.08E-05
160138_at	Mxi1	Max interacting protein 1	1.27	9.09E-04
94505_at	Peli1	pellino 1	1.27	6.76E-04
97812_at	Ranbp9	RAN binding protein 9	1.27	6.77E-03
160176_at	Nfu1	NFU1 iron-sulfur cluster scaffold homolog (S. cerevisiae)	1.27	1.73E-04
160271_at	0610007C21Rik	RIKEN cDNA 0610007C21 gene	1.27	1.32E-04
97918_at	Rell1	RELT-like 1	1.27	1.03E-04
96345_at	Ergic3	ERGIC and golgi 3	1.27	9.72E-05
95474_at	F2r	coagulation factor II (thrombin) receptor	1.27	4.75E-05
98144_f_at	Tspan31	tetraspanin 31	1.27	3.62E-04
100608_at	Sptlc1	serine palmitoyltransferase, long chain base subunit 1	1.27	1.07E-04
94488_at	Vta1	Vps20-associated 1 homolog (S. cerevisiae)	1.27	5.69E-05
95049_at	Snrpd2	small nuclear ribonucleoprotein D2	1.27	1.92E-04
94396_at	Ing1	inhibitor of growth family, member 1	1.26	2.79E-04
101031_at	Surf1	surfeit gene 1	1.26	4.83E-05
94206_at	Grcc10	gene rich cluster, C10 gene	1.26	6.53E-05
100597_at	Gyg	glycogenin	1.26	4.17E-05
93466_at	Exoc4	exocyst complex component 4	1.26	3.96E-04
92247_at	Arhgap5	Rho GTPase activating protein 5	1.26	2.66E-04
99194_at	Pnpt1	polyribonucleotide nucleotidyltransferase 1	1.26	4.75E-05
93994_at	Chpt1	choline phosphotransferase 1	1.26	9.40E-04
93039_at	Pgcp	plasma glutamate carboxypeptidase	1.26	8.39E-05
98143_at	Fut8	fucosyltransferase 8	1.26	6.31E-05
104627_at	Cds2	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2	1.26	2.68E-05
102848_f_at	2610524H06Rik	RIKEN cDNA 2610524H06 gene	1.26	1.04E-04
96188_at	Adar	adenosine deaminase, RNA-specific	1.26	1.12E-04
95110_at	Ppil2	peptidylprolyl isomerase (cyclophilin)-like 2	1.26	8.18E-05
98904_at	Mrpl35	mitochondrial ribosomal protein L35	1.26	1.08E-04
93949_at	Gnb4	guanine nucleotide binding protein (G protein), beta 4	1.26	2.19E-04
101990_at	Ldhb	lactate dehydrogenase B	1.26	1.54E-04
161060_i_at	Ddx51	DEAD (Asp-Glu-Ala-Asp) box polypeptide 51	1.26	5.52E-03
99935_at	Tjp1	tight junction protein 1	1.26	5.15E-04
103038_at	Guca1a	guanylate cyclase activator 1a (retina)	1.26	6.58E-05
96300_f_at	Rps27	ribosomal protein S27	1.26	4.56E-05
161629_i_at	Afp	alpha fetoprotein	1.26	7.92E-03
95282_at	Hsp90aa1	heat shock protein 90, alpha (cytosolic),	1.26	7.37E-05

		class A member 1		
98514_at	Tfpi	tissue factor pathway inhibitor	1.26	8.10E-05
95709_at	Vkorc1	vitamin K epoxide reductase complex, subunit 1	1.26	5.02E-05
101064_at	Plrg1	pleiotropic regulator 1, PRL1 homolog (Arabidopsis)	1.26	1.03E-04
94366_at	2310079N02Rik	RIKEN cDNA 2310079N02 gene	1.26	1.37E-04
93614_at	Rragd	Ras-related GTP binding D	1.26	3.67E-04
104709_at	Sec23a	SEC23A (S. cerevisiae)	1.26	8.93E-05
97195_at	Gnai1	guanine nucleotide binding protein (G protein), alpha inhibiting 1	1.26	4.83E-03
98120_at	Mrpl27	mitochondrial ribosomal protein L27	1.26	6.74E-05
93735_f_at	Psmc3	proteasome (prosome, macropain) 26S subunit, ATPase 3	1.26	1.37E-04
97380_at	Zc3h14	zinc finger CCCH type containing 14	1.26	9.68E-05
95046_s_at	Eif2b4	eukaryotic translation initiation factor 2B, subunit 4 delta	1.25	3.27E-04
98544_at	Guk1	guanylate kinase 1	1.25	7.79E-05
98398_s_at	Apobec1	apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1	1.25	5.35E-05
94073_at	Polr2g	polymerase (RNA) II (DNA directed) polypeptide G	1.25	9.65E-05
103371_at	Slc39a7	solute carrier family 39 (zinc transporter), member 7	1.25	5.79E-05
97292_at	2810407C02Rik	RIKEN cDNA 2810407C02 gene	1.25	9.23E-05
100450_r_at	Acvrl1	activin A receptor, type II-like 1	1.25	8.54E-05
93711_at	Sec23a	SEC23A (S. cerevisiae)	1.25	5.77E-04
103762_at	Gtf2f1	general transcription factor IIF, polypeptide 1	1.25	1.81E-04
94302_at	Psmd4	proteasome (prosome, macropain) 26S subunit, non-ATPase, 4	1.25	6.35E-05
100733_at	Psma2	proteasome (prosome, macropain) subunit, alpha type 2	1.25	1.80E-04
102910_at	Abcb1a	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	1.25	1.23E-03
100772_g_at	Blnk	B-cell linker	1.25	3.85E-05
93252_at	Bcap31	B-cell receptor-associated protein 31	1.25	4.91E-05
92958_at	Foxo3	forkhead box O3	1.25	5.24E-05
98026_g_at	Evi2a	ecotropic viral integration site 2a	1.25	1.36E-04
100600_at	Cd24a	CD24a antigen	1.25	1.16E-03
99623_s_at	Olfm1	olfactomedin 1	1.25	3.93E-05
94428_at	Ilvbl	ilvB (bacterial acetolactate synthase)-like	1.25	4.92E-05
102056_f_at	2610002J02Rik	RIKEN cDNA 2610002J02 gene	1.25	7.73E-04
99544_at	Dguok	deoxyguanosine kinase	1.25	7.57E-04
96076_at	Stx5a	syntaxin 5A	1.25	3.20E-05
160149_at	Rab10	RAB10, member RAS oncogene family	1.25	2.06E-04
96773_at	Erp44	endoplasmic reticulum protein 44	1.25	4.62E-04
160455_s_at	Zwint	ZW10 interactor	1.25	1.33E-04
160704_at	1110067D22Rik	RIKEN cDNA 1110067D22 gene	1.25	4.65E-03

103946_at	Pstpip1	proline-serine-threonine phosphatase-interacting protein 1	1.25	2.13E-04
97958_at	Zmynd8	zinc finger, MYND-type containing 8	1.25	7.31E-05

Appendix 3: Micro array experiment and analysis

Temporal gene expression analysis of BMDMs

Experiment

Briefly, BMDMs were either mock treated or infected with mCMV at a multiplicity of infection (MOI) of 1 or treated with 10 U IFNγ (Boelinger Manhaim Corp). Every 30 min for each treatment, cells were lysed with Trizol and stored at -80ºC (a full description of the experiment will be discussed elsewhere).

RNA was extracted from each sample using Trizol RNA extraction protocol. Mock samples were pooled and labeled with Cy3 while the lyzed or IFNγ treated samples were labeled with Cy5 using a modification of the Agilent Fluorescent protocol, using half of the standard Cy3/Cy5 labeled dUTP concentration. The Cy3 labeled pooled control was hybridized with each of the 75 Cy5 labeled samples according to the Agilent Low RNA Input protocol. The dual hybridizations were carried out on Mouse Agilent V2 array (G4121A, 20868 annotated probes), and were scanned on an Agilent Technologies scanner. Agilent feature extraction software (V.A7.5.1) was used to extract numeric data for further analysis.

Processing microarray data

Data of each sample was first background corrected, and then transformed to log (base 2) scale. Any non-positive values in the background corrected data were replaced with unity before performing the log-transformation. Exploratory plots do not suggest any intensity dependent non-linear trend in the data. Therefore subset median normalization based on 42 positive controls needed to be used to remove any chip-to-chip variation within the data. More specifically, suppose $(m_1, m_{2,\ldots,} m_{50})$ represent the medians of the positive controls for the 50 arrays, and m0 is their mean. Then the normalizing constants for the 50 samples are defined as $(m_0\text{-}m_1, m_0\text{-}m_2 \ldots, m_0\text{-}m_{50})$. The data of each sample are adjusted by adding these constants to the log (base 2) intensity data of the respective samples.

The normalised data was filtered using ROC analysis based on 42 positive and 111 negative controls. For each array, a threshold value was calculated corresponding to the 80% sensitivity level of the corresponding ROC curve. A gene is declared on in a particular sample (time point)

if its log2 expression value exceeds that threshold. Genes are then selected for further analysis if they were found to be on in 5 or more consecutive time points in a particular experimental condition. These genes were also selected for inclusion in Tabs. S4 and S5.

Statistical analysis of time course

The method below36 provides statistical test for identifying within condition temporal differential expression as well as test for identifying differential expression between conditions. The framework of the model is defined as

$$y_{ij} = \mu_i(t_j) + \varepsilon_{ij} \qquad (1)$$

with y_{ij} being the log (base 2) expression level of gene i in sample j and the sample j is observed at time point t_j , where there are i = 1,2,...,M genes on each array and J =1,2,...,N time points/samples for each experimental conditions. The population average time curve is represented by a linear regression of a p-dimensional basis:

$$\mu_i(t) = \alpha_i + \beta_i^T S(t) = \alpha_i + \beta_{i1}S_1(t) + \beta_{i2}S_2(t) + ... + \beta_{ip}S_p(t) \qquad (2)$$

where α_i is the gene-specific intercept term, $\beta_i = \beta_{i1}, \beta_{i2},..., \beta_{ip})^T$ is a p-dimensional vector of regression parameters, and $S(t) = S_1(t), S_2(t), ..., S_p(t))^T$ is a known p-dimensional basis.

A polynomial of degree p or more flexible natural cubic splines has been suggested as a choice of basis. For identifying temporal differential expression, the null hypothesis, restricting $\mu_i(t)$ to be constant, can be tested against the alternative hypothesis that $\mu_i(t)$ is a curve. Thus, mathematically, the hypothesis of no temporal differential expression for gene i can be stated as

H_0: $\beta_i = 0$, or equivalently, H_0: $\mu_i = \alpha_i$.

The observed statistics and null statistics were used to estimate a q-value for each gene, which estimate the false discovery rate for calling the gene significant. In order to select very highly significant temporal changes, we considered a q-value cutoff of 10^{-6}.

Cholesterol gene expression profiling of SFV HSV1 VV and AD infected macrophages

Experiment:

The avirulent Semliki Forest virus strain A774 (SFV A7), HSV1 (strain), Vaccinia virus and Adenovirus 2 were used to infect BMDMs and were kindly donated by Dr Hesper of the DPM.

RNA was isolated from cultured macrophages using the TRIzol method according to the manufacturer's instructions (Invitrogen, San Diego, CA, USA). RNA concentration and purity were obtained by spectrophotometry and RNA integrity monitored using the Agilent 2100 bioanalyzer system (Agilent, Palo Alto, CA, USA). Five micrograms of total RNA isolated from macrophage cultures were used to synthesise biotinylated cRNA target which was hybridised to the Mouse Genome 430 2.0 GeneChip (Affymetrix, Santa Clara, CA, USA). RNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen) into double stranded cDNA using oligo dT primers that contained a T7 promoter. cDNA was extracted with phenol-chloroform and a Phase Lock Gel (Eppendorf, Hamburg, Germany) and precipitated with ethanol and ammonium acetate. Biotinylated cRNA was synthesised using the cDNA as a template in an in vitro transcription reaction using the BioArray HighYield RNA Transcript Labelling Kit (Enzo Life Sciences Inc., Farmingdale, NY, USA) as described by the manufacturer. The resulting biotinylated target cRNA was purified using RNeasy columns according to the manufacturers instructions (QIAgen Ltd., Crawley, UK) and quantified by spectrophotometry. 15µg of purified biotinylated cRNA was fragmented by heating for 35 mins at 94^{o}C in the presence of magnesium ions, spiked with eukaryotic hybridisation control and hybridised to Mouse Genome 430 2.0 microarrays overnight at 45^{o}C. After hybridisation the array was washed, stained with phycoerythrin coupled streptavidin and processed on the Affymetrix GeneChip Fluidics Workstation 400 using the EukGE-Ws2v4 protocol. Microarrays were then scanned using the Agilent 2500A GeneArray Scanner (Agilent).

Data processing and statistical analysis:

Data from hybridised chips were acquired using proprietary Affymetrix platform scanners and GCOS software (Affymetrix). The numeric data were processed and subsequently analysed with the Bioconductor package for the R statistical programming environment. Raw data distributions and summary statistics were assessed for quality. Data were then background corrected, quantile normalised and probe-set summarised using the RMA algorithm. Prior to statistical analysis, a non-specific filter was applied to remove genes that were not expressed on any of the samples in the experiment. Null hypotheses for each gene were based on the comparison between mock arrays and each of the 3 biological conditions; they were tested using an empirical Bayes test, providing good robustness for small sample sizes. In order to adjust for multiple testing issues,

the false discovery rate was controlled using the Benjamini-Hochberg p-value adjustment method. Genes were interpreted on the basis of differential expression between mock and each of the 3 groups, and the corresponding statistical significance. Visualisation of data was performed using GeneSpring GX v7.3 (Agilent).

Appendix 4:Lipid associated genes list

LMPD ID	Gene Symbol	Uniprto ID	Unigene ID	David ID	AffyID	AgilentID	Lipid Category	Full name
LMP000004	St6galnac 1	Q9QZ39	MM.383719	11828	#N/A	A_51_P504028	Sphingolipids (SP)	ST6 (ALPHA-N-ACETYL-NEURAMINYL-2,3-BETA-GALACTOSYL-1,3)-N-ACETYLGALACT...
LMP000007	St3gal5	O88829	MM.38248	39223	#N/A	A_51_P483473	Sphingolipids (SP)	ST3 BETA-GALACTOSIDE ALPHA-2,3-SIALYLTRANSFERASE 5
LMP000008	Skip	Q8C5L6	MM.1458	9636	#N/A	#N/A	Glycerophospholipids (GP)	PUTATIVE PHOSPHATASE
LMP000009	Dgke	Q9R1C6	MM.153695	10953	#N/A	A_51_P410744	Glycerolipids (GL) / Glycerophospholipids (GP)	DIACYLGLYCEROL KINASE, EPSILON
LMP000012	Pik4cb	Q8BKC8	MM.386837	149041	#N/A	A_51_P202320	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 4-KINASE, CATALYTIC, BETA POLYPEPTIDE
LMP000013	Pip5k1a	P70182	MM.296409	3871	101649_at;103573_at	A_51_P441974	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL-4-PHOSPHATE 5-KINASE, TYPE 1 BETA
LMP000018	Lipa	Q9Z0M5	MM.157545	145282	#N/A	A_51_P459661	Sterol Lipids (ST)	LYSOSOMAL ACID LIPASE 1
LMP000024	Flot1	O08917	MM.2931	117626	95095_at	A_51_P423088	Not classified	FLOTILLIN 1
LMP000025	Tbxa2r	P30987	MM.4545	144071	92685_at	A_51_P473086	Fatty acids/Eicosanoids (FA)	THROMBOXANE A2 RECEPTOR
LMP000026	Slc27a2	O35488	MM.290044	27168	100967_at	A_51_P484551	Fatty acids/Eicosanoids (FA)	SOLUTE CARRIER FAMILY 27 (FATTY ACID TRANSPORTER), MEMBER 2
LMP000043	Pik3r1	Q99LY6	MM.259333	37643	96592_at	#N/A	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE, REGULATORY SUBUNIT, POLYPEPTIDE 1 (P85 ...
LMP000044	Anxa11	P97384	MM.294083	145144	102815_at	A_51_P280597	Glycerophospholipids (GP)	ANNEXIN A11
LMP000049	Hsd17b7	Q8C5N9	MM.12882	4622	94177_at	A_51_P250934	Sterol Lipids (ST)	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 7
LMP000057	Hsd17b1	P51656	MM.188939	4220	#N/A	A_51_P243435	Sterol Lipids (ST)	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 1
LMP000059	Osbpl8	Q69ZJ4	MM.220204	144350	#N/A	A_51_P286551	Sterol Lipids (ST)	OXYSTEROL BINDING PROTEIN-LIKE 8
LMP000061	Hsd17b2	P51658	MM.276466	144377	101891_at	A_51_P441914	Sterol Lipids (ST)	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 2
LMP000064	Pla2g5	P97391	MM.23347	8353	101328_at;94665_at	A_51_P207892	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP V
LMP000066	Ltb4r	O88855	MM.20853	11092	#N/A	#N/A	Fatty acids/Eicosanoids (FA)	LEUKOTRIENE B4 RECEPTOR 1
LMP000067	Osbp2	Q6PE09	MM.61022	10914	#N/A	A_51_P348636	Sterol Lipids (ST)	OXYSTEROL BINDING PROTEIN 2
LMP000070	Mcat	Q8R3F5	MM.37560	6647	#N/A	A_51_P314852	Fatty acids/Eicosanoids (FA)	CDNA SEQUENCE BC025519
LMP000074	Hsd17b4	P51660	MM.277857	3265	97515_at	A_51_P445662	Sterol Lipids (ST) / Fatty acids/Eicosanoids (FA)	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 4
LMP000076	Hsd11b2	P51661	MM.5079	149447	100493_at;161517_at	A_51_P410205	Sterol Lipids (ST)	HYDROXYSTEROID 11-BETA DEHYDROGENASE 2
LMP000079	Pla2g4c	Q64GA5	MM.223639	3309	#N/A	#N/A	Not classified	PHOSPHOLIPASE A2, GROUP IVC (CYTOSOLIC, CALCIUM-INDEPENDENT)

LMP000084	Lip1	Q6PDR1	MM.157545	145282	102123_at	#N/A	Sterol Lipids (ST)	LYSOSOMAL ACID LIPASE 1
LMP000085	Abo	Q8BZH3	MM.160386	149058	#N/A	A_51_P241499	Glycerolipids (GL) / Sphingolipids (SP)	ABO BLOOD GROUP (TRANSFERASE A, ALPHA 1-3-N-ACETYLGALACTOSAMINYLTRANSF...
LMP000086	Ndufab1	Q9CR21	MM.347976	117374	161122_f_at; 96909_at	A_51_P208801	Fatty acids/Eicosanoids (FA) / Prenol Lipids (PR)	RIKEN CDNA 2310039H15 GENE
LMP000087	Pclo	Q9QYX7	MM.332218	144565	97753_at	A_51_P436669	Not classified	PICCOLO (PRESYNAPTIC CYTOMATRIX PROTEIN)
LMP000089	Pbp2	Q8VIN1	MM.293018	37032	#N/A	A_51_P324450	Not classified	PHOSPHATIDYLETHANOLAMINE BINDING PROTEIN 2
LMP000091	Lrp10	Q7TQH7	MM.28465	144229	161625_r_at; 96186_at	A_51_P430357	Not classified	LOW-DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 10
LMP000093	Soat1	Q61263	MM.28099	149465	160767_at,95887_at	A_51_P391754	Sterol Lipids (ST)	STEROL O-ACYLTRANSFERASE 1
LMP000096	Pla2g2f	Q8CE14	MM.331989	37041	#N/A	A_51_P118255	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP IIF
LMP000097	Apoc4	Q61268	MM.344270	13194	101463_at	A_51_P460332	Not classified	APOLIPOPROTEIN C-IV
LMP000099	Anxa9	Q9JHQ0	MM.218844	38836	#N/A	A_51_P451482	Not classified	ANNEXIN A9
LMP000101	Cyp11a1	Q9QZ82	MM.302865	4084	#N/A	A_51_P309731	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 11, SUBFAMILY A, POLYPEPTIDE 1
LMP000112	Edg4	Q6P290	MM.23253	7002	#N/A	A_51_P410665	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION, LYSOPHOSPHATIDIC ACID G-PROTEIN-COUPLED R...
LMP000115	Alox12e	P55249	MM.274093	144032	102269_at;161947_f_at	A_51_P471659	Fatty acids/Eicosanoids (FA)	ARACHIDONATE LIPOXYGENASE, EPIDERMAL
LMP000116	Nr5a2	P45448	MM.16794	37660	102932_at	A_51_P514449	Sterol Lipids (ST)	NUCLEAR RECEPTOR SUBFAMILY 5, GROUP A, MEMBER 2
LMP000118	Cpt1a	P97742	MM.18522	37793	161248_r_at; 93320_at	A_51_P427674	Fatty acids/Eicosanoids (FA)	CARNITINE PALMITOYLTRANSFERASE 1A, LIVER
LMP000120	Pscd3	O08967	MM.281003	144332	103433_at;103434_at	A_51_P488937	Glycerophospholipids (GP)	PLECKSTRIN HOMOLOGY, SEC7 AND COILED-COIL DOMAINS 3
LMP000121	Elovl2	Q9JLJ4	MM.2567	27222	94393_r_at	A_51_P292582	Fatty acids/Eicosanoids (FA)	ELONGATION OF VERY LONG CHAIN FATTY ACIDS (FEN1/ELO2, SUR4/ELO3, YEAST...
LMP000122	Fads2	Q9Z0R9	MM.38901	12871	#N/A	A_51_P364609	Fatty acids/Eicosanoids (FA)	FATTY ACID DESATURASE 2
LMP000127	Sphk1	O88885	MM.20944	11179	103839_at	A_51_P501248	Sphingolipids (SP)	SPHINGOSINE KINASE 1
LMP000128	Sphk1	O88886	MM.20944	11179	103839_at	A_51_P501248	Sphingolipids (SP)	SPHINGOSINE KINASE 1
LMP000131	Slco2a1	Q9CQU9	MM.207106	38158	#N/A	A_51_P315931	Fatty acids/Eicosanoids (FA)	SOLUTE CARRIER ORGANIC ANION TRANSPORTER FAMILY, MEMBER 2A1
LMP000135	Aldh8a1	Q8BH00	MM.90181	16565	#N/A	A_51_P509384	Not classified	ALDEHYDE DEHYDROGENASE 8 FAMILY, MEMBER A1
LMP000137	Serpina6	Q06770	MM.290079	26257	161326_f_at, 96227_at	A_51_P133562	Sterol Lipids (ST)	SERINE (OR CYSTEINE) PEPTIDASE INHIBITOR, CLADE A, MEMBER 6
LMP000139	Apoa2	P09813	MM.288374	144830	161924_f_at; 99648_at	A_51_P128973	Sterol Lipids (ST)	APOLIPOPROTEIN A-II
LMP000141	Hexa	P29416	MM.2284	27886	162488_at;94840_at	A_51_P282663	Sphingolipids (SP)	HEXOSAMINIDASE A

LMP000142	Bpnt1	Q9Z0S1	MM.227549	36023	103336_r_at	A_51_P512503	Glycerophospholipids (GP)	BISPHOSPHATE 3'-NUCLEOTIDASE 1
LMP000144	Apoc1	P34928	MM.182440	7402	93354_at	A_51_P164504	Not classified	APOLIPOPROTEIN C-I
LMP000155	Ednra	Q61614	MM.283168	15439	#N/A	A_51_P457734	Not classified	ENDOTHELIN RECEPTOR TYPE A
LMP000160	Pctp	P53808	MM.5062	9516	101173_at	A_51_P113477	Not classified	PHOSPHATIDYLCHOLINE TRANSFER PROTEIN
LMP000162	Elovl6	Q8CE45	MM.314113	38130	103665_at;94418_at	A_51_P463440	Fatty acids/Eicosanoids (FA)	ELOVL FAMILY MEMBER 6, ELONGATION OF LONG CHAIN FATTY ACIDS (YEAST)
LMP000164	Cyp2j5	O54749	MM.12838	16763	92814_at	A_51_P307872	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY J, POLYPEPTIDE 5
LMP000169	Hexb	P20060	MM.27816	14150	97232_at	A_51_P453111	Sphingolipids (SP)	HEXOSAMINIDASE B
LMP000177	Hlcs	Q920N2	MM.30921	9998	#N/A	A_51_P176912	Fatty acids/Eicosanoids (FA)	HOLOCARBOXYLASE SYNTHETASE (BIOTIN-[PROPRIONY-COENZYME A-CARBOXYLASE ...
LMP000180	Pitpna	P53810	NA	149711	#N/A	A_51_P466371	Not classified	PHOSPHATIDYLINOSITOL TRANSFER PROTEIN, ALPHA
LMP000181	Rxra	P28700	MM.24624	145920	92234_at,92235_g_at	A_51_P307370	Sterol Lipids (ST)	RETINOID X RECEPTOR ALPHA
LMP000185	Cyp2j6	O54750	MM.98200	8098	92813_at	A_51_P506328	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY J, POLYPEPTIDE 6
LMP000187	Rxrb	P28704	MM.1243	144990	102398_at	A_51_P314830	Sterol Lipids (ST)	RETINOID X RECEPTOR BETA
LMP000188	Rxrg	P28705	MM.3475	149387	92237_at	A_51_P513311	Sterol Lipids (ST)	RETINOID X RECEPTOR GAMMA
LMP000191	Lipc	P27656	MM.30056	150635	98962_at	A_51_P263993	Glycerophospholipids (GP)	LIPASE, HEPATIC
LMP000195	Cept1	Q8BGS7	MM.14816	145071	#N/A	A_51_P313460	Not classified	CHOLINE/ETHANOLAMINEPHOSPHOTRANSFERASE 1
LMP000196	Edg3	Q9Z0U9	MM.136736	3654	92352_at	A_51_P137522	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION, SPHINGOLIPID G-PROTEIN-COUPLED RECEPTOR, ...
LMP000197	Osbp	Q52KH7	MM.291279	35712	#N/A	A_51_P393768	Sterol Lipids (ST)	OXYSTEROL BINDING PROTEIN
LMP000200	ND2	Q9MD59	NA	10664	#N/A	#N/A	Not classified	NADH DEHYDROGENASE SUBUNIT 2
LMP000202	Isyna1	Q9JHU9	MM.29357	13815	#N/A	#N/A	Not classified	MYO-INOSITOL 1-PHOSPHATE SYNTHASE A1
LMP000203	Acsl4	Q8BW44	MM.143689	17019	#N/A	A_51_P268154	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE LONG-CHAIN FAMILY MEMBER 4
LMP000213	Slc10a3	P21129	MM.3979	38063	#N/A	A_51_P423041	Not classified	UBIQUITIN-LIKE 4
LMP000218	Pik3r3	Q64143	MM.253819	148193	#N/A	A_51_P222863	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3 KINASE, REGULATORY SUBUNIT, POLYPEPTIDE 3 (P55)
LMP000219	Rarg	P18911	MM.383991	5803	102419_at 100700_s_at; 100701_r_at; 101665_at;101666_at;161418_r_at	A_51_P372853	Prenol Lipids (PR) / Sterol Lipids (ST)	RETINOIC ACID RECEPTOR, GAMMA
LMP000224	Nr5a1	P97782	MM.31387	145521	94140_at;94792_at	A_51_P315964	Sterol Lipids (ST)	FUSHI TARAZU 1 FACTOR HOMOLOG, (DROSOPHILA)
LMP000225	Msr1	P30204	MM.239291	149493		A_51_P404846	Not classified	MACROPHAGE SCAVENGER RECEPTOR 1

LMP000226	Paqr7	Q80ZE4	MM.142343	145415	#N/A	A_51_P430973	Sterol Lipids (ST)	PROGESTIN AND ADIPOQ RECEPTOR FAMILY MEMBER VII
LMP000227	Paqr8	Q80ZE5	MM.40780	20294	#N/A	A_51_P422540	Sterol Lipids (ST)	PROGESTIN AND ADIPOQ RECEPTOR FAMILY MEMBER VIII
LMP000228	Fut4	Q11127	MM.63450	144114	99406_at	A_51_P441726	Glycerolipids (GL) / Glycerophospholipids (GP) / Sphingolipids (SP)	FUCOSYLTRANSFERASE 4
LMP000237	Pla2g7	Q8BKM3	MM.9277	17346	101923_at	A_51_P447835	Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP VII (PLATELET-ACTIVATING FACTOR ACETYLHYDROLAS...
LMP000239	Mecr	Q9DCS3	MM.192706	31172	#N/A	A_51_P386080	Fatty acids/Eicosanoids (FA)	MITOCHONDRIAL TRANS-2-ENOYL-COA REDUCTASE
LMP000242	Acot5	Q6Q2Z6	MM.123934	27213	#N/A	A_51_P355996	Fatty acids/Eicosanoids (FA)	ACYL-COA THIOESTERASE 5
LMP000248	Lrat	Q9JI60	MM.33921	6861	#N/A	A_51_P350033	Glycerophospholipids (GP) / Prenol Lipids (PR)	LECITHIN-RETINOL ACYLTRANSFERASE (PHOSPHATIDYLCHOLINE-RETINOL-O-ACYLTR...
LMP000251	Fut7	Q11131	MM.1203	26667	94138_s_at	A_51_P445479	Glycerolipids (GL) / Glycerophospholipids (GP) / Sphingolipids (SP)	FUCOSYLTRANSFERASE 7
LMP000252	mt-Nd4	Q9MD77	NA	27202	#N/A	#N/A	Not classified	ACTIVATED SPLEEN CDNA, RIKEN FULL-LENGTH ENRICHED LIBRARY, CLONE:F8302
LMP000253	B3galt5	Q9JI67	MM.154783	24703	#N/A	A_51_P296583;A_51_P301435	Sphingolipids (SP)	UDP-GAL:BETAGLCNAC BETA 1,3-GALACTOSYLTRANSFERASE, POLYPEPTIDE 5
LMP000255	Cyp4f14	Q9EP75	MM.10976	19282	#N/A	A_51_P452768	Not classified	CYTOCHROME P450, FAMILY 4, SUBFAMILY F, POLYPEPTIDE 14
LMP000256	Agpat3	Q9D517	MM.141230	145440	160807_at	A_51_P425490	Not classified	1-ACYLGLYCEROL-3-PHOSPHATE O-ACYLTRANSFERASE 3
LMP000265	Atp8b3	Q6UQ17	MM.52511	16782	#N/A	A_51_P371500	Not classified	ATPASE, CLASS I, TYPE 8B, MEMBER 3
LMP000266	Akr1e1	Q9DCT1	MM.251908	8656	103068_at	A_51_P195316	Glycerophospholipids (GP) / Sterol Lipids (ST)	ALDO-KETO REDUCTASE FAMILY 1, MEMBER E1
LMP000269	Acot8	P58137	MM.277878	4064	#N/A	A_51_P454078	Fatty acids/Eicosanoids (FA)	ACYL-COA THIOESTERASE 8
LMP000270	Ggt1	Q60928	MM.4559	7923	100085_at	A_51_P468071	Fatty acids/Eicosanoids (FA)	GAMMA-GLUTAMYLTRANSFERASE 1
LMP000275	A4galt	Q67BJ4	MM.323805	144170	#N/A	#N/A	Sphingolipids (SP)	ALPHA 1,4-GALACTOSYLTRANSFERASE
LMP000276	mt-Nd5	Q9MD82	NA	10662	#N/A	#N/A	Not classified	NADH DEHYDROGENASE SUBUNIT 5
LMP000278	Nqo2	Q9JI75	MM.264036	11348	#N/A	A_51_P229536	Sterol Lipids (ST)	NAD(P)H DEHYDROGENASE, QUINONE 2
LMP000287	Cyp26b1	Q811W2	MM.255246	9519	#N/A	A_51_P100396;A_51_P501844	Prenol Lipids (PR)	CYTOCHROME P450, FAMILY 26, SUBFAMILY B, POLYPEPTIDE 1
LMP000288	Paqr5	Q9DCU0	MM.273267	37896	#N/A	A_51_P258768	Sterol Lipids (ST)	PROGESTIN AND ADIPOQ RECEPTOR FAMILY MEMBER V
LMP000291	Sphk1	Q8CI15	MM.20944	11179	103839_at	A_51_P501248	Sphingolipids (SP)	SPHINGOSINE KINASE 1
LMP000292	Nr1h5	Q811W9	MM.331269	13241	#N/A	A_51_P226667	Not classified	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP H, MEMBER 5
LMP000293	Pnliprp2	P17892	MM.212333	146794	160070_at	A_51_P314669	Glycerophospholipids (GP)	PANCREATIC LIPASE-RELATED PROTEIN 2

LMP000295	Pla2g1b	Q9Z0Y2	MM.20190	22565	160120_i_at	A_51_P280893	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP IB, PANCREAS
LMP000296	Nr1h3	Q9Z0Y9	MM.22690	144164	104381_at	A_51_P448778	Sterol Lipids (ST)	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP H, MEMBER 3
LMP000300	Apoa1	Q00623	MM.26743	18836	96094_at	A_51_P408082	Sterol Lipids (ST)	APOLIPOPROTEIN A-I
LMP000309	Itpka	Q8R071	MM.65337	27434	#N/A	A_51_P273609	Glycerophospholipids (GP)	INOSITOL 1,4,5-TRISPHOSPHATE 3-KINASE A
LMP000310	Nr1h5	Q811X0	MM.331269	13241	#N/A	A_51_P226667	Not classified	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP H, MEMBER 5
LMP000312	Nr1h5	Q811X1	MM.331269	13241	#N/A	A_51_P226667	Not classified	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP H, MEMBER 5
LMP000313	Nr1h5	Q811X2	MM.331269	13241	#N/A	A_51_P226667	Not classified	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP H, MEMBER 5
LMP000314	Syt16	Q7TN83	MM.311393	148305	#N/A	A_51_P122866	Not classified	SYNAPTOTAGMIN XVI
LMP000315	Cyp7a1	Q64505	MM.57029	17149	99404_at	A_51_P290981	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 7, SUBFAMILY A, POLYPEPTIDE 1
LMP000317	Syt14	Q7TN84	MM.170401	147194	#N/A	A_51_P372565	Not classified	SYNAPTOTAGMIN XIV
LMP000320	Dgat2	Q9DCV3	MM.180189	45478	#N/A	A_51_P396003	Glycerolipids (GL)	DIACYLGLYCEROL O-ACYLTRANSFERASE 2
LMP000321	Plcxd1	Q8CHS4	MM.297057	19242	#N/A	A_51_P322658	Not classified	PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C, X DOMAIN CONTAINING 1
LMP000328	Scp2	P32020	MM.379011	143786	93278_at	A_51_P444052	Sterol Lipids (ST)	STEROL CARRIER PROTEIN 2, LIVER
LMP000330	Acot8	Q8BZR4	MM.277878	4064	#N/A	A_51_P454078	Fatty acids/Eicosanoids (FA)	ACYL-COA THIOESTERASE 8
LMP000331	Prkca	P20444	MM.222178	31943	102299_at	A_51_P118007	Glycerolipids (GL) / Glycerophospholipids (GP)	PROTEIN KINASE C, ALPHA
LMP000332	Sgpp1	Q9JI99	MM.280199	147197	94501_at	A_51_P469992	Sphingolipids (SP)	SPHINGOSINE-1-PHOSPHATE PHOSPHATASE 1
LMP000338	Gk	Q64516	MM.246682	144099	#N/A	#N/A	Glycerophospholipids (GP)	GLYCEROL KINASE
LMP000339	Nr1i3	Q8CI30	MM.3077	27185	102171_r_at; 104506_at;104507_g_at	A_51_P358256	Sterol Lipids (ST)	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP I, MEMBER 3
LMP000342	Atp5g2	P56383	MM.381662	7015	102134_f_at; 92570_at	A_51_P417399	Not classified	ATP SYNTHASE, H+ TRANSPORTING, MITOCHONDRIAL F0 COMPLEX, SUBUNIT C (SU...
LMP000343	Slc25a29	Q8BL03	MM.266264	145827	#N/A	A_51_P288479	Fatty acids/Eicosanoids (FA)	SOLUTE CARRIER FAMILY 25 (MITOCHONDRIAL CARRIER, PALMITOYLCARNITINE TR...
LMP000357	Egln2	Q91YE2	MM.29978	146982	96338_at	A_51_P385415	Not classified	EXPRESSED SEQUENCE C85656
LMP000361	Impa1	Q80ZJ2	MM.183042	145170	101498_at	A_51_P242056	Glycerophospholipids (GP)	INOSITOL (MYO)-1(OR 4)-MONOPHOSPHATASE 1
LMP000363	Gpd2	Q64521	MM.3711	26998	162210_r_at; 98984_f_at	A_51_P342481	Glycerophospholipids (GP)	GLYCEROL PHOSPHATE DEHYDROGENASE 2, MITOCHONDRIAL
LMP000370	Pla2g7	Q60963	MM.9277	17346	101923_at	A_51_P447835	Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP VII (PLATELET-ACTIVATING FACTOR ACETYLHYDROLAS...
LMP000371	Hsd3b5	Q61694	MM.17910	12345	94795_at	A_51_P496162	Sterol Lipids (ST)	HYDROXYSTEROID DEHYDROGENASE-5, DELTA<5>-3-BETA
LMP000373	Cyb5a	P56395	MM.31018	33638	#N/A	#N/A	Fatty acids/Eicosanoids (FA)	RIKEN CDNA 0610009N12 GENE

LMP000374	Liph	Q8CI45	MM.33192	16830	#N/A	A_51_P122660	Not classified	LIPASE, MEMBER H
LMP000375	Echs1	Q8BH95	MM.24452	19848	95426_at	A_51_P409039	Fatty acids/Eicosanoids (FA)	ENOYL COENZYME A HYDRATASE, SHORT CHAIN, 1, MITOCHONDRIAL
LMP000379	Fads3	Q6IQY1	MM.253875	144010	#N/A	A_51_P464029	Fatty acids/Eicosanoids (FA)	FATTY ACID DESATURASE 3
LMP000382	Cyp2d9	P11714	MM.378904	148470	162174_at;94539_f_at	A_51_P478303	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY D, POLYPEPTIDE 9
LMP000390	Crabp1	P62965	MM.34797	27997	98108_at	A_51_P378978	Prenol Lipids (PR)	CELLULAR RETINOIC ACID BINDING PROTEIN I
LMP000391	Hadha	Q8BKS7	MM.200497	33608	#N/A	A_51_P331549	Fatty acids/Eicosanoids (FA)	HYDROXYACYL-COENZYME A DEHYDROGENASE/3-KETOACYL-COENZYME A THIOLASE/EN...
LMP000394	Cerk	Q52KP2	MM.222685	146813	101426_at	A_51_P146360	Glycerolipids (GL) / Sphingolipids (SP)	CERAMIDE KINASE
LMP000396	Prkar2b	P31324	MM.25594	17895	#N/A	A_51_P221062;A_51_P507546	Fatty acids/Eicosanoids (FA)	PROTEIN KINASE, CAMP DEPENDENT REGULATORY, TYPE II BETA
LMP000409	Inpp1	P49442	MM.917	147030	161407_i_at;93942_at	A_51_P361465	Glycerophospholipids (GP)	INOSITOL POLYPHOSPHATE-1-PHOSPHATASE
LMP000424	Mulk	Q9ESW4	MM.32840	146910	#N/A	#N/A	Sphingolipids (SP)	MULTIPLE SUBSTRATE LIPID KINASE
LMP000428	Scd1	P13516	MM.267377	143718	161445_at,94056_at,94057_g_at,94058_r_at,94424_at	A_51_P408569	Fatty acids/Eicosanoids (FA)	STEAROYL-COENZYME A DESATURASE 1
LMP000432	Fcer1a	P20489	MM.5266	14709	101209_at	A_51_P429770	Fatty acids/Eicosanoids (FA)	FC RECEPTOR, IGE, HIGH AFFINITY I, ALPHA POLYPEPTIDE
LMP000433	Crls1	Q80ZM8	MM.357342	37126	#N/A	A_51_P373187	Not classified	RIKEN CDNA 0610009I22 GENE
LMP000434	Cyp7b1	Q60991	MM.316000	9309	161345_f_at;92898_at	A_51_P461429	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 7, SUBFAMILY B, POLYPEPTIDE 1
LMP000437	Alox12l	P39654	MM.4584	9805	#N/A	#N/A	Fatty acids/Eicosanoids (FA)	ARACHIDONATE 15-LIPOXYGENASE
LMP000439	Alox12	P39655	MM.12286	148931	102290_at	A_51_P520306	Fatty acids/Eicosanoids (FA)	ARACHIDONATE 12-LIPOXYGENASE
LMP000440	Adipoq	Q60994	MM.3969	147321	#N/A	A_51_P458451	Fatty acids/Eicosanoids (FA)	ADIPONECTIN, C1Q AND COLLAGEN DOMAIN CONTAINING
LMP000457	Sc4mol	Q9CRA4	MM.30119	22983	160388_at	A_51_P209372	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	STEROL-C4-METHYL OXIDASE-LIKE
LMP000463	Pisd	Q8BSF4	MM.273765	6970	#N/A	A_51_P461947	Glycerophospholipids (GP)	PHOSPHATIDYLSERINE DECARBOXYLASE
LMP000470	Zdhhc2	P59267	MM.34326	146241	#N/A	A_51_P117226	Fatty acids/Eicosanoids (FA)	ZINC FINGER, DHHC DOMAIN CONTAINING 2
LMP000474	Plcb3	Q8CI86	MM.273204	148650	101358_at	A_51_P236042	Glycerophospholipids (GP)	PHOSPHOLIPASE C, BETA 3
LMP000483	Rarb	P22605	MM.259318	20242	#N/A	A_51_P202440	Prenol Lipids (PR) / Sterol Lipids (ST)	RETINOIC ACID RECEPTOR, BETA
LMP000486	Ggta1	P23336	MM.281124	7347	102993_at	A_51_P349988	Glycerolipids (GL)	GLYCOPROTEIN GALACTOSYLTRANSFERASE ALPHA 1, 3
LMP000489	Acot7	Q91V12	MM.296191	3939	#N/A	A_51_P328652	Fatty acids/Eicosanoids (FA)	ACYL-COA THIOESTERASE 7

LMP000501	Calm4	Q9JM83	MM.21075	8375	93744_at	A_51_P460613	Glycerophospholipids (GP)	RIKEN CDNA 2310037J09 GENE
LMP000503	Lep	P41160	MM.277072	22348	98443_at;98444_g_at	A_51_P225546	Sterol Lipids (ST)	LEPTIN
LMP000510	Sult2a2	P50236	MM.260026	9546	#N/A	#N/A	Sterol Lipids (ST)	SULFOTRANSFERASE FAMILY 2A, DEHYDROEPIANDROSTERONE (DHEA)-PREFERRING, ...
LMP000511	Prkce	P16054	MM.24614	145670	94161_at	A_51_P352782;A_51_P436429	Glycerolipids (GL)	PROTEIN KINASE C, EPSILON
LMP000513	Sqle	P52019	MM.296169	36009	161274_at;94322_at	A_51_P450487	Prenol Lipids (PR) / Sterol Lipids (ST)	SQUALENE EPOXIDASE
LMP000523	Pla2g3	Q8BL71	MM.100476	43776	#N/A	A_51_P402868	Not classified	PHOSPHOLIPASE A2, GROUP III
LMP000524	Cbr1	P48758	MM.26940	37417	96110_at	A_51_P262739	Fatty acids/Eicosanoids (FA)	CARBONYL REDUCTASE 1
LMP000526	Ocrl	Q6NVF0	MM.210343	38837	#N/A	A_51_P159492	Not classified	OCULOCEREBRORENAL SYNDROME OF LOWE
LMP000529	Auh	Q9JLZ3	MM.252034	9232	96650_at	A_51_P372472	Fatty acids/Eicosanoids (FA)	AU RNA BINDING PROTEIN/ENOYL-COENZYME A HYDRATASE
LMP000538	Sphk2	Q9JIA7	MM.24222	26770	162381_f_at	A_51_P119302	Glycerolipids (GL) / Sphingolipids (SP)	SPHINGOSINE KINASE 2
LMP000544	Mlycd	Q99J39	MM.255499	45373	103622_at	A_51_P222283	Fatty acids/Eicosanoids (FA)	MALONYL-COA DECARBOXYLASE
LMP000549	Cav1	P49817	MM.28278	147850	#N/A	A_51_P107302	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	CAVEOLIN, CAVEOLAE PROTEIN 1
LMP000554	Plcb1	Q9Z1B3	MM.378986	146620	92465_at;92466_at;92467_g_at	A_51_P284665	Glycerophospholipids (GP)	PHOSPHOLIPASE C, BETA 1
LMP000556	Plcd1	Q9Z1B4	MM.23963	16634	#N/A	A_51_P239766	Glycerophospholipids (GP)	PHOSPHOLIPASE C, DELTA 1
LMP000558	Stat5a	P42230	MM.277403	15573	#N/A	A_51_P152765	Sterol Lipids (ST)	SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 5A
LMP000559	Pnlip	Q9D950	MM.20407	17651	#N/A	A_51_P520552	Sterol Lipids (ST)	PANCREATIC LIPASE
LMP000560	Stat5b	P42232	MM.34064	10891	100422_i_at;100423_f_at	A_51_P281938	Sterol Lipids (ST)	SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 5B
LMP000572	Rbp7	Q9EPC5	MM.46023	17791	#N/A	A_51_P508902	Prenol Lipids (PR)	RETINOL BINDING PROTEIN 7, CELLULAR
LMP000576	Agpat5	Q9D1E8	MM.24117	26777	#N/A	A_51_P361286	Glycerophospholipids (GP)	1-ACYLGLYCEROL-3-PHOSPHATE O-ACYLTRANSFERASE 5 (LYSOPHOSPHATIDIC ACID ...
LMP000577	Mogat2	Q80W94	MM.208030	13083	#N/A	A_51_P411345	Glycerolipids (GL)	MONOACYLGLYCEROL O-ACYLTRANSFERASE 2
LMP000583	Pdhx	Q8BKZ9	MM.315011	146520	#N/A	A_51_P149944	Not classified	PYRUVATE DEHYDROGENASE COMPLEX, COMPONENT X
LMP000591	St3gal5	Q9CZ65	MM.38248	39223	#N/A	A_51_P483473	Sphingolipids (SP)	ST3 BETA-GALACTOSIDE ALPHA-2,3-SIALYLTRANSFERASE 5
LMP000595	Prkab2	Q6PAM0	MM.31175	9154	#N/A	#N/A	Not classified	PROTEIN KINASE, AMP-ACTIVATED, BETA 2 NON-CATALYTIC SUBUNIT
LMP000596	Pccb	Q99MN9	MM.335385	19392	160128_at	A_51_P418259	Fatty acids/Eicosanoids (FA)	PROPIONYL COENZYME A CARBOXYLASE, BETA POLYPEPTIDE

LMP000607	Mttp	O08601	MM.2941	144926	104448_at	A_51_P178887	Glycerolipids (GL)	MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN
LMP000610	Cbr2	P08074	MM.21454	14431	93808_at	A_51_P238425	Fatty acids/Eicosanoids (FA)	CARBONYL REDUCTASE 2
LMP000613	Pmvk	Q9D1G2	MM.34242	146543	#N/A	A_51_P492408	Sterol Lipids (ST)	PHOSPHOMEVALONATE KINASE
LMP000614	Ihpk3	Q8BWD2	MM.209679	12680	#N/A	A_51_P163238	Not classified	INOSITOL HEXAPHOSPHATE KINASE 3
LMP000616	Pib5pa	P59644	MM.24313	144173	103732_at	A_51_P180669	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL (4,5) BISPHOSPHATE 5-PHOSPHATASE, A
LMP000626	Anxa2	P07356	MM.238343	16639	100569_at	A_51_P165335	Not classified	ANNEXIN A2
LMP000631	Cpt1b	Q924X2	MM.358582	34408	#N/A	A_51_P232913	Fatty acids/Eicosanoids (FA)	CARNITINE PALMITOYLTRANSFERASE 1B, MUSCLE
LMP000634	Lrp8	Q924X6	MM.331760	143820	#N/A	A_51_P145041	Not classified	LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 8, APOLIPOPROTEIN E R...
LMP000638	Akr1c13	Q8VC28	NA	20604	95015_at	#N/A	Glycerophospholipids (GP) / Sterol Lipids (ST)	ALDO-KETO REDUCTASE FAMILY 1, MEMBER C13
LMP000642	Prmt1	Q9JIF0	MM.27545	147345	#N/A	A_51_P389531	Sterol Lipids (ST)	PROTEIN ARGININE N-METHYLTRANSFERASE 1
LMP000644	Clc	P97400	NA	22164	#N/A	#N/A	Not classified	CHARCOT-LEYDEN CRYSTAL PROTEIN
LMP000645	Cyp2d10	P24456	NA	3215	98612_at	A_51_P111192	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY D, POLYPEPTIDE 10
LMP000646	Gcnt2	P97402	MM.314757	26665	102095_f_at; 95392_at	A_51_P135742	Glycerolipids (GL)	GLUCOSAMINYL (N-ACETYL) TRANSFERASE 2, I-BRANCHING ENZYME
LMP000652	Acot5	Q91YQ6	MM.123934	27213	#N/A	A_51_P355996	Fatty acids/Eicosanoids (FA)	ACYL-COA THIOESTERASE 5
LMP000653	Hhat	Q8BWF5	MM.145857	26910	#N/A	A_51_P445677	Fatty acids/Eicosanoids (FA)	HEDGEHOG ACYLTRANSFERASE
LMP000660	Apob48r	Q8VBT6	MM.170665	18796	#N/A	#N/A	Not classified	APOLIPOPROTEIN B48 RECEPTOR
LMP000663	Atp6v0a1	Q9Z1G4	MM.340818	17112	103275_at	A_51_P142080	Not classified	ATPASE, H+ TRANSPORTING, LYSOSOMAL V0 SUBUNIT A1
LMP000665	Itpr3	P70227	MM.328900	3786	#N/A	A_51_P104897	Glycerophospholipids (GP)	INOSITOL 1,4,5-TRIPHOSPHATE RECEPTOR 3
LMP000668	Cyp26a1	O55127	MM.42230	146537	#N/A	A_51_P401568	Prenol Lipids (PR)	CYTOCHROME P450, FAMILY 26, SUBFAMILY A, POLYPEPTIDE 1
LMP000670	Cyp2a4	P15392	MM.154643	16767	102847_s_at	A_51_P377154	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY A, POLYPEPTIDE 4
LMP000672	Mfge8	P21956	MM.1451	37289	92880_at	A_51_P255682	Glycerophospholipids (GP)	MILK FAT GLOBULE-EGF FACTOR 8 PROTEIN
LMP000675	Plcb4	Q91UZ1	MM.38009	148146	#N/A	A_51_P211659;A_51_P225695	Glycerophospholipids (GP)	PHOSPHOLIPASE C, BETA 4
LMP000679	Lass2	Q924Z4	MM.181009	7967	160396_at	A_51_P517375	Not classified	LONGEVITY ASSURANCE HOMOLOG 2 (S. CEREVISIAE)
LMP000684	Lipc	Q8VC44	MM.30056	31093	98962_at	A_51_P263993	Glycerolipids (GL)	LIPASE, HEPATIC
LMP000685	Sec14l4	Q8R0F9	MM.337476	37247	#N/A	A_51_P194853	Not classified	SEC14-LIKE 4 (S. CEREVISIAE)
LMP000691	Acot1	O55137	MM.1978	143852	#N/A	A_51_P451574	Fatty acids/Eicosanoids (FA)	ACYL-COA THIOESTERASE 1
LMP000696	Vldlr	P98156	MM.4141	17613	160865_at;96534_at	A_51_P278333;A_51_P500044	Sterol Lipids (ST)	VERY LOW DENSITY LIPOPROTEIN RECEPTOR

LMP000699	Lrp8	Q921B5	MM.331760	143820	#N/A	A_51_P145041	Not classified	LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 8, APOLIPOPROTEIN E R...
LMP000706	Fads1	Q8R0G8	MM.30158	3941	#N/A	A_51_P358112	Fatty acids/Eicosanoids (FA)	FATTY ACID DESATURASE 1
LMP000709	Dgkb	Q6NS52	MM.242576	146614	#N/A	A_51_P515612	Glycerolipids (GL)	DIACYLGLYCEROL KINASE, BETA
LMP000710	Ebp	P70245	MM.27183	38190	96627_at	A_51_P429682	Sterol Lipids (ST)	PHENYLALKYLAMINE CA2+ ANTAGONIST (EMOPAMIL) BINDING PROTEIN
LMP000714	Gpaa1	Q9WTK3	MM.5903	5313	100911_at;161682_f_at	A_51_P273203	Glycerophospholipids (GP)	GPI ANCHOR ATTACHMENT PROTEIN 1
LMP000715	Rbm14	Q62019	MM.276338	25411	100138_f_at, 100546_at,162067_at	A_51_P116616	Not classified	RNA BINDING MOTIF PROTEIN 14
LMP000721	Inpp5e	Q9JII1	MM.250359	17140	#N/A	A_51_P136324	Glycerophospholipids (GP)	INOSITOL POLYPHOSPHATE-5-PHOSPHATASE E
LMP000724	Cyp3a41	Q9JMA7	MM.379071	7396	#N/A	A_51_P341203	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 3, SUBFAMILY A, POLYPEPTIDE 41
LMP000725	Akr1a1	Q9JII6	MM.30085	32755	#N/A	#N/A	Glycerolipids (GL)	ALDO-KETO REDUCTASE FAMILY 1, MEMBER A4 (ALDEHYDE REDUCTASE)
LMP000729	Sult1e1	P49891	MM.89655	149873	#N/A	A_51_P493649	Sterol Lipids (ST)	SULFOTRANSFERASE FAMILY 1E, MEMBER 1
LMP000732	Rbm14	Q8C2Q3	MM.276338	25411	100138_f_at, 100546_at,162067_at	A_51_P116616	Not classified	RNA BINDING MOTIF PROTEIN 14
LMP000735	Cyp51	Q8BSQ7	MM.140158	6612	94916_at	A_51_P485791	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 51
LMP000738	Cept1	Q8VC64	MM.14816	145071	#N/A	A_51_P313460	Not classified	CHOLINE/ETHANOLAMINEPHOSPHOTRANSFERASE 1
LMP000740	Hmgcs1	Q8JZK9	MM.61526	29332	94325_at	A_51_P146941	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	3-HYDROXY-3-METHYLGLUTARYL-COENZYME A SYNTHASE 1
LMP000742	Hsd3b1	P24815	MM.140811	136198	103072_at;161261_f_at	A_51_P421422	Sterol Lipids (ST)	HYDROXYSTEROID DEHYDROGENASE-1, DELTA<5>-3-BETA
LMP000746	Lypla2	Q9WTL7	MM.34302	7014	98429_at	A_51_P520084	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	LYSOPHOSPHOLIPASE 2
LMP000747	Dbi	P31786	MM.2785	26248	97248_at	A_51_P399697	Not classified	DIAZEPAM BINDING INHIBITOR
LMP000752	Soat2	O88908	MM.358862	39251	97132_at;99431_at	A_51_P196995	/ Sterol Lipids (ST)	STEROL O-ACYLTRANSFERASE 2
LMP000755	Pemt	Q8R0I1	MM.2731	144334	161893_i_at; 94987_at	A_51_P193095	Not classified	PHOSPHATIDYLETHANOLAMINE N-METHYLTRANSFERASE
LMP000757	Ppt1	Q8VBX5	MM.277719	147223	99646_at	A_51_P114307	Fatty acids/Eicosanoids (FA)	PALMITOYL-PROTEIN THIOESTERASE 1
LMP000758	Ptafr	Q62035	MM.89389	3572	94157_i_at;94158_f_at;94159_at	A_51_P292250;A_51_P430014	Glycerophospholipids (GP)	PLATELET-ACTIVATING FACTOR RECEPTOR
LMP000759	Ptgdr	P70263	MM.5105	36867	#N/A	#N/A	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN D RECEPTOR
LMP000769	Gpihbp1	Q9D1N2	MM.46367	145573	#N/A	#N/A	Sterol Lipids (ST)	GPI-ANCHORED HDL-BINDING PROTEIN 1
LMP000773	Acox3	Q9EPL9	MM.291503	146997	#N/A	A_51_P252768	Fatty acids/Eicosanoids (FA) / Prenol Lipids (PR)	ACYL-COENZYME A OXIDASE 3, PRISTANOYL

LMP000775	St6galnac4	Q9R2B6	MM.27446	6968	#N/A	A_51_P284891	Sphingolipids (SP)	ST6 (ALPHA-N-ACETYL-NEURAMINYL-2,3-BETA-GALACTOSYL-1,3)-N-ACETYLGALACT...
LMP000780	Pla2g12b	Q8VC81	MM.30268	37683	#N/A	A_51_P184385	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP XIIB
LMP000781	Ggps1	Q9WTN0	MM.148039	16902	104222_f_at; 98970_at	A_51_P179605;A_51_P469898	Prenol Lipids (PR) / Sterol Lipids (ST)	GERANYLGERANYL DIPHOSPHATE SYNTHASE 1
LMP000781	Ggps2	Q9WTN1	MM.148039	67972.45323	104222_f_at; 98970_at	A_51_P179605;A_51_P469899	Prenol Lipids (PR) / Sterol Lipids (ST)	GERANYLGERANYL DIPHOSPHATE SYNTHASE 2
LMP000782	Glb1	P23780	MM.290516	13271	103647_at;161754_f_at	A_51_P327559	Glycerolipids (GL) / Sphingolipids (SP)	GALACTOSIDASE, BETA 1
LMP000786	Srebf1	Q9WTN3	MM.278701	17082	93264_at	A_51_P401921	Sterol Lipids (ST)	STEROL REGULATORY ELEMENT BINDING FACTOR 1
LMP000792	Cds1	P98191	MM.46764	3318	#N/A	A_51_P479688	Glycerolipids (GL) / Glycerophospholipids (GP)	CDP-DIACYLGLYCEROL SYNTHASE 1
LMP000793	Pla2g2f	Q9QZT4	MM.331989	37041	#N/A	A_51_P118255	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP IIF
LMP000794	Gnpat	P98192	MM.29114	107088	94562_at	A_51_P224575	Glycerophospholipids (GP)	GLYCERONEPHOSPHATE O-ACYLTRANSFERASE
LMP000799	Lcn5	Q9R2C6	MM.12867	8935	99236_at	A_51_P191875	Prenol Lipids (PR)	LIPOCALIN 5
LMP000802	Ptger2	Q62053	MM.4630	31104	98768_at	A_51_P249215	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN E RECEPTOR 2 (SUBTYPE EP2)
LMP000805	Tbxas1	P36423	MM.4054	22953	162136_r_at; 92387_at	A_51_P448667	Fatty acids/Eicosanoids (FA)	THROMBOXANE A SYNTHASE 1, PLATELET
LMP000806	Plekha4	Q8VC98	MM.274158	147078	#N/A	A_51_P501858	Not classified	PLECKSTRIN HOMOLOGY DOMAIN CONTAINING, FAMILY A (PHOSPHOINOSITIDE BIND...
LMP000808	Pik3cd	O35904	MM.229108	147660	103866_at;92390_at	A_51_P142320	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE CATALYTIC DELTA POLYPEPTIDE
LMP000809	Prkcg	P63318	MM.368265	28552	#N/A	#N/A	Glycerolipids (GL) / Glycerophospholipids (GP)	PROTEIN KINASE C, GAMMA
LMP000812	Ptger1	P35375	MM.347482	13051	99998_at	A_51_P144303	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN E RECEPTOR 1 (SUBTYPE EP1)
LMP000813	Ldlr	Q8CAV5	MM.3213	8818	160832_at	A_51_P274173	Sterol Lipids (ST)	LOW DENSITY LIPOPROTEIN RECEPTOR
LMP000816	Ptgs2	Q05769	MM.292547	37761	104647_at	A_51_P254855	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN-ENDOPEROXIDE SYNTHASE 2
LMP000818	Lpgat1	Q91YX5	MM.277958	5370	#N/A	A_51_P148069;A_51_P453351	Not classified	LYSOPHOSPHATIDYLGLYCEROL ACYLTRANSFERASE 1
LMP000820	Coq7	P97478	MM.20634	26734	93582_at	A_51_P161682	Prenol Lipids (PR)	DEMETHYL-Q 7
LMP000822	Cxcl16	Q8BSU2	MM.359802	16564	#N/A	A_51_P374198	Not classified	CHEMOKINE (C-X-C MOTIF) LIGAND 16
LMP000825	Lrpap1	P55302	MM.277661	15928	100086_at;161988_f_at	A_51_P513941	Not classified	LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN ASSOCIATED PROTEIN 1
LMP000833	Pebp1	P70296	MM.195898	27999	#N/A	A_51_P171700	Not classified	PHOSPHATIDYLETHANOLAMINE BINDING PROTEIN 1
LMP000841	Acot4	Q8BWN8	MM.219001	12942	#N/A	A_51_P396141	Fatty acids/Eicosanoids (FA)	ACYL-COA THIOESTERASE 4
LMP000847	Acaa1a	Q921H8	MM.205266	27176	#N/A	A_51_P327075	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	ACETYL-COENZYME A ACYLTRANSFERASE 1A
LMP000852	Cyp1a2	P00186	MM.15537	36875	102998_at	A_51_P450140	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 1, SUBFAMILY A,

LMP000856	Plcg2	Q8CIH5	MM.192699	59878	#N/A	A_51_P279163	Glycerophospholipids (GP)	PHOSPHOLIPASE C, GAMMA 2
LMP000859	Apoa2	Q60615	MM.288374	144830	161924_f_at; 99648_at	A_51_P128973	Sterol Lipids (ST)	APOLIPOPROTEIN A-II
LMP000862	Prkci	Q62074	MM.291554	145800	#N/A	A_51_P387713;A_51_P406671	Glycerolipids (GL)	PROTEIN KINASE C, IOTA
LMP000865	Plcg1	Q62077	MM.44463	9403	#N/A	A_51_P325919;A_51_P377035	Glycerophospholipids (GP)	CELL DIFFERENTIATION AND EMBRYONIC DEVELOPMENT
LMP000867	Ppard	P35396	MM.328914	37622	94198_at	A_51_P271556	Sterol Lipids (ST)	PEROXISOME PROLIFERATOR ACTIVATOR RECEPTOR DELTA
LMP000871	Pld2	P97813	MM.260177	146888	94371_at	A_51_P357759	Not classified	PHOSPHOLIPASE D2
LMP000873	Pecr	Q99MZ7	MM.281738	9938	#N/A	A_51_P291749	Fatty acids/Eicosanoids (FA)	RIKEN CDNA 2400003B18 GENE
LMP000875	Cysltr1	Q99JA4	MM.287166	19380	#N/A	A_51_P140017	Fatty acids/Eicosanoids (FA)	CYSTEINYL LEUKOTRIENE RECEPTOR 1
LMP000876	Acsm1	Q91VA0	MM.135543	4182	#N/A	A_51_P376139	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE MEDIUM-CHAIN FAMILY MEMBER 1
LMP000877	Pla2g6	P97819	MM.155620	12642	97965_at	A_51_P464860	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP VI
LMP000878	Carkl	Q9D5J6	MM.200905	33612	#N/A	A_51_P318804	Glycerophospholipids (GP)	CARBOHYDRATE KINASE-LIKE
LMP000879	Srd5a2	Q99N99	MM.38933	144889	#N/A	A_51_P497006	Sterol Lipids (ST)	STEROID 5 ALPHA-REDUCTASE 2
LMP000880	Shbg	P97497	MM.1431	19648	100702_at	A_51_P423549	Sterol Lipids (ST)	SEX HORMONE BINDING GLOBULIN
LMP000881	Cpne3	Q8BT60	MM.38390	31109	104274_at	A_51_P175146	Not classified	COPINE III
LMP000887	Zdhhc1	Q8R0N9	MM.100917	13100	#N/A	A_51_P514898	Fatty acids/Eicosanoids (FA)	ZINC FINGER, DHHC DOMAIN CONTAINING 1
LMP000889	Chka	O54804	NA	146571	#N/A	A_51_P142923	Glycerophospholipids (GP)	CHOLINE KINASE ALPHA
LMP000890	Dlst	Q8BHN4	MM.296221	146625	160289_s_at; 97880_at	A_51_P290139	Not classified	DIHYDROLIPOAMIDE S-SUCCINYLTRANSFERASE (E2 COMPONENT OF 2-OXO-GLUTARAT...
LMP000892	Samd8	Q9DA37	MM.102765	15663	#N/A	A_51_P246077	Sphingolipids (SP)	STERILE ALPHA MOTIF DOMAIN CONTAINING 8
LMP000895	Alox15b	O35936	MM.289672	28220	99848_at	#N/A	Fatty acids/Eicosanoids (FA)	ARACHIDONATE 8-LIPOXYGENASE
LMP000897	Neu2	Q9JMH3	MM.45670	3707	#N/A	A_51_P460143	Sphingolipids (SP)	NEURAMINIDASE 2
LMP000898	Acat2	Q8CAY6	MM.360538	3712	101006_at 101527_at;101944_at;101945_g_at;101946_at;161774_f_at;97207_f_at	#N/A	Fatty acids/Eicosanoids (FA)	T-COMPLEX PROTEIN 1, RELATED SEQUENCE 1
LMP000901	Lypla1	P97823	MM.299955	10098		A_51_P475741	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	LYSOPHOSPHOLIPASE 1
LMP000902	Neu3	Q9JMH7	MM.103703	28706	#N/A	A_51_P210106	Sphingolipids (SP)	NEURAMINIDASE 3
LMP000910	Stard5	Q9EPQ7	MM.357953	25400	#N/A	A_51_P319141	Sterol Lipids (ST)	DNA SEGMENT, CHR 7, ERATO DOI 152, EXPRESSED
LMP000911	Ttpa	Q8BWP5	MM.379065	26891	#N/A	A_51_P258817	Prenol Lipids (PR)	TOCOPHEROL (ALPHA) TRANSFER PROTEIN

LMP000913	Olr1	Q9EQ09	MM.293626	4873	93167_f_at	A_51_P462192	Not classified	OXIDIZED LOW DENSITY LIPOPROTEIN (LECTIN-LIKE) RECEPTOR 1
LMP000915	Acsl5	Q8JZR0	MM.292056	26342	#N/A	A_51_P472173	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE LONG-CHAIN FAMILY MEMBER 5
LMP000926	Cpt2	P52825	MM.307620	148638	161978_r_at; 95646_at	A_51_P403388	Fatty acids/Eicosanoids (FA)	CARNITINE PALMITOYLTRANSFERASE 2
LMP000928	Elovl3	O35949	MM.21806	3845	103469_at	A_51_P324636	Fatty acids/Eicosanoids (FA)	ELONGATION OF VERY LONG CHAIN FATTY ACIDS (FEN1/ELO2, SUR4/ELO3, YEAST...
LMP000929	Pla2g12a	Q9EPR2	MM.151951	148707	#N/A	A_51_P207550	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP XIIA
LMP000934	Nr1h4	Q60641	MM.3095	135585	97969_at 101981_at;16	A_51_P464669	Sterol Lipids (ST)	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP H, MEMBER 4
LMP000938	Nr1h2	Q60644	MM.968	11964	1680_r_at	A_51_P400203	Sterol Lipids (ST)	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP H, MEMBER 2
LMP000941	Amacr	Q543Q9	MM.2787	145128	95588_at	A_51_P118885	Prenol Lipids (PR)	ALPHA-METHYLACYL-COA RACEMASE
LMP000942	Gm2a	Q60648	MM.287807	4322	99141_at	A_51_P466180	Sphingolipids (SP)	GM2 GANGLIOSIDE ACTIVATOR PROTEIN
LMP000943	Atp10a	O54827	MM.135129	24608	#N/A	A_51_P320022	Not classified	ATPASE, CLASS V, TYPE 10A
LMP000945	Apom	Q9Z1R3	MM.2161	143971	162148_r_at; 93840_at	A_51_P461178	Not classified	APOLIPOPROTEIN M
LMP000946	Sdha	Q8K2B3	MM.158231	12673	94080_at 104431_at;16	A_51_P410823	Prenol Lipids (PR)	SUCCINATE DEHYDROGENASE COMPLEX, SUBUNIT A, FLAVOPROTEIN (FP)
LMP000951	Prkcq	Q02111	MM.329993	146652	1853_f_at	A_51_P338935	Glycerolipids (GL)	PROTEIN KINASE C, THETA
LMP000954	Pigw	Q8C398	MM.288689	146505	#N/A	A_51_P414927	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL GLYCAN, CLASS W
LMP000956	Rbp2	Q08652	MM.12825	145779	92811_at	A_51_P399143	Prenol Lipids (PR)	RETINOL BINDING PROTEIN 2, CELLULAR
LMP000963	B4galt5	Q9JMK0	MM.200886	25450	#N/A	A_51_P324535	Glycerolipids (GL) / Glycerophospholipids (GP) / Sphingolipids (SP)	UDP-GAL:BETAGLCNAC BETA 1,4-GALACTOSYLTRANSFERASE, POLYPEPTIDE 5
LMP000969	Edg7	Q9EQ31	MM.155520	33597	#N/A	A_51_P134452	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION, LYSOPHOSPHATIDIC ACID G-PROTEIN-COUPLED R...
LMP000970	Slco2a1	Q9EPT5	MM.207106	38158	#N/A	A_51_P315931	Fatty acids/Eicosanoids (FA)	SOLUTE CARRIER ORGANIC ANION TRANSPORTER FAMILY, MEMBER 2A1
LMP000975	Fabp3	P11404	MM.22220	145246	94214_at	A_51_P167535	Glycerophospholipids (GP)	FATTY ACID BINDING PROTEIN 3, MUSCLE AND HEART
LMP000977	Fabp4	P04117	MM.582	27888	100567_at	A_51_P336830	Not classified	FATTY ACID BINDING PROTEIN 4, ADIPOCYTE
LMP000984	Plcxd3	Q8BLJ3	MM.308996	9062	#N/A	#N/A	Not classified	PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C, X DOMAIN CONTAINING 3
LMP000988	Adh5	P28474	MM.3874	9136	98625_s_at	A_51_P404275	Fatty acids/Eicosanoids (FA) / Glycerolipids (GL) / Prenol Lipids (PR) / Sterol Lipids (ST)	ALCOHOL DEHYDROGENASE 5 (CLASS III), CHI POLYPEPTIDE
LMP000989	Pla2g2c	P48076	MM.5189	99994	99326_at	A_51_P427052	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP IIC

LMP000993	Pip5k3	Q9Z1T6	MM.38370	16869	97712_at	A_51_P393355	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL-3-PHOSPHATE/PHOSPHATIDYLINOSITOL 5-KINASE, TYPE I...
LMP000995	Acaa2	Q8BWT1	MM.245724	29019	95064_at	A_51_P125260	Fatty acids/Eicosanoids (FA)	ACETYL-COENZYME A ACYLTRANSFERASE 2 (MITOCHONDRIAL 3-OXOACYL-COENZYME ...
LMP000997	Mvd	Q99JF5	MM.28146	145978	160770_at	A_51_P355943	Prenol Lipids (PR) / Sterol Lipids (ST)	MEVALONATE (DIPHOSPHO) DECARBOXYLASE
LMP000999	St3gal2	Q11204	MM.200388	13089	#N/A	A_51_P305537	Sphingolipids (SP)	ST3 BETA-GALACTOSIDE ALPHA-2,3-SIALYLTRANSFERASE 2
LMP001001	Rara	P11416	MM.103336	26670	92901_at	A_51_P389724	Prenol Lipids (PR) / Sterol Lipids (ST)	RETINOIC ACID RECEPTOR, ALPHA
LMP001003	Mlstd1	Q7TNT2	MM.322130	146460	#N/A	A_51_P285986	Fatty acids/Eicosanoids (FA)	MALE STERILITY DOMAIN CONTAINING 1
LMP001011	Gk2	Q9WU65	MM.61206	8004	#N/A	A_51_P194658	Not classified	GLYCEROL KINASE 2
LMP001017	Nr1h5	Q80ST6	MM.331269	13241	#N/A	A_51_P226667	Not classified	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP H, MEMBER 5
LMP001018	St6galnac6	Q8JZW3	MM.88831	4643	#N/A	A_51_P284997	Sphingolipids (SP)	ST6 (ALPHA-N-ACETYL-NEURAMINYL-2,3-BETA-GALACTOSYL-1,3)-N-ACETYLGALACT...
LMP001019	Ugt1	P70691	NA	3809	#N/A	#N/A	Sterol Lipids (ST)	UDP GLUCURONOSYLTRANSFERASE 1 FAMILY, POLYPEPTIDE A2
LMP001024	Akr1c6	P70694	MM.196666	6796	92556_at	A_51_P189082	Sterol Lipids (ST)	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 5
LMP001031	Inpp4a	Q9EPW0	MM.150420	9035	#N/A	A_51_P400737;A_51_P500200	Glycerophospholipids (GP)	INOSITOL POLYPHOSPHATE-4-PHOSPHATASE, TYPE I
LMP001033	Pnpla5	Q9D603	MM.159565	3627	#N/A	A_51_P334804	Not classified	PATATIN-LIKE PHOSPHOLIPASE DOMAIN CONTAINING 5
LMP001036	Adipor1	Q91VH1	MM.259976	21906	#N/A	A_51_P485243	Fatty acids/Eicosanoids (FA)	ADIPONECTIN RECEPTOR 1
LMP001052	Fdft1	Q8BPF5	MM.386760	3360	97518_at	A_51_P485945	Prenol Lipids (PR) / Sterol Lipids (ST)	FARNESYL DIPHOSPHATE FARNESYL TRANSFERASE 1
LMP001054	Pemt	Q7TNW6	MM.2731	144334	161893_i_at; 94987_at	A_51_P193095	Glycerophospholipids (GP)	PHOSPHATIDYLETHANOLAMINE N-METHYLTRANSFERASE
LMP001057	Lss	Q8BLN5	MM.55075	4544	160737_at	A_51_P296487	Sterol Lipids (ST)	LANOSTEROL SYNTHASE
LMP001063	Cyp17a1	P27786	MM.1262	27096	102416_at;161721_f_at	A_51_P116813	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 17, SUBFAMILY A, POLYPEPTIDE 1
LMP001064	Ptdss2	Q9Z1X2	MM.293591	37986	160853_at	A_51_P185713	Glycerophospholipids (GP) / Sphingolipids (SP)	PHOSPHATIDYLSERINE SYNTHASE 2
LMP001065	Mbtps1	Q9WTZ2	MM.206934	18084	95754_at	A_51_P382588	Sterol Lipids (ST)	MEMBRANE-BOUND TRANSCRIPTION FACTOR PEPTIDASE, SITE 1
LMP001066	Rlbp1	Q9Z275	MM.41653	36177	92435_at	A_51_P211798	Prenol Lipids (PR)	RETINALDEHYDE BINDING PROTEIN 1
LMP001068	2310016F22Rik	Q6PF90	MM.243758	145658	#N/A	A_51_P500215	Not classified	RIKEN CDNA 2310016F22 GENE
LMP001071	Pik3c3	Q6PF93	MM.194127	3591	#N/A	#N/A	Glycerophospholipids (GP)	PHOSPHOINOSITIDE-3-KINASE, CLASS 3
LMP001082	Pla2g4a	P47713	MM.4186	27154	99513_at	A_51_P402760	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP IVA (CYTOSOLIC, CALCIUM-DEPENDENT)
LMP001085	Hsd3b4	Q61767	MM.14309	20317	92869_at	A_51_P352005	Sterol Lipids (ST)	HYDROXYSTEROID DEHYDROGENASE-4, DELTA<5>-3-BETA

LMP001088	Pld1	Q9Z280	MM.212039	37042	101627_at;101628_g_at;92474_at;92475_g_at	A_51_P159203	Glycerophospholipids (GP)	PHOSPHOLIPASE D1
LMP001098	Rbp4	Q00724	MM.2605	146821	96047_at	A_51_P374752	Prenol Lipids (PR)	RETINOL BINDING PROTEIN 4, PLASMA
LMP001101	Stard4	Q80SX0	MM.127058	10716	#N/A	A_51_P105445	Sterol Lipids (ST)	RIKEN CDNA 4632419C16 GENE
LMP001103	Zdhhc3	Q8R173	MM.28300	9165	97857_at	A_51_P258432	Fatty acids/Eicosanoids (FA)	ZINC FINGER, DHHC DOMAIN CONTAINING 3
LMP001106	Sgpl1	Q8R0X7	MM.200373	144501	96126_at,96127_at	A_51_P391694	Sphingolipids (SP)	SPHINGOSINE PHOSPHATE LYASE 1
LMP001107	Cel	Q64285	MM.236017	90772	99939_at	A_51_P242205	Glycerolipids (GL) /Sphingolipids (SP) / Sterol Lipids (ST)	CARBOXYL ESTER LIPASE
LMP001110	Esr1	P19785	MM.9213	3256	161113_at;93945_at;93946_at;93947_g_at	A_51_P474658	Sterol Lipids (ST)	ESTROGEN RECEPTOR 1 (ALPHA)
LMP001113	Plcd3	Q8K2J0	MM.264743	9346	#N/A	A_51_P278163	Glycerophospholipids (GP)	PHOSPHOLIPASE C, DELTA 3
LMP001115	Apoc2	Q05020	MM.28394	37971	97887_at	A_51_P334979	Not classified	APOLIPOPROTEIN C-II
LMP001119	Degs1	O09005	MM.29648	18914	#N/A	A_51_P498890	Sphingolipids (SP)	DEGENERATIVE SPERMATOCYTE HOMOLOG 1 (DROSOPHILA)
LMP001129	Cckbr	P56481	MM.44513	22183	101344_at	A_51_P506201	Not classified	CHOLECYSTOKININ B RECEPTOR
LMP001130	Acadm	P45952	MM.10530	32812	92581_at	A_51_P319879	Fatty acids/Eicosanoids (FA)	ACETYL-COENZYME A DEHYDROGENASE, MEDIUM CHAIN
LMP001135	Plcb2	Q8BI81	MM.379966	6990	#N/A	A_51_P260350;A_51_P429382	Glycerophospholipids (GP)	PHOSPHOLIPASE C, BETA 2
LMP001138	Aldh2	P47738	MM.284446	8123	96057_at,96058_s_at	A_51_P140182	Fatty acids/Eicosanoids (FA) / Glycerolipids (GL) / Sterol Lipids (ST)	ALDEHYDE DEHYDROGENASE 2, MITOCHONDRIAL
LMP001139	Prkcd	P28867	MM.2314	9520	104531_at;160698_s_at	A_51_P460734	Glycerolipids (GL)	PROTEIN KINASE C, DELTA
LMP001143	Alox5ap	P30355	MM.19844	3373	#N/A	A_51_P235687	Fatty acids/Eicosanoids (FA)	ARACHIDONATE 5-LIPOXYGENASE ACTIVATING PROTEIN
LMP001148	Aldh3a2	P47740	MM.145091	144762	161401_f_at, 99559_at	A_51_P464175	Fatty acids/Eicosanoids (FA) / Glycerolipids (GL) / Sterol Lipids (ST)	ALDEHYDE DEHYDROGENASE FAMILY 3, SUBFAMILY A2
LMP001150	Acaa1b	Q8VCH0	NA	147437	#N/A	#N/A	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	ACETYL-COENZYME A ACYLTRANSFERASE 1B
LMP001151	Olah	Q8R197	MM.13808	27450	#N/A	A_51_P369906	Fatty acids/Eicosanoids (FA)	THIOESTERASE DOMAIN CONTAINING 1
LMP001159	Bcdo2	Q99NF1	MM.379309	7224	#N/A	A_51_P424168	Not classified	BETA-CAROTENE 9', 10'-DIOXYGENASE 2
LMP001167	Gc	P21614	MM.196595	17654	99197_at	A_51_P349961	Sterol Lipids (ST)	GROUP SPECIFIC COMPONENT
LMP001168	Acsl1	P41216	MM.210323	5059	#N/A	A_51_P463452;A_51_P496432	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE LONG-CHAIN FAMILY MEMBER 1

LMP001177	Prkcb1	P68404	NA	35234	#N/A	A_51_P485098	Glycerolipids (GL) / Glycerophospholipids (GP)	PROTEIN KINASE C, BETA 1
LMP001181	Scd4	Q6T707	MM.313583	108193	#N/A	#N/A	Fatty acids/Eicosanoids (FA)	STEAROYL-COENZYME A DESATURASE 4
LMP001185	Liph	Q8CIV3	MM.33192	16830	#N/A	A_51_P122660	Not classified	LIPASE, MEMBER H
LMP001204	Pdss1	Q9CZQ1	MM.249752	148145	#N/A	A_51_P127841	Not classified	PRENYL (SOLANESYL) DIPHOSPHATE SYNTHASE, SUBUNIT 1
LMP001209	Abca1	P41233	MM.277376	31084	94354_at;97198_at	A_51_P214977	Sterol Lipids (ST)	ATP-BINDING CASSETTE, SUB-FAMILY A (ABC1), MEMBER 1
LMP001232	Ggtla1	Q80WV0	MM.257927	20280	102968_at	A_51_P201709	Fatty acids/Eicosanoids (FA)	GAMMA-GLUTAMYLTRANSFERASE-LIKE ACTIVITY 1
LMP001238	Nqo1	Q64669	MM.252	149538	100725_at;94350_f_at;94351_r_at	A_51_P424338	Sterol Lipids (ST)	NAD(P)H DEHYDROGENASE, QUINONE 1
LMP001242	Plcz1	Q9DAC8	MM.50808	37226	#N/A	A_51_P297441	Glycerophospholipids (GP)	PHOSPHOLIPASE C, ZETA 1
LMP001252	Itpkc	Q7TS72	MM.30963	145969	#N/A	A_51_P507622	Not classified	INOSITOL 1,4,5-TRISPHOSPHATE 3-KINASE C
LMP001255	Atp8b1	Q6R964	MM.270043	144899	#N/A	A_51_P192329;A_51_P494297	Sterol Lipids (ST)	ATPASE, CLASS I, TYPE 8B, MEMBER 1
LMP001257	Ugt8	Q64676	MM.306021	146732	98872_at	#N/A	Glycerolipids (GL)/Sphingolipids (SP)	UDP GALACTOSYLTRANSFERASE 8A
LMP001259	Dgat2l4	Q6E1M8	MM.118171	4847	#N/A	A_51_P474151	Fatty acids/Eicosanoids (FA)	DIACYLGLYCEROL O-ACYLTRANSFERASE 2-LIKE 4
LMP001264	Nr5a1	P33242	MM.31387	145521	100700_s_at;100701_r_at;101665_at;101666_at;161418_r_at	A_51_P315964	Sterol Lipids (ST)	FUSHI TARAZU 1 FACTOR HOMOLOG, (DROSOPHILA)
LMP001273	Asah3	Q8R4X1	MM.218784	4147	#N/A	A_51_P510239	Sphingolipids (SP)	RIKEN CDNA 2310024P18 GENE
LMP001276	Hadha	Q5U5Y5	MM.200497	33608	#N/A	A_51_P331549	Fatty acids/Eicosanoids (FA)	HYDROXYACYL-COENZYME A DEHYDROGENASE/3-KETOACYL-COENZYME A THIOLASE/EN...
LMP001278	St8sia1	Q64687	MM.383300	5135	#N/A	A_51_P455356	Glycerolipids (GL) / Sphingolipids (SP)	ST8 ALPHA-N-ACETYL-NEURAMINIDE ALPHA-2,8-SIALYLTRANSFERASE 1
LMP001279	Pcyt1a	P49586	MM.209300	145052	161074_at;99035_at	A_51_P294705	Glycerophospholipids (GP)	PHOSPHATE CYTIDYLYLTRANSFERASE 1, CHOLINE, ALPHA ISOFORM
LMP001285	Pdpk1	Q9Z2A0	MM.10504	36860	102063_at;160578_at	A_51_P207140	Glycerophospholipids (GP)	3-PHOSPHOINOSITIDE DEPENDENT PROTEIN KINASE-1
LMP001286	Inpp5d	Q9WUC2	MM.15105	3846	#N/A	A_51_P337125	Glycerophospholipids (GP)	INOSITOL POLYPHOSPHATE-5-PHOSPHATASE D
LMP001287	Cyp2e1	Q05421	MM.21758	4103	93996_at	A_51_P283456	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY E, POLYPEPTIDE 1
LMP001288	Dgat1	Q9Z2A7	MM.22633	6880	104371_at;162316_f_at	A_51_P510059	Glycerolipids (GL)	DIACYLGLYCEROL O-ACYLTRANSFERASE 1
LMP001290	Ggtla1	Q9Z2A9	MM.257927	20280	102968_at	A_51_P201709	Fatty acids/Eicosanoids (FA)	GAMMA-GLUTAMYLTRANSFERASE-LIKE ACTIVITY 1
LMP001296	Esrrb	Q8C7A6	MM.235550	26436	100301_at	A_51_P419047	Sterol Lipids (ST)	ESTROGEN RECEPTOR RELATED 2

LMP001311	Pik3cb	Q8BTI9	MM.213128	10735	#N/A	A_51_P479914	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE, CATALYTIC, BETA POLYPEPTIDE
LMP001316	Eif2ak3	Q9Z2B5	MM.247167	113355	103537_at	A_51_P253642	Glycerophospholipids (GP)	EUKARYOTIC TRANSLATION INITIATION FACTOR 2 ALPHA KINASE 3
LMP001317	Pip5k1c	O70161	NA	6253	94119_at	A_51_P149852	Glycerophospholipids (GP)	PIP5KIGAMMA
LMP001324	Pla2g2a	P31482	MM.4675	143998	92735_at	#N/A	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP IIA (PLATELETS, SYNOVIAL FLUID)
LMP001327	Itpr1	P11881	MM.227912	43495	161661_i_at; 93895_s_at;94977_at	A_51_P269663	Glycerophospholipids (GP)	INOSITOL 1,4,5-TRIPHOSPHATE RECEPTOR 1
LMP001328	Pik3ca	P42337	MM.260521	19965	92452_at	A_51_P166682	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE, CATALYTIC, ALPHA POLYPEPTIDE
LMP001329	Hsd3b7	Q9EQC1	MM.157103	18865	160104_at	A_51_P435911	Sterol Lipids (ST)	HYDROXY-DELTA-5-STEROID DEHYDROGENASE, 3 BETA- AND STEROID DELTA-ISOME...
LMP001331	Pik3c2g	O70167	MM.333471	23796	94108_at	A_51_P191077	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE, C2 DOMAIN CONTAINING, GAMMA POLYPEPTIDE
LMP001341	Ggtla1	Q8C7B4	MM.257927	20280	102968_at	A_51_P201709	Fatty acids/Eicosanoids (FA)	GAMMA-GLUTAMYLTRANSFERASE-LIKE ACTIVITY 1
LMP001344	Ehhadh	Q91W49	MM.28100	146125	#N/A	A_51_P462918	Fatty acids/Eicosanoids (FA)	ENOYL-COENZYME A, HYDRATASE/3-HYDROXYACYL COENZYME A DEHYDROGENASE
LMP001352	Pip5k2a	O70172	MM.313977	148824	101865_at;162503_f_at;95358_at 94398_s_at20/01/200994399_at;102884_at;161678_at;96848_at	A_51_P518747	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL-4-PHOSPHATE 5-KINASE, TYPE II, ALPHA
LMP001353	Inpp5b	Q8K337	MM.296202	147335		A_51_P166695	Glycerophospholipids (GP)	INOSITOL POLYPHOSPHATE-5-PHOSPHATASE B
LMP001356	Acsl3	Q9CZW4	NA	29539	#N/A	A_51_P511560	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE LONG-CHAIN FAMILY MEMBER 3
LMP001363	Lta4h	P24527	MM.271071	14994	100540_at	A_51_P101955	Fatty acids/Eicosanoids (FA)	LEUKOTRIENE A4 HYDROLASE
LMP001365	Stard4	Q99JV5	MM.127058	10716	#N/A	A_51_P105445	Sterol Lipids (ST)	RIKEN CDNA 4632419C16 GENE
LMP001371	Chpt1	Q8C025	MM.288897	30110	#N/A	A_51_P240857	Not classified	CHOLINE PHOSPHOTRANSFERASE 1
LMP001375	Acsl6	Q8C028	MM.267478	148310	#N/A	A_51_P380699;A_51_P518822	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE LONG-CHAIN FAMILY MEMBER 6
LMP001377	Tmem23	Q8VCQ6	MM.171256	15383	#N/A	#N/A	Sphingolipids (SP)	TRANSMEMBRANE PROTEIN 23
LMP001382	Hsd17b12	O70503	MM.22505	146726	94276_at	A_51_P470612	Glycerophospholipids (GP) / Sterol Lipids (ST)	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 12
LMP001384	Ncf1	Q09014	MM.4149	144386	97763_at	A_51_P207031	Fatty acids/Eicosanoids (FA)	NEUTROPHIL CYTOSOLIC FACTOR 1
LMP001385	Hexdc	Q6NSQ8	MM.386870	13666	#N/A	#N/A	Not classified	HEXOSAMINIDASE (GLYCOSYL HYDROLASE FAMILY 20, CATALYTIC DOMAIN) CONTAI...
LMP001388	Slc10a1	O08705	MM.341781	26753	100339_at,100340_at,100341_g_at	A_51_P410525	Sterol Lipids (ST)	SOLUTE CARRIER FAMILY 10 (SODIUM/BILE ACID COTRANSPORTER FAMILY), MEMB...

LMP001393	Prdx6	O08709	MM.186185	3244	100332_s_at; 100622_at	A_51_P496023	Fatty acids/Eicosanoids (FA)	PEROXIREDOXIN 6
LMP001395	Dlst	Q9D2G2	MM.296221	146625	160289_s_at; 97880_at	A_51_P290139	Not classified	DIHYDROLIPOAMIDE S-SUCCINYLTRANSFERASE (E2 COMPONENT OF 2-OXO-GLUTARAT...
LMP001398	Synj2	Q9D2G5	MM.236068	145707	100676_at;97962_at	A_51_P208953;A_51_P264433	Glycerophospholipids (GP)	SYNAPTOJANIN 2
LMP001405	Abca4	O35600	MM.3918	37684	97730_at	A_51_P413614	Not classified	ATP-BINDING CASSETTE, SUB-FAMILY A (ABC1), MEMBER 4
LMP001407	Pik3r1	P70304	MM.259333	37643	96592_at	#N/A	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE, REGULATORY SUBUNIT, POLYPEPTIDE 1 (P85 ...
LMP001408	Plcb3	P51432	MM.273204	148650	101358_at	A_51_P236042	Glycerophospholipids (GP)	PHOSPHOLIPASE C, BETA 3
LMP001409	Ltc4s	Q8K355	MM.245151	45402	92401_at	A_51_P354792	Fatty acids/Eicosanoids (FA)	LEUKOTRIENE C4 SYNTHASE
LMP001410	Npc1	O35604	MM.3484	17029	98114_at	A_51_P349341	Sterol Lipids (ST)	NIEMANN PICK TYPE C1
LMP001416	Fabp9	O08716	MM.26654	36000	160729_f_at; 94927_at	A_51_P234996	Not classified	FATTY ACID BINDING PROTEIN 9, TESTIS
LMP001419	Prkcz	Q02956	MM.28561	27837	161964_r_at; 96198_at;96571_at	A_51_P314631	Glycerolipids (GL)	PROTEIN KINASE C, ZETA
LMP001420	Apoa4	P06728	MM.4533	147861	100078_at	A_51_P327496	Sterol Lipids (ST)	APOLIPOPROTEIN A-IV
LMP001422	Lpin1	Q91ZP3	MM.153625	12995	98892_at	A_51_P394984	Not classified	LIPIN 1
LMP001423	4632408 A20Rik	Q8BXE0	MM.49245	145642	#N/A	#N/A	Fatty acids/Eicosanoids (FA)	RIKEN CDNA 4632408A20 GENE
LMP001426	Aldh1a1	P24549	MM.250866	149907	100068_at,161956_at	A_51_P334942	Prenol Lipids (PR)	ALDEHYDE DEHYDROGENASE FAMILY 1, SUBFAMILY A1
LMP001432	Pparg	Q6TQE4	MM.3020	148555	97926_s_at	A_51_P106799	Sterol Lipids (ST)	PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA
LMP001442	Acsm3	Q9Z2F3	MM.334199	37109	#N/A	A_51_P487175	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE MEDIUM-CHAIN FAMILY MEMBER 3
LMP001444	Apoc3	P33622	MM.178973	15124	#N/A	A_51_P310629	Not classified	APOLIPOPROTEIN C-III
LMP001450	Hadhb	Q99JY0	MM.291463	26354	#N/A	A_51_P217990	Fatty acids/Eicosanoids (FA)	HYDROXYACYL-COENZYME A DEHYDROGENASE/3-KETOACYL-COENZYME A THIOLASE/EN...
LMP001457	Ppap2b	Q99JY8	MM.348326	12962	#N/A	A_51_P452997;A_51_P503433	Glycerophospholipids (GP)	PHOSPHATIDIC ACID PHOSPHATASE TYPE 2B
LMP001458	Acads	Q91W85	MM.18759	14602	103401_at	A_51_P452807	Fatty acids/Eicosanoids (FA)	ACYL-COENZYME A DEHYDROGENASE, SHORT CHAIN
LMP001459	Gba2	Q8BTN9	MM.229444	9138	92784_at	A_51_P261359	Not classified	GLUCOSIDASE BETA 2
LMP001460	Gpam	Q8VCT2	MM.210196	146028	101867_at	#N/A	Fatty acids/Eicosanoids (FA) / Glycerolipids (GL) / Glycerophospholipids (GP)	GLYCEROL-3-PHOSPHATE ACYLTRANSFERASE, MITOCHONDRIAL
LMP001461	Zdhhc17	Q8BIE0	MM.339281	15248	#N/A	A_51_P348672	Fatty acids/Eicosanoids (FA)	HUNTINGTIN INTERACTING PROTEIN 14
LMP001465	Acad10	Q8K370	MM.45423	27723	#N/A	A_51_P187507	Fatty acids/Eicosanoids (FA)	ACYL-COENZYME A DEHYDROGENASE FAMILY, MEMBER 10

LMP001477	Nr1i3	O35627	MM.3077	27185	102171_r_at; 104506_at;104507_g_at	A_51_P358256	Sterol Lipids (ST)	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP I, MEMBER 3
LMP001478	Chkb	O55229	MM.227738	4925	#N/A	A_51_P159771	Glycerophospholipids (GP)	CHOLINE KINASE BETA
LMP001483	Chpt1	Q91W91	MM.288897	30110	#N/A	A_51_P240857	Not classified	CHOLINE PHOSPHOTRANSFERASE 1
LMP001484	Apoa5	Q8C7G5	MM.29738	37790	95727_at;95728_g_at;95729_at	A_51_P259930	Not classified	APOLIPOPROTEIN A-V
LMP001491	Prkd1	Q62101	MM.386771	20045	#N/A	#N/A	Glycerolipids (GL)	PROTEIN KINASE C, MU
LMP001492	Fabp9	Q9DAL2	MM.26654	36000	160729_f_at; 94927_at	A_51_P234996	Not classified	FATTY ACID BINDING PROTEIN 9, TESTIS
LMP001494	Vamp2	P63044	MM.28643	146844	98925_at;98926_at	A_51_P446927	Not classified	VESICLE-ASSOCIATED MEMBRANE PROTEIN 2
LMP001499	Fabp5	Q05816	MM.741	35192	160544_at	A_51_P387764	Glycerophospholipids (GP)	FATTY ACID BINDING PROTEIN 5, EPIDERMAL
LMP001500	Anxa3	O35639	MM.7214	36967	101393_at	A_51_P451032	Not classified	ANNEXIN A3
LMP001503	Dld	O08749	MM.3131	147715	97502_at	A_51_P184282	Not classified	DIHYDROLIPOAMIDE DEHYDROGENASE
LMP001505	Cyp2r1	Q6VVW9	MM.108037	148005	#N/A	A_51_P118046	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 2, SUBFAMILY R, POLYPEPTIDE 1
LMP001507	Asah3l	Q8VD53	MM.266817	16613	#N/A	A_51_P244408;A_51_P249305	Not classified	RIKEN CDNA 2410116I05 GENE
LMP001508	Rdh5	O55240	MM.378996	148291	101431_at	A_51_P479618	Prenol Lipids (PR)	RETINOL DEHYDROGENASE 5
LMP001516	Plcl2	Q8K394	MM.217362	132312	#N/A	A_51_P219444	Glycerophospholipids (GP)	PHOSPHOLIPASE C-LIKE 2
LMP001521	Hadh2	O08756	NA	13192	101045_at	#N/A	Fatty acids/Eicosanoids (FA)	3-HYDROXYACYL-COA DEHYDROGENASE TYPE II
LMP001523	Pigo	Q9JJI6	MM.143738	147400	#N/A	A_51_P469545	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL GLYCAN, CLASS O
LMP001524	Dgka	O88673	MM.291235	25737	103596_at	A_51_P319093	Glycerolipids (GL) / Glycerophospholipids (GP)	DIACYLGLYCEROL KINASE, ALPHA
LMP001529	Edg5	Q8C3Q7	MM.46493	148695	99372_at	A_51_P499876	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION, SPHINGOLIPID G-PROTEIN-COUPLED RECEPTOR, ...
LMP001534	Nmt2	Q8CFK1	MM.65021	8003	102229_at	A_51_P465512	Fatty acids/Eicosanoids (FA)	N-MYRISTOYLTRANSFERASE 2
LMP001542	Gpr44	Q9Z2J6	MM.353637	7375	101282_at	A_51_P406315	Fatty acids/Eicosanoids (FA)	G PROTEIN-COUPLED RECEPTOR 44
LMP001546	Neu1	O35657	MM.8856	3440	#N/A	A_51_P428032	Sphingolipids (SP)	NEURAMINIDASE 1
LMP001553	Dgkg	Q8C413	MM.379296	26711	#N/A	A_51_P494037	Glycerolipids (GL) / Glycerophospholipids (GP)	DIACYLGLYCEROL KINASE, GAMMA
LMP001555	Akr1d1	Q8VCX1	MM.262635	16140	#N/A	A_51_P391626	Sterol Lipids (ST)	ALDO-KETO REDUCTASE FAMILY 1, MEMBER D1
LMP001557	Fabp2	P55050	MM.28398	35211	97889_at	A_51_P313581	Not classified	FATTY ACID BINDING PROTEIN 2, INTESTINAL
LMP001563	Pdk4	O70571	MM.235547	32788	102049_at	A_51_P350453	Not classified	PYRUVATE DEHYDROGENASE KINASE, ISOENZYME 4
LMP001564	Slc10a5	Q5PT54	MM.358910	145580	#N/A	#N/A	Not classified	SOLUTE CARRIER FAMILY 10 (SODIUM/BILE ACID COTRANSPORTER FAMILY), MEMB...

LMP001565	Smpd2	O70572	MM.953	27591	101940_at	A_51_P234421	Sphingolipids (SP)	SPHINGOMYELIN PHOSPHODIESTERASE 2, NEUTRAL
LMP001566	Ar	P19091	MM.39005	149722	92667_at	A_51_P496031	Sterol Lipids (ST)	ANDROGEN RECEPTOR
LMP001573	Pparg	P37238	MM.3020	148555	97926_s_at	A_51_P106799	Sterol Lipids (ST)	PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA
LMP001577	Fasn	P19096	MM.236443	100298	98575_at	A_51_P321126	Fatty acids/Eicosanoids (FA)	FATTY ACID SYNTHASE
LMP001583	Pik3cg	Q9EQL1	MM.101369	34398	#N/A	A_51_P507832	Glycerophospholipids (GP)	PHOSPHOINOSITIDE-3-KINASE, CATALYTIC, GAMMA POLYPEPTIDE
LMP001584	Mogat1	Q91ZV4	MM.41325	148046	#N/A	A_51_P112151	Glycerolipids (GL)	MONOACYLGLYCEROL O-ACYLTRANSFERASE 1
LMP001586	Ugcg	O88693	MM.198803	13231	94197_at;96623_at	A_51_P332717;A_51_P481342	Sphingolipids (SP)	UDP-GLUCOSE CERAMIDE GLUCOSYLTRANSFERASE
LMP001590	Pcyt2	Q922E4	MM.21439	4597	#N/A	A_51_P432504	Glycerophospholipids (GP)	PHOSPHATE CYTIDYLYLTRANSFERASE 2, ETHANOLAMINE
LMP001597	Alox12b	O70582	MM.340329	30278	102216_at	A_51_P447258	Fatty acids/Eicosanoids (FA)	ARACHIDONATE 12-LIPOXYGENASE, 12R TYPE
LMP001598	Pltp	P55065	MM.6105	27082	100927_at	A_51_P226655	Not classified	PHOSPHOLIPID TRANSFER PROTEIN
LMP001599	Aldh1a2	Q62148	MM.42016	8489	101707_at	A_51_P166824	Prenol Lipids (PR)	ALDEHYDE DEHYDROGENASE FAMILY 1, SUBFAMILY A2
LMP001602	Mgll	O35678	MM.272197	38143	97510_at;97511_at	A_51_P139923	Glycerolipids (GL)	MONOGLYCERIDE LIPASE
LMP001606	Ltb4r2	Q9JJL9	MM.159670	36058	#N/A	A_51_P282210	Fatty acids/Eicosanoids (FA)	LEUKOTRIENE B4 RECEPTOR 2
LMP001611	Atp8a1	P70704	MM.153230	145172	100315_at	A_51_P119636;A_51_P383949	Not classified	ATPASE, AMINOPHOSPHOLIPID TRANSPORTER (APLT), CLASS I, TYPE 8A, MEMBER...
LMP001613	Hadh	Q61425	MM.260164	7444	#N/A	A_51_P196590	Fatty acids/Eicosanoids (FA)	L-3-HYDROXYACYL-COENZYME A DEHYDROGENASE, SHORT CHAIN
LMP001616	Hsd17b3	P70385	MM.5109	13867	101184_at	A_51_P275207	Sterol Lipids (ST)	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 3
LMP001623	Scap	Q6GQT6	MM.288741	149121	#N/A	#N/A	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	SREBP CLEAVAGE ACTIVATING PROTEIN
LMP001649	Prkaa1	Q5EG47	MM.207004	145495	#N/A	A_51_P158922	Fatty acids/Eicosanoids (FA)	PROTEIN KINASE, AMP-ACTIVATED, ALPHA 1 CATALYTIC SUBUNIT
LMP001654	Plcl4	Q8BXN5	MM.379458	27196	#N/A	#N/A	Not classified	PHOSPHOLIPASE C-LIKE 4
LMP001655	Prmt3	Q922H1	MM.33202	37373	#N/A	A_51_P101316	Sterol Lipids (ST)	RIKEN CDNA 2010005E20 GENE
LMP001656	Pdk3	Q922H2	MM.12775	40814	92810_at	A_51_P392293	Not classified	PYRUVATE DEHYDROGENASE KINASE, ISOENZYME 3
LMP001661	Slc27a1	Q60714	MM.38165	38056	93486_at	A_51_P117477	Fatty acids/Eicosanoids (FA)	SOLUTE CARRIER FAMILY 27 (FATTY ACID TRANSPORTER), MEMBER 1
LMP001670	Aloxe3	Q9WV07	MM.41989	25401	97784_at	A_51_P136143	Fatty acids/Eicosanoids (FA)	ARACHIDONATE LIPOXYGENASE 3
LMP001672	Lass4	Q9D6J1	MM.35511	11305	#N/A	A_51_P296528	Sphingolipids (SP)	LONGEVITY ASSURANCE HOMOLOG 4 (S. CEREVISIAE)
LMP001677	Aacs	Q9D2R0	MM.296918	10113	#N/A	A_51_P453043	Not classified	ACETOACETYL-COA SYNTHETASE
LMP001681	Zdhhc17	Q80TN5	MM.339281	15248	#N/A	A_51_P348672	Fatty acids/Eicosanoids (FA)	HUNTINGTIN INTERACTING PROTEIN 14

LMP001682	Dlat	Q8BMF4	MM.285076	14301	96745_at;96746_at	A_51_P265106	Not classified	DIHYDROLIPOAMIDE S-ACETYLTRANSFERASE (E2 COMPONENT OF PYRUVATE DEHYDRO...
LMP001685	Plch1	Q8CFQ2	MM.316391	147017	#N/A	#N/A	Not classified	PHOSPHOLIPASE C-LIKE 3
LMP001687	Edg5	P52592	MM.46493	148695	99372_at	A_51_P499876	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION, SPHINGOLIPID G-PROTEIN-COUPLED RECEPTOR, ...
LMP001688	Peci	Q9WUR2	MM.28883	145921	94485_at	A_51_P394665	Fatty acids/Eicosanoids (FA)	PEROXISOMAL DELTA3, DELTA2-ENOYL-COENZYME A ISOMERASE
LMP001693	Pik3r1	Q8C7P2	MM.259333	37643	96592_at	#N/A	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE, REGULATORY SUBUNIT, POLYPEPTIDE 1 (P85 ...
LMP001695	Lass5	Q9D6K9	MM.9550	3879	#N/A	A_51_P481546	Sphingolipids (SP)	LONGEVITY ASSURANCE HOMOLOG 5 (S. CEREVISIAE)
LMP001697	Lpl	P11152	MM.1514	144445	160083_at;95611_at	A_51_P259296	Glycerophospholipids (GP)	LIPOPROTEIN LIPASE
LMP001700	Nr1i2	O54915	MM.8509	146098	93696_at	A_51_P266958	Sterol Lipids (ST)	NUCLEAR RECEPTOR SUBFAMILY 1, GROUP I, MEMBER 2
LMP001705	Ppap2a	Q61469	MM.317186	147429	96043_at;98508_s_at	A_51_P189105	Glycerolipids (GL) / Glycerophospholipids (GP) / Sphingolipids (SP)	HYDROGEN PEROXIDE INDUCIBLE PROTEIN 53
LMP001708	Etnk1	Q8BXQ0	MM.272548	144874	#N/A	A_51_P229600	Not classified	ETHANOLAMINE KINASE 1
LMP001711	Pigt	Q8BXQ2	MM.28228	3997	#N/A	A_51_P338803	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL GLYCAN, CLASS T
LMP001712	Apol6	Q9D6L7	MM.34173	149208	#N/A	A_51_P404815	Not classified	APOLIPOPROTEIN L, 6
LMP001715	Daglb	Q91WC9	MM.332549	35027	#N/A	A_51_P122582	Not classified	RIKEN CDNA E330036I19 GENE
LMP001716	Pnpla7	Q8BTY7	MM.29046	17154	#N/A	A_51_P276235	Not classified	CDNA SEQUENCE BC027342
LMP001718	Edd1	Q80TP3	MM.275426	13282	#N/A	A_51_P102174;A_51_P275915;A_51_P332303	Not classified	E3 UBIQUITIN PROTEIN LIGASE, HECT DOMAIN CONTAINING, 1
LMP001725	Fabp7	P51880	MM.3644	15073	98967_at	A_51_P290074	Not classified	FATTY ACID BINDING PROTEIN 7, BRAIN
LMP001727	Picalm	Q7M6Y3	MM.235175	148598	#N/A	A_51_P183400	Not classified	PHOSPHATIDYLINOSITOL BINDING CLATHRIN ASSEMBLY PROTEIN
LMP001731	Chka	Q99KD4	MM.225505	27079	#N/A	A_51_P142923	Glycerophospholipids (GP)	CHOLINE KINASE ALPHA
LMP001735	Gpr6	Q6YNI2	MM.290693	146015	#N/A	#N/A	Glycerophospholipids (GP) / Sphingolipids (SP)	G PROTEIN-COUPLED RECEPTOR 6
LMP001737	Pip5k2b	Q80XI4	MM.39700	3280	#N/A	A_51_P267375	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL-4-PHOSPHATE 5-KINASE, TYPE II, BETA
LMP001743	Mvk	Q9R008	MM.28088	37172	95632_f_at;95633_r_at	A_51_P169527	Sterol Lipids (ST)	MEVALONATE KINASE
LMP001747	Lbp	Q61805	MM.218846	90678	96123_at	A_51_P454008	Not classified	LIPOPOLYSACCHARIDE BINDING PROTEIN
LMP001751	Gcdh	Q60759	MM.2475	28290	160194_at;162189_r_at;95561_at	A_51_P260098	Fatty acids/Eicosanoids (FA)	GLUTARYL-COENZYME A DEHYDROGENASE
LMP001756	Itpr2	Q9Z329	MM.333496	18040	101441_i_at;101442_f_at;	#N/A		INOSITOL 1,4,5-TRIPHOSPHATE RECEPTOR 2

LMP001757	Akr1b7	P21300	MM.90151	17178	102826_at;161918_at	A_51_P331288	Glycerolipids (GL)	ALDO-KETO REDUCTASE FAMILY 1, MEMBER B7
LMP001758	Bcmo1	Q9JJS6	MM.174133	34393	#N/A	A_51_P374137	Prenol Lipids (PR)	BETA,BETA-CAROTENE 15,15'-DIOXYGENASE
LMP001759	Fvt1	Q6GV12	MM.284775	20232	#N/A	A_51_P107782	Sphingolipids (SP)	FOLLICULAR LYMPHOMA VARIANT TRANSLOCATION 1
LMP001760	Fn3k	Q9ER35	MM.266448	10634	#N/A	A_51_P398525	Glycerophospholipids (GP)	FRUCTOSAMINE 3 KINASE
LMP001766	Piga	Q64323	MM.3781	149597	161433_f_at;92304_at	A_51_P458839	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL GLYCAN, CLASS A
LMP001771	Ppap2c	Q9DAX2	MM.28873	147433	#N/A	A_51_P361812	Glycerophospholipids (GP)	PHOSPHATIDIC ACID PHOSPHATASE TYPE 2C
LMP001772	Adfp	P43883	MM.381	39259	161443_r_at;98589_at	A_51_P258150	Fatty acids/Eicosanoids (FA)	ADIPOSE DIFFERENTIATION RELATED PROTEIN
LMP001774	St6galnac3	Q9WUV2	MM.296453	22157	#N/A	A_51_P479724	Sphingolipids (SP)	ST6 (ALPHA-N-ACETYL-NEURAMINYL-2,3-BETA-GALACTOSYL-1,3)-N-ACETYLGALACT...
LMP001776	Asah1	Q9WV54	MM.22547	33203	94282_at	A_51_P509098	Sphingolipids (SP)	N-ACYLSPHINGOSINE AMIDOHYDROLASE 1
LMP001781	Hsd3b3	Q5FW73	MM.158717	3543	98401_at	A_51_P397056	Sterol Lipids (ST)	HYDROXYSTEROID DEHYDROGENASE-3, DELTA<5>-3-BETA
LMP001783	Prkag1	O54950	MM.6670	144449	100632_at	A_51_P121818	Fatty acids/Eicosanoids (FA)	PROTEIN KINASE, AMP-ACTIVATED, GAMMA 1 NON-CATALYTIC SUBUNIT
LMP001796	Pdk2	Q9JK42	MM.29768	17110	#N/A	A_51_P426274	Not classified	PYRUVATE DEHYDROGENASE KINASE, ISOENZYME 2
LMP001799	Acox2	Q9QXD1	MM.28700	3284	#N/A	A_51_P206704	Fatty acids/Eicosanoids (FA)	ACYL-COENZYME A OXIDASE 2, BRANCHED CHAIN
LMP001802	Sh3glb1	Q9JK48	MM.271775	146667	103569_at,98945_at	A_51_P223315	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	SH3-DOMAIN GRB2-LIKE B1 (ENDOPHILIN)
LMP001805	Glb1	Q8C847	MM.290516	13271	103647_at;161754_f_at	A_51_P327559	Glycerolipids (GL) / Sphingolipids (SP)	GALACTOSIDASE, BETA 1
LMP001806	Dgkg	Q91WG7	MM.379296	26711	#N/A	A_51_P494037	Glycerolipids (GL) / Glycerophospholipids (GP)	DIACYLGLYCEROL KINASE, GAMMA
LMP001811	Acad11	Q80XL6	MM.347362	13037	#N/A	A_51_P299260	Not classified	ACYL-COENZYME A DEHYDROGENASE FAMILY, MEMBER 11
LMP001813	St3gal1	P54751	MM.371457	37431	#N/A	A_51_P301804	Sphingolipids (SP)	ST3 BETA-GALACTOSIDE ALPHA-2,3-SIALYLTRANSFERASE 1
LMP001816	Zdhhc17	Q8BIT5	MM.339281	15248	#N/A	A_51_P348672	Fatty acids/Eicosanoids (FA)	HUNTINGTIN INTERACTING PROTEIN 14
LMP001823	Hacl1	Q9QXE0	MM.38887	36032	#N/A	A_51_P314065	Not classified	PHYTANOYL-COA 2-HYDROXYLASE 2
LMP001824	9130022K13Rik	Q9D2Y0	MM.232636	17766	#N/A	#N/A	Not classified	RIKEN CDNA 9130022K13 GENE
LMP001827	Osbpl5	Q9ER64	MM.21199	22790	103311_at	A_51_P200309	Sterol Lipids (ST)	OXYSTEROL BINDING PROTEIN-LIKE 5
LMP001828	Mlycd	Q9QXE6	MM.255499	45373	103622_at	A_51_P222283	Fatty acids/Eicosanoids (FA)	MALONYL-COA DECARBOXYLASE
LMP001842	Acsm3	Q91WI1	MM.334199	37109	#N/A	A_51_P487175	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE MEDIUM-CHAIN FAMILY MEMBER 3
LMP001847	Plin	Q8C0F5	MM.254917	37305	#N/A	A_51_P244497	Not classified	PERILIPIN
LMP001851	Hnf4a	Q8CFY1	MM.202383	16277	#N/A	A_51_P198473	Sterol Lipids (ST)	HEPATIC NUCLEAR FACTOR 4, ALPHA

LMP001855	Scd4	Q8CFY4	MM.313583	108193	#N/A	#N/A	Fatty acids/Eicosanoids (FA)	STEAROYL-COENZYME A DESATURASE 4
LMP001856	Cox10	Q8CFY5	MM.340211	144335	#N/A	A_51_P276198	Prenol Lipids (PR)	COX10 HOMOLOG, CYTOCHROME C OXIDASE ASSEMBLY PROTEIN, HEME A: FARNESYL...
LMP001857	Cyp39a1	Q8CFY8	MM.17991	10876	#N/A	A_51_P515446	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 39, SUBFAMILY A, POLYPEPTIDE 1
LMP001858	Pla2g4f	Q50L41	MM.24880	19813	#N/A	#N/A	Not classified	PHOSPHOLIPASE A2, GROUP IVF
LMP001859	Pla2g4e	Q50L42	MM.330505	147200	#N/A	A_51_P362498	Not classified	PHOSPHOLIPASE A2, GROUP IVE
LMP001861	Pla2g4d	Q50L43	NA	13717	#N/A	#N/A	Not classified	PHOSPHOLIPASE A2, GROUP IVD
LMP001863	Chm	Q9QXG2	MM.257316	26939	#N/A	A_51_P296512	Prenol Lipids (PR)	CHOROIDERMIA
LMP001873	Npc1l1	Q6T3U4	MM.212492	19653	#N/A	A_51_P262645	Sterol Lipids (ST)	NPC1-LIKE 1
LMP001877	Inpp5b	O54997	MM.296202	147335	94398_s_at20/01/200994399_at;102884_at;161678_at;96848_at	A_51_P166695	Glycerophospholipids (GP)	INOSITOL POLYPHOSPHATE-5-PHOSPHATASE B
LMP001883	smpd3	Q9JJY3	MM.23298	27939	#N/A	A_51_P383459	Sphingolipids (SP)	SPHINGOMYELIN PHOSPHODIESTERASE 3, NEUTRAL
LMP001895	Acsl6	Q8R1X1	MM.267478	148310	#N/A	A_51_P380699;A_51_P518822	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE LONG-CHAIN FAMILY MEMBER 6
LMP001899	Prkab1	Q9R078	MM.200912	148605	160808_at	#N/A	Fatty acids/Eicosanoids (FA)	PROTEIN KINASE, AMP-ACTIVATED, BETA 1 NON-CATALYTIC SUBUNIT
LMP001903	Acbd3	Q8BMP6	MM.272981	3381	#N/A	A_51_P326762	Sterol Lipids (ST)	ACYL-COENZYME A BINDING DOMAIN CONTAINING 3
LMP001906	Pigf	O09101	MM.219685	149069	101466_at	A_51_P264053	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL GLYCAN, CLASS F
LMP001911	Edg1	Q8C4A3	MM.982	26540	#N/A	#N/A	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION SPHINGOLIPID G-PROTEIN-COUPLED RECEPTOR 1
LMP001915	Ipmk	Q7TT16	MM.245867	148510	#N/A	A_51_P472799	Glycerophospholipids (GP)	INOSITOL POLYPHOSPHATE MULTIKINASE
LMP001922	Stard6	P59096	MM.83623	19307	#N/A	A_51_P397152	Sterol Lipids (ST)	STAR-RELATED LIPID TRANSFER (START) DOMAIN CONTAINING 6
LMP001925	Pafah2	Q8VDG7	MM.22116	4648	#N/A	A_51_P345896	Glycerophospholipids (GP)	PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE 2
LMP001930	Akr1c18	Q8K023	MM.41337	4187	#N/A	A_51_P256170	Sterol Lipids (ST)	ALDO-KETO REDUCTASE FAMILY 1, MEMBER C18
LMP001931	Agpat2	Q8K3K7	MM.24244	147074	#N/A	A_51_P238565	Glycerolipids (GL) / Glycerophospholipids (GP)	1-ACYLGLYCEROL-3-PHOSPHATE O-ACYLTRANSFERASE 2 (LYSOPHOSPHATIDIC ACID ...
LMP001933	Ptgds	O09114	MM.1008	147644	102105_f_at;92545_f_at;92546_r_at	A_51_P157403	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN D2 SYNTHASE (BRAIN)
LMP001934	Ptgs1	P22437	MM.275434	18555	161694_f_at,95597_at	A_51_P279100	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN-ENDOPEROXIDE SYNTHASE 1
LMP001944	Azgp1	Q64726	MM.30061	8857	96867_at	A_51_P456721	Not classified	ALPHA-2-GLYCOPROTEIN 1, ZINC

LMP001948	Ptger4	P32240	MM.18509	144520	103362_at	A_51_P207988	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN E RECEPTOR 4 (SUBTYPE EP4)
LMP001957	Pla2g3	Q6AXH0	MM.100476	43776	#N/A	A_51_P402868	Not classified	PHOSPHOLIPASE A2, GROUP III
LMP001961	Coq3	Q8BMS4	MM.5662	22118	99365_at	A_51_P378336	Prenol Lipids (PR)	COENZYME Q3 HOMOLOG, METHYLTRANSFERASE (YEAST)
LMP001973	Gpd1	P13707	MM.252391	37800	161753_f_at; 92592_at	A_51_P293849	Glycerophospholipids (GP)	GLYCEROL-3-PHOSPHATE DEHYDROGENASE 1 (SOLUBLE)
LMP001985	Hdlbp	Q8VDJ3	MM.30012	35186	96359_at	A_51_P416591	Sterol Lipids (ST)	DNA SEGMENT, CHR 1, ERATO DOI 101, EXPRESSED
LMP001989	B3gnt5	Q810C6	MM.33935	31746	#N/A	A_51_P389156	Glycerolipids (GL) / Sphingolipids (SP)	UDP-GLCNAC:BETAGAL BETA-1,3-N-ACETYLGLUCOSAMINYLTRANSFERASE 5
LMP001990	Hhat	Q8BMT9	MM.145857	26910	#N/A	A_51_P445677	Fatty acids/Eicosanoids (FA)	HEDGEHOG ACYLTRANSFERASE
LMP001998	Sftpb	P50405	MM.46033	13393	160891_at	A_51_P269134	Sphingolipids (SP)	SURFACTANT ASSOCIATED PROTEIN B
LMP002000	Ptgfr	P43117	MM.331442	146259	161713_f_at; 97769_at	A_51_P511329	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN F RECEPTOR
LMP002005	Vdr	Q922X0	MM.245084	5037	99964_at;99965_at	A_51_P506843	Sterol Lipids (ST)	VITAMIN D RECEPTOR
LMP002010	Acsl4	Q5D071	MM.143689	17019	#N/A	A_51_P268154	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE LONG-CHAIN FAMILY MEMBER 4
LMP002026	Ptgs1	Q6QDC8	MM.275434	18555	161694_f_at, 95597_at	A_51_P279100	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN-ENDOPEROXIDE SYNTHASE 1
LMP002033	Fut1	O09160	MM.56933	15895	99381_at	A_51_P222604	Glycerolipids (GL) / Sphingolipids (SP)	FUCOSYLTRANSFERASE 1
LMP002034	Nr2f2	P43135	MM.158143	29270	103052_r_at	A_51_P147684	Sterol Lipids (ST)	NUCLEAR RECEPTOR SUBFAMILY 2, GROUP F, MEMBER 2
LMP002036	Apoe	P08226	MM.305152	15454	161321_i_at; 95356_at	A_51_P171999	Not classified	APOLIPOPROTEIN E
LMP002037	Sts	P50427	MM.5129	19966	98860_at;98861_at	A_51_P377526	Sterol Lipids (ST)	STEROID SULFATASE
LMP002038	Arsa	P50428	MM.620	38094	100931_at;161286_f_at	A_51_P310649	Sphingolipids (SP)	ARYLSULFATASE A
LMP002042	Dgkd	Q80Y60	MM.277217	9628	#N/A	#N/A	Glycerolipids (GL)	DIACYLGLYCEROL KINASE, DELTA
LMP002053	Fabp6	P51162	MM.142716	13437	160886_i_at; 99977_at	A_51_P309066	Sterol Lipids (ST)	FATTY ACID BINDING PROTEIN 6, ILEAL (GASTROTROPIN)
LMP002055	Cyp21	P03940	MM.57192	144658	#N/A	#N/A	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 21, SUBFAMILY A, POLYPEPTIDE 1
LMP002056	Adfp	Q8K3Q8	MM.381	39259	161443_r_at; 98589_at	A_51_P258150	Fatty acids/Eicosanoids (FA)	ADIPOSE DIFFERENTIATION RELATED PROTEIN
LMP002059	Amacr	O09174	NA	24913	95588_at	A_51_P118885	Prenol Lipids (PR)	2-METHYLACYL-COA RACEMASE
LMP002067	Chpt1	Q6SXV1	MM.288897	30110	#N/A	A_51_P240857	Not classified	CHOLINE PHOSPHOTRANSFERASE 1
LMP002070	Mtmr12	Q8C0P1	MM.54460	4003	#N/A	A_51_P308308	Not classified	VMYOTUBULARIN RELATED PROTEIN 12
LMP002075	Plcd4	Q8K3R3	MM.290731	149111	#N/A	#N/A	Glycerophospholipids (GP)	PHOSPHOLIPASE C, DELTA 4

LMP002080	Acadl	P51174	MM.2445	144354	95425_at	A_51_P149455	Fatty acids/Eicosanoids (FA)	ACETYL-COENZYME A DEHYDROGENASE, LONG-CHAIN
LMP002081	B4galt1	P15535	MM.15622	146299	103002_at	A_51_P155257	Glycerolipids (GL) / Glycerophospholipids (GP) / Sphingolipids (SP)	UDP-GAL:BETAGLCNAC BETA 1,4-GALACTOSYLTRANSFERASE, POLYPEPTIDE 1
LMP002085	Pon1	Q91X30	MM.237657	17183	96895_at	A_51_P108659	Sterol Lipids (ST)	PARAOXONASE 1
LMP002089	Cyp11b2	P15539	MM.377079	146066	#N/A	A_51_P508159	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 11, SUBFAMILY B, POLYPEPTIDE 2
LMP002095	Akr1c14	Q91WT7	MM.26838	7965	#N/A	A_51_P284177	Sterol Lipids (ST)	RIKEN CDNA 9030611N15 GENE
LMP002097	Hnf4a	P49698	MM.202383	16277	#N/A	A_51_P198473	Sterol Lipids (ST)	HEPATIC NUCLEAR FACTOR 4, ALPHA
LMP002099	Aldh7a1	Q9DBF1	MM.30250	4048	161783_at;97449_at;97450_s_at	A_51_P470414	Fatty acids/Eicosanoids (FA) / Glycerolipids (GL) / Sterol Lipids (ST)	DNA SEGMENT, CHR 18, WAYNE STATE UNIVERSITY 181, EXPRESSED
LMP002107	Cds2	Q99L43	MM.284503	37638	104627_at	A_51_P183121;A_51_P356353	Glycerophospholipids (GP)	CDP-DIACYLGLYCEROL SYNTHASE (PHOSPHATIDATE CYTIDYLYLTRANSFERASE) 2
LMP002113	Zdhhc7	Q91WU6	MM.240076	7125	#N/A	A_51_P154933	Fatty acids/Eicosanoids (FA)	ZINC FINGER, DHHC DOMAIN CONTAINING 7
LMP002121	Cdipt	Q8VDP6	MM.28219	19021	97829_at	A_51_P408471	Glycerophospholipids (GP)	CDP-DIACYLGLYCEROL--INOSITOL 3-PHOSPHATIDYLTRANSFERASE (PHOSPHATIDYLIN...
LMP002132	Acox1	Q8BYC3	MM.383812	16948	101515_at;162158_r_at	A_51_P366704	Fatty acids/Eicosanoids (FA)	ACYL-COENZYME A OXIDASE 1, PALMITOYL
LMP002135	Hmgcl	Q8QZS6	MM.22668	4058	161970_f_at;94324_f_at	A_51_P308961	Fatty acids/Eicosanoids (FA)	3-HYDROXY-3-METHYLGLUTARYL-COENZYME A LYASE
LMP002137	Edg8	Q91X56	MM.190619	24978	#N/A	A_51_P480168	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION, SPHINGOLIPID G-PROTEIN-COUPLED RECEPTOR, ...
LMP002140	Adipor2	Q8BQS5	MM.291826	6586	#N/A	A_51_P257151	Fatty acids/Eicosanoids (FA)	EST AI115388
LMP002142	Gpld1	Q9DBH3	MM.291831	148323	161399_r_at;95566_at	A_51_P402583	Glycerophospholipids (GP)	GLYCOSYLPHOSPHATIDYLINOSITOL SPECIFIC PHOSPHOLIPASE D1
LMP002146	Acsl6	Q5ICG6	MM.267478	148310	#N/A	A_51_P380699;A_51_P518822	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE LONG-CHAIN FAMILY MEMBER 6
LMP002148	Pla2g2d	Q9WVF6	MM.71913	66677	#N/A	A_51_P232281	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP IID
LMP002151	Rara	Q5BLJ8	MM.103336	149784	92901_at	A_51_P389724	Prenol Lipids (PR) / / Sterol Lipids (ST)	RETINOIC ACID RECEPTOR, ALPHA
LMP002153	Thrap5	Q6PGF3	MM.260089	13263	#N/A	A_51_P297552	Not classified	THYROID HORMONE RECEPTOR ASSOCIATED PROTEIN 5
LMP002154	Dapp1	Q9QXT1	MM.254835	148780	#N/A	A_51_P244824	Glycerophospholipids (GP)	DUAL ADAPTOR FOR PHOSPHOTYROSINE AND 3-PHOSPHOINOSITIDES 1
LMP002157	Acat1	Q8QZT1	MM.293233	19679	#N/A	A_51_P319449	Fatty acids/Eicosanoids (FA)	ACETYL-COENZYME A ACETYLTRANSFERASE 1
LMP002159	Ard1a	Q9QY36	MM.305796	143996	#N/A	#N/A	Glycerophospholipids (GP)	N-ACETYLTRANSFERASE ARD1 HOMOLOG (S. CEREVISIAE)
LMP002161	Pnpla3	Q91WW7	MM.86557	14518	#N/A	A_51_P389265	Glycerolipids (GL)	ADIPONUTRIN

LMP002163	Plekha1	Q8BUL6	MM.323554	145652	#N/A	A_51_P237367	Not classified	PLECKSTRIN HOMOLOGY DOMAIN CONTAINING, FAMILY A (PHOSPHOINOSITIDE BIND...
LMP002167	Alox5	P48999	MM.41072	14970	#N/A	A_51_P247249;A_51_P473051	Fatty acids/Eicosanoids (FA)	ARACHIDONATE 5-LIPOXYGENASE
LMP002169	Ppargc1b	Q8VHJ7	MM.32269	145357	#N/A	A_51_P294891	Not classified	PEROXISOME PROLIFERATIVE ACTIVATED RECEPTOR, GAMMA, COACTIVATOR 1 BETA
LMP002170	Slc10a4	Q8BJC7	MM.253661	9142	#N/A	A_51_P448479	Not classified	SOLUTE CARRIER FAMILY 10 (SODIUM/BILE ACID COTRANSPORTER FAMILY), MEMB...
LMP002171	Sptlc1	O35704	MM.240336	9616	100608_at	A_51_P345593	Fatty acids/Eicosanoids (FA) / Sphingolipids (SP)	SERINE PALMITOYLTRANSFERASE, LONG CHAIN BASE SUBUNIT 1
LMP002173	Lipg	Q9WVG5	MM.299647	17095	#N/A	A_51_P354782	Glycerolipids (GL)	LIPASE, ENDOTHELIAL
LMP002175	Grk6	O70293	MM.10193	144592	#N/A	#N/A	Glycerophospholipids (GP)	G PROTEIN-COUPLED RECEPTOR KINASE 6
LMP002178	Phyh	O35386	MM.27066	148871	96608_at	A_51_P276063	Fatty acids/Eicosanoids (FA) / Prenol Lipids (PR)	PHYTANOYL-COA HYDROXYLASE
LMP002179	Ttpa	Q8QZU5	MM.379065	26891	#N/A	A_51_P258817	Prenol Lipids (PR)	TOCOPHEROL (ALPHA) TRANSFER PROTEIN
LMP002181	Hsd17b7	O88736	MM.12882	4622	94177_at	A_51_P250934	Sterol Lipids (ST)	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 7
LMP002183	Lipf	Q9CPP7	MM.329816	3503	#N/A	A_51_P201174	Glycerolipids (GL)	LIPASE, GASTRIC
LMP002188	Apob	Q8CGG8	MM.221239	27630	#N/A	A_51_P380650;A_51_P413088;A_51_P436689;A_51_P470542	Glycerolipids (GL) / Sterol Lipids (ST)	APOLIPOPROTEIN B
LMP002198	Dci	Q8QZV3	MM.291743	7147	98527_at	A_51_P105589	Fatty acids/Eicosanoids (FA)	DODECENOYL-COENZYME A DELTA ISOMERASE (3,2 TRANS-ENOYL-COENYME A ISOME...
LMP002199	Dgkb	Q8BUN1	MM.242576	146614	#N/A	A_51_P515612	Glycerolipids (GL)	DIACYLGLYCEROL KINASE, BETA
LMP002203	Gpd2	Q8VDT0	MM.3711	26998	162210_r_at; 98984_f_at	A_51_P342481	Glycerophospholipids (GP)	GLYCEROL PHOSPHATE DEHYDROGENASE 2, MITOCHONDRIAL
LMP002204	Anxa1	P10107	MM.248360	13826	161703_f_at; 93037_i_at;93038_f_at	A_51_P283590	Not classified	ANNEXIN A1
LMP002205	Acot12	Q9DBK0	MM.275963	145958	#N/A	A_51_P414305	Fatty acids/Eicosanoids (FA)	ACYL-COA THIOESTERASE 12
LMP002214	Star	P51557	MM.293314	40769	161784_f_at; 92213_at	A_51_P274436	Sterol Lipids (ST)	STEROIDOGENIC ACUTE REGULATORY PROTEIN
LMP002216	Slc10a6	Q9CXB2	MM.7446	59888	#N/A	A_51_P173678	Not classified	SOLUTE CARRIER FAMILY 10 (SODIUM/BILE ACID COTRANSPORTER FAMILY), MEMB...
LMP002218	Acad8	Q9D7B6	MM.289244	24625	#N/A	A_51_P244052	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	ACYL-COENZYME A DEHYDROGENASE FAMILY, MEMBER 8
LMP002220	Degs2	Q8R2F2	MM.207605	10639	#N/A	A_51_P122649	Sphingolipids (SP)	DEGENERATIVE SPERMATOCYTE HOMOLOG 2 (DROSOPHILA), LIPID DESATURASE
LMP002222	2310016 F22Rik	Q8VDU3	MM.379359	144986	#N/A	A_51_P500215	Not classified	CDNA SEQUENCE BC020489
LMP002223	Acox1	Q8C168	MM.383812	16948	101515_at;162158_r_at	A_51_P366704	Fatty acids/Eicosanoids (FA)	ACYL-COENZYME A OXIDASE 1, PALMITOYL
LMP002224	Acadsb	Q9DBL1	MM.334274	30263	#N/A	A_51_P435068	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	ACYL-COENZYME A DEHYDROGENASE, SHORT/BRANCHED CHAIN

LMP002227	Pik3r1	P26450	MM.259333	37643	96592_at	#N/A	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE, REGULATORY SUBUNIT, POLYPEPTIDE 1 (P85 ...
LMP002228	Abhd5	Q9DBL9	MM.280254	144532	#N/A	A_51_P407165	Not classified	ABHYDROLASE DOMAIN CONTAINING 5
LMP002232	Gla	P51569	MM.1114	14848	102341_at	A_51_P153265	Glycerolipids (GL) / Sphingolipids (SP)	GALACTOSIDASE, ALPHA
LMP002236	Pla2g12b	Q99P27	MM.30268	37683	#N/A	A_51_P184385	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP XIIB
LMP002238	Ihpk1	Q6PD10	MM.276155	26873	162092_f_at; 94244_at	A_51_P103718;A_51_P213765	Glycerophospholipids (GP)	INOSITOL HEXAPHOSPHATE KINASE 1
LMP002240	Pla2g10	Q9QXX3	MM.4214	4590	161034_at	A_51_P380451	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP X
LMP002242	Lass6	Q8C172	MM.222222	22082	#N/A	A_51_P335269	Not classified	LONGEVITY ASSURANCE HOMOLOG 6 (S. CEREVISIAE)
LMP002245	Acox3	Q8C178	MM.291503	146997	#N/A	A_51_P252768	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	ACYL-COENZYME A OXIDASE 3, PRISTANOYL
LMP002248	Adh1	P00329	MM.2409	20047	94906_at	A_51_P428555	Fatty acids/Eicosanoids (FA) / Prenol Lipids (PR) / Sterol Lipids (ST) / Glycerolipids (GL)	ALCOHOL DEHYDROGENASE 1 (CLASS I)
LMP002250	Ehhadh	Q9DBM2	MM.28100	146125	#N/A	A_51_P462918	Fatty acids/Eicosanoids (FA)	ENOYL-COENZYME A, HYDRATASE/3-HYDROXYACYL COENZYME A DEHYDROGENASE
LMP002261	B4galt6	Q9WVK5	MM.26364	10133	102936_at	A_51_P122268	Glycerolipids (GL) / Glycerophospholipids (GP) / Sphingolipids (SP)	UDP-GAL:BETAGLCNAC BETA 1,4-GALACTOSYLTRANSFERASE, POLYPEPTIDE 6
LMP002262	Ugt2b5	P17717	MM.29157	146553	96796_f_at	#N/A	Sterol Lipids (ST)	UDP GLUCURONOSYLTRANSFERASE 2 FAMILY, POLYPEPTIDE B5
LMP002267	Cyp46a1	Q9WVK8	MM.41911	32016	#N/A	A_51_P457658	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 46, SUBFAMILY A, POLYPEPTIDE 1
LMP002272	4632408 A20Rik	Q8BYI3	MM.49245	145642	#N/A	#N/A	Fatty acids/Eicosanoids (FA)	RIKEN CDNA 4632408A20 GENE
LMP002273	Pigs	Q6PD26	MM.295908	14415	#N/A	#N/A	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL GLYCAN, CLASS S
LMP002274	Acox1	Q9R0H0	MM.383812	16948	101515_at;162158_r_at	A_51_P366704	Fatty acids/Eicosanoids (FA)	ACYL-COENZYME A OXIDASE 1, PALMITOYL
LMP002276	Tnr	Q8BYI9	MM.383195	147060	#N/A	#N/A	Sphingolipids (SP)	TENASCIN R
LMP002282	Shh	Q62226	MM.57202	6746	101831_at	A_51_P350419	Sterol Lipids (ST)	SONIC HEDGEHOG
LMP002287	Apobec1	P51908	MM.3333	35220	98398_s_at	A_51_P160754	Not classified	APOLIPOPROTEIN B EDITING COMPLEX 1
LMP002290	Pdk1	Q8BFP9	MM.34411	147286	#N/A	A_51_P406429	Not classified	PYRUVATE DEHYDROGENASE KINASE, ISOENZYME 1
LMP002294	Cyp39a1	Q9JKJ9	MM.17991	10876	#N/A	A_51_P515446	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 39, SUBFAMILY A, POLYPEPTIDE 1
LMP002299	Ddhd1	Q80YA3	MM.121918	22102	#N/A	A_51_P454441	Not classified	DDHD DOMAIN CONTAINING 1
LMP002301	Srd5a1	Q8BUR8	MM.315983	12273	#N/A	A_51_P420415	Sterol Lipids (ST)	STEROID 5 ALPHA-REDUCTASE 1
LMP002302	Pik3r1	Q80UI5	MM.259333	37643	96592_at	#N/A	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE, REGULATORY SUBUNIT, POLYPEPTIDE 1 (P85 ...

LMP002303	Apod	P51910	MM.2082	144426	93592_at	A_51_P366811	Not classified	APOLIPOPROTEIN D
LMP002304	Hmgcl	P38060	MM.22668	4058	161970_f_at; 94324_f_at	A_51_P308961	Fatty acids/Eicosanoids (FA)	3-HYDROXY-3-METHYLGLUTARYL-COENZYME A LYASE
LMP002306	Inpp5d	Q61181	MM.15105	3846	#N/A	A_51_P337125	Glycerophospholipids (GP)	INOSITOL POLYPHOSPHATE-5-PHOSPHATASE D
LMP002309	Gpr68	Q8BFQ3	MM.32160	16856	#N/A	A_51_P296815	Not classified	G PROTEIN-COUPLED RECEPTOR 68
LMP002321	Rdh12	Q8BYK4	MM.274373	144082	#N/A	A_51_P141960	Not classified	RETINOL DEHYDROGENASE 12
LMP002323	Pla2g3	Q8BV23	MM.100476	43776	#N/A	A_51_P402868	Not classified	PHOSPHOLIPASE A2, GROUP III
LMP002328	Acot11	Q8VHQ9	MM.274924	23811	#N/A	A_51_P141741;A_51_P226453	Not classified	THIOESTERASE, ADIPOSE ASSOCIATED
LMP002337	Pik3c2a	Q61194	MM.3810	38192	92311_s_at;92312_at	A_51_P159172	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE, C2 DOMAIN CONTAINING, ALPHA POLYPEPTIDE
LMP002340	Syt1	P46096	MM.336111	108131	93005_at	A_51_P297896;A_51_P298873	Not classified	SYNAPTOTAGMIN I
LMP002342	Cyp7a1	Q8BFR7	MM.57029	17149	99404_at	A_51_P290981	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 7, SUBFAMILY A, POLYPEPTIDE 1
LMP002346	B4galnt2	Q09199	MM.340702	33558	#N/A	A_51_P404405	Sphingolipids (SP) / Glycerophospholipids (GP) / Glycerolipids (GL)	BETA-1,4-N-ACETYL-GALACTOSAMINYL TRANSFERASE 2
LMP002352	Atp5g1	P48202	MM.371547	21238	161324_r_at; 161487_f_at; 96032_at	A_51_P183300	Not classified	ATP SYNTHASE, H+ TRANSPORTING, MITOCHONDRIAL F0 COMPLEX, SUBUNIT C (SU...
LMP002355	Plin	Q8CGN5	MM.254917	37305	#N/A	A_51_P244497	Not classified	PERILIPIN
LMP002356	Dbt	P53395	MM.3636	17113	98966_at	A_51_P350883	Glycerophospholipids (GP)	DIHYDROLIPOAMIDE BRANCHED CHAIN TRANSACYLASE E2
LMP002357	Akr1b1	P45376	MM.451	3746	#N/A	#N/A	Glycerolipids (GL)	ALDO-KETO REDUCTASE FAMILY 1, MEMBER B3 (ALDOSE REDUCTASE)
LMP002358	Inppl1	Q6P549	MM.5028	16981	102988_at	A_51_P142334	Glycerophospholipids (GP)	INOSITOL POLYPHOSPHATE PHOSPHATASE-LIKE 1
LMP002360	Akr1b8	P45377	MM.5378	17166	100884_at	A_51_P128987	Glycerolipids (GL)	ALDO-KETO REDUCTASE FAMILY 1, MEMBER B8
LMP002362	Dpagt1	P42867	MM.18353	36965	103352_at;161420_r_at	A_51_P422208	Prenol Lipids (PR)	DOLICHYL-PHOSPHATE (UDP-N-ACETYLGLUCOSAMINE) ACETYLGLUCOSAMINEPHOSPHOT...
LMP002382	Esrrb	Q61539	MM.235550	149415	100301_at	A_51_P419047	Sterol Lipids (ST)	ESTROGEN RECEPTOR RELATED 2
LMP002389	Oxsm	Q9D404	MM.197960	23143	#N/A	A_51_P312749	Fatty acids/Eicosanoids (FA)	3-OXOACYL-ACP SYNTHASE, MITOCHONDRIAL
LMP002390	Scd2	P13011	MM.193096	4054	162077_f_at, 95758_at	A_51_P129464	Fatty acids/Eicosanoids (FA)	STEAROYL-COENZYME A DESATURASE 2
LMP002394	Atp8b3	Q9CPY8	MM.52511	16782	#N/A	A_51_P371500	Not classified	ATPASE, CLASS I, TYPE 8B, MEMBER 3
LMP002396	Pgr	Q00175	MM.12798	146799	98726_at	A_51_P213476	Sterol Lipids (ST)	PROGESTERONE RECEPTOR
LMP002398	Pnpla8	Q9DC20	MM.54126	14954	#N/A	A_51_P201104	Not classified	RIKEN CDNA 1200006O19 GENE
LMP002399	Gbgt1	Q8VI38	MM.213199	26263	#N/A	A_51_P394281	Sphingolipids (SP)	GLOBOSIDE ALPHA-1,3-N-ACETYLGALACTOSAMINYLTRANSFERASE 1

LMP002400	Stard3	Q61542	MM.265546	30268	162228_f_at; 95607_at	A_51_P154973	Sterol Lipids (ST)	START DOMAIN CONTAINING 3
LMP002414	Hmgcr	Q01237	MM.316652	148912	104285_at;99 425_at	A_51_P507410	Sterol Lipids (ST)	3-HYDROXY-3-METHYLGLUTARYL-COENZYME A REDUCTASE
LMP002416	Phca	Q9D099	MM.334041	144791	#N/A	A_51_P130757	Sphingolipids (SP)	PHYTOCERAMIDASE, ALKALINE
LMP002421	Edg1	Q9DC35	MM.982	26540	#N/A	#N/A	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION SPHINGOLIPID G-PROTEIN-COUPLED RECEPTOR 1
LMP002423	Chpt1	Q8K0H2	MM.288897	30110	#N/A	A_51_P240857	Not classified	CHOLINE PHOSPHOTRANSFERASE 1
LMP002430	Acox3	Q7TPP6	MM.291503	146997	#N/A 101072_at;10	A_51_P252768	Fatty acids/Eicosanoids (FA) / Prenol Lipids (PR)	ACYL-COENZYME A OXIDASE 3, PRISTANOYL
LMP002434	Idi1	P58044	MM.29847	23815	3667_at;9626 9_at	A_51_P329711	Prenol Lipids (PR) / Sterol Lipids (ST)	ISOPENTENYL-DIPHOSPHATE DELTA ISOMERASE
LMP002440	Cyp19a1	P28649	MM.5199	7249	101200_at	A_51_P474555	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 19, SUBFAMILY A, POLYPEPTIDE 1
LMP002441	Esr2	Q8BG65	MM.2561	10899	96514_at	A_51_P279803	Sterol Lipids (ST)	ESTROGEN RECEPTOR 2 (BETA)
LMP002442	Lpin1	Q8CD95	MM.153625	12995	98892_at	A_51_P394984	Not classified	LIPIN 1
LMP002445	Edg4	Q9JL06	MM.23253	7002	#N/A	A_51_P410665	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION, LYSOPHOSPHATIDIC ACID G-PROTEIN-COUPLED R...
LMP002447	Nsddr	Q6WQJ1	MM.329718	31996	#N/A	#N/A	Not classified	5'-NUCLEOTIDASE DOMAIN CONTAINING 3
LMP002450	Plch1	Q7TPQ1	MM.316391	147017	#N/A	#N/A	Not classified	PHOSPHOLIPASE C-LIKE 3
LMP002451	Fads3	Q8C4Y5	MM.253875	144010	#N/A	A_51_P464029	Fatty acids/Eicosanoids (FA)	FATTY ACID DESATURASE 3
LMP002452	Star	Q6P910	MM.293314	40769	161784_f_at; 92213_at	A_51_P274436	Sterol Lipids (ST)	STEROIDOGENIC ACUTE REGULATORY PROTEIN
LMP002453	Dgkz	Q80UP3	MM.314923	27299	#N/A	A_51_P226049;A_ 51_P505156	Glycerolipids (GL)	DIACYLGLYCEROL KINASE ZETA
LMP002456	Crot	Q9DC50	MM.28197	28137	#N/A	A_51_P489153	Fatty acids/Eicosanoids (FA)	CARNITINE O-OCTANOYLTRANSFERASE
LMP002458	Galc	P54818	MM.5120	15929	161732_at;93 131_at	A_51_P428688	Sphingolipids (SP)	GALACTOSYLCERAMIDASE
LMP002464	Plekha3	Q9ERS4	MM.41636	34426	#N/A	A_51_P108525	Glycerophospholipids (GP)	PLECKSTRIN HOMOLOGY DOMAIN-CONTAINING, FAMILY A (PHOSPHOINOSITIDE BIND...
LMP002465	Plekha2	Q9ERS5	MM.261122	28336	#N/A	A_51_P280785;A_ 51_P489107	Glycerophospholipids (GP)	PLECKSTRIN HOMOLOGY DOMAIN-CONTAINING, FAMILY A (PHOSPHOINOSITIDE BIND...
LMP002467	Ncoa6	Q9JL19	MM.27592	11429	95351_at;955 25_at	A_51_P521128	Not classified	NUCLEAR RECEPTOR COACTIVATOR 6
LMP002473	Ptges3	Q9R0Q7	MM.22421	14581	#N/A	A_51_P165960;A_ 51_P474367	Not classified	PROSTAGLANDIN E SYNTHASE 3 (CYTOSOLIC)
LMP002475	Pla1a	Q8VI78	MM.279805	145809	#N/A	A_51_P381618	Not classified	PHOSPHOLIPASE A1 MEMBER A
LMP002476	Ldlr	P35951	MM.3213	8818	160832_at	A_51_P274173	Sterol Lipids (ST)	LOW DENSITY LIPOPROTEIN RECEPTOR
LMP002477	Pemt	Q61907	MM.2731	144334	161893_i_at; 94987_at	A_51_P193095	Glycerophospholipids (GP)	PHOSPHATIDYLETHANOLAMINE N-METHYLTRANSFERASE

LMP002478	Edg2	P61793	MM.4772	26792	100435_at	A_51_P475574	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION, LYSOPHOSPHATIDIC ACID G-PROTEIN-COUPLED R...
LMP002490	Fut2	Q9JL27	MM.290046	17765	101760_at	A_51_P393098	Glycerolipids (GL) / Sphingolipids (SP)	FUCOSYLTRANSFERASE 2
LMP002494	Pgr	Q8BZ29	MM.12798	146799	98726_at	A_51_P213476	Sterol Lipids (ST)	PROGESTERONE RECEPTOR
LMP002495	Acsl4	Q9QUJ7	MM.143689	17019	#N/A	A_51_P268154	Fatty acids/Eicosanoids (FA)	ACYL-COA SYNTHETASE LONG-CHAIN FAMILY MEMBER 4
LMP002498	Pnpla2	Q643S0	MM.29998	26846	#N/A	A_51_P197213	Not classified	PATATIN-LIKE PHOSPHOLIPASE DOMAIN CONTAINING 2
LMP002499	Pnpla6	Q9R114	MM.23085	4926	#N/A	A_51_P301891	Not classified	NEUROPATHY TARGET ESTERASE
LMP002502	Ltc4s 4833405	Q60860	MM.245151	45402	92401_at	A_51_P354792	Fatty acids/Eicosanoids (FA)	LEUKOTRIENE C4 SYNTHASE
LMP002514	L16Rik	Q8BFZ6	MM.313743	37398	#N/A	#N/A	Prenol Lipids (PR)	RIKEN CDNA 4833405L16 GENE
LMP002515	Plcz1	Q8K4D7	MM.50808	37226	#N/A	A_51_P297441	Glycerophospholipids (GP)	PHOSPHOLIPASE C, ZETA 1
LMP002524	Tlr4	Q9QUK6	MM.38049	12229	#N/A	A_51_P300806	Not classified	TOLL-LIKE RECEPTOR 4
LMP002528	Nat6	Q9R123	MM.10305	9303	104439_at	A_51_P268145	Glycerophospholipids (GP)	N-ACETYLTRANSFERASE 6
LMP002533	Vdr	P48281	MM.245084	5037	99964_at;99965_at	A_51_P506843	Sterol Lipids (ST)	VITAMIN D RECEPTOR
LMP002534	Ugt1a6	Q64435	MM.300095	146268	#N/A	#N/A	Sterol Lipids (ST)	UDP GLUCURONOSYLTRANSFERASE 1 FAMILY, POLYPEPTIDE A6A
LMP002535	Impad1	Q80V26	MM.218889	35267	#N/A	A_51_P220799;A_51_P293017	Not classified	INOSITOL MONOPHOSPHATASE DOMAIN CONTAINING 1
LMP002536	Inpp5a	Q8BNK3	MM.277096	5838	#N/A	A_51_P364014	Not classified	INOSITOL POLYPHOSPHATE-5-PHOSPHATASE A
LMP002538	Adh7	Q64437	MM.8473	10625	93695_at	A_51_P233801	Fatty acids/Eicosanoids (FA) / Glycerolipids (GL) / Sterol Lipids (ST)	ALCOHOL DEHYDROGENASE 7 (CLASS IV), MU OR SIGMA POLYPEPTIDE
LMP002539	Pnlip	Q6P8U6	MM.20407	17651	#N/A	A_51_P520552	Sterol Lipids (ST)	PANCREATIC LIPASE
LMP002542	Acaca	Q705X8	MM.352014	36995	#N/A	A_51_P191939;A_51_P439426	Fatty acids/Eicosanoids (FA)	ACETYL-COENZYME A CARBOXYLASE ALPHA
LMP002543	Rdh1	Q8CGV4	MM.235814	8096	#N/A	#N/A	Prenol Lipids (PR) / Sterol Lipids (ST)	RETINOL DEHYDROGENASE 1 (ALL TRANS)
LMP002546	Fdft1	P53798	MM.386760	3360	97518_at	A_51_P485945	Prenol Lipids (PR) / Sterol Lipids (ST)	FARNESYL DIPHOSPHATE FARNESYL TRANSFERASE 1
LMP002551	Ppara	P23204	MM.212789	16946	102668_at	A_51_P348334	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR ALPHA
LMP002553	Pla2g2e	Q9QUL3	MM.296007	14170	#N/A	A_51_P311159	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP IIE
LMP002557	Cyp24a1	Q64441	MM.6575	146497	162070_r_at; 93435_at	A_51_P231099	Sterol Lipids (ST)	CYTOCHROME P450, FAMILY 24, SUBFAMILY A, POLYPEPTIDE 1
LMP002560	Ncoa2	Q7TPU7	MM.2537	12954	96508_at	A_51_P478593	Not classified	NUCLEAR RECEPTOR COACTIVATOR 2

LMP002561	Lypla3	Q8VEB4	MM.284770	146865	#N/A	A_51_P137467	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP) / Sphingolipids (SP) / Sterol Lipids (ST)	LYSOPHOSPHOLIPASE 3
LMP002565	Ptdss1	Q99LH2	MM.281464	3506	101931_at;161864_f_at	A_51_P442056	Glycerophospholipids (GP)	PHOSPHATIDYLSERINE SYNTHASE 1
LMP002573	Prmt2	Q9R144	MM.32020	149274	#N/A	A_51_P219064	Sterol Lipids (ST)	PROTEIN ARGININE N-METHYLTRANSFERASE 2
LMP002581	Cyp2c29	Q64458	MM.20764	147212	93585_at	A_51_P103706	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY C, POLYPEPTIDE 29
LMP002582	Pgrmc2	Q80UU9	MM.40321	22116	#N/A	A_51_P413916	Sterol Lipids (ST)	PROGESTERONE RECEPTOR MEMBRANE COMPONENT 2
LMP002583	Dgkq	Q8BK42	MM.260921	20152	#N/A	A_51_P329869	Glycerolipids (GL)	DIACYLGLYCEROL KINASE, THETA
LMP002584	Cyp3a11	Q64459	MM.332844	98682	93770_at	A_51_P355301	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 3, SUBFAMILY A, POLYPEPTIDE 11
LMP002586	Hmgcs2	P54869	MM.289131	6700	92590_at	A_51_P116039	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	3-HYDROXY-3-METHYLGLUTARYL-COENZYME A SYNTHASE 2
LMP002589	Gltp	Q9JL62	MM.275766	26293	#N/A	A_51_P255271	Not classified	GLYCOLIPID TRANSFER PROTEIN
LMP002590	Tmepai	Q9D7R2	MM.73682	13837	#N/A	A_51_P134475;A_51_P449777	Not classified	TRANSMEMBRANE, PROSTATE ANDROGEN INDUCED RNA
LMP002591	Rdh11	Q9QYF1	MM.291799	16643	99591_i_at;99592_f_at	A_51_P469160	Not classified	RETINOL DEHYDROGENASE 11
LMP002592	Pigc	Q9CXR4	MM.45106	12872	#N/A	A_51_P307800	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL GLYCAN, CLASSC
LMP002598	Sdhb	Q9CQA3	MM.246965	5189	95053_s_at	A_51_P234853	Prenol Lipids (PR)	SUCCINATE DEHYDROGENASE COMPLEX, SUBUNIT B, IRON SULFUR (IP)
LMP002608	Cyp3a13	Q64464	MM.289886	19495	99463_at	A_51_P114941	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 3, SUBFAMILY A, POLYPEPTIDE 13
LMP002609	Cyp2c37	P56654	MM.220317	148321	99083_at	A_51_P498882	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2. SUBFAMILY C, POLYPEPTIDE 37
LMP002611	Cyp2c38	P56655	MM.42100	8638	102084_f_at	A_51_P342207	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY C, POLYPEPTIDE 38
LMP002612	Cyp2c39	P56656	MM.42101	147076	98295_at	A_51_P304111	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY C, POLYPEPTIDE 39
LMP002614	Cyp2c40	P56657	MM.358585	37664	96334_f_at	A_51_P502599	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY C, POLYPEPTIDE 40
LMP002621	Wbscr22	Q9CY21	MM.347936	4185	#N/A	A_51_P456590	Sterol Lipids (ST)	WILLIAMS BEUREN SYNDROME CHROMOSOME REGION 22
LMP002624	Gyk	Q8C8X0	MM.246682	144099	97525_at	A_51_P297671	Glycerolipids (GL)	GLYCEROL KINASE
LMP002630	Rbp1	Q00915	MM.279741	20055	104716_at	A_51_P423484	Prenol Lipids (PR)	RETINOL BINDING PROTEIN 1, CELLULAR
LMP002633	Ptger2	Q8BZ75	MM.4630	31104	98768_at	A_51_P249215	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN E RECEPTOR 2 (SUBTYPE EP2)
LMP002636	Apol2	Q8BZ78	MM.125650	5737	#N/A	A_51_P115471	Not classified	APOLIPOPROTEIN L, 2
LMP002642	Osbp	Q570Y8	MM.291279	35712	#N/A	A_51_P393768	Sterol Lipids (ST)	OXYSTEROL BINDING PROTEIN
LMP002644	Srd5a1	Q68FF9	MM.315983	12273	#N/A	A_51_P420415	Sterol Lipids (ST)	STEROID 5 ALPHA-REDUCTASE 1

LMP002646	Fabp1	P12710	MM.22126	21351	162342_at;94075_at	A_51_P487818	Fatty acids/Eicosanoids (FA)	FATTY ACID BINDING PROTEIN 1, LIVER
LMP002655	Clk1	P22518	MM.1761	144442	#N/A	A_51_P193573	Glycerophospholipids (GP)	CDC-LIKE KINASE 1
LMP002656	Clps	Q9CQC2	MM.21160	13665	160132_at	A_51_P164459	Not classified	RIKEN CDNA 2200003J09 GENE
LMP002661	Cyp3a16	Q64481	MM.378905	28372	101638_s_at;101639_r_at	A_51_P482050	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 3, SUBFAMILY A, POLYPEPTIDE 16
LMP002662	Ihpk2	Q80V72	MM.276336	9692	#N/A	A_51_P257058	Not classified	INOSITOL HEXAPHOSPHATE KINASE 2
LMP002670	Ptges	Q8BNP8	MM.28768	59852	104406_at	A_51_P312328	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN E SYNTHASE
LMP002673	Dci	P42125	MM.291743	7147	98527_at	A_51_P105589	Fatty acids/Eicosanoids (FA)	DODECENOYL-COENZYME A DELTA ISOMERASE (3,2 TRANS-ENOYL-COENYME A ISOME...
LMP002684	Angptl3	Q9R182	MM.28341	31181	#N/A	A_51_P481679	Not classified	ANGIOPOIETIN-LIKE 3
LMP002688	Crat	P47934	MM.20396	24681	103646_at;161989_f_at	A_51_P440807	Fatty acids/Eicosanoids (FA)	CARNITINE ACETYLTRANSFERASE
LMP002691	Coq6	Q8BJY5	MM.280062	144323	#N/A	A_51_P116479	Prenol Lipids (PR)	COENZYME Q6 HOMOLOG (YEAST)
LMP002693	St6galnac6	Q8CDC3	MM.88831	4643	#N/A	A_51_P284997	Sphingolipids (SP)	ST6 (ALPHA-N-ACETYL-NEURAMINYL-2,3-BETA-GALACTOSYL-1,3)-N-ACETYLGALACT...
LMP002695	St6galnac5	Q9QYJ1	MM.40915	37257	#N/A	A_51_P421724	Sphingolipids (SP)	ST6 (ALPHA-N-ACETYL-NEURAMINYL-2,3-BETA-GALACTOSYL-1,3)-N-ACETYLGALACT...
LMP002698	Ptger3	P30557	MM.30424	146974	96588_at;96589_at	A_51_P520718	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN E RECEPTOR 3 (SUBTYPE EP3)
LMP002709	Pla2g10	Q8K130	MM.4214	4590	161034_at	A_51_P380451	Fatty acids/Eicosanoids (FA) / Glycerophospholipids (GP)	PHOSPHOLIPASE A2, GROUP X
LMP002710	Lcat	P16301	MM.1593	16934	103023_at;161759_r_at	A_51_P336068	Glycerophospholipids (GP) / Sterol Lipids (ST)	LECITHIN CHOLESTEROL ACYLTRANSFERASE
LMP002711	Osbp2	Q5QNQ6	MM.61022	10914	#N/A	A_51_P348636	Sterol Lipids (ST)	OXYSTEROL BINDING PROTEIN 2
LMP002716	Smpd1	Q04519	MM.4628	19432	100099_at,161789_r_at	A_51_P429209	Sphingolipids (SP)	SPHINGOMYELIN PHOSPHODIESTERASE 1, ACID LYSOSOMAL
LMP002725	Aadac	Q99PG0	MM.24547	34406	95439_at	A_51_P394115	Not classified	ARYLACETAMIDE DEACETYLASE (ESTERASE)
LMP002728	Hsd17b8	P50171	MM.275452	146466	#N/A	#N/A	Sterol Lipids (ST)	H2-K REGION EXPRESSED GENE 6
LMP002729	Hsd11b1	P50172	MM.28328	37378	97867_at	A_51_P127297	Sterol Lipids (ST)	HYDROXYSTEROID 11-BETA DEHYDROGENASE 1
LMP002731	Ptgis	O35074	MM.2339	27536	104538_at	A_51_P372819	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN I2 (PROSTACYCLIN) SYNTHASE
LMP002732	Ptger3	Q6PDF2	MM.30424	146974	96588_at;96589_at	A_51_P520718	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN E RECEPTOR 3 (SUBTYPE EP3)
LMP002742	Dgkq	Q6P5E8	MM.260921	20152	#N/A	A_51_P329869	Glycerolipids (GL)	DIACYLGLYCEROL KINASE, THETA
LMP002743	Nmt1	O70310	MM.10265	30273	102047_at;102980_at	A_51_P180825	Fatty acids/Eicosanoids (FA)	N-MYRISTOYLTRANSFERASE 1
LMP002744	Nmt2	O70311	MM.65021	8003	102229_at	A_51_P465512	Fatty acids/Eicosanoids (FA)	N-MYRISTOYLTRANSFERASE 2
LMP002745	Sult2b1	O35400	MM.271634	9560	101406_at	A_51_P403170	Sterol Lipids (ST)	SULFOTRANSFERASE FAMILY, CYTOSOLIC, 2B, MEMBER 1

LMP002753	Agpat1	O35083	MM.8684	145459	93720_at	A_51_P322612	Glycerophospholipids (GP)	1-ACYLGLYCEROL-3-PHOSPHATE O-ACYLTRANSFERASE 1 (LYSOPHOSPHATIDIC ACID ...
LMP002755	Scgb1a1	Q06318	MM.2258	14448	94291_at	A_51_P128575	Sterol Lipids (ST)	SECRETOGLOBIN, FAMILY 1A, MEMBER 1 (UTEROGLOBIN)
LMP002757	Ache	P21836	MM.255464	16860	104650_at	A_51_P117627	Glycerophospholipids (GP)	ACETYLCHOLINESTERASE
LMP002761	Gal3st1	Q9JHE4	MM.103414	3839	#N/A	A_51_P354354	Glycerolipids (GL) / Sphingolipids (SP)	GALACTOSE-3-O-SULFOTRANSFERASE 1
LMP002763	Pigk	Q9CXY9	MM.331447	8124	#N/A	A_51_P197174	Glycerophospholipids (GP)	RIKEN CDNA 3000001O05 GENE
LMP002764	Prkch	P23298	MM.341677	107086	99916_at	A_51_P355852	Glycerolipids (GL)	PROTEIN KINASE C, ETA
LMP002765	Rarg	P20787	MM.1273	5803	102419_at	A_51_P372853	Prenol Lipids (PR) / Sterol Lipids (ST)	RETINOIC ACID RECEPTOR, GAMMA
LMP002772	B3galt4	Q9Z0F0	MM.11132	144732	102267_at	A_51_P167105	Sphingolipids (SP)	UDP-GAL:BETAGALNAC BETA 1,3-GALACTOSYLTRANSFERASE, POLYPEPTIDE 4
LMP002774	Cpt1c	Q8BGD5	MM.231465	26746	#N/A	A_51_P177562	Fatty acids/Eicosanoids (FA)	CARNITINE PALMITOYLTRANSFERASE 1C
LMP002781	Gpx4	O70325	NA	28391	94897_at	A_51_P462448	Not classified	PHOSPHOLIPID HYDROPEROXIDE GLUTATHIONE PEROXIDASE, MITOCHONDRIAL PRECU
LMP002785	Lias	Q99M04	MM.195776	16736	98909_at	A_51_P430644	Not classified	LIPOIC ACID SYNTHETASE
LMP002789	Plcb1	Q6PDH1	MM.378986	146620	92465_at;92466_at;92467_g_at	A_51_P284665	Glycerophospholipids (GP)	PHOSPHOLIPASE C, BETA 1
LMP002790	Nr3c1	P06537	MM.129481	143770	98818_at	A_51_P355693	Sterol Lipids (ST)	NUCLEAR RECEPTOR SUBFAMILY 3, GROUP C, MEMBER 1
LMP002797	Ptgds2	Q9JHF7	MM.143720	37483	#N/A	A_51_P416315	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN D2 SYNTHASE 2, HEMATOPOIETIC
LMP002800	Hmgcs1	Q8C5F4	MM.61526	29332	94325_at	A_51_P146941	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	3-HYDROXY-3-METHYLGLUTARYL-COENZYME A SYNTHASE 1
LMP002803	Pccb	Q7TMF4	MM.335385	19392	160128_at	A_51_P418259	Fatty acids/Eicosanoids (FA)	PROPIONYL COENZYME A CARBOXYLASE, BETA POLYPEPTIDE
LMP002808	Pgrmc1	O55022	MM.9052	148833	101585_at	A_51_P144581	Sterol Lipids (ST)	PROGESTERONE RECEPTOR MEMBRANE COMPONENT 1
LMP002809	Impa1	O55023	MM.183042	145170	101498_at	A_51_P242056	Glycerophospholipids (GP)	INOSITOL (MYO)-1(OR 4)-MONOPHOSPHATASE 1
LMP002810	Pik4ca	Q6DIC7	MM.5718	3385	104208_at;99438_at	A_51_P204366	Not classified	PHOSPHATIDYLINOSITOL 4-KINASE, CATALYTIC, ALPHA POLYPEPTIDE
LMP002811	Edg1	O08530	MM.982	26540	#N/A	#N/A	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION SPHINGOLIPID G-PROTEIN-COUPLED RECEPTOR 1
LMP002812	Dgkk	Q6DIC8	MM.335953	5408	#N/A	#N/A	Not classified	DIACYLGLYCEROL KINASE KAPPA
LMP002813	St8sia5	P70126	MM.224750	40813	#N/A	A_51_P406734	Glycerolipids (GL) / Sphingolipids (SP)	SIALYLTRANSFERASE 8 (ALPHA-2, 8-SIALYLTRANSFERASE) E
LMP002817	Bckdk	O55028	MM.8903	7830	101557_at	A_51_P404413	Not classified	BRANCHED CHAIN KETOACID DEHYDROGENASE KINASE
LMP002819	Sgms2	Q9D4B1	MM.273360	12698	#N/A	A_51_P211998	Sphingolipids (SP)	RIKEN CDNA 4933405A16 GENE

LMP002821	Hsd3b2	P26149	MM.196405	37169	101659_at	A_51_P226567	Sterol Lipids (ST)	HYDROXYSTEROID DEHYDROGENASE-2, DELTA<5>-3-BETA
LMP002822	Esr2	O08537	NA	45332	96514_at	A_51_P279803	Sterol Lipids (ST)	ER-BETA
LMP002828	Pik3cg	Q9JHG7	MM.101369	34398	#N/A	A_51_P507832	Glycerophospholipids (GP)	PHOSPHOINOSITIDE-3-KINASE, CATALYTIC, GAMMA POLYPEPTIDE
LMP002830	Dhcr7	O88455	MM.249342	27932	98989_at	A_51_P290986	Sterol Lipids (ST)	7-DEHYDROCHOLESTEROL REDUCTASE
LMP002831	Cysltr2	Q920A1	MM.158324	27684	#N/A	A_51_P132013	Fatty acids/Eicosanoids (FA)	CYSTEINYL LEUKOTRIENE RECEPTOR 2
LMP002832	Gpx4	Q91XR9	MM.332810	144833	94897_at	A_51_P462448	Not classified	GLUTATHIONE PEROXIDASE 4
LMP002839	Ppargc1a	O70343	MM.259072	13095	#N/A	A_51_P279038	Not classified	PEROXISOME PROLIFERATIVE ACTIVATED RECEPTOR, GAMMA, COACTIVATOR 1 ALPH...
LMP002842	Cerk	Q8K4Q7	MM.222685	146813	101426_at	A_51_P146360	Glycerolipids (GL) / Sphingolipids (SP)	CERAMIDE KINASE
LMP002845	Lipe	Q8CDI9	MM.349647	9071	103083_at	A_51_P435366	Glycerolipids (GL) / Sterol Lipids (ST)	LIPASE, HORMONE SENSITIVE
LMP002847	Cyp2b9	P12790	MM.14413	12602	101862_at	A_51_P467076	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY B, POLYPEPTIDE 9
LMP002849	Cyp2b10	P12791	MM.218749	34409	#N/A	A_51_P182370	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY B, POLYPEPTIDE 10
LMP002855	St3gal3	P97325	MM.251002	16664	#N/A	A_51_P496551	Glycerolipids (GL)	ST3 BETA-GALACTOSIDE ALPHA-2,3-SIALYLTRANSFERASE 3
LMP002856	Oxct1	Q9D0K2	MM.13445	17968	#N/A	A_51_P107321	Fatty acids/Eicosanoids (FA)	3-OXOACID COA TRANSFERASE 1
LMP002857	Fut1	P97327	MM.56933	15895	99381_at	A_51_P222604	Glycerolipids (GL) / Sphingolipids (SP)	FUCOSYLTRANSFERASE 1
LMP002864	Ptgir	P43252	MM.287572	26885	98811_at	A_51_P202408	Fatty acids/Eicosanoids (FA)	PROSTAGLANDIN I RECEPTOR (IP)
LMP002866	Acadvl	P50544	MM.18630	11234	161623_at;93334_at	A_51_P518340	Fatty acids/Eicosanoids (FA) / Sterol Lipids (ST)	ACYL-COENZYME A DEHYDROGENASE, VERY LONG CHAIN
LMP002871	Scd3	Q99PL7	MM.371599	27373	#N/A	A_51_P446045	Fatty acids/Eicosanoids (FA)	STEAROYL-COENZYME A DESATURASE 3
LMP002873	Ppt2	O35448	MM.358666	7298	98426_at	A_51_P391885	Fatty acids/Eicosanoids (FA)	PALMITOYL-PROTEIN THIOESTERASE 2
LMP002880	Plce1	Q8K4S1	MM.34031	28123	#N/A	A_51_P137029;A_51_P451529	Glycerophospholipids (GP)	PHOSPHOLIPASE C-LIKE PROTEIN
LMP002881	Pnpla2	Q9DCF6	MM.29998	26846	#N/A	A_51_P197213	Not classified	PATATIN-LIKE PHOSPHOLIPASE DOMAIN CONTAINING 2
LMP002885	Gpld1	O70362	MM.291831	148323	161399_r_at;95566_at	A_51_P402583	Glycerophospholipids (GP)	GLYCOSYLPHOSPHATIDYLINOSITOL SPECIFIC PHOSPHOLIPASE D1
LMP002888	Acads	Q07417	MM.18759	14602	103401_at	A_51_P452807	Fatty acids/Eicosanoids (FA)	ACYL-COENZYME A DEHYDROGENASE, SHORT CHAIN
LMP002889	Crabp2	P22935	MM.4757	14840	100127_at	A_51_P521283	Not classified	CELLULAR RETINOIC ACID BINDING PROTEIN II
LMP002895	Ech1	O35459	MM.291776	7668	93754_at	A_51_P421846	Fatty acids/Eicosanoids (FA)	ENOYL COENZYME A HYDRATASE 1, PEROXISOMAL
LMP002898	Pip5k2c	Q91XU3	MM.22682	8237	160693_at;161763_r_at	A_51_P466049	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL-4-PHOSPHATE 5-KINASE, TYPE II, GAMMA
LMP002900	Acot3	Q9QYR7	MM.202331	8835	#N/A	A_51_P420489	Fatty acids/Eicosanoids (FA)	ACYL-COA THIOESTERASE 3

LMP002901	Acot2	Q9QYR9	MM.371675	10787	#N/A	#N/A	Fatty acids/Eicosanoids (FA)	ACYL-COA THIOESTERASE 2
LMP002903	Pik3cd	Q8BS14	MM.229108	147660	103866_at;92390_at	A_51_P142320	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE CATALYTIC DELTA POLYPEPTIDE
LMP002905	Scd3	Q8BNZ5	MM.371599	27373	#N/A	A_51_P446045	Fatty acids/Eicosanoids (FA)	STEAROYL-COENZYME A DESATURASE 3
LMP002907	B4galnt1	Q09200	MM.386762	146222	#N/A	A_51_P233928	Sphingolipids (SP)	BETA-1,4-N-ACETYL-GALACTOSAMINYL TRANSFERASE 1
LMP002908	St8sia6	Q8K4T1	MM.330004	149022	#N/A	A_51_P471791	Glycerolipids (GL)	ST8 ALPHA-N-ACETYL-NEURAMINIDE ALPHA-2,8-SIALYLTRANSFERASE 6
LMP002910	Pafah1b3	Q61205	MM.597	33579	100576_at;161218_r_at	A_51_P267024	Glycerophospholipids (GP)	PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE, ISOFORM 1B, ALPHA1 SUBUNIT
LMP002911	Pafah1b2	Q61206	MM.200859	147176	160187_at;99023_at	A_51_P461844	Glycerophospholipids (GP)	PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE, ISOFORM 1B, ALPHA2 SUBUNIT
LMP002912	Psap	Q61207	MM.277498	37075	161476_at;97114_at;97560_at	A_51_P136820	Sphingolipids (SP)	PROSAPOSIN
LMP002919	Gba	P17439	MM.5031	37752	100488_at;162295_at	A_51_P423119	Sphingolipids (SP)	GLUCOSIDASE, BETA, ACID
LMP002923	Hsd3b6	O35469	MM.14435	10683	102729_f_at	A_51_P466312	Sterol Lipids (ST)	HYDROXYSTEROID DEHYDROGENASE-6, DELTA<5>-3-BETA
LMP002929	Plcd1	Q8R3B1	MM.23963	16634	#N/A	A_51_P239766	Glycerophospholipids (GP)	PHOSPHOLIPASE C, DELTA 1
LMP002930	Fdps	Q920E5	MM.335450	9629	160424_f_at;162217_r_at;162281_at;99098_at	A_51_P379801	Sterol Lipids (ST)	FARNESYL DIPHOSPHATE SYNTHETASE
LMP002932	Nr3c2	Q8VII8	MM.324393	35995	#N/A	#N/A	Sterol Lipids (ST)	NUCLEAR RECEPTOR SUBFAMILY 3, GROUP C, MEMBER 2
LMP002934	Itpkb	Q8BKB2	MM.383297	38142	#N/A	A_51_P250307	Not classified	INOSITOL 1,4,5-TRISPHOSPHATE 3-KINASE B
LMP002936	Zdhhc15	Q8BGJ0	MM.30574	144594	#N/A	A_51_P405375	Fatty acids/Eicosanoids (FA)	ZINC FINGER, DHHC DOMAIN CONTAINING 15
LMP002938	Edg6	Q9Z0L1	MM.33065	23039	104687_at	A_51_P307944	Glycerophospholipids (GP) / Sphingolipids (SP)	ENDOTHELIAL DIFFERENTIATION, G-PROTEIN-COUPLED RECEPTOR 6
LMP002939	Slc10a2	P70172	MM.3500	147628	97150_at	A_51_P243755	Sterol Lipids (ST)	SOLUTE CARRIER FAMILY 10, MEMBER 2
LMP002940	Cyp2b19	O55071	MM.14098	7650	102690_at	A_51_P352763	Fatty acids/Eicosanoids (FA)	CYTOCHROME P450, FAMILY 2, SUBFAMILY B, POLYPEPTIDE 19
LMP002944	Esrra	O08580	NA	13011	102145_f_at;103964_at	A_51_P248580	Sterol Lipids (ST)	ERR-ALPHA
LMP002949	Pik3r2	O08908	MM.12945	15466	102759_at	A_51_P417725	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL 3-KINASE, REGULATORY SUBUNIT, POLYPEPTIDE 2 (P85 ...
LMP002953	Pten	O08586	MM.245395	145820	160614_at;94988_at	A_51_P275350	Glycerophospholipids (GP)	PHOSPHATASE AND TENSIN HOMOLOG
LMP002954	Sc5dl	O88822	MM.32700	147456	#N/A	#N/A	Sterol Lipids (ST)	STEROL-C5-DESATURASE (FUNGAL ERG3, DELTA-5-DESATURASE) HOMOLOG (S. CER...

LMP002957	Sptlc2	P97363	MM.565	19280	100893_at 100594_at;10	A_51_P321331	Fatty acids/Eicosanoids (FA) / Sphingolipids (SP)	SERINE PALMITOYLTRANSFERASE, LONG CHAIN BASE SUBUNIT 2
LMP002959	Pigq	Q9QYT7	MM.362054	12583	1646_at;1620 27_f_at	A_51_P232107	Glycerophospholipids (GP)	PHOSPHATIDYLINOSITOL GLYCAN, CLASS Q
LMP002961	Pdss2	Q33DR3	MM.363225	11230	#N/A	A_51_P513181	Not classified	PRENYL (SOLANESYL) DIPHOSPHATE SYNTHASE, SUBUNIT 2
LMP002965	Rarres1	Q8BVL6	MM.38002	13381	#N/A	A_51_P401184	Not classified	RETINOIC ACID RECEPTOR RESPONDER (TAZAROTENE INDUCED) 1
LMP002969	Arsg	Q3TYD4	MM.211850	145084	#N/A	A_51_P221263	Not classified	ARYLSULFATASE G
LMP002970	Rdh1	Q8VIJ7	MM.235814	8096	#N/A	#N/A	Not classified	RETINOL DEHYDROGENASE 1 (ALL TRANS)
LMP002974	Pip5k1b	P70181	MM.217214	34377	104663_at	A_51_P410260	Not classified	PHOSPHATIDYLINOSITOL-4-PHOSPHATE 5-KINASE, TYPE 1 ALPHA
LMP002980	Faah	O08914	MM.256025	3449	96795_at	A_51_P386660	Not classified	FATTY ACID AMIDE HYDROLASE
LMP002991	Slc22a16	Q3LVC1	MM.332570	4792	#N/A	A_51_P144134	Not classified	SOLUTE CARRIER FAMILY 22 (ORGANIC CATION TRANSPORTER), MEMBER 16
LMP002995	Cyp4a10	O88833	MM.10742	28356	92600_f_at;9 8353_at	A_51_P262890	Not classified	CYTOCHROME P450, FAMILY 4, SUBFAMILY A, POLYPEPTIDE 10
LMP002996	St3gal4	Q91Y74	MM.275973	148317	#N/A	A_51_P135132	Not classified	ST3 BETA-GALACTOSIDE ALPHA-2,3-SIALYLTRANSFERASE 4
LMP003000	Acsbg1	Q6ZQ79	MM.20592	147390	#N/A	A_51_P457636	Not classified	ACYL-COA SYNTHETASE BUBBLEGUM FAMILY MEMBER 1
LMP003004	Slc18a2	Q8BRU6	MM.268797	4891	#N/A	A_51_P469551	Not classified	SOLUTE CARRIER FAMILY 18 (VESICULAR MONOAMINE), MEMBER 2
LMP003007	Gdpd5	Q640M6	MM.286317	144041	#N/A	A_51_P468463	Not classified	GLYCEROPHOSPHODIESTER PHOSPHODIESTERASE DOMAIN CONTAINING 5
LMP003017	Acsm3	Q3UNX5	MM.334199	37109	#N/A	A_51_P487175	Not classified	ACYL-COA SYNTHETASE MEDIUM-CHAIN FAMILY MEMBER 3
LMP003018	Fnta	Q61239	MM.3496	36170	98121_at	A_51_P112114	Not classified	FARNESYLTRANSFERASE, CAAX BOX, ALPHA
LMP003023	Gdpd3	Q99LY2	MM.246881	16918	#N/A	A_51_P426872	Not classified	GLYCEROPHOSPHODIESTER PHOSPHODIESTERASE DOMAIN CONTAINING 3
LMP003037	Wipi1	Q8R3E3	MM.35817	145763	#N/A	A_51_P158044	Not classified	WD REPEAT DOMAIN, PHOSPHOINOSITIDE INTERACTING 1
LMP003044	Prkag3	Q8BGM7	MM.166501	147675	#N/A	A_51_P327129	Not classified	PROTEIN KINASE, AMP-ACTIVATED, GAMMA 3 NON-CATATLYTIC SUBUNIT
LMP003048	Agpat4	Q8K4X7	MM.258300	11990	#N/A	A_51_P346165	Not classified	1-ACYLGLYCEROL-3-PHOSPHATE O-ACYLTRANSFERASE 1 (LYSOPHOSPHATIDIC ACID ...
LMP003067	Plekha6	Q7TQG1	MM.253559	145922	#N/A	A_51_P310850	Not classified	HYPOTHETICAL PROTEIN BC031133
LMP003072	Enpp6	Q8BGN3	MM.211429	27969	#N/A	A_51_P359806	Not classified	ECTONUCLEOTIDE PYROPHOSPHATASE/PHOSPHODIESTERASE 6
LMP003096	Plaa	P27612	MM.22724	14412	160169_at;94 825_at	A_51_P378348	Not classified	PHOSPHOLIPASE A2, ACTIVATING PROTEIN
LMP003111	Inpp4b	Q6P1Y8	NA	11030	#N/A	#N/A	Not classified	INOSITOL POLYPHOSPHATE-4-PHOSPHATASE, TYPE II

LMP003113	Oxct2b	Q2HJ06	MM.189660		#N/A	#N/A	Not classified	
LMP003117	Abpd	Q9JI02	MM.296883	36043	#N/A	A_51_P491627	Not classified	ANDROGEN BINDING PROTEIN DELTA
LMP003122	Dhrs3	O88876	MM.14063	6980	102797_at	A_51_P282947	Not classified	DEHYDROGENASE/REDUCTASE (SDR FAMILY) MEMBER 3
LMP003124	Adh4	Q9QYY9	MM.158750	28017	103982_s_at; 103983_at	A_51_P189442	Not classified	ALCOHOL DEHYDROGENASE 4 (CLASS II), PI POLYPEPTIDE
LMP003125	Pik3ap1	Q3TBW6	MM.222266	40772	#N/A	A_51_P199199;A_51_P270339	Not classified	PHOSPHOINOSITIDE-3-KINASE ADAPTOR PROTEIN 1
LMP003133	Hs2st1	Q8R3H7	MM.12863	27100	102306_at	A_51_P469522	Not classified	HEPARAN SULFATE 2-O-SULFOTRANSFERASE 1
LMP003136	Nudt12	Q9DCN1	MM.36507	31775	#N/A 161655_at;161896_at;162175_at;96008_at	A_51_P136441	Not classified	NUDIX (NUCLEOSIDE DIPHOSPHATE LINKED MOIETY X)-TYPE MOTIF 12
LMP003140	Dad1	P61804	MM.319038	5491		A_51_P189905	Not classified	DEFENDER AGAINST CELL DEATH 1
LMP003146	Ephx2	P34914	MM.15295	26589	93051_at	A_51_P116940	Not classified	EPOXIDE HYDROLASE 2, CYTOPLASMIC
LMP003156	Aldh9a1	Q9JLJ2	MM.330055	17185	96243_f_at	A_51_P106211	Not classified	ALDEHYDE DEHYDROGENASE 9, SUBFAMILY A1
LMP003170	Galntl5	Q9D4M9	MM.141471	27551	#N/A	A_51_P378006	Not classified	UDP-N-ACETYL-ALPHA-D-GALACTOSAMINE:POLYPEPTIDE N-ACETYLGALACTOSAMINYLT...
LMP003172	Mboat2	Q8R3I2	MM.167671	23850	#N/A	A_51_P433281	Not classified	O-ACYLTRANSFERASE (MEMBRANE BOUND) DOMAIN CONTAINING 2
LMP003178	Elovl6	Q920L5	MM.314113	38130	103665_at;94418_at	A_51_P463440	Not classified	ELOVL FAMILY MEMBER 6, ELONGATION OF LONG CHAIN FATTY ACIDS (YEAST)
LMP003186	Pcyt1b	Q811Q9	MM.166467	31075	#N/A	A_51_P353125	Not classified	PHOSPHATE CYTIDYLYLTRANSFERASE 1, CHOLINE, BETA ISOFORM
LMP003187	Ddost	O54734	MM.7236	146315	101074_at;162020_at	A_51_P216075	Not classified	DOLICHYL-DI-PHOSPHOOLIGOSACCHARIDE-PROTEIN GLYCOTRANSFERASE
LMP003188	Acsm2	Q3US47	MM.268448	147809	#N/A	A_51_P331732	Not classified	ACYL-COA SYNTHETASE MEDIUM-CHAIN FAMILY MEMBER 2
LMP003204	Rchy1	Q9CR50	MM.159453	9660	#N/A	A_51_P374306	Not classified	RING FINGER AND CHY ZINC FINGER DOMAIN CONTAINING 1
LMP003209	Sra1	Q80VJ2	MM.29058	12945	160323_at	A_51_P226053	Not classified	STEROID RECEPTOR RNA ACTIVATOR 1
LMP003225	Neu4	Q8BZL1	MM.214565	145694	#N/A	A_51_P507709	Not classified	SIALIDASE 4
LMP003228	Inppl1	Q9JLL7	MM.5028	16981	102988_at	A_51_P142334	Not classified	INOSITOL POLYPHOSPHATE PHOSPHATASE-LIKE 1
LMP003230	Gdpd2	Q9ESM6	MM.283495	145944	#N/A 102696_s_at; 102697_at;104557_at	A_51_P355954	Not classified	GLYCEROPHOSPHODIESTER PHOSPHODIESTERASE DOMAIN CONTAINING 2
LMP003239	Pitpnb	P53811	MM.200516	27419		A_51_P491195	Not classified	PHOSPHATIDYLINOSITOL TRANSFER PROTEIN, BETA
LMP003243	Ffar1	Q76JU9	MM.347605	35188	#N/A	#N/A	Not classified	FREE FATTY ACID RECEPTOR 1

LMP003246	Rarres2	Q9DD06	MM.28231	12587	#N/A	A_51_P350311	Not classified	RETINOIC ACID RECEPTOR RESPONDER (TAZAROTENE INDUCED) 2
LMP003254	Plch1	Q4KWH5	MM.316391	147017	#N/A	#N/A	Not classified	PHOSPHOLIPASE C-LIKE 3
LMP003256	Nudt3	Q9JI46	MM.144699	146076	101977_at	A_51_P440657	Not classified	NUDIX (NUCLEOTIDE DIPHOSPHATE LINKED MOIETY X)-TYPE MOTIF 3
LMP003296	Mmd	Q9CQY7	MM.277518	37579	#N/A	A_51_P431470	Not classified	MONOCYTE TO MACROPHAGE DIFFERENTIATION-ASSOCIATED
LMP003303	Echdc2	Q3U553	MM.270783	28069	#N/A	A_51_P477414	Not classified	ENOYL COENZYME A HYDRATASE DOMAIN CONTAINING 2
LMP003324	Aldh1a3	Q9JHW9	MM.140988	18871	98372_at	A_51_P499482	Not classified	ALDEHYDE DEHYDROGENASE FAMILY 1, SUBFAMILY A3
LMP003334	Apoh	Q01339	MM.2266	19219	94318_at	A_51_P342387	Not classified	APOLIPOPROTEIN H
LMP003356	Gpx1	P11352	MM.1090	3915	94132_at	A_51_P119725	Not classified	GLUTATHIONE PEROXIDASE 1
LMP003363	Pllp	Q9DCU2	MM.279977	36199	#N/A	A_51_P154867;A_51_P201187	Not classified	PLASMA MEMBRANE PROTEOLIPID
LMP003369	Cyp2b20	Q62397	MM.218749	34409	102701_at	#N/A	Not classified	CYTOCHROME P450, FAMILY 2, SUBFAMILY B, POLYPEPTIDE 10
LMP003393	Slc37a1	Q8R070	MM.311395	26509	#N/A	A_51_P215489	Not classified	SOLUTE CARRIER FAMILY 37 (GLYCEROL-3-PHOSPHATE TRANSPORTER), MEMBER 1
LMP003394	Ugt1a9	Q6XL44	NA	27051	#N/A	#N/A	Not classified	UDP GLUCURONOSYLTRANSFERASE 1 FAMILY, POLYPEPTIDE A9
LMP003411	2310076 L09Rik	Q8BVZ1	MM.254985	143951	#N/A	A_51_P262757	Not classified	RIKEN CDNA 2310076L09 GENE
LMP003421	Plp2	Q9R1Q7	MM.18565	40189	93323_at	A_51_P243525	Not classified	PROTEOLIPID PROTEIN 2
LMP003423	Lcn5	Q3UW42	MM.12867	8935	99236_at	A_51_P191875	Not classified	LIPOCALIN 5
LMP003426	B4galt7	Q8R087	MM.139825	26245	#N/A	A_51_P458891	Not classified	XYLOSYLPROTEIN BETA1,4-GALACTOSYLTRANSFERASE, POLYPEPTIDE 7 (GALACTOSY...
LMP003427	Plscr2	Q9DCW2	MM.10306	144912	102053_at	A_51_P251023	Not classified	PHOSPHOLIPID SCRAMBLASE 2
LMP003430	AB11235 0	Q8BH82	MM.153292	144088	#N/A	A_51_P392943	Not classified	CDNA SEQUENCE AB112350
LMP003467	Mboat1	Q8BH98	MM.89682	146319	#N/A	A_51_P349495	Not classified	O-ACYLTRANSFERASE (MEMBRANE BOUND) DOMAIN CONTAINING 1
LMP003475	Pla2g3	Q8BZT7	MM.100476	43776	#N/A	A_51_P402868	Not classified	PHOSPHOLIPASE A2, GROUP III
LMP003477	Pign	Q9R1S3	MM.268911	4140	92296_at	A_51_P221274	Not classified	PHOSPHATIDYLINOSITOL GLYCAN, CLASS N
LMP003478	b3galnt1	Q920V1	MM.153710	17009	#N/A	A_51_P318637	Not classified	UDP-GAL:BETAGLCNAC BETA 1,3-GALACTOSYLTRANSFERASE, POLYPEPTIDE 3
LMP003488	Ncor1	Q60974	MM.271814	27157	101536_at	A_51_P457744;A_51_P489043	Not classified	NUCLEAR RECEPTOR CO-REPRESSOR 1
LMP003516	Plscr4	P58196	MM.55289	145369	#N/A	A_51_P111049	Not classified	EXPRESSED SEQUENCE AV245873
LMP003520	Ptges	Q9JM51	MM.28768	59852	104406_at	A_51_P312328	Not classified	PROSTAGLANDIN E SYNTHASE

LMP003532	Lcn8	Q924P3	MM.196708	4961	#N/A	A_51_P379873	Not classified	LIPOCALIN 8
LMP003549	Apob	Q3UH74	MM.221239	27630	#N/A	A_51_P380650;A_51_P413088;A_51_P436689;A_51_P	Not classified	APOLIPOPROTEIN B
LMP003566	Zmpste24	Q80W54	MM.34399	16150	#N/A	A_51_P467720	Not classified	ZINC METALLOPEPTIDASE, STE24 HOMOLOG (S. CEREVISIAE)
LMP003572	Zdhhc9	P59268	MM.207367	12723	#N/A	A_51_P131514	Not classified	ZINC FINGER, DHHC DOMAIN CONTAINING 9
LMP003574	Pla2g4b	Q4QQM1	MM.41467	143633	#N/A	A_51_P288549	Not classified	PHOSPHOLIPASE A2, GROUP IVB (CYTOSOLIC)
LMP003575	Pag1	Q3U1F9	MM.214484	146141	#N/A	A_51_P335945	Not classified	PHOSPHOPROTEIN ASSOCIATED WITH GLYCOSPHINGOLIPID MICRODOMAINS 1
LMP003591	Phb2	O35129	MM.36241	35216	#N/A	A_51_P205833	Not classified	PROHIBITIN 2
LMP003604	Plcl1	Q3USB7	NA	15791	#N/A	A_51_P422457	Not classified	PHOSPHOLIPASE C-LIKE 1
LMP003612	Vkorc1	Q9CRC0	MM.29703	45498	#N/A	A_51_P395555	Not classified	VITAMIN K EPOXIDE REDUCTASE COMPLEX, SUBUNIT 1
LMP003617	Prey	Q9D1C3	MM.44201	28365	#N/A	#N/A	Not classified	PHOSPHATIDYLINOSITOL GLYCAN, CLASS Y
LMP003618	Dhcr24	Q6ZQK9	MM.133370	27995	160369_at	A_51_P242043;A_51_P482711	Not classified	24-DEHYDROCHOLESTEROL REDUCTASE
LMP003619	Slc33a1	Q99J27	MM.135619	14637	160201_r_at	A_51_P164400	Not classified	SOLUTE CARRIER FAMILY 33 (ACETYL-COA TRANSPORTER), MEMBER 1
LMP003623	Plekha8	Q80W71	MM.35416	15309	#N/A	A_51_P288756	Not classified	PLECKSTRIN HOMOLOGY DOMAIN CONTAINING, FAMILY A (PHOSPHOINOSITIDE BIND...
LMP003636	Stt3a	P46978	MM.2863	9312	#N/A	A_51_P318401	Not classified	STT3, SUBUNIT OF THE OLIGOSACCHARYLTRANSFERASE COMPLEX, HOMOLOG A (S. ...
LMP003644	Ang1	P21570	NA	13832	#N/A	A_51_P391159	Not classified	ANGIOGENIN, RIBONUCLEASE A FAMILY, MEMBER 1
LMP003653	Serinc1	Q9QZI8	MM.29344	129009	#N/A	A_51_P358225	Not classified	SERINE INCORPORATOR 1
LMP003654	Prmt8	Q6PAK3	MM.39750	37978	#N/A	#N/A	Not classified	PROTEIN ARGININE N-METHYLTRANSFERASE 8
LMP003662	Agpat7	Q6NVG1			#N/A	#N/A	Not classified	
LMP003676	Grlf1	Q91YM2	MM.28646	15184	#N/A	A_51_P506748	Not classified	GLUCOCORTICOID RECEPTOR DNA BINDING FACTOR 1
LMP003705	Pla1a	Q99J51	MM.279805	145809	#N/A	A_51_P381618	Not classified	PHOSPHOLIPASE A1 MEMBER A
LMP003717	Ugt1a1	Q63886	NA	3809	161844_at;99580_s_at	#N/A	Not classified	UDP GLUCURONOSYLTRANSFERASE 1 FAMILY, POLYPEPTIDE A2
LMP003723	Apon	Q8VC08	MM.30121	6041	#N/A	A_51_P100625	Not classified	APOLIPOPROTEIN N
LMP003725	Rdh13	Q8CEE7	MM.1627	14066	161882_f_at, 94765_at	A_51_P513071	Not classified	RETINOL DEHYDROGENASE 13 (ALL-TRANS AND 9-CIS)
LMP003738	Apbh	O35176	MM.6205	45473	#N/A	A_51_P151211	Not classified	ANDROGEN-BINDING PROTEIN ETA
LMP003749	Prmt6	Q6NZB1	MM.36115	147034	#N/A	A_51_P331212	Not classified	PROTEIN ARGININE N-METHYLTRANSFERASE 6
LMP003778	Ppt1	O88531	MM.358818	147223	99646_at	A_51_P114307	Not classified	PALMITOYL-PROTEIN THIOESTERASE 1

LMP003784	Slc27a6	Q14C50	MM.258517		#N/A	A_51_P211616	Not classified	
LMP003786	Acp6	Q8BP40	MM.101368	8370	162336_r_at;	A_51_P444335	Not classified	LYSOPHOSPHATIDIC ACID PHOSPHATASE
LMP003789	Gmeb2	P58929	MM.220423	13085	#N/A	A_51_P101228;A_51_P346304	Not classified	GLUCOCORTICOID MODULATORY ELEMENT BINDING PROTEIN 2
LMP003795	Osbp	Q3B7Z2	MM.291279	35712	#N/A	A_51_P393768	Not classified	OXYSTEROL BINDING PROTEIN
LMP003816	Cyp2d11	P24457	NA		101287_s_at	#N/A	Not classified	CYTOCHROME P450, FAMILY 2, SUBFAMILY D, POLYPEPTIDE 11
LMP003821	Apof	Q91V80	MM.26513	4028	96074_at	A_51_P495825	Not classified	APOLIPOPROTEIN F
LMP003823	Mcee	Q9D1I5	MM.10093	19896	#N/A	A_51_P320481	Not classified	METHYLMALONYL COA EPIMERASE
LMP003824	Liph	Q3UWA2	MM.33192	16830	#N/A	A_51_P122660	Not classified	LIPASE, MEMBER H
LMP003828	Calm1	P62204	MM.288630	7000	96522_at	A_51_P465023;A_51_P503933	Not classified	CALMODULIN 1
LMP003829	Pigh	Q5M9N4	MM.281044	145839	101443_at	#N/A	Not classified	PHOSPHATIDYLINOSITOL GLYCAN, CLASS H
LMP003838	Cyp4f3	Q99N16	MM.160020	6807	#N/A	#N/A	Not classified	CYTOCHROME P450, FAMILY 4, SUBFAMILY F, POLYPEPTIDE 18
LMP003840	Mccc1	Q99MR8	MM.249016	6991	94940_at	A_51_P282974	Not classified	METHYLCROTONOYL-COENZYME A CARBOXYLASE 1 (ALPHA)
LMP003841	Cyp4f13	Q99N19	MM.254838	145867	162058_f_at	A_51_P210603	Not classified	RIKEN CDNA 0610030I10 GENE
LMP003844	Egln3	Q91UZ4	MM.133037	4004	#N/A	A_51_P274852	Not classified	EGL NINE HOMOLOG 3 (C. ELEGANS)
LMP003846	Ltb4dh	Q91YR9	MM.34497	17035	98440_at	A_51_P444437	Not classified	LEUKOTRIENE B4 12-HYDROXYDEHYDROGENASE
LMP003848	Impa2	Q91UZ5	MM.34079	18883	98420_at	A_51_P299287	Not classified	INOSITOL (MYO)-1(OR 4)-MONOPHOSPHATASE 2
LMP003860	Prkd3	Q8K1Y2	MM.252776	9184	#N/A	#N/A	Not classified	PROTEIN KINASE C, NU
LMP003861	Pgs1	Q8BHF7	MM.28864	7791	#N/A	A_51_P515349	Not classified	RIKEN CDNA 2610019F11 GENE
LMP003876	Slc27a3	O88561	NA	7439	92686_at	A_51_P133247	Not classified	LONG-CHAIN FATTY ACID TRANSPORT PROTEIN 3
LMP003883	Coq9	Q8K1Z0	MM.169234	12511	#N/A	A_51_P134542	Not classified	COENZYME Q9 HOMOLOG (YEAST)
LMP003885	Esco2	Q8CIB9	MM.249280	10638	#N/A	A_51_P195034	Not classified	ESTABLISHMENT OF COHESION 1 HOMOLOG 2 (S. CEREVISIAE)
LMP003908	Pon1	P52430	MM.237657	17183	96895_at	A_51_P108659	Not classified	PARAOXONASE 1
LMP003912	Pla2r1	Q62028	MM.5092	144395	#N/A	A_51_P451335	Not classified	PHOSPHOLIPASE A2 RECEPTOR 1
LMP003927	Pigm	Q8C2R7	MM.26612	148214	162405_at;96527_at	A_51_P219822	Not classified	PHOSPHATIDYLINOSITOL GLYCAN, CLASS M
LMP003932	Akr1c20	Q8VC77	MM.37605	37054	#N/A	A_51_P276800;A_51_P382764	Not classified	ALDO-KETO REDUCTASE FAMILY 1, MEMBER C20
LMP003937	Elovl5	Q8BHI7	MM.19130	148870	93496_at	A_51_P505493	Not classified	ELOVL FAMILY MEMBER 5, ELONGATION OF LONG CHAIN FATTY ACIDS (YEAST)
LMP003961	Centg1	Q3UHD9	NA	143809	#N/A	A_51_P488118	Not classified	CENTAURIN, GAMMA 1
LMP003977	Pitpnm3	Q3UHE1	NA	9374	#N/A	A_51_P118527	Not classified	EXPRESSED SEQUENCE AI848332

LMP003983	Slc22a21	Q9WTN6	MM.154284	24671	#N/A	A_51_P407774	Not classified	SOLUTE CARRIER FAMILY 22 (ORGANIC CATION TRANSPORTER), MEMBER 21
LMP003995	Hnrpll	Q921F4	MM.64579	8251	#N/A	A_51_P390676	Not classified	HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN L-LIKE
LMP004000	Dhrsx	Q8VBZ0	MM.358892	26684	93238_at	#N/A	Not classified	DEHYDROGENASE/REDUCTASE (SDR FAMILY) X CHROMOSOME
LMP004002	Btd	Q8CIF4	MM.282679	4746	95469_at	A_51_P196158	Not classified	BIOTINIDASE
LMP004003	Acad9	Q8JZN5	MM.260997	37611	#N/A	A_51_P341379	Not classified	ACYL-COENZYME A DEHYDROGENASE FAMILY, MEMBER 9
LMP004020	Ptges2	Q8BWM0	MM.28048	144328	160765_at	A_51_P349008	Not classified	PROSTAGLANDIN E SYNTHASE 2
LMP004033	Prmt5	Q8CIG8	MM.196585	9358	#N/A	#N/A	Not classified	PROTEIN ARGININE N-METHYLTRANSFERASE 5
LMP004042	B4galt3	Q91YY2	MM.274011	16742	160915_at;99346_at	A_51_P117494	Not classified	UDP-GAL:BETAGLCNAC BETA 1,4-GALACTOSYLTRANSFERASE, POLYPEPTIDE 3
LMP004045	Dpm3	Q9D1Q4	MM.272927	19465	101084_f_at	A_51_P518566	Not classified	DOLICHYL-PHOSPHATE MANNOSYLTRANSFERASE POLYPEPTIDE 3
LMP004092	Pon3	Q62087	MM.9122	16861	93940_at	A_51_P256665	Not classified	PARAOXONASE 3
LMP004100	Cyp8b1	O88962	MM.20889	148953	103284_at	A_51_P266618	Not classified	CYTOCHROME P450, FAMILY 8, SUBFAMILY B, POLYPEPTIDE 1
LMP004105	Cyp51	Q9JIP8	MM.140158	6612	94916_at	A_51_P485791	Not classified	CYTOCHROME P450, FAMILY 51
LMP004109	Dhrs8	Q9EQ06	MM.46019	27061	102370_at	#N/A	Not classified	DEHYDROGENASE/REDUCTASE (SDR FAMILY) MEMBER 8
LMP004113	B3gnt1	Q8BWP8	MM.29628	148600	160137_at;161267_f_at;98291_at	A_51_P305320	Not classified	UDP-GLCNAC:BETAGAL BETA-1,3-N-ACETYLGLUCOSAMINYLTRANSFERASE 6
LMP004120	Gpr23	Q8BLG2	MM.90147	6868	#N/A	#N/A	Not classified	G PROTEIN-COUPLED RECEPTOR 23
LMP004129	Plscr1	Q9JJ00	MM.14627	145307	102839_at	A_51_P229676	Not classified	PHOSPHOLIPID SCRAMBLASE 1
LMP004132	B4galt4	Q9JJ04	MM.182377	26462	#N/A	A_51_P238383	Not classified	UDP-GAL:BETAGLCNAC BETA 1,4-GALACTOSYLTRANSFERASE, POLYPEPTIDE 4
LMP004136	Srebf2	Q925C2	MM.38016	32779	#N/A	A_51_P377514	Not classified	STEROL REGULATORY ELEMENT BINDING FACTOR 2
LMP004151	Pitpnm1	O35954	MM.1860	23007	#N/A	A_51_P103000	Not classified	PHOSPHATIDYLINOSITOL MEMBRANE-ASSOCIATED 1
LMP004170	Hemk1	Q921L7	MM.259467	9425	#N/A	A_51_P475244	Not classified	HEMK METHYLTRANSFERASE FAMILY MEMBER 1
LMP004186	B3gnt2	Q9Z222	MM.258094	27086	#N/A	A_51_P335653	Not classified	UDP-GLCNAC:BETAGAL BETA-1,3-N-ACETYLGLUCOSAMINYLTRANSFERASE 1
LMP004193	Mccc2	Q3ULD5	MM.137327	42151	#N/A	A_51_P211880	Not classified	METHYLCROTONOYL-COENZYME A CARBOXYLASE 2 (BETA)
LMP004196	Agpat6	Q8K2C8	MM.200898	147434	#N/A	A_51_P335710	Not classified	PUTATIVE LYSOPHOSPHATIDIC ACID ACYLTRANSFERASE
LMP004199	Slc27a4	Q91VE0	MM.330113	145393	97957_at	A_51_P408609	Not classified	SOLUTE CARRIER FAMILY 27 (FATTY ACID TRANSPORTER), MEMBER 4
LMP004209	9230116B18Rik	Q9D258	MM.99733	4406	#N/A	#N/A	Not classified	RIKEN CDNA 9230116B18 GENE

LMP004234	Elovl7	Q8BX38	MM.286127	8496	#N/A	A_51_P189594	Not classified	ELOVL FAMILY MEMBER 7, ELONGATION OF LONG CHAIN FATTY ACIDS (YEAST)
LMP004241	Ugt1	Q62452	NA	3809	#N/A	#N/A	Not classified	UDP GLUCURONOSYLTRANSFERASE 1 FAMILY, POLYPEPTIDE A2
LMP004243	Gpbar1	Q80SS6	MM.246587	27649	#N/A	A_51_P175311	Not classified	G PROTEIN-COUPLED BILE ACID RECEPTOR 1
LMP004249	Clu	Q06890	MM.200608	149786	161294_f_at; 95286_at	A_51_P367720	Not classified	CLUSTERIN
LMP004294	Gdpd1	Q9CRY7	MM.281887	6797	95470_at	A_51_P487439	Not classified	GLYCEROPHOSPHODIESTER PHOSPHODIESTERASE DOMAIN CONTAINING 1
LMP004297	Hpgd	Q8VCC1	MM.18832	6998	93351_at	A_51_P458778	Not classified	HYDROXYPROSTAGLANDIN DEHYDROGENASE 15 (NAD)
LMP004298	Abpg	Q8JZX1	MM.155750	7323	#N/A	#N/A	Not classified	ANDROGEN BINDING PROTEIN GAMMA
LMP004300	Ces1	Q8VCC2	MM.22720	144720	103519_at	A_51_P253484	Not classified	CARBOXYLESTERASE 1
LMP004304	Pdia3	P27773	MM.263177	30857	#N/A	A_51_P168412	Not classified	PROTEIN DISULFIDE ISOMERASE ASSOCIATED 3
LMP004314	Pcca	Q91ZA3	MM.23876	3471	#N/A	A_51_P484254	Not classified	EXPRESSED SEQUENCE C79630
LMP004322	Alg1	Q921Q3	MM.34581	144280	#N/A	A_51_P329795	Not classified	ASPARAGINE-LINKED GLYCOSYLATION 1 HOMOLOG (YEAST, BETA-1,4-MANNOSYLTRA...
LMP004346	Pi4k2a	Q2TBE6	MM.117037		#N/A	A_51_P345467	Not classified	
LMP004347	Ahcyl1	Q80SW1	MM.220328	147253	97217_at	A_51_P206243;A_51_P296328	Not classified	S-ADENOSYLHOMOCYSTEINE HYDROLASE-LIKE 1
LMP004348	Smpdl3b	P58242	MM.287187	27147	#N/A	A_51_P422839	Not classified	SPHINGOMYELIN PHOSPHODIESTERASE, ACID-LIKE 3B
LMP004358	Fntb	Q8K2I1	MM.151174	4883	96218_at	A_51_P517680	Not classified	EXPRESSED SEQUENCE AA409500
LMP004374	Rxrip110	Q5U5Q9	MM.259301	3384	99464_at	#N/A	Not classified	RETINOID X RECEPTOR INTERACTING PROTEIN 110
LMP004382	Bckdhb	Q6P3A8	MM.12819	7352	102302_at	A_51_P102782	Not classified	BRANCHED CHAIN KETOACID DEHYDROGENASE E1, BETA POLYPEPTIDE
LMP004388	Ncoa3	O09000	MM.1011	3245	102024_at	A_51_P454207	Not classified	NUCLEAR RECEPTOR COACTIVATOR 3
LMP004394	Plscr3	Q9JIZ9	MM.28874	3366	#N/A	A_51_P302456	Not classified	PHOSPHOLIPID SCRAMBLASE 3
LMP004404	Rbp3	P49194	MM.151562	27112	#N/A	A_51_P331690	Not classified	RETINOL BINDING PROTEIN 3, INTERSTITIAL
LMP004426	Cnr1	P47746	MM.7992	26801	99892_at	A_51_P403378	Not classified	CANNABINOID RECEPTOR 1 (BRAIN)
LMP004429	Rdh10	Q8VCH7	MM.274376	21146	#N/A	A_51_P184261	Not classified	RETINOL DEHYDROGENASE 10 (ALL-TRANS)
LMP004430	Nrip1	Q8CBD1	MM.74711	37797	#N/A	A_51_P152747	Not classified	NUCLEAR RECEPTOR INTERACTING PROTEIN 1
LMP004448	Rad54l2	Q99NG0	MM.246689	149135	#N/A	A_51_P162812	Not classified	RAD54 LIKE 2 (S. CEREVISIAE)
LMP004460	Pitpnc1	Q8BTD8	MM.340968	9512	#N/A	A_51_P260478;A_51_P443857	Not classified	PHOSPHATIDYLINOSITOL TRANSFER PROTEIN, CYTOPLASMIC 1
LMP004462	Sdpr	Q63918	MM.255909	19312	160373_i_at	A_51_P277336	Not classified	SERUM DEPRIVATION RESPONSE
LMP004479	Ggtl3	Q99JP7	MM.41757	3520	#N/A	A_51_P265303	Not classified	GAMMA-GLUTAMYLTRANSFERASE-LIKE 3

LMP004483	Acsbg2	Q2XU92	MM.179421	31538	#N/A	#N/A	Not classified	ACYL-COA SYNTHETASE BUBBLEGUM FAMILY MEMBER 2
LMP004490	Ffar2	Q8VCK6	MM.97338	11914	#N/A	A_51_P176083	Not classified	FREE FATTY ACID RECEPTOR 2
LMP004523	Tinagl1	Q99JR5	MM.15801	26882	#N/A	#N/A	Not classified	TUBULOINTERSTITIAL NEPHRITIS ANTIGEN-LIKE
LMP004550	Dpm1	O70152	MM.370658	7766	92828_at	A_51_P397834	Not classified	DOLICHOL-PHOSPHATE (BETA-D) MANNOSYLTRANSFERASE 1
LMP004551	Nsmaf	O35242	MM.3059	36172	160826_at;161700_i_at	A_51_P310076	Not classified	NEUTRAL SPHINGOMYELINASE (N-SMASE) ACTIVATION ASSOCIATED FACTOR
LMP004564	Acat3	Q80X81	MM.229342	4898	#N/A	A_51_P139745	Not classified	ACETYL-COENZYME A ACETYLTRANSFERASE 3
LMP004573	Rdh9	Q76LB8	MM.379285	5481	#N/A	A_51_P177764	Not classified	RETINOL DEHYDROGENASE 9
LMP004592	Naga	Q9QWR8	MM.20325	31946	103637_at	A_51_P117130	Not classified	N-ACETYL GALACTOSAMINIDASE, ALPHA
LMP004594	Acbd4	Q80X94	MM.225881	25421	#N/A	A_51_P292757	Not classified	ACYL-COENZYME A BINDING DOMAIN CONTAINING 4
LMP004596	Mtmr12	Q80TA6	MM.54460	4003	#N/A	A_51_P308308	Not classified	VMYOTUBULARIN RELATED PROTEIN 12
LMP004603	Tm7sf2	Q71KT5	MM.380396	5863	92437_at;95383_at	A_51_P246568	Not classified	TRANSMEMBRANE 7 SUPERFAMILY MEMBER 2
LMP004625	Dhrs9	Q58NB6	MM.211655	26489	#N/A	A_51_P149126	Not classified	DEHYDROGENASE/REDUCTASE (SDR FAMILY) MEMBER 9
LMP004629	Pafah1b1	P63005	MM.56337	6989	100560_at	A_51_P291388	Not classified	PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE, ISOFORM 1B, BETA1 SUBUNIT
LMP004638	Tmem55a	Q9CZX7	MM.279158	19676	#N/A	A_51_P495485	Not classified	TRANSMEMBRANE PROTEIN 55A
LMP004641	Nrk1	Q91W63	MM.211595	15822	#N/A	#N/A	Not classified	CDNA SEQUENCE BC016495
LMP004645	Hsd17b13	Q8VCR2	MM.284944	146945	#N/A	#N/A	Not classified	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 13
LMP004658	Ncoa2	Q61026	MM.2537	12954	96508_at	A_51_P478593	Not classified	NUCLEAR RECEPTOR COACTIVATOR 2
LMP004667	Rragc	Q99K70	MM.220922	144004	#N/A	A_51_P346826	Not classified	RAS-RELATED GTP BINDING C
LMP004672	Paqr4	Q9JJE4	MM.274942	32008	#N/A	A_51_P109050	Not classified	PROGESTIN AND ADIPOQ RECEPTOR FAMILY MEMBER IV
LMP004688	4632408A20Rik	Q32Q92	MM.49245	145642	#N/A	#N/A	Not classified	RIKEN CDNA 4632408A20 GENE
LMP004690	Gpr65	Q61038	MM.378924	146191	#N/A	A_51_P108459	Not classified	G-PROTEIN COUPLED RECEPTOR 65
LMP004697	Aoah	O35298	MM.314046	143944	99838_at	A_51_P517105	Not classified	ACYLOXYACYL HYDROLASE
LMP004699	Psat1	Q99K85	MM.289936	146132	96295_at	A_51_P317428	Not classified	PHOSPHOSERINE AMINOTRANSFERASE 1
LMP004702	Col4a3bp	Q9EQG9	MM.24125	28302	#N/A	A_51_P134574;A_51_P238533	Not classified	PROCOLLAGEN, TYPE IV, ALPHA 3 (GOODPASTURE ANTIGEN) BINDING PROTEIN
LMP004709	Ces3	Q8VCT4	MM.292803	148789	101538_i_at;101539_f_at	A_51_P375969	Not classified	CARBOXYLESTERASE 3
LMP004721	Dnaja1	P63037	MM.27897	5439	97261_at	A_51_P303353	Not classified	DNAJ (HSP40) HOMOLOG, SUBFAMILY A, MEMBER 1

LMP004732	Zdhhc18	Q5Y5T2	NA	5284	#N/A	#N/A	Not classified	ZINC FINGER, DHHC DOMAIN CONTAINING 18
LMP004768	Pigz	Q8BTP0	MM.347625	12682	#N/A	A_51_P285673	Not classified	PHOSPHATIDYLINOSITOL GLYCAN, CLASS Z
LMP004782	Suclg2	Q9Z2I8	MM.371585	18366	160428_at	A_51_P478672	Not classified	SUCCINATE-COENZYME A LIGASE, GDP-FORMING, BETA SUBUNIT
LMP004783	Sucla2	Q9Z2I9	MM.38951	146413	93501_f_at;93502_r_at	A_51_P111554	Not classified	SUCCINATE-COENZYME A LIGASE, ADP-FORMING, BETA SUBUNIT
LMP004789	2310016F22Rik	Q8C7I4	MM.243758	145658	#N/A	A_51_P500215	Not classified	RIKEN CDNA 2310016F22 GENE
LMP004799	Nat5	P61600	MM.151168	16805	#N/A	A_51_P424828	Not classified	N-ACETYLTRANSFERASE 5 (ARD1 HOMOLOG, S. CEREVISIAE)
LMP004801	Lipe	P54310	MM.349647	9071	103083_at	A_51_P435366	Not classified	LIPASE, HORMONE SENSITIVE
LMP004802	Pik3r4	Q8VD65	MM.274830	16591	#N/A	A_51_P110029	Not classified	PHOSPHATIDYLINOSITOL 3 KINASE, REGULATORY SUBUNIT, POLYPEPTIDE 4, P150
LMP004803	Abhd4	Q8VD66	MM.28771	19407	#N/A	A_51_P406020	Not classified	ABHYDROLASE DOMAIN CONTAINING 4
LMP004805	4930544G21Rik	Q6NSW3	MM.154303	27566	#N/A	A_51_P482398	Not classified	RIKEN CDNA 4930544G21 GENE
LMP004806	Cyp4a12b	O35650	NA	36002	#N/A	#N/A	Not classified	CDNA SEQUENCE BC060945
LMP004818	Icmt	Q9EQK7	MM.277464	8390	160336_at	A_51_P283464	Not classified	ISOPRENYLCYSTEINE CARBOXYL METHYLTRANSFERASE
LMP004831	Pigr	O70570	MM.276414	146814	99926_at	A_51_P239737	Not classified	POLYMERIC IMMUNOGLOBULIN RECEPTOR
LMP004832	Ncoa1	P70365	MM.301039	17056	103229_at	A_51_P502888	Not classified	NUCLEAR RECEPTOR COACTIVATOR 1
LMP004839	Suclg1	Q9WUM5	MM.29845	144589	96268_at	A_51_P491227	Not classified	SUCCINATE-COA LIGASE, GDP-FORMING, ALPHA SUBUNIT
LMP004846	Stt3b	Q3TDQ1	MM.296158	16905	#N/A	A_51_P149621;A_51_P218548	Not classified	STT3, SUBUNIT OF THE OLIGOSACCHARYLTRANSFERASE COMPLEX, HOMOLOG B (S. ...
LMP004850	Acbd5	Q7TSC2	MM.181973	15148	#N/A	A_51_P279308	Not classified	ACYL-COENZYME A BINDING DOMAIN CONTAINING 5
LMP004863	Coq2	Q66JT7	MM.260661	137823	#N/A	A_51_P447752	Not classified	COENZYME Q2 HOMOLOG, PRENYLTRANSFERASE (YEAST)
LMP004865	Atp6v0c	P63082	MM.30155	27977	96919_at	A_51_P127107	Not classified	ATPASE, H+ TRANSPORTING, LYSOSOMAL V0 SUBUNIT C
LMP004872	Minpp1	Q9Z2L6	MM.255116	148776	99640_at	A_51_P353008	Not classified	MULTIPLE INOSITOL POLYPHOSPHATE HISTIDINE PHOSPHATASE 1
LMP004883	Tmem55b	Q3TWL2	MM.239117	6316	#N/A	A_51_P232508	Not classified	TRANSMEMBRANE PROTEIN 55B
LMP004886	Rabggtb	P53612	MM.277831	25461	95077_at	A_51_P314763	Not classified	RAB GERANYLGERANYL TRANSFERASE, B SUBUNIT
LMP004896	Fprl1	O08790	MM.378919	30565	100741_at	A_51_P510982	Not classified	FORMYL PEPTIDE RECEPTOR-LIKE 1
LMP004917	Abpz	Q7M747	MM.261648	144524	#N/A	#N/A	Not classified	ANDROGEN BINDING PROTEIN ZETA
LMP004923	Srd5a2l	Q9WUP4	MM.289446	105184	#N/A	A_51_P334730	Not classified	STEROID 5 ALPHA-REDUCTASE 2-LIKE

LMP004928	Oxct2a	Q9JJN4	MM.270287	26829	#N/A	A_51_P125062	Not classified	3-OXOACID COA TRANSFERASE 2A
LMP004946	Phyhd1	Q9DB26	MM.297949	37282	#N/A	A_51_P357606	Not classified	PHYTANOYL-COA DIOXYGENASE DOMAIN CONTAINING 1
LMP004969	B3galt1	O54904	MM.226435	21347	101725_at	A_51_P342916	Not classified	UDP-GAL:BETAGLCNAC BETA 1,3-GALACTOSYLTRANSFERASE, POLYPEPTIDE 1
LMP004971	B3galt2	O54905	MM.285580	26626	92341_at	A_51_P148216	Not classified	UDP-GAL:BETAGLCNAC BETA 1,3-GALACTOSYLTRANSFERASE, POLYPEPTIDE 2
LMP004979	Rdh16	O54909	MM.100276	147819	#N/A	A_51_P455256	Not classified	RETINOL DEHYDROGENASE 16
LMP004985	Cyp2g1	Q9WV19	MM.102312	32101	#N/A	A_51_P137448	Not classified	CYTOCHROME P450, FAMILY 2, SUBFAMILY G, POLYPEPTIDE 1
LMP004990	Ppapdc3	Q91WB2	MM.257236	16065	#N/A	A_51_P321374	Not classified	PHOSPHATIDIC ACID PHOSPHATASE TYPE 2 DOMAIN CONTAINING 3
LMP004993	Abpa	Q91WB5	MM.8025	15299	94628_r_at	A_51_P344136	Not classified	ANDROGEN BINDING PROTEIN ALPHA
LMP005002	Mlstd2	Q922J9	MM.206919	15015	#N/A	A_51_P186092	Not classified	MALE STERILITY DOMAIN CONTAINING 2
LMP005013	Slc22a4	Q9Z306	MM.274590	32849	92497_at	#N/A		SOLUTE CARRIER FAMILY 22 (ORGANIC CATION TRANSPORTER), MEMBER 4
LMP005015	Pigb	Q9JJQ0	MM.139905	147667	#N/A	A_51_P427483	Not classified	PHOSPHATIDYLINOSITOL GLYCAN, CLASS B
LMP005019	Acsl6	Q91WC3	MM.267478	148310	#N/A	A_51_P380699;A_51_P518822	Not classified	ACYL-COA SYNTHETASE LONG-CHAIN FAMILY MEMBER 6
LMP005028	Pik3r6	Q3U6Q4	MM.234573	32759	#N/A	#N/A	Not classified	EXPRESSED SEQUENCE BB220380
LMP005074	Dpm2	Q9Z324	MM.22001	25680	94052_at	#N/A		DOLICHOL-PHOSPHATE (BETA-D) MANNOSYLTRANSFERASE 2
LMP005075	Gdpd4	Q3TT99	MM.358889	143835	#N/A	A_51_P241690	Not classified	GLYCEROPHOSPHODIESTER PHOSPHODIESTERASE DOMAIN CONTAINING 4
LMP005117	Coq6	Q8R1S0	MM.280062	144323	#N/A	A_51_P116479	Not classified	COENZYME Q6 HOMOLOG (YEAST)
LMP005122	Mettl2	Q8BMK1	MM.270091	22972	#N/A	A_51_P401745	Not classified	METHYLTRANSFERASE LIKE 2
LMP005131	B3gnt3	Q5JCS9	MM.290837	5751	#N/A	A_51_P240760	Not classified	UDP-GLCNAC:BETAGAL BETA-1,3-N-ACETYLGLUCOSAMINYLTRANSFERASE 3
LMP005137	Decr2	Q9WV68	MM.292869	37132	#N/A	A_51_P443293	Not classified	2-4-DIENOYL-COENZYME A REDUCTASE 2, PEROXISOMAL
LMP005147	Ces2	Q91WG0	MM.28191	19326	#N/A	A_51_P513571	Not classified	CARBOXYLESTERASE 2
LMP005150	Lsr	Q99KG5	MM.4067	146044	#N/A	A_51_P436839	Not classified	LIPOLYSIS STIMULATED LIPOPROTEIN RECEPTOR
LMP005152	Prkag2	Q91WG5	MM.33649	5057	#N/A	A_51_P460872	Not classified	RIKEN CDNA 2410051C13 GENE
LMP005157	Gcnt3	Q5JCT0	MM.195555	31071	#N/A	A_51_P412369	Not classified	GLUCOSAMINYL (N-ACETYL) TRANSFERASE 3, MUCIN TYPE
LMP005176	Gusb	P12265	MM.371672	17517	#N/A	A_51_P211491	Not classified	BETA-GLUCURONIDASE STRUCTURAL
LMP005181	Pik3r5	Q5SW28	MM.244960	11284	#N/A	A_51_P173331	Not classified	PHOSPHOINOSITIDE-3-KINASE, REGULATORY SUBUNIT 5, P101
LMP005187	Akr7a2	Q8CG76	MM.287397	26238	#N/A	#N/A	Not classified	ALDO-KETO REDUCTASE FAMILY 7, MEMBER A5 (AFLATOXIN ALDEHYDE REDUCTASE)

LMP005197	Fkbp4	P30416	MM.12758	45371	92808_f_at;92809_r_at	A_51_P185175	Not classified	FK506 BINDING PROTEIN 4
LMP005206	4833424O15Rik	Q8BJ52	MM.72187	144924	#N/A	A_51_P128786	Not classified	RIKEN CDNA 4833424O15 GENE
LMP005211	Pnpla2	Q8BJ56	MM.29998	26846	#N/A	A_51_P197213	Not classified	PATATIN-LIKE PHOSPHOLIPASE DOMAIN CONTAINING 2
LMP005214	Ptgfrn	Q9WV91	MM.24807	36916	99894_at	A_51_P354062	Not classified	PROSTAGLANDIN F2 RECEPTOR NEGATIVE REGULATOR
LMP005223	Acss2	Q9QXG4	MM.255026	6835	#N/A	A_51_P488399	Not classified	ACETYL-COA SYNTHETASE
LMP005241	B4galt2	Q9Z2Y2	MM.123843	7411	104005_at	#N/A		UDP-GAL:BETAGLCNAC BETA 1,4-GALACTOSYLTRANSFERASE, POLYPEPTIDE 2
LMP005250	Slc10a4	Q3UEZ8	MM.253661	9142	#N/A	A_51_P448479	Not classified	SOLUTE CARRIER FAMILY 10 (SODIUM/BILE ACID COTRANSPORTER FAMILY), MEMB...
LMP005269	Slc25a20	Q9Z2Z6	MM.29666	147040	95695_at	#N/A		SOLUTE CARRIER FAMILY 25 (MITOCHONDRIAL CARNITINE/ACYLCARNITINE TRANSL...
LMP005280	Cyp4a12	Q91WL5	MM.276106	22145	101307_at	A_51_P433360	Not classified	SIMILAR TO CYTOCHROME P450 4A8 (CYPIVA8) (P450-KP1) (P450-PP1)
LMP005283	Agps	Q8C0I1	MM.31227	12617	#N/A	A_51_P399504	Not classified	ALKYLGLYCERONE PHOSPHATE SYNTHASE
LMP005318	Hsd17b9	Q9R092	MM.26719	7364	#N/A	#N/A	Not classified	HYDROXYSTEROID (17-BETA) DEHYDROGENASE 9
LMP005329	Nr2c1	Q505F1	MM.107483	147601	92190_at	A_51_P197849	Not classified	NUCLEAR RECEPTOR SUBFAMILY 2, GROUP C, MEMBER 1
LMP005339	Acot10	Q32MW2	MM.352763	145870	#N/A	#N/A	Not classified	ACYL-COA THIOESTERASE 10
LMP005340	AY053573	Q8K3L9	MM.331466	30297	#N/A	#N/A	Not classified	CDNA SEQUENCE AY053573
LMP005355	Fads6	Q810B5	MM.21995	14041	#N/A	#N/A	Not classified	FATTY ACID DESATURASE DOMAIN FAMILY, MEMBER 6
LMP005370	Ywhah	P68510	MM.332314	28860	97535_at	A_51_P306527	Not classified	TYROSINE 3-MONOOXYGENASE/TRYPTOPHAN 5-MONOOXYGENASE ACTIVATION PROTEIN...
LMP005377	Nudt10	P0C027	MM.41198	146851	#N/A	#N/A	Not classified	NUDIX (NUCLEOSIDE DIPHOSPHATE LINKED MOIETY X)-TYPE MOTIF 11
LMP005378	Nudt11	P0C028	MM.41198	146851	#N/A	A_51_P490217	Not classified	NUDIX (NUCLEOSIDE DIPHOSPHATE LINKED MOIETY X)-TYPE MOTIF 11
LMP005391	Plp1	P60202	MM.1268	16768	#N/A	A_51_P127392	Not classified	PROTEOLIPID PROTEIN (MYELIN) 1
LMP005403	Prmt7	Q922X9	MM.251804	149196	#N/A	A_51_P298538	Not classified	PROTEIN ARGININE N-METHYLTRANSFERASE 7
LMP005410	Cyp11b1	Q3UQH5	NA	28328	#N/A	#N/A	Not classified	CYTOCHROME P450, FAMILY 11, SUBFAMILY B, POLYPEPTIDE 1
LMP005416	Dhrs1	Q99L04	MM.21623	6294	93843_at	A_51_P293901	Not classified	DEHYDROGENASE/REDUCTASE (SDR FAMILY) MEMBER 1
LMP005421	Miox	Q9QXN5	MM.158200	127058	#N/A	A_51_P385258	Not classified	MYO-INOSITOL OXYGENASE
LMP005422	Cyp3a25	O09158	MM.301900	17429	104024_at	A_51_P489367	Not classified	CYTOCHROME P450, FAMILY 3, SUBFAMILY A, POLYPEPTIDE 25
LMP005440	Abpe	Q6UGQ3	MM.333771	37224	#N/A	#N/A	Not classified	ANDROGEN BINDING PROTEIN EPSILON

LMP005442	Lycat	Q3UN02	MM.329194	10462	#N/A	#N/A	Not classified	LYSOCARDIOLIPIN ACYLTRANSFERASE
LMP005450	Apol6	Q3UN08	MM.34173	149208	#N/A	A_51_P404815	Not classified	APOLIPOPROTEIN L, 6
LMP005459	Akr1c21	Q91WR5	MM.27085	26838	160733_at	A_51_P208472	Not classified	ALDO-KETO REDUCTASE FAMILY 1, MEMBER C21
LMP005462	Gpx6	Q91WR8	MM.46195	144846	#N/A	A_51_P299149	Not classified	GLUTATHIONE PEROXIDASE 6
LMP005471	Srd5a1	Q505K7	MM.315983	12273	#N/A	A_51_P420415	Not classified	STEROID 5 ALPHA-REDUCTASE 1
LMP005479	Gpx5	P21765	MM.1332	149254	161986_f_at; 94692_at	A_51_P267644	Not classified	GLUTATHIONE PEROXIDASE 5
LMP005502	Baat	Q91X34	MM.2859	38265	104273_at	A_51_P245123	Not classified	BILE ACID-COENZYME A: AMINO ACID N-ACYLTRANSFERASE
LMP005511	D030013I16Rik	Q3TPQ0	NA	147591	#N/A	#N/A	Not classified	RIKEN CDNA D030013I16 GENE
LMP005524	Mall	Q91X49	MM.133036	13736	#N/A	A_51_P221441	Not classified	MAL, T-CELL DIFFERENTIATION PROTEIN-LIKE
LMP005529	Cyp27a1	Q9DBG1	MM.85083	37251	#N/A	A_51_P112821	Not classified	RIKEN CDNA 1300013A03 GENE
LMP005530	Atp8b1	Q3U010	MM.270043	144899	#N/A	A_51_P192329;A_51_P494297	Not classified	ATPASE, CLASS I, TYPE 8B, MEMBER 1
LMP005543	Hibch	Q8QZS1	MM.222063	37162	#N/A	A_51_P178735	Not classified	3-HYDROXYISOBUTYRYL-COENZYME A HYDROLASE
LMP005546	Ffar3	Q3UFD7	MM.291167	18381	#N/A	#N/A	Not classified	G PROTEIN-COUPLED RECEPTOR 41
LMP005549	Plcl4	Q3LUA7	MM.379458	27196	#N/A	#N/A	Not classified	PHOSPHOLIPASE C-LIKE 4
LMP005558	Ptpmt1	Q66GT5	MM.23926	15355	#N/A	A_51_P226341	Not classified	PROTEIN TYROSINE PHOSPHATASE, MITOCHONDRIAL 1
LMP005573	Abcb11	Q9QY30	MM.303187	7543	#N/A	A_51_P309338	Not classified	ATP-BINDING CASSETTE, SUB-FAMILY B (MDR/TAP), MEMBER 11
LMP005576	Sgk3	Q9ERE3	MM.336410	43494	#N/A	A_51_P306151	Not classified	SERUM/GLUCOCORTICOID REGULATED KINASE 3
LMP005578	Abcc1	O35379	MM.196634	143756	99329_at	A_51_P152633	Not classified	ATP-BINDING CASSETTE, SUB-FAMILY C (CFTR/MRP), MEMBER 1
LMP005582	Sgpp2	Q810K3	MM.276248	148455	#N/A	A_51_P267169	Not classified	SPHINGOSINE-1-PHOSPHATE PHOSPHOTASE 2
LMP005596	Carm1	Q9WVG6	MM.230844	23321	99169_at	A_51_P370390	Not classified	PROTEIN ARGININE N-METHYLTRANSFERASE 4
LMP005609	Ldlrap1	Q8C142	MM.27486	3956	#N/A	A_51_P404644	Not classified	LOW DENSITY LIPOPROTEIN RECEPTOR ADAPTOR PROTEIN 1
LMP005610	Slc37a2	Q8C144	MM.325350	15004	#N/A	A_51_P259773	Not classified	SOLUTE CARRIER FAMILY 37 (GLYCEROL-3-PHOSPHATE TRANSPORTER), MEMBER 2
LMP005653	Cyp4a14	O35728	MM.250901	36636	101103_at	A_51_P238576	Not classified	CYTOCHROME P450, FAMILY 4, SUBFAMILY A, POLYPEPTIDE 14
LMP005660	Cyp2c44	Q8QZW4	MM.329866	16956	#N/A	A_51_P209782	Not classified	CYTOCHROME P450, FAMILY 2, SUBFAMILY C, POLYPEPTIDE 44
LMP005674	Ppapdc1	Q3UMZ3	MM.217337	11389	#N/A	A_51_P281593	Not classified	PHOSPHATIDIC ACID PHOSPHATASE TYPE 2 DOMAIN CONTAINING 1
LMP005684	Ptdsr	Q9ERI5	MM.348004	146930	95486_at	#N/A	Not classified	PHOSPHATIDYLSERINE RECEPTOR
LMP005685	Rdh14	Q9ERI6	MM.119343	37882	#N/A	A_51_P370380	Not classified	RIKEN CDNA 3110030G19 GENE

LMP005686	Itgb1bp3	Q9D7C9	MM.81562	14672	#N/A	A_51_P369766	Not classified	INTEGRIN BETA 1 BINDING PROTEIN 3
LMP005704	Paqr9	Q6TCG2	MM.151485	27619	#N/A	#N/A	Not classified	PROGESTIN AND ADIPOQ RECEPTOR FAMILY MEMBER IX
LMP005707	Paqr6	Q6TCG5	MM.267521	148920	#N/A	A_51_P475628	Not classified	PROGESTIN AND ADIPOQ RECEPTOR FAMILY MEMBER VI
LMP005709	Paqr3	Q6TCG8	MM.332505	37307	#N/A	A_51_P418749	Not classified	PROGESTIN AND ADIPOQ RECEPTOR FAMILY MEMBER III
LMP005716	Echdc1	Q8C185	MM.41728	14258	#N/A	A_51_P120448	Not classified	ENOYL COENZYME A HYDRATASE DOMAIN CONTAINING 1
LMP005719	Phospho 1	Q8R2H9	MM.133075	27481	#N/A	A_51_P149257	Not classified	PHOSPHATASE, ORPHAN 1
LMP005721	Ldlrad3	Q8CCS0	MM.35116	146405	#N/A	A_51_P283826	Not classified	EXPRESSED SEQUENCE AI194318
LMP005729	Fpr1	P33766	MM.56951	32253	99387_at	A_51_P312485	Not classified	FORMYL PEPTIDE RECEPTOR 1
LMP005741	Acacb	Q6JIZ0	MM.81793	37148	#N/A	A_51_P239236	Not classified	ACETYL-COENZYME A CARBOXYLASE BETA
LMP005747	Slc27a5	Q4LDG0	MM.10984	19519	92612_at	A_51_P289742	Not classified	SOLUTE CARRIER FAMILY 27 (FATTY ACID TRANSPORTER), MEMBER 5
LMP005757	Pld4	Q8BG07	MM.203915	16896	#N/A	A_51_P506148	Not classified	PHOSPHOLIPASE D FAMILY, MEMBER 4
LMP005762	Pdhb	Q9D051	MM.301527	146539	94806_at	A_51_P321921	Not classified	PYRUVATE DEHYDROGENASE (LIPOAMIDE) BETA
LMP005773	Gpx3	P46412	MM.200916	26447	101676_at	A_51_P292008	Not classified	GLUTATHIONE PEROXIDASE 3
LMP005784	Syt2	P46097	MM.5102	4628	101183_at	A_51_P330749	Not classified	SYNAPTOTAGMIN II
LMP005789	Acbd6	Q9D061	MM.288249	13166	104346_at	A_51_P208919	Not classified	RIKEN CDNA 0610010G04 GENE
LMP005791	Decr1	Q9CQ62	MM.24395	16636	160711_at	A_51_P208555	Not classified	2,4-DIENOYL COA REDUCTASE 1, MITOCHONDRIAL
LMP005820	Pi4k2b	Q9D072	MM.248647	149242	#N/A	A_51_P230987	Not classified	PHOSPHATIDYLINOSITOL 4-KINASE TYPE 2 BETA
LMP005837	Coq5	Q9CXI0	MM.290142	147239	#N/A	A_51_P125183	Not classified	COENZYME Q5 HOMOLOG, METHYLTRANSFERASE (YEAST)
LMP005842	Itpk1	Q8BYN3	MM.347546	37298	#N/A	#N/A	Not classified	INOSITOL 1,3,4-TRIPHOSPHATE 5/6 KINASE
LMP005848	B3gnt4	Q923H4	MM.373617	22165	#N/A	A_51_P225565	Not classified	UDP-GLCNAC:BETAGAL BETA-1,3-N-ACETYLGLUCOSAMINYLTRANSFERASE 4
LMP005850	Pigv	Q7TPN3	MM.217004	144457	#N/A	A_51_P116427	Not classified	PHOSPHATIDYLINOSITOL GLYCAN, CLASS V
LMP005853	Cyp21a2-p	Q60810	NA	16694	#N/A	#N/A	Not classified	SIMILAR TO CYTOCHROME P450 XXI (STEROID 21-HYDROXYLASE) (21-OHASE) (P4
LMP005870	Pnpla7	Q3UFP1	MM.29046	17154	#N/A	A_51_P276235	Not classified	CDNA SEQUENCE BC027342
LMP005876	Echdc3	Q9D7J9	MM.38342	37628	#N/A	A_51_P475378	Not classified	ENOYL COENZYME A HYDRATASE DOMAIN CONTAINING 3
LMP005904	Dhrs4	Q99LB2	MM.27427	5440	96678_at	A_51_P352374	Not classified	DEHYDROGENASE/REDUCTASE (SDR FAMILY) MEMBER 4
LMP005922	Fads3	Q3U3X6	MM.253875	144010	#N/A	A_51_P464029	Not classified	FATTY ACID DESATURASE 3
LMP005931	Cryz	P47199	MM.347513	37139	98131_at	A_51_P435198	Not classified	CRYSTALLIN, ZETA

LMP005936	Prkd2	Q8BZ03	MM.1881	27297	#N/A	A_51_P499441	Not classified	PROTEIN KINASE D2
LMP005942	Pggt1b	Q8BUY9	MM.262096	39624	#N/A	A_51_P184477	Not classified	PROTEIN GERANYLGERANYLTRANSFERASE TYPE I, BETA SUBUNIT
LMP005945	Plb1	Q3TTY0	MM.160067	11097	#N/A	A_51_P108031;A_51_P238803	Not classified	PHOSPHOLIPASE B1
LMP005986	Ubiad1	Q9DC60	MM.292503	9131	#N/A	A_51_P331661	Not classified	UBIA PRENYLTRANSFERASE DOMAIN CONTAINING 1
LMP005996	Fads1	Q3U494	MM.30158	3941	#N/A	A_51_P358112	Not classified	FATTY ACID DESATURASE 1
LMP006002	Cd36	Q08857	MM.18628	20611	161704_r_at, 93332_at	A_51_P375138	Not classified	CD36 ANTIGEN
LMP006007	Plcd3	Q69Z55	MM.264743	9346	#N/A	A_51_P278163	Not classified	PHOSPHOLIPASE C, DELTA 3
LMP006009	Myst3	Q8BZ21	MM.182776	3650	#N/A	A_51_P295277	Not classified	MYST HISTONE ACETYLTRANSFERASE (MONOCYTIC LEUKEMIA) 3
LMP006016	Chat	Q03059	MM.190503	27416	#N/A	A_51_P285243	Not classified	CHOLINE ACETYLTRANSFERASE
LMP006027	Ippk	Q6P1C1	NA	8772	#N/A	A_51_P100361	Not classified	INOSITOL 1,3,4,5,6-PENTAKISPHOSPHATE 2-KINASE
LMP006035	Esco1	Q69Z69	MM.210996	37260	#N/A	A_51_P270350	Not classified	ESTABLISHMENT OF COHESION 1 HOMOLOG 1 (S. CEREVISIAE)
LMP006064	Dlat	Q8R339	MM.285076	14301	96745_at;96746_at	A_51_P265106	Not classified	DIHYDROLIPOAMIDE S-ACETYLTRANSFERASE (E2 COMPONENT OF PYRUVATE DEHYDRO...
LMP006065	Pigl	Q5SX19	MM.374899	24617	#N/A	#N/A	Not classified	PHOSPHATIDYLINOSITOL GLYCAN, CLASS L
LMP006077	Gde1	Q9JL56	MM.273142	27496	#N/A	#N/A	Not classified	MEMBRANE INTERACTING PROTEIN OF RGS16
LMP006085	Nudt4	Q8R2U6	MM.24397	144575	#N/A	A_51_P189418	Not classified	NUDIX (NUCLEOSIDE DIPHOSPHATE LINKED MOIETY X)-TYPE MOTIF 4
LMP006098	Dhrs7	Q9CXR1	MM.289653	9210	95620_at	A_51_P312437	Not classified	DEHYDROGENASE/REDUCTASE (SDR FAMILY) MEMBER 7
LMP006108	Med4	Q9CQA5	MM.282888	7517	#N/A	A_51_P373369	Not classified	MEDIATOR OF RNA POLYMERASE II TRANSCRIPTION, SUBUNIT 4 HOMOLOG (YEAST)
LMP006111	Cyp2b13	Q64460	NA	37039	102820_at	A_51_P184750;A_51_P492339	Not classified	CYTOCHROME P450, FAMILY 2, SUBFAMILY B, POLYPEPTIDE 13
LMP006123	Ogt	Q8CGY8	MM.259191	38165	94818_at	A_51_P261107	Not classified	O-LINKED N-ACETYLGLUCOSAMINE (GLCNAC) TRANSFERASE (UDP-N-ACETYLGLUCOSA...
LMP006131	Gpx7	Q99LJ6	MM.20164	26861	#N/A	A_51_P461364	Not classified	GLUTATHIONE PEROXIDASE 7
LMP006146	Acaca	Q5SWU9	MM.31374	36995	#N/A	A_51_P191939;A_51_P439426	Not classified	ACETYL-COENZYME A CARBOXYLASE ALPHA
LMP006151	Plekha5	Q6ZPK1	MM.247670	14771	#N/A	A_51_P500862	Not classified	EST AI428202
LMP006152	Bckdha	P50136	MM.25848	37474	96035_at	A_51_P466148	Not classified	BRANCHED CHAIN KETOACID DEHYDROGENASE E1, ALPHA POLYPEPTIDE
LMP006153	Enpp7	Q3TIW9	NA	146231	#N/A	#N/A	Not classified	ECTONUCLEOTIDE PYROPHOSPHATASE/PHOSPHODIESTERASE 7
LMP006163	Acot9	Q9R0X4	MM.268710	136320	#N/A	A_51_P280165	Not classified	ACYL-COA THIOESTERASE 9
LMP006175	Chst12	Q99LL3	MM.28934	8923	#N/A	A_51_P464021	Not classified	CARBOHYDRATE SULFOTRANSFERASE 12

LMP006194	Cnr2	P47936	MM.297251	16881	102674_at	A_51_P208511	Not classified	CANNABINOID RECEPTOR 2 (MACROPHAGE)
LMP006197	Pnpla1	Q3V1D5	MM.328925	148364	#N/A	#N/A	Not classified	SIMILAR TO PATATIN-LIKE PHOSPHOLIPASE DOMAIN CONTAINING 1
LMP006200	Phyhip	Q8K0S0	MM.192598	37122	#N/A	A_51_P368210	Not classified	PHYTANOYL-COA HYDROXYLASE INTERACTING PROTEIN
LMP006218	Asahl	Q9D7V9	MM.28890	4414	#N/A	A_51_P242201	Not classified	N-ACYLSPHINGOSINE AMIDOHYDROLASE (ACID CERAMIDASE)-LIKE
LMP006238	2210418 G03Rik	Q3U4B4	MM.75602	16174	#N/A	#N/A	Not classified	RIKEN CDNA 2210418G03 GENE
LMP006250	Gpx2	Q9JHC0	MM.371561	27037	99810_at	A_51_P486810	Not classified	GLUTATHIONE PEROXIDASE 2
LMP006256	Aasdhppt	Q9CQF6	MM.33970	37143	#N/A	A_51_P265096	Not classified	AMINOADIPATE-SEMIALDEHYDE DEHYDROGENASE-PHOSPHOPANTETHEINYL TRANSFERAS...
LMP006257	Pcyox1	Q9CQF9	MM.30849	15472	#N/A	A_51_P508029	Not classified	PRENYLCYSTEINE OXIDASE 1
LMP006266	Pld5	Q3UNN8	MM.338001	36834	#N/A	#N/A	Not classified	PHOSPHOLIPASE D FAMILY, MEMBER 5
LMP006267	Hoxa1	P09022	MM.197	14559	95297_at	A_51_P351975	Not classified	HOMEO BOX A1
LMP006283	St3gal6	Q8VIB3	MM.212742	3529	#N/A	A_51_P281333	Not classified	ST3 BETA-GALACTOSIDE ALPHA-2,3-SIALYLTRANSFERASE 6
LMP006292	Ugcgl1	Q6P5E4	MM.261022	9195	#N/A	A_51_P148838;A_51_P236775	Not classified	RIKEN CDNA 0910001L17 GENE
LMP006298	Retsat	Q64FW2	MM.305108	26782	#N/A	A_51_P319070	Not classified	RIKEN CDNA 0610039N19 GENE
LMP006300	Fa2h	Q5RL53	MM.41083	36913	#N/A	A_51_P477692	Not classified	HYPOTHETICAL PROTEIN LOC234737
LMP006309	Pld3	O35405	MM.6483	37501	100607_at	A_51_P361478	Not classified	PHOSPHOLIPASE D FAMILY, MEMBER 3
LMP006310	Slc22a5	Q9Z0E8	MM.42253	5874	98322_at	A_51_P165060	Not classified	SOLUTE CARRIER FAMILY 22 (ORGANIC CATION TRANSPORTER), MEMBER 5
LMP006313	Cyp27b1	O35084	MM.6216	3409	99836_at	A_51_P287221	Not classified	CYTOCHROME P450, FAMILY 27, SUBFAMILY B, POLYPEPTIDE 1
LMP006314	Asah2	Q9JHE3	MM.104900	16288	#N/A	A_51_P305708	Not classified	N-ACYLSPHINGOSINE AMIDOHYDROLASE 2
LMP006329	Ch25h	Q9Z0F5	MM.30824	145585	104509_at	A_51_P112966	Not classified	CHOLESTEROL 25-HYDROXYLASE
LMP006330	Mut	P16332	MM.259884	144428	99613_at	A_51_P193935	Not classified	METHYLMALONYL-COENZYME A MUTASE
LMP006338	Aytl2	Q3TFD2	MM.284649	3869	#N/A	A_51_P405089	Not classified	RIKEN CDNA 2900035H07 GENE
LMP006340	Pitpnm2	Q6ZPQ6	MM.44261	11278	#N/A	A_51_P112529	Not classified	PHOSPHATIDYLINOSITOL TRANSFER PROTEIN, MEMBRANE-ASSOCIATED 2
LMP006357	Wisp2	Q9Z0G4	MM.13828	148069	94704_at	A_51_P390804	Not classified	WNT1 INDUCIBLE SIGNALING PATHWAY PROTEIN 2
LMP006364	Pigp	Q9JHG1	MM.328717	32992	#N/A	A_51_P182144	Not classified	PHOSPHATIDYLINOSITOL GLYCAN, CLASS P
LMP006370	Smpd4	Q6ZPR5	MM.34659	148099	#N/A	A_51_P159813	Not classified	RIKEN CDNA 4122402O22 GENE
LMP006373	Rdh7	O88451	MM.6696	23037	100634_at	A_51_P122170	Not classified	RETINOL DEHYDROGENASE 7
LMP006375	Inpp5f	Q6ZQ16	MM.58882	41244	#N/A	A_51_P176054	Not classified	INOSITOL POLYPHOSPHATE-5-PHOSPHATASE F

LMP006407	Psph	Q99LS3	MM.271784	30317	92589_at	A_51_P430263	Not classified	PHOSPHOSERINE PHOSPHATASE
LMP006411	4933403 G17Rik	Q9D4C4	MM.158548	149013	#N/A	A_51_P174133	Not classified	RIKEN CDNA 4933403G17 GENE
LMP006418	Mettl6	Q8BVH9	MM.291731	148873	#N/A	A_51_P288961	Not classified	METHYLTRANSFERASE LIKE 6
LMP006424	Pak1ip1	Q9DCE5	MM.24789	6977	#N/A	A_51_P200151	Not classified	PAK1 INTERACTING PROTEIN 1
LMP006425	Hexdc	Q3U4H6	MM.386870	13666	#N/A	#N/A	Not classified	HEXOSAMINIDASE (GLYCOSYL HYDROLASE FAMILY 20, CATALYTIC DOMAIN) CONTAI...
LMP006446	Ivd	Q9JHI5	MM.6635	14962	#N/A	A_51_P425674	Not classified	ISOVALERYL COENZYME A DEHYDROGENASE
LMP006461	Ppapdc1 a	Q0VBU9			#N/A	#N/A	Not classified	
LMP006469	Dgkd	Q6A0B7	MM.277217	9628	#N/A	#N/A	Not classified	DIACYLGLYCEROL KINASE, DELTA
LMP006481	Smpdl3a	P70158	MM.2379	26391	#N/A	A_51_P153053	Not classified	SPHINGOMYELIN PHOSPHODIESTERASE, ACID-LIKE 3A
LMP006483	Cubn	Q9JLB4	MM.313915	144219	#N/A	A_51_P145691	Not classified	CUBILIN (INTRINSIC FACTOR-COBALAMIN RECEPTOR)
LMP006487	4833405 L16Rik	Q4FZF0	MM.313743	37398	#N/A	#N/A	Not classified	RIKEN CDNA 4833405L16 GENE
LMP006508	Gba2	Q69ZF3	MM.229444	9138	92784_at	A_51_P261359	Not classified	GLUCOSIDASE BETA 2
LMP006516	Ppapdc2	Q9D4F2	NA	14242	#N/A	A_51_P433026	Not classified	PHOSPHATIDIC ACID PHOSPHATASE TYPE 2 DOMAIN CONTAINING 2
LMP006518	Rabggta	Q9JHK4	MM.87216	28273	#N/A	A_51_P481832	Not classified	RAB GERANYLGERANYL TRANSFERASE, A SUBUNIT
LMP006519	Pigx	Q99LV7	MM.37613	19984	#N/A	A_51_P399217	Not classified	PHOSPHATIDYLINOSITOL GLYCAN, CLASS X
LMP006522	Nsd1	O88491	MM.386965	3641	#N/A	A_51_P477809	Not classified	NUCLEAR RECEPTOR-BINDING SET-DOMAIN PROTEIN 1
LMP006525	Fut9	O88819	MM.39101	148200	#N/A	A_51_P250733;A_51_P417758	Not classified	FUCOSYLTRANSFERASE 9
LMP006536	Rftn1	Q6A0D4	MM.41854	5341	#N/A	A_51_P408800	Not classified	RIKEN CDNA 2310015N21 GENE
LMP006537	A3galt2	Q3V1N9	MM.300900	19569	#N/A	#N/A	Not classified	ALPHA 1,3-GALACTOSYLTRANSFERASE 2 (ISOGLOBOTRIAOSYLCERAMIDE SYNTHASE)
LMP006548	Pdss1	Q33DR2	MM.249752	148145	#N/A	A_51_P127841	Not classified	PRENYL (SOLANESYL) DIPHOSPHATE SYNTHASE, SUBUNIT 1