

Autophagy in Atherosclerosis

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Table of content

Table of content	ii
Abstract	v
Acknowledgments	vii
List of Figures	ix
List of Tables	x
Abbreviations	xi
1 Introduction	1
1.1 Atherosclerosis	1
1.1.1 Atherosclerosis Progression	2
1.1.1.1 Foam Cell Formation	5
1.1.1.1.1 Macrophage Foam Cells	6
1.1.1.1.2 Smooth Muscle Cells Foam Cells	7
1.1.1.1.3 The Fate of the Cholesterol	9
1.1.2 Atherosclerosis Regression	9
1.1.2.1 Resolution of Residual Inflammation	10
1.1.2.2 Reverse Cholesterol Transport	11
1.1.2.2.1 Mechanisms of Cholesterol Hydrolysis and Efflux	12
1.1.2.2.2 Capacity for Cholesterol Efflux of Macrophages and Smooth Muscle Cells	14
1.1.2.2.3 Cholesterol Transport and Excretion	15
1.1.2.2.4 ApoA-I and HDL dysfunction	16
1.2 Autophagy	17
1.3 Macroautophagy	18
1.3.1.1 Autophagosome Formation and Lysosomal Fusion	19
1.3.1.1.1 Initiation	19
1.3.1.1.2 Nucleation	19
1.3.1.1.3 Expansion	20
1.3.1.1.4 Fusion	22
1.3.1.2 Bulk or Selective Autophagy	23
1.3.1.2.1 mTOR Signaling	24
1.3.1.2.2 Selective Autophagy Mechanisms	25
1.3.2 Microautophagy	27
1.3.2.1 Non-selective Microautophagy	27
1.3.2.2 Selective Microautophagy	27
1.3.3 Chaperone-Mediated Autophagy	28
1.3.4 Autophagy in Disease	29
1.3.4.1 Regulation of Autophagy in Foam Cells	30
1.3.4.2 Macrophage Autophagy	32
1.3.4.2.1 Smooth Muscle Cell Autophagy	33
1.3.4.2.2 Lysosomal Dysfunction	34

1.3.4.2.3	Lipid Metabolism and Cholesterol Efflux	34
1.4	Lipid Droplets.....	35
1.4.1	Lipid Droplet Biogenesis	35
1.4.2	Lipid Droplet Degradation	37
1.5	Open Questions in the Role of Autophagy in Atherosclerosis	38
1.6	Objectives.....	39
1.7	References	40
2	Manuscript 1: Identification of Novel Lipid Droplet Factors that Regulate Lipophagy and Cholesterol Efflux in Macrophage Foam Cells	58
2.1	Abstract	59
2.2	Introduction.....	60
2.3	Results.....	62
2.3.1	Lipid droplet ubiquitination occurs during lipophagy.....	62
2.3.2	Identification of putative lipophagy factors on the surface of lipid droplets.....	64
2.3.3	Knockdown of candidate selective lipophagy factors reduces cholesterol efflux.....	70
2.3.4	Genetic ablation of selective lipophagy factors in <i>Saccharomyces cerevisiae</i> impairs lipophagy	75
2.3.5	Single LDs and clusters of LDs are tagged for autophagic degradation....	80
2.4	Discussion	83
2.5	Materials and Methods	94
2.6	Acknowledgements	102
2.7	Conflict of Interest.....	103
2.8	References	103
3	Manuscript 2: Autophagy is Differentially Regulated in Leukocyte and Nonleukocyte Foam Cells during Atherosclerosis	109
3.1	Abstract	110
3.2	Introduction.....	111
3.3	Methods.....	114
3.4	Results.....	116
3.4.1	Autophagy becomes impaired in leukocyte and nonleukocyte foam cells during atherogenesis.....	116
3.4.2	Autophagy flux is primarily defective in atherosclerotic nonleukocyte foam cells	120
3.4.3	Autophagy activation in VSMC, but not macrophage, foam cells promotes cholesterol efflux	129
3.4.4	Elevated expression of ABCG1 as compared to ABCA1 in nonleukocyte foam cells	133
3.5	Discussion	135
3.6	Acknowledgements	145
3.7	Sources of Funding	146
3.8	Disclosures	146
3.9	References	146

4	Manuscript 3: Trehalose Promotes Atherosclerosis Regression in Female Mice	151
4.1	Abstract	152
4.2	Introduction	152
4.3	Materials and Methods	155
4.4	Results	160
4.4.1	The administration of trehalose did not alter body composition nor circulating cholesterol in female mice	160
4.4.2	Trehalose promotes plaque regression in female mice	164
4.4.3	Differential regulation of autophagy in male and female mice	166
4.4.4	Trehalose reduces inflammation in female mice	168
4.5	Discussion	172
4.6	Conflict of Interest	176
4.7	Author Contributions	176
4.8	Funding	176
4.9	Acknowledgements	176
4.10	References	177
5	Discussion	181
5.1	Lipophagy in Macrophage Foam Cells	181
5.2	Defective Autophagy During Atherosclerosis Progression	184
5.3	Autophagy During Atherosclerosis Regression	187
5.4	Concluding Remarks	188
5.5	References	190
6	Appendix	206
6.1	Supplemental Figures Manuscript #1	206
6.2	Supplemental Material Manuscript #2	287
6.2.1	Expanded Materials & Methods	287
6.2.2	Online Figures	297
6.2.3	Major Resources Table	301
6.3	Supplementary Materials Manuscript #3	306
6.3.1	Supplementary Data	306
6.3.2	Supplementary Table	308
7	Copyrights	309
8	Curriculum Vitae	311

Abstract

Cardiovascular disease is the leading cause of death worldwide. The underlying cause of mortality from cardiovascular disease is the rupture of cholesterol-rich plaques which develops in the artery walls during atherosclerosis. Atherosclerotic plaques are comprised of lipid-laden cells, called foam cells. Overtime, the accumulation of foam cells within the plaque impedes blood flow to the downstream blood vessel. As time progresses, plaque become unstable and at risk of rupture or erosion leading to myocardial infarction, stroke, or death. Current therapies for the treatment of atherosclerosis include cholesterol-lowering drugs which can prevent plaque progression but fail to regress existing plaques in its totality.

Autophagy is a highly conserved constitutive cellular process which promotes cellular homeostasis by degrading excess proteins and damaged organelles. It has been previously demonstrated that macrophage autophagy was atheroprotective by reducing inflammation and promoting the clearance of apoptotic cells through efferocytosis. In addition, selective autophagy of lipid droplets, or lipophagy, was demonstrated to account for half of apolipoproteinA-I cholesterol efflux in macrophages foam cells highlighting the importance of functional autophagy during atherosclerosis.

The goal of this thesis was to elucidate how autophagy in foam cells participates in atherogenesis with a particular interest in how autophagy controls lipid catabolism to promote the removal of cholesterol from the plaque. The first part of this work uncovered novel receptors important in mediating macrophage foam cell lipophagy. In addition, this study demonstrated that lipid droplets can undergo both macroautophagy and

microautophagy. The second part of this work investigated the functionality of autophagy in the two main foam cell population of the atherosclerotic plaque being the macrophage foam cells and the vascular smooth muscle cell foam cells. Unlike macrophages which continue to have functional autophagy at both early and late stages of atherosclerosis, vascular smooth muscle cell foam cells have defective autophagy throughout atherosclerosis progression. Moreover, vascular smooth muscle cells foam cells have a very poor capacity to perform cholesterol efflux. The third part of this work explored how promoting autophagy using trehalose impacted atherosclerosis regression. The promotion of autophagy increased plaque regression in female mice, but not male mice. Altogether, these studies highlighted the critical role of autophagy in maintaining cellular homeostasis to prevent disease progression.

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List of Figures

Figure 1-1: Atherosclerosis Progression Overview	2
Figure 1-2: Overview of Reverse Cholesterol Transport.....	12
Figure 1-3: Mechanism of Lipid Uptake and Cholesterol Efflux	14
Figure 1-4: Autophagy Pathways	18
Figure 1-5: Mechanism of Macroautophagy	23
Figure 2-1: LC3, SQSTM1 and Ubiquitin Localize to Lipid Droplets in Macrophage	64
Figure 2-2: Proteins Enriched on Lipid Droplets Following Lipophagy Inhibition	69
Figure 2-3: Lipophagy Candidate Genes are Dysregulated in Atherosclerosis and Regulate Foam Cell Cholesterol Efflux	74
Figure 2-4: Selective Autophagy Receptors (SARs) OPTN, NBR1 and SQSTM1 Selectively Localize to Foam Cell Lipid Droplets	75
Figure 2-5: Genetic Ablation of Selective Lipophagy Factors Impairs Lipophagy in Yeast	79
Figure 2-6: Single Lipid Droplets and Lipid Droplet Clusters are Tagged for Lipophagy	82
Figure 3-1: Impaired Autophagy in Foam Cells During Atherosclerosis	119
Figure 3-2: Autophagy Flux is Impaired in Nonleukocyte Foam Cells During Atherogenesis	123
Figure 3-3: VSMC-Derived Foam Cells Fail to Initiate Autophagy Upon Lipid Loading	125
Figure 3-4: Autophagy Components are Differentially Expressed Between Leukocyte and Nonleukocyte Foam Cells in Atherosclerotic Plaque	128
Figure 3-5: Autophagy-Mediated Cholesterol Efflux is Impaired in VSMC Foam Cells	132
Figure 3-6: VSMC Derived Foam Cells Predominantly Express ABCG1	135
Figure 4-1: Trehalose Does Not Alter Body Composition or Circulating Cholesterol ...	163
Figure 4-2: Trehalose Promotes Plaque Size Stabilization in Female Mice	165
Figure 4-3: Autophagy is Differentially Regulated Between Female and Male Mice ...	168
Figure 4-4: Trehalose Reduces Inflammation in Female Mice	171
Figure 6-1: LC3, SQSTM1 and Ubiquitin Localize to Lipid Droplets in Oleic Acid-Loaded Macrophage Foam Cells	206
Figure 6-2. Motifs Enriched in Foam Cells Following Autophagy Inhibition	207
Figure 6-3. Ubiquitin and SARs Colocalize on Lipid Droplets in agLDL-Loaded Macrophage Foam Cells	208
Figure 6-4. Faa4-GFP Cleavage in Various Yeast Strains	209
Figure 6-5. Faa4-GFP Entry Into Vacuoles	210
Figure 6-6: Gating Scheme for Leukocyte and Nonleukocyte Derived Foam Cells.....	297
Figure 6-7. Pipeline for Quantification of Autophagy Markers in Leukocyte and Nonleukocyte Foam Cells by Immunofluorescence Staining.....	298
Figure 6-8. Transcriptomic analysis of autophagy markers in the atherosclerotic plaque	299
Figure 6-9. Embryonic-Derived VSMC and Macrophage Express Associated Lineage Markers	300
Figure 6-10: Circulating Leukocytes	306
Figure 6.11: Autophagy Flux is Equivalent in Mouse Macrophages Isolated From Male As Compared to Female Mice.....	307

List of Tables

Table 2.1: Proteins on Human Macrophage Foam Cell Lipid Droplets	67
Table 6.1. Complete List of Proteins on Human Macrophage Foam Cell Lipid Droplets	211
Table 6.2. Gene Ontology (GO) Enrichment Analysis of Lipid Droplets Enriched Following Chloroquine Treatment	236
Table 6.3. Efflux of 3H-Cholesterol from agLDL-Loaded Mouse Peritoneal Macrophages Transfected with siRNA Against Indicated Target Genes.....	283
Table 6.4: List of Antibodies	308

Abbreviations

AAV	Adeno-associated viral
ABCA1	ATP binding cassette A1
ABCG1	ATP binding cassette G1
ACAT	Acyl-coenzyme A:cholesterol acyltransferase
acLDL	Acetylated LDL
ADGRL3	Adhesion G protein-coupled receptor L3
agLDL	Aggregated low density lipoprotein
AMPK	AMP-activated protein kinase
ApoA-I	Apolipoprotein A-I
ApoB	Apolipoprotein B
ApoE	Apolipoprotein E
aSMA	Alpha smooth muscle actin
ATG	Autophagy genes
ATK	Protein kinase B
AUP1	AUP1 lipid droplet regulating VLDL assembly factor
BECN1	Beclin1
BMDM	Bone-marrow derived macrophages
BNIP3L	BCL2/adenovirus E1B interacting protein 3-like
BSA	Bovine serum albumin
CAD	Cardiovascular disease
CALCOCO2	Calcium binding and coiled-coil domain 2
CANTOS	Canakinumab Anti-inflammatory Thrombosis Outcome Study
CATHD	Cathepsin D
CCL5	Chemokine ligand 5
CD36	Cluster of differentiation 36
CD38	Cluster of differentiation 38
CD45	Cluster of differentiation 45
CD68	Cluster of differentiation 68
CEH	Cholesteryl ester hydrolase
CETP	Cholesteryl ester transfer protein
CHIP	E3 ubiquitin-protein ligase CHIP
CIRBP	Cold inducible RNA binding protein
CNN1	Calponin 1
COLGALT1	Collagen beta(1-O)galactosyltransferase 1
COPII	Coat protein complex ii
CORO1A	Coronin 1A
CTSB	Cathepsin B
CXCL1	C-X-C motif chemokine ligand 1
DAMP	Damage associated molecular pattern
DAP1	Death associated protein
DIT	Diffuse intimal thickening
DMA	Deletion mutant array
ER	Endoplasmic reticulum
ESC	Embryonic stem cell
ESCRT	Endosomal sorting complexes required for transport
FAA4	Long chain fatty acyl-coa synthetase
FBS	Fetal bovine serum

FIP200	FAK family kinase-interacting protein of 200 kd
FUS	Fused in sarcoma
GABARAP	GABA type A receptor-associated protein
GABARAPL	Gamma-aminobutyric acid receptor-associated protein-like
GRAMD1A	GRAM domain-containing 1A
HDL	High density lipoprotein
HMG-CoA	Hydroxymethylglutaryl-coenzyme A
HMGB1	High mobility group box 1
HMGB2	High mobility group box 2
HOP	Homeodomain-only protein
HOPS	Homotypic fusion and protein sorting
HSP40	DnaJ homolog subfamily B member 1
HSP90AA1	Heat shock protein 90, alpha (cytosolic) , class A member 1
HSPA5	Heat shock protein family A (Hsp70) member 5
HSPA8/HSC70	Heat shock protein 8
HSPB1	Heat shock protein 1
HSPH1	Heat shock 105kda/110kda protein 1
ICAM-1	Intracellular adhesion molecule 1
IL-1 β	Interleukin-1 β
IL-3	Interleukin 3
INSIG1	Insulin induced gene 1
LAL/LIPA	Lysosomal acid lipase
LAMP1	Lysosomal associated membrane protein 1
LAMP2	Lysosomal associated membrane protein 2
LC3	Microtubule-associated proteins 1A/1B light chain 3
LCAT	Lecithin:cholesterol acyltransferase
LD	Lipid droplet
LDAH	Lipid droplet associated hydrolase
LDL	Low density lipoprotein
LDLR	Low density lipoprotein receptor
LGALS3/Mac2	Galactin 3
LIPE	Hormone-sensitive lipase
LIR	LC3-interacting region
LOX-1	Lectin-like oxidl receptor-1
LRP1	LDL receptor-related protein 1
LXR	Liver x receptor
MACE	Major adverse cardiovascular events
MACROH2A1	MacroH2A.1 histone
MAGL	Monoacylglycerol lipase
MAP1LC3	Microtubule-associated protein 1 light chain 3
MCOLN1	Mucolipin 1
MCP-1	Monocyte chemoattractant protein-1
mESC	Mouse embryonic stem cells
modLDL	Modified LDL
mTOR	Mammalian target of rapamycin
mTORC1	Mammalian target of rapamycin complex 1
MYH11	Myosin heavy chain 11
m β -CD	Methyl-beta-cyclodextrin
M ϕ	Macrophage
NBR1	NBR1, autophagy cargo receptor

NCEH1	Neutral cholesterol ester hydrolase 1
NCP	NPC intracellular cholesterol transporter
NF- κ B	Nuclear factor kappa b
NLRP3	NLR family pyrin domain containing 3
NPC1	Neimann-pick c1
OPTN	Optineurin
ORP	Oxysterol binding proteins
oxLDL	Oxidized low density lipoprotein
P/S	Penicillin-streptomycin
P150	Pi3-kinase p150 subunit
PAS	Phagophore assembly site
PCSK9	Proprotein convertase subtilisin/kexin type 9
PE	Phosphatidylethanolamine
PI3K	Phosphoinositide-3-kinase
PI3KC3	Class III phosphatidylinositol3-kinase I
PI3P	Phosphatidylinositol 3-phosphate
PLEKHM1	Pleckstrin homology and RUN domain containing M1
PLIN	Perilipin
PLIN2	Perilipin 2
PLIN3	Perilipin 3
PNPLA2	Patatin like phospholipase domain containing 2
PNPLA5	Patatin-like phospholipase domain-containing protein 5
PRDX1	Peroxiredoxin-1
PRMT2	Protein arginine methyltransferase 2
PSAP	Prosaposin
RA	Retinoic
RAB	RAB, member RAS oncogene family
RAB7	Ras-related protein Rab-7a
RAPTOR	Regulatory-associated protein of mtor
RBBP7	Retinoblastoma binding protein 7, chromatin remodeling factor
RCT	Reverse cholesterol transport
RICTOR	Rapamycin insensitive companion of mtor
ROS	Reactive oxygen species
S1P	Site-1 protease
S2P	Site-2 protease
SAR	Selective autophagy receptors
SCAP	SREBP cleavage-activating protein
SCARB2	Scavenger receptor class B, member 2
SCC	Surface-connected compartment
SGA	Synthetic genetic array
SIRT	Sirtuins
SNAP29	Synaptosome associated protein 29
SNARE	Soluble N-ethylmaleimide-sensitive-factor attachment protein receptor
SQSTM1/p62	Sequestome-1
SR	Scavenger receptors
SREBP2	Sterol regulatory element-binding protein 2
STAT6	Signal transducer and activator of transcription 6
STX17	Syntaxin 17
TAX1BP1	Tax1-binding protein 1
TFEB	Transcription factor EB

TICE	Transintestinal cholesterol efflux
TOLLIP	Toll-interacting protein
TPM1	Tropomyosin 1
TRIM	Tripartite motif
UBE2G2	Ubiquitin conjugating enzyme E2 G2
ULK1	Unc-51 like autophagy activating kinase 1
UVRAG	UV radiation resistance associated gene
VAMP8	Vesicle associated membrane protein 8
VCAM-1	Vascular cell adhesion protein 1
VDAC2	Voltage dependent anion channel 2
VIM	Vimentin
VLDL	Very low density lipoprotein
VPS13A	Vacuolar sorting protein 13A
VSMC	Vascular smooth muscle cell
WIPI2	WD repeat domain phosphoinositide-interacting protein 2

1 Introduction

1.1 Atherosclerosis

Cardiovascular disease is the leading cause of death worldwide^{1,2}. The main contributor to cardiovascular disease, atherosclerosis, is characterized by a deposition of lipid-rich plaques within arteries impeding blood flow. Overtime, plaques can become vulnerable, prone to erosion and rupture leading to myocardial infarction, stroke, or death^{3,4}. The key risk factor for developing atherosclerosis is high levels of circulating cholesterol held within apolipoprotein B (apoB) particles^{5,6}. To date, therapies offered are capable of reducing disease progression via the means of cholesterol lowering but are unable to completely reverse the plaque buildup⁷. Since the discovery of cholesterol-lowering compounds back in 1976⁸ followed by their clinical approval in 1987⁹, statins have been the standard of care for patients with hypercholesterolemia and remain one of the most prescribed drugs in North America to this day¹⁰. The main mechanism of action by which statins achieve cholesterol lowering is by inhibiting HMG-CoA reductase, the rate-limiting enzyme part of the cholesterol biosynthesis pathway. Therefore, statins prevent cellular production of cholesterol and force the uptake of circulating cholesterol from the circulation⁸. Atherosclerosis develops over a lifetime and patients can stay asymptomatic until their last decades of life. For those patients who are symptomatic (i.e., chest pain, angina, myocardial infarction, or stroke), treatment options available include undergoing a catheter guided stent placement to widen the affected artery/arteries or the very invasive

coronary artery bypass graft surgery^{11,12}. There is currently an unmet need to provide these patients with therapies which can promote the complete reversal of these plaques.

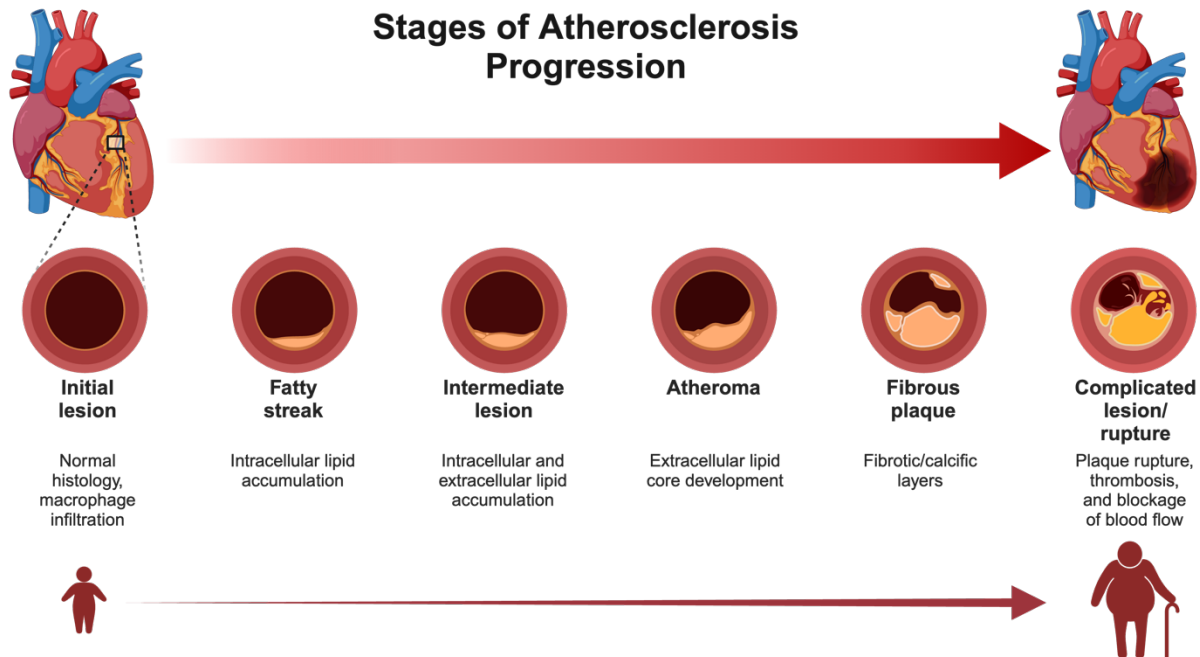


Figure 1-1: Atherosclerosis Progression Overview

Figure adapted from BioRender.

1.1.1 Atherosclerosis Progression

High levels of circulating apoB containing lipoproteins, such as low-density lipoprotein (LDL), promotes the entry of LDL into the intimal space of medium to large bore arteries where disturbed flow can be observed including the coronary and carotid arteries^{11,12}. The entry of LDL particles within the intimal space can occur via two pathways: paracellular and transcellular¹³⁻¹⁵. In these areas of disturbed flow, the endothelial junctions are permeable allowing for LDL particles to enter the intimal space through these loose cellular junctions¹⁵. During the transcellular pathway otherwise known as transcytosis, the LDL particles are endocytosed on the luminal side of the endothelial cells and delivered

to the abluminal membrane where it will be excreted into the intimal space^{14,16}. LDL will be retained in the extracellular matrix via charge-charge interaction between the apoB protein found on the LDL particle and the proteoglycans of the intimal space¹⁷⁻¹⁹. The extracellular milieu of the intima will cause LDL to become modified (modLDL). The proteoglycans promote LDL aggregation and free radicals oxidize the proteins and lipids at the surface of LDL leading to the formation of aggregated LDL (agLDL) or oxidized LDL (oxLDL) respectively²⁰⁻²². The major form of modLDL found within the plaque is agLDL²³. The modLDL elicit an immune response resulting in the production of chemokines from the vessel wall such as MCP-1, CCL5 and CXCL1 to promote the recruitment of circulating monocytes to the intimal space²⁴. Moreover, endothelial cells upregulate the adhesion molecules ICAM-1 and VCAM-1 which promotes the adhesion of circulating monocytes to the endothelium²⁵. Once attached to the endothelium, monocytes will enter the intima via extravasation where they will differentiate into macrophages²⁶. Uptake of these modLDL found in the extracellular milieu by macrophages stimulates the formation of foam cells²⁷. In addition to macrophages, smooth muscle cells can also uptake cholesterol and become foam cells²⁸⁻³¹. This buildup of foam cells further exacerbates the local inflammation allowing for additional recruitment of circulating monocytes and leads to a greater accumulation of foam cells causing plaque progression²⁶.

As the plaque progresses, the macrophages found within the plaque become less effective at performing some of their key effector functions including efferocytosis causing an accumulation of apoptotic cells^{32,33}. These apoptotic cells can undergo secondary necrosis which contributes to the development of necrotic cores^{26,34}. Necrotic cores are an acellular area of the plaque and contain cellular debris and cholesterol crystals which

promote inflammation. Necrotic cores are a marker of non-stable plaques³⁴. In addition to necrotic cores, proliferating smooth muscle cells secrete metalloproteinases which degrades the collagen found in the extracellular space, rendering the plaque unstable³.

It is important to note the differences between the pathogenesis of atherosclerosis between mouse and humans. First, in humans, medium and large arteries undergo diffuse intimal thickening (DIT). This process begins at a very early age. The first evidence of DIT has been demonstrated in arteries of humans aged as young as two years old^{35,36}. During DIT, smooth muscle cells secrete proteoglycans³⁷ which enhances lipoprotein retention in the vessel wall. Atherosclerosis will not develop in arteries that do not exhibit DIT³⁵. Notably, DIT does not occur in mice. In addition, lipoprotein profiles greatly differ between mice and humans³⁸. As mice do not express CETP, they hold the majority of their cholesterol in high-density lipoproteins (HDL) since they are unable to exchange cholesterol to other apoB lipoproteins such as very low-density lipoprotein (VLDL) or LDL. In contrast, humans hold most of their cholesterol in larger particles such as LDL³⁹. Therefore, mice do not readily develop atherosclerosis without alterations to the levels of apoB containing lipoproteins. Most popular mouse models to induce atherosclerosis consist of apolipoprotein E (ApoE) or LDL receptor (LDLR) knockout mice which lead to a significant increase in VLDL or LDL cholesterol respectively³⁸. A new model was recently introduced which doesn't require genetic deletion. Injections of a gain-of-function PCSK9 adeno-associated virus targeted for the liver promoted the development of atherosclerosis in wildtype mice. This mouse model more closely mimics the LDLR knockout mouse as the expression of this gain-of-function PSCK9 promotes the

degradation of LDLR from the liver and causes a sustained increase in circulating LDL cholesterol⁴⁰.

1.1.1.1 Foam Cell Formation

Intracellular cholesterol concentration is a tightly regulated process, through the INSIG1/SCAP/SREBP2 signalling cascade^{41,42}. Located at the endoplasmic reticulum (ER), where cholesterol concentration in the cells is at the lowest, the INSIG/SCAP/SREBP2 protein complex sense the cholesterol levels in the ER membranes⁴³. Under low intracellular cholesterol concentration, INSIG dissociates from SCAP/SREBP2 allowing for the complex to translocate from the ER membrane to the Golgi^{44,45}. The Golgi contains two proteases, S1P⁴⁶ and S2P⁴⁷, which can cleave SREBP2 thereby releasing SREBP2 from the membrane. Cleaved SREBP2 can then act as a transcription factor and induce the expression of genes involved in cholesterol uptake such as LDLR and cholesterol synthesis⁴⁸. In addition, SREBP2 can repress the expression of genes implicated in the efflux of cholesterol out of cells such as ABCA1 and ABCG1⁴⁹.

On the other hand, when cells have excess cholesterol, INSIG1 anchors the SCAP/SREBP2 complex at the ER preventing SREBP2 cleavage and its activation as a transcription factor. Excess cholesterol cannot be metabolized and therefore need to be excreted or stored to minimize cytotoxicity⁴¹. Cholesterol can bind to LXR to promote the transcription of genes linked to cholesterol efflux ABCA1 and ABCG1. In addition, cells downregulate the amount of LDLR at their cell surface to decrease LDL uptake⁵⁰.

The uptake of modified forms of LDL found within the intimal layer, unlike native LDL, is not regulated. Found by Brown and Goldstein as receptors which mediates the uptake of modLDL regardless of the levels of cholesterol in the cells⁵¹, scavenger receptors (SRs) are a vast family of receptors that are pattern recognition receptors responsible for the clearance of non-self (ie bacteria) and modified self-targets (modLDL)⁵².

1.1.1.1.1 Macrophage Foam Cells

Macrophages, being professional phagocytes express several of the classes of scavenger receptors. The majority of oxLDL uptake from macrophages is mediated through SR-AI and CD36 (SR class B)⁵³. Other scavenger receptors such as LOX-1 can also facilitate oxLDL uptake in macrophages. In addition, SR-B1 activity can be bidirectional meaning it can both mediate the uptake and excretion of cholesterol in macrophages.⁵⁴

Unlike oxLDL, aggregates of LDL are not internalized via scavenger receptors. In macrophages, the presence of agLDL leads to the formation of “surface-connected compartment” (SCC). SCC are complex invaginations of the plasma membrane where agLDL is sequestered as part of a unique endocytosis pathway termed patocytosis⁵⁵⁻⁵⁷. Recently, the Maxfield laboratory demonstrated that macrophages excrete lysosomal enzymes through lysosomal exocytosis to degrade the agLDL by forming lysosomal synapses around the agLDL. The lysosomal synapse is acidified following the fusion of the lysosome with the plasma membrane allowing for the lysosomal enzymes released to perform their functions. Amongst those enzymes released are LAL and CTSD. CTSD can hydrolyze ApoB while LAL can break down cholesterol esters found within the LDL

particle releasing free cholesterol^{23,58-60}. This free cholesterol is available for uptake by macrophages and neighbouring cells⁶¹.

Lastly, macrophages uptake cholesterol via micropinocytosis allowing for the internalization of native LDL and oxLDL⁶². It is unclear at this time if micropinocytosis contributes to agLDL internalization or if agLDL internalization occurs solely through pinocytosis.

1.1.1.1.2 Smooth Muscle Cells Foam Cells

Although the first reports of smooth muscle cells containing high amounts of intracellular lipid droplets were reported back in the 1960⁶³, the importance of their contribution to the foam cell population hasn't gained importance until recently. In both human and mouse smooth muscle cells, the accumulation of intracellular lipid droplets leads to a significant decrease in traditional smooth muscle cell markers including α SMA, MYH11, CNN1, TPM1 and begin expressing traditional macrophages markers such as CD68, Mac2/LGALS3^{30,31,64-66} which made their identification nearly impossible. Lineage tracing studies of smooth muscle cells revealed not only that arterial smooth muscle cells undergo phenotypic dedifferentiation in the plaque to promote smooth muscle cell proliferation, but that the majority of foam cells in the plaque were from vascular origins^{28,30,67}. In 2019, Wang et al. found that the pan-leukocyte marker, CD45, was not expressed in smooth muscle cell-derived foam cells. By using CD45 in conjunction with a lipid marker such as BODIPY now allows for the identification of the origin of foam cells without the need of a lineage tracing mouse models²⁸.

The internalization of cholesterol by smooth muscle cells is not as well described as in macrophages. Naïve smooth muscle cells do not express nearly as much scavenger receptors as macrophages and seem to require a priming event to promote cholesterol uptake⁶⁸. Pretreatment of smooth muscle cells with enzymatically modified LDL can upregulate LOX-1 expression⁶⁹. In addition, IL-1 β has been demonstrated to upregulate LDLR and promote lipid uptake in smooth muscle cells regardless of intracellular cholesterol concentration⁷⁰. Furthermore, exposure to oxLDL increases the expression of LOX-1, thereby enhancing oxLDL uptake⁷¹. Llorente-Cortes and al. showed agLDL uptake by smooth muscle cells is mediated by LRP1^{72,73}. The scavenger receptors SR-A and CD36 are poorly expressed in SMC^{68,74}.

We cannot ignore the potential crosstalk between macrophages and smooth muscle cells and how these interactions may promote smooth muscle cell foam cell development and vice versa. Co-culture of macrophages with smooth muscle cells⁷⁵ or the incubation of macrophage conditioned media⁷⁶⁻⁷⁹ increases the cholesterol content in smooth muscle cells treated with modLDL suggesting that macrophages secrete into the medium factors required for the smooth muscle cells to uptake modLDL. Alternatively, macrophages may digest some of the modLDL extracellularly causing the release of free cholesterol which could be taken up by nearby smooth muscle cells. In addition, it was suggested that macrophages release lipid droplets as a form of cholesterol efflux and that smooth muscle cells could uptake these lipid droplets.⁸⁰⁻⁸²

1.1.1.1.3 The Fate of the Cholesterol

After the modLDL enters the cells via endocytosis, it will make its way through the endocytic pathway⁸³ with the final destination being the lysosome. Inside the lysosome, proteases will degrade the apoB proteins while LAL will hydrolyze cholesterol esters into free cholesterol and free fatty acids^{84,85}. The free cholesterol is exported out of the lysosome through the coordinated actions of NPC1 and NPC2 transporters⁸⁶. The free cholesterol can be shuttled to the plasma membrane for efflux out of the cells via ABCA1 or ABCG1 and be transferred onto lipid poor apoA-I or HDL respectively⁸⁷. With the unregulated uptake of cholesterol through the scavenger receptors, the high quantity of free cholesterol quickly leads to the efflux capacity of the cells to become overloaded, and the excess free cholesterol must be re-esterified to minimize cytotoxicity⁸⁸. The excess free cholesterol is shuttled to the ER where ACAT will re-esterify the free cholesterol to a fatty acid and cholesterol esters will be incorporated into lipid droplets⁸⁹.

1.1.2 Atherosclerosis Regression

Atherosclerosis regression can be characterized by a reduction in plaque size, lipid content, macrophages, inflammation, or necrotic area and/or an increase in collagen deposition or fibrous cap thickness^{90,91}. Evidence of atherosclerosis regression in humans has been documented dating as far back as 1920⁹². It wasn't until the late 1950 with a re-emergence in early 1970 that the first animal models of atherosclerosis regression were developed and demonstrated a reduction plaque size and complexity in rabbits⁹³, swine^{94,95} and non-human primates⁹⁶⁻⁹⁹. These preclinical models of atherosclerosis

regression included the removal of excess cholesterol from the diet or infusions with liposomes to promote cholesterol efflux.

With the introduction of cholesterol-lowering therapies, plaque progression has been significantly reduced^{100,101}, and in some instances, although very modest, plaque size reduction has been observed¹⁰²⁻¹⁰⁶. While the reduction in plaque size is modest, there is a reduction in major adverse cardiovascular events (MACE), but ultimately these therapies do not fully resolve the disease^{12,107}. Two key processes which have been proven to promote regression are: 1) resolution of residual inflammation 2) reverse cholesterol transport⁹¹.

1.1.2.1 Resolution of Residual Inflammation

Atherosclerosis is not only a disease of hypercholesterolemia, but also of chronic sterile inflammation¹⁰⁸. Although the use of statins greatly reduced the occurrence of MACE¹⁰⁹, patients with high residual inflammation are at a greater risk^{110,111}. In 2017, the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) trial using monoclonal antibodies targeting IL-1 β demonstrated that a reduction in systemic inflammation, measured by a decrease in high-sensitivity C-reactive protein, lowered the risks of myocardial infarction, stroke and death in patients with high residual inflammation^{112,113}.

In mice, a shift from the pro-inflammatory phenotype towards a pro-resolving phenotype in macrophages is linked to plaque regression¹¹⁴. Pro-resolving macrophages have better efferocytic capacity¹¹⁵. The conversion of Ly6C^{hi} monocytes into pro-resolving macrophages via STAT-6 signalling is required to mitigate the inflammation and promote

regression¹¹⁶. The loss of PRMT2 in macrophages promoted inflammation and reduced regression¹¹⁷. Overall, a reduction in inflammation in macrophages is required to induce plaque regression.

1.1.2.2 Reverse Cholesterol Transport

Briefly, the process of reverse cholesterol transport starts with the removal of intracellular cholesterol from the cells to a circulating cholesterol acceptor followed by delivery to the liver for excretion¹¹⁸. The rate-limiting step in this process is the removal of intracellular cholesterol from the peripheral cells¹¹⁹. Increased cholesterol efflux capacity has been shown to be inversely correlated with coronary artery disease, regardless of HDL cholesterol levels highlighting the importance of cellular cholesterol metabolism in reverse cholesterol transport¹²⁰.

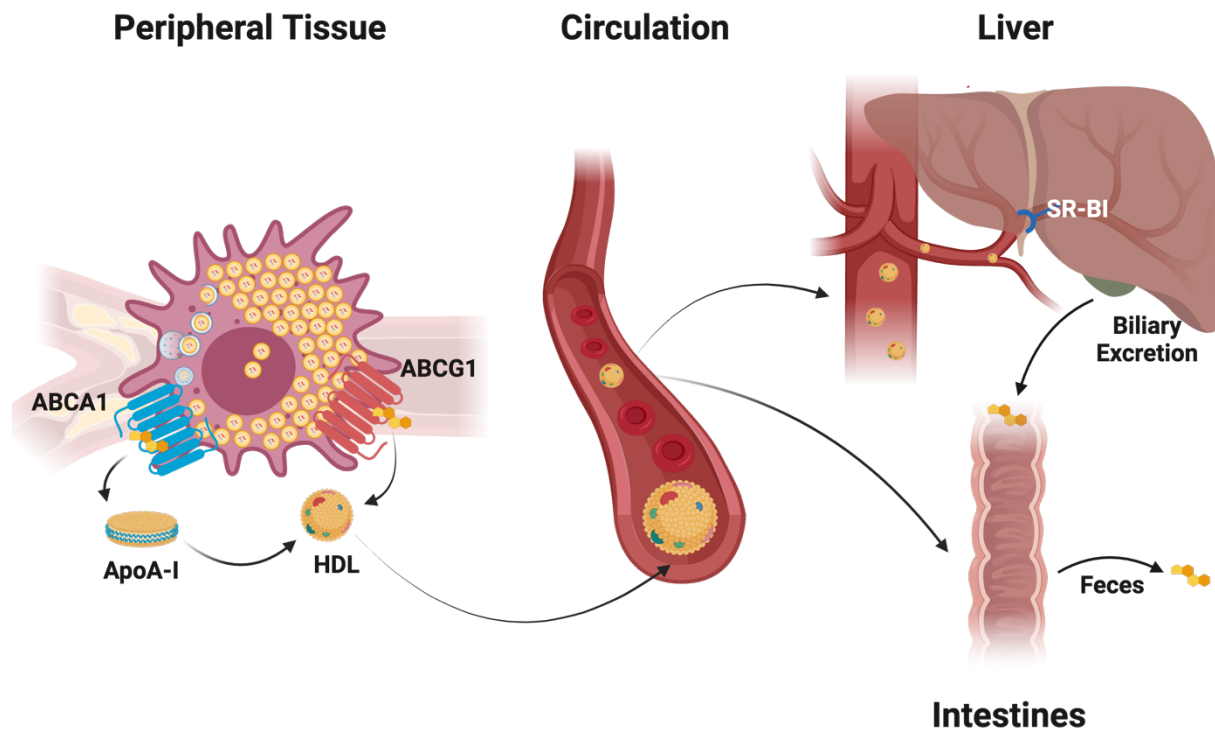


Figure 1-2: Overview of Reverse Cholesterol Transport

Briefly during reverse cholesterol transport, cholesterol is hydrolyzed from the lipid droplets and is transported to HDL particles by cholesterol transporters ABCA1 and ABCG1. The HDL particle will enter the circulation and be transported to the liver or the intestines for excretion in the feces. Figure created in BioRender.

1.1.2.2.1 Mechanisms of Cholesterol Hydrolysis and Efflux

As previously discussed, excess cholesterol is rapidly esterified and stored into lipid droplets. The first step in promoting cholesterol efflux out of cells is the hydrolysis of the cholesterol esters back into free cholesterol where it can be transported to the plasma membrane. The hydrolysis of lipid droplets can occur in two different pathways: neutral and acidic lipolysis. During neutral lipolysis, neutral lipases including NCEH1¹²¹⁻¹²³, LIPE^{124,125} and CEH¹²⁶ promote the hydrolysis of cholesterol esters into free cholesterol within the cytoplasm. On the other hand, acidic lipolysis occurs in the lysosome. Delivery

of lipid droplets to the lysosome ensues via selective autophagy, or lipophagy^{127,128}, discussed in more detail below.

The mechanism by which free cholesterol is transported from the lysosome or the surface of the lipid droplets to the cholesterol transporters ABCA1 and ABCG1 is still an active area of investigation. Due to the lack of solubility of free cholesterol in aqueous solution, free cholesterol is either moved between membranes by vesicular transport or through the binding of carrier proteins. Carrier proteins such as the oxysterol binding proteins (ORPs) have been implicated in contributing to cholesterol efflux. ORP1S has been demonstrated to play a role in controlling the movement of cholesterol from the late endosome/lysosome to the plasma membrane thereby increasing cholesterol efflux to ApoA-I¹²⁹ while ORP2 can promote efflux to both HDL and ApoA-I¹³⁰.

Although depicted as being at the plasma membrane, ABCA1 and ABCG1 can be shuttled to the endocytic compartment to promote their function and interactions with various cholesterol pools. Endocytosis of ABCA1/apoA-I complexes can bind free cholesterol found within the late endosome/lysosomal compartment before being transported back to the cells surface in a process called retroendocytosis¹³¹⁻¹³³. This mechanism is key to the creation of nascent HDL particles from lipid poor apoA-I¹³³. These nascent HDL particles can be further lipidated by interactions with ABCG1⁸⁷. ABCG1 has been found both at the plasma membrane¹³⁴⁻¹³⁶ within lipid rafts, but also at the Golgi and the endosome¹³⁷. ABCG1 present within the endosomes promotes the incorporation of cholesterol in the inner phospholipid leaflet of the endosome which becomes part of the outer phospholipid leaflet following the endosome's fusion with the plasma membrane^{137,138}. ABCG1 is

capable of cholesterol efflux to all kinds of lipidated HDL particles, but not lipid poor ApoA-I^{136,138}. In addition, SR-B1 can bind HDL to mediate efflux of free cholesterol found in the nearby plasma membrane. Lastly, passive diffusion to HDL is another method of cholesterol efflux¹³⁹.

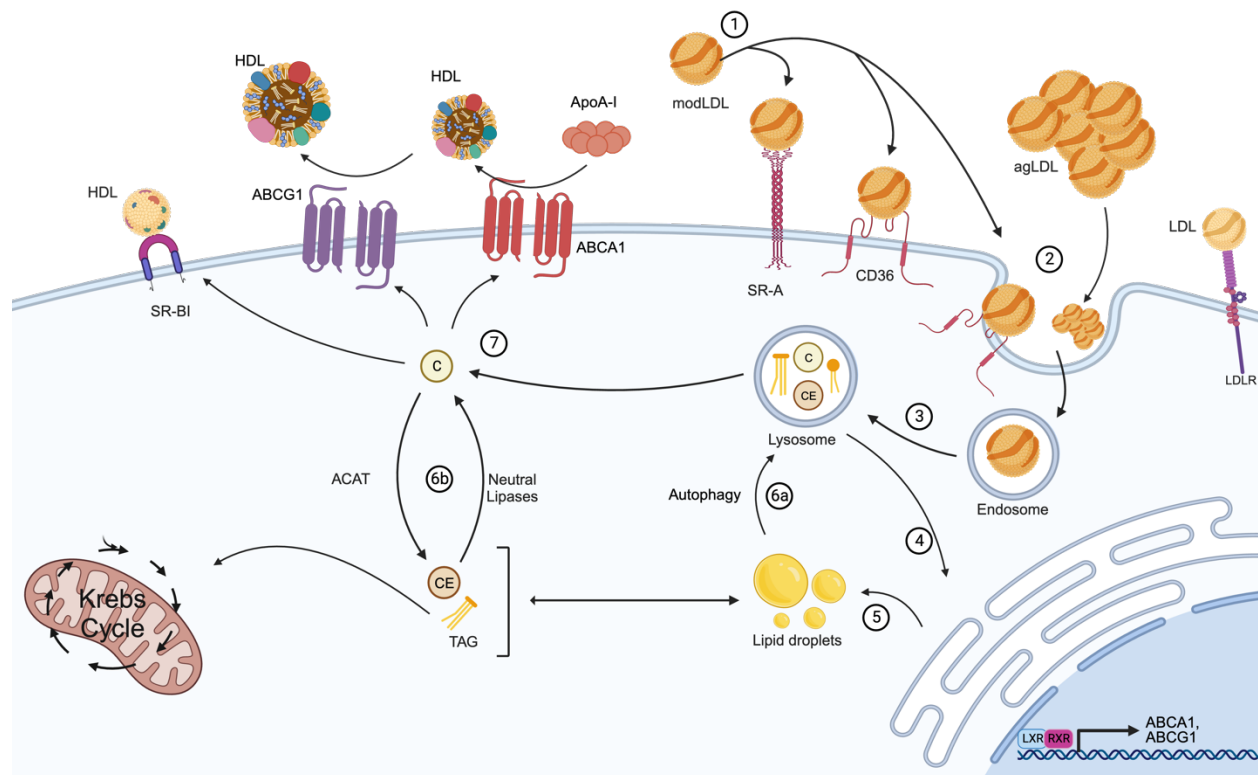


Figure 1-3: Mechanism of Lipid Uptake and Cholesterol Efflux

Modified lipids such as oxLDL binds to scavenger receptors at the cells surface (1). Modified lipoproteins undergo receptor-mediated endocytosis (2). Following endocytosis, the lipoprotein will end in the lysosome where it will be degraded into free cholesterol (3) and will either be shuttled to the endoplasmic reticulum (4) or to cholesterol transporter (7) for cholesterol efflux. The excess free cholesterol at the ER will be re-esterified into lipid droplets (5). Lipid droplets can be hydrolyzed by neutral lipases (6b) or via autophagy (6a) to generate free cholesterol for efflux (7). Figure created in BioRender.

1.1.2.2.2 Capacity for Cholesterol Efflux of Macrophages and Smooth Muscle Cells

Both macrophages foam cells and smooth muscle cells foam cells express the cholesterol transporters ABCA1 and ABCG1, albeit at different levels and therefore their capacity to

perform cholesterol efflux greatly differs^{140,141}. We recently demonstrated that macrophage foam cells had higher ABCA1 and ABCG1 expression resulting in significantly higher levels of cholesterol efflux as compared to smooth muscle cells foam cells¹⁴⁰. Expression of ABCA1 is decreased in intimal smooth muscle cells and fail to properly bind apoA-I to produce HDL unlike medial smooth muscle cells¹⁴² suggesting impaired cholesterol efflux in the smooth muscle cells of the plaque. The kinetic analysis of cholesterol efflux to apoA-I is very slow in smooth muscle cells as compared to macrophages^{141,143}. Overall, smooth muscle cells foam cells have low capacity to promote cholesterol efflux to apoA-I, but can efflux cholesterol to HDL^{140,141,144,145}. Contrasting with smooth muscle cells, macrophages rely heavily on ABCA1 mediated cholesterol efflux to apoA-I¹⁴⁶. While macrophages express SR-B1, cholesterol efflux in vivo is mediated through ABCA1 and ABCG1¹⁴⁷. One could speculate that apoA-I mediated cholesterol efflux from macrophages is necessary for nearby smooth muscle cells to have access to HDL particles to efflux cholesterol via ABCG1.

1.1.2.2.3 Cholesterol Transport and Excretion

Once the free cholesterol has been transferred onto HDL, the particle will leave the tissue, in this case the plaque, via the lymphatic system followed by its re-entry into the circulation.^{148 149,150} Without proper lymphatic circulation returning HDL to the blood, reverse cholesterol transport is greatly reduced¹⁵⁰.

In the circulation, once the free cholesterol has been transferred onto nascent HDL by ABCA1, the free cholesterol is esterified by LCAT where it will build the hydrophobic core of the mature spherical HDL particle called HDL₃. Accumulation of free cholesterol onto

HDL₃ promotes its growth via the esterification of the free cholesterol into HDL₂ particles. In addition, HDL₃ can exchange cholesterol for triglycerides with HDL₂ via the action of CETP. Both HDL₂ and HDL₃ can exchange cholesterol for triglycerides with circulating VLDL or LDL¹⁵¹. Finally, cholesterol esters can be taken up by interaction between HDL and SR-B1 at the surface of the liver or through the internalization of LDL and VLDL via the hepatic LDLR¹¹⁸.

Once in the liver, excess cholesterol can bind to bile acids for excretion through the feces¹⁵². More recently, a new mechanism of cholesterol excretion called transintestinal cholesterol efflux (TICE) where VLDL or LDL, but not HDL¹⁵³ transfers cholesterol to the enterocytes for excretion in the feces^{154,155}. The mechanisms behind TICE are not well described yet¹⁵⁶.

1.1.2.2.4 ApoA-I and HDL dysfunction

During atherosclerosis, there is evidence that the function and trafficking of apoA-I and HDL are dysfunctional. Prolonged high-fat diet feeding¹⁵⁷, a reduction in LDLR¹⁵⁸ or hypercholesterolemia¹⁵⁹ can lead to lymphatic vessels function impairment thereby preventing HDL particles from leaving the atheroma and enter the circulation.

The profile of the cholesterol acceptors in the plaque greatly differs from the one in the circulation. Although the content of apoA-I in human plaque is more than 100-time fold higher than in the circulation, it is highly oxidated and therefore dysfunctional¹⁶⁰. Similar to native LDL, HDL can undergo modification due to the harsh environment of the plaque. Oxidation of HDL particles severely impede its capacity to accept cholesterol and mediate efflux^{161,162}. Furthermore, modification of apoA-I/HDL can promote its entrapment in the

extracellular matrix further reducing cholesterol efflux out of the plaque¹⁶⁰. In addition, oxidized HDL has pro-inflammatory properties promoting NF- κ B activation and an increase in adhesion molecules expression¹⁶³⁻¹⁶⁵.

Of interest, autophagy, can promote cholesterol efflux and mitigate inflammation therefore modulating autophagy may be of relevance in the treatment of atherosclerosis.

1.2 Autophagy

Autophagy is a highly conserved constitutive homeostatic process which promotes degradation of intracellular components via the lysosome. In 1955, Christian de Duve discovered the presence of acidic compartment in the cells using ultracentrifugation mediated cell fractionation and named these vacuoles lysosomes^{166,167}. A few years later, he coined the term autophagy for self-eat and heterophagy for eating other when referring to lysosomal functions¹⁶⁸. Although the notion of autophagic degradation had been described in 1955, it was very difficult to study autophagy, with the most reliable method being electron microscopy. It took almost 40 years for the first autophagy genes (ATGs) to be identified in yeast^{169,170}. Thus far, there has been 40 core ATG¹⁷¹ identified conserved across species. Basally, under homeostatic condition, autophagy promotes the degradation of long-lived proteins and organelles¹⁷².

The process of autophagy can be further subdivided as a whole according to the mode of delivery of the cargo to the lysosome: Macroautophagy, microautophagy and chaperone mediated autophagy¹⁷³. During macroautophagy, the cargo is sequestered by an autophagosome before delivery to the lysosome¹⁷⁴. Direct engulfment of the cargo by the lysosome is microautophagy^{175,176}. During chaperone-mediated autophagy, a specific

peptide sequence is recognized on the protein by chaperones with allows for lysosomal translocation^{177,178}.

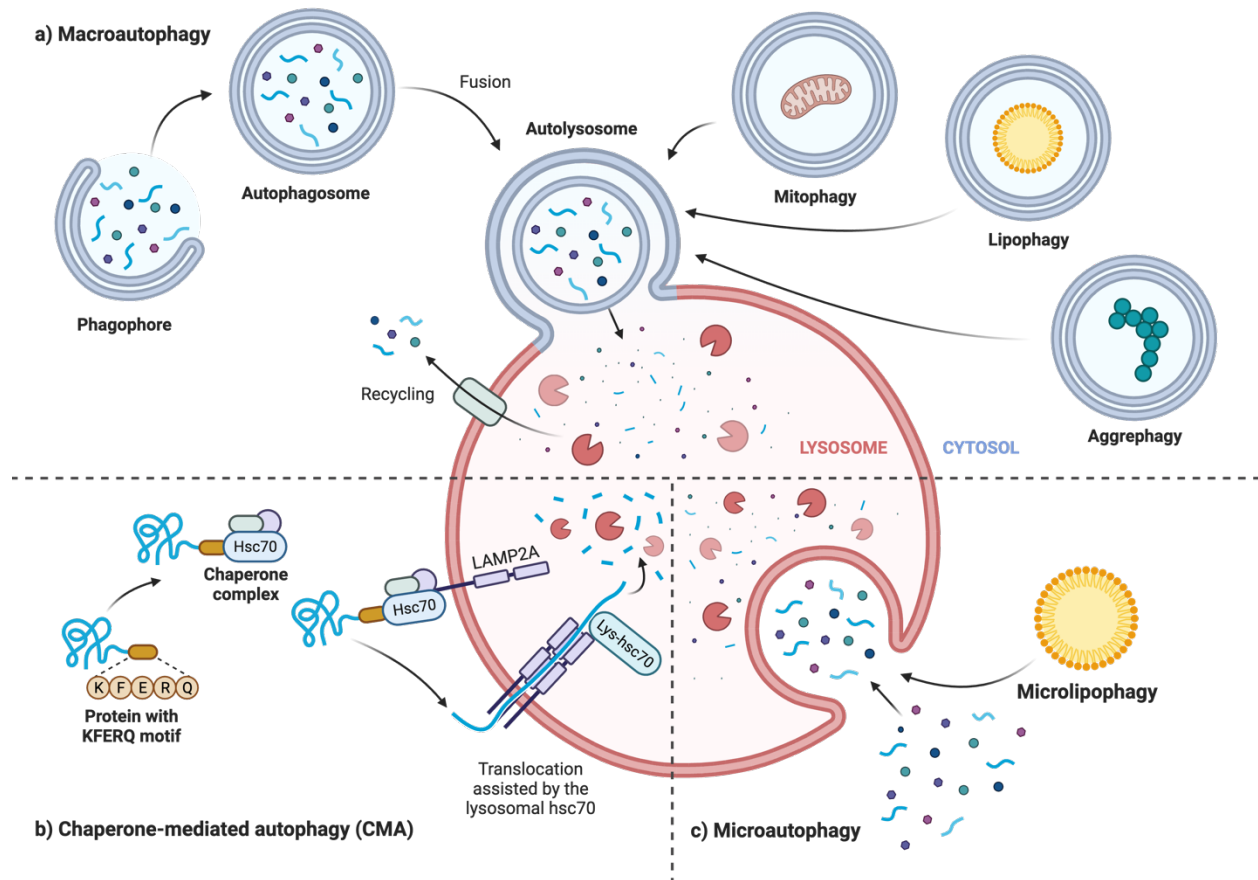


Figure 1-4: Autophagy Pathways

Schematic of the three main autophagy pathways (A) macroautophagy, (B) chaperone-mediated autophagy and (C) microautophagy. Figure created with BioRender.

1.3 Macroautophagy

Macroautophagy (hereby referred to as autophagy) is the form of autophagy that has been characterized the most so far. Briefly, during autophagy, a cargo (bulk or specific) is engulfed by a double phospholipid bilayer membrane called autophagosome^{174,179}. Following the cargo's complete sequestration, the autophagosome outer membrane will fuse with a nearby lysosome forming an autolysosome where the hydrolytic enzymes

present within the lysosomal lumen will degrade the content¹⁸⁰⁻¹⁸². The process of autophagosome formation is complex and can be subdivided in four key steps: initiation, nucleation, expansion, and fusion.

1.3.1.1 Autophagosome Formation and Lysosomal Fusion

1.3.1.1.1 Initiation

The ULK1 complex is comprised of ULK1, ATG13, FIP200 and ATG101¹⁸³. Its activity is under the control of mTORC1 mediated phosphorylation¹⁸⁴. When mTORC1 is active and bound to the complex, ULK1 and ATG13 are phosphorylated rendering the complex inactive^{183,185,186}. Upon mTORC1 inactivation, mTORC1 will dissociate from ULK1. The ULK1 complex becomes dephosphorylated, but the phosphatase required is still unknown. Dephosphorylated ULK1 can autophosphorylate itself to enhance its own kinase activity¹⁸⁷. ULK1 can phosphorylate FIP200 and ATG13 allowing for a functional ULK complex¹⁸⁶.

1.3.1.1.2 Nucleation

Following the activation of the ULK1 complex, the nucleation of the phagophore is initiated. The ULK1 complex relocates to the phagophore assembly site (PAS) at the ER¹⁸⁸⁻¹⁹⁰. The ULK1 complex binds and phosphorylates the class III phosphatidylinositol 3-kinase I (PI3KC3) comprised of VPS34, BECN1, p115, and ATG14^{191,192}. The active PI3KC3 complex I produces phosphatidylinositol 3-phosphate (PI3P). The generation of PI3P at the PAS drives the remodeling of the ER membrane to form the omegasome.¹⁹³ The formation of the omegasome promotes the recruitment and binding of WIPI2 to

PI3Ps. Lastly, WIPI2 recruits the autophagosome conjugation system of ATG5/ATG12/ATG16L1 complex through its interactions with ATG16L1.^{194,195}

1.3.1.1.3 Expansion

The expansion of the autophagosome is mediated through a sequence of two ubiquitin-like reaction with the ubiquitin-like substrates ATG12 and ATG8 family¹⁷⁴. In mammalian cells, the ATG8 homologs are a family comprised of LC3 (LC3A, LC3B, LC3C) and GABARAPS (GABARAP, GABARAPL1, GABARAPL2 and GABARAPL3). Both LC3 and GABARAPs are required during autophagosome formation, and both undergo lipidation. The LC3 family is required during the initial autophagosome formation while the GABARAP family is required at a later stage¹⁹⁶. The first ubiquitin reaction allows for the creation of the ATG5/ATG12 complex. First, the ubiquitin-like ATG12 is activated by the E1-like enzyme ATG7 and is then transferred onto the E2-like enzyme ATG10. Next, ATG12 is covalently bonded to ATG5 forming the ATG5/ATG12 complex¹⁹⁷. The ATG5/ATG12 complex binds ATG16L1¹⁹⁸ to form a bigger complex and is recruited to the PAS via the interaction of ATG16L1 with WIPI2¹⁹⁹. The second ubiquitin-like reaction involves the ubiquitin substrate LC3/GABARAP (hereby referred to as LC3 only for simplicity). LC3 is first produced as pro-LC3 which is cleaved into LC3 by ATG4. LC3 is activated by ATG7 and transferred onto the E2-like enzyme ATG3. The ATG5/ATG12/ATG16L1 complex functions as the E3-like enzyme in this secondary ubiquitin-like reaction allowing for the conjugation of LC3 to a phosphatidylethanolamine (PE) found at the surface of nearby membranes. This conjugation of LC3 to PE will

continue to occur until the autophagosome is completed^{200,201}. Upon completion, the ATG5/ATG12/ATG16L1 complex will dissociate from the autophagosome¹⁹⁶.

Needless to say, phagophore expansion requires an enormous quantity of phospholipids/membranes to fully form. It has been estimated that for the generation of a 400 nm autophagosome, over 3 000 000 lipids need to be delivered to PAS¹⁹¹. Although some phospholipid synthesis occurs at the PAS²⁰²⁻²⁰⁴, the cells cannot rapidly generate enough phospholipids to sustain autophagosome formation. Instead, the cell re-routes membranes from other organelles to produce autophagosomes. This intricate mechanism is far from being fully elucidated. ATG9 is one of the only transmembrane proteins of the nascent autophagosome and is required for phagophore expansion. ATG9 is thought to mediate the trafficking of donor membrane to the autophagosome²⁰⁵. ATG9 can be found in the membranes of the Golgi, endosomes as well as small vesicles clusters with the latter serving as a potential source of membranes²⁰⁶⁻²⁰⁸. Although ATG9 is membrane bound, it is not present within the autophagosome itself²⁰⁸. Additionally, lipid droplets can be used as a source of lipids for autophagosome formation²⁰⁹ in part through the action PNPLA5²¹⁰. Other membranes are thought to come from COPII vesicles²¹¹, the outer mitochondrial membrane²¹², plasma membrane²¹³ and ER-mitochondria contact sites²¹⁴. Lastly, carrier proteins can serve to transport lipids to the PAS. Thus far, three proteins serving as lipid carrier proteins have been demonstrated to be involved in autophagosome formation: GRAMD1A^{215,216}, VPS13A²¹⁷ and ATG2A²¹⁸.

Once the autophagosome membrane have been elongated, membrane scission²¹⁹ will allow for the closure of the autophagosome. This process is mediated by the ESCRT

machinery. First, through an unknown mechanism, ESCRT-I is recruited to the membrane which allows for the recruitment of the ESCRT-III where both complexes will mediate membrane scission^{191,220,221}.

1.3.1.1.4 Fusion

Fusion between the autophagosomes and the lysosome occurs with the help of tethering complexes and SNARE proteins. RAB7 found at the surface of the lysosomes recruits effector protein PLEKHM1 found on the autophagosome²²². The tethering complex HOPS is recruited to promote the interaction of the SNARE complex. The SNARE complex required for the fusion is comprised of STX17 and SNAP29 on the autophagosome and VAMP8 on the lysosome^{223,224}. Lastly, ATG14 binds to STX17 and stabilizes the interaction with the SNARE protein on the lysosome SNAP29 to promote the fusion²²⁵.

After complete fusion between the autophagosome outer membrane and the lysosome, the autolysosome will start acidifying itself²²⁶ in order to promote lysis of the cargo. First, lysosomal enzymes must degrade the inner autophagosome membrane before being able to access the engulfed cytoplasmic content.

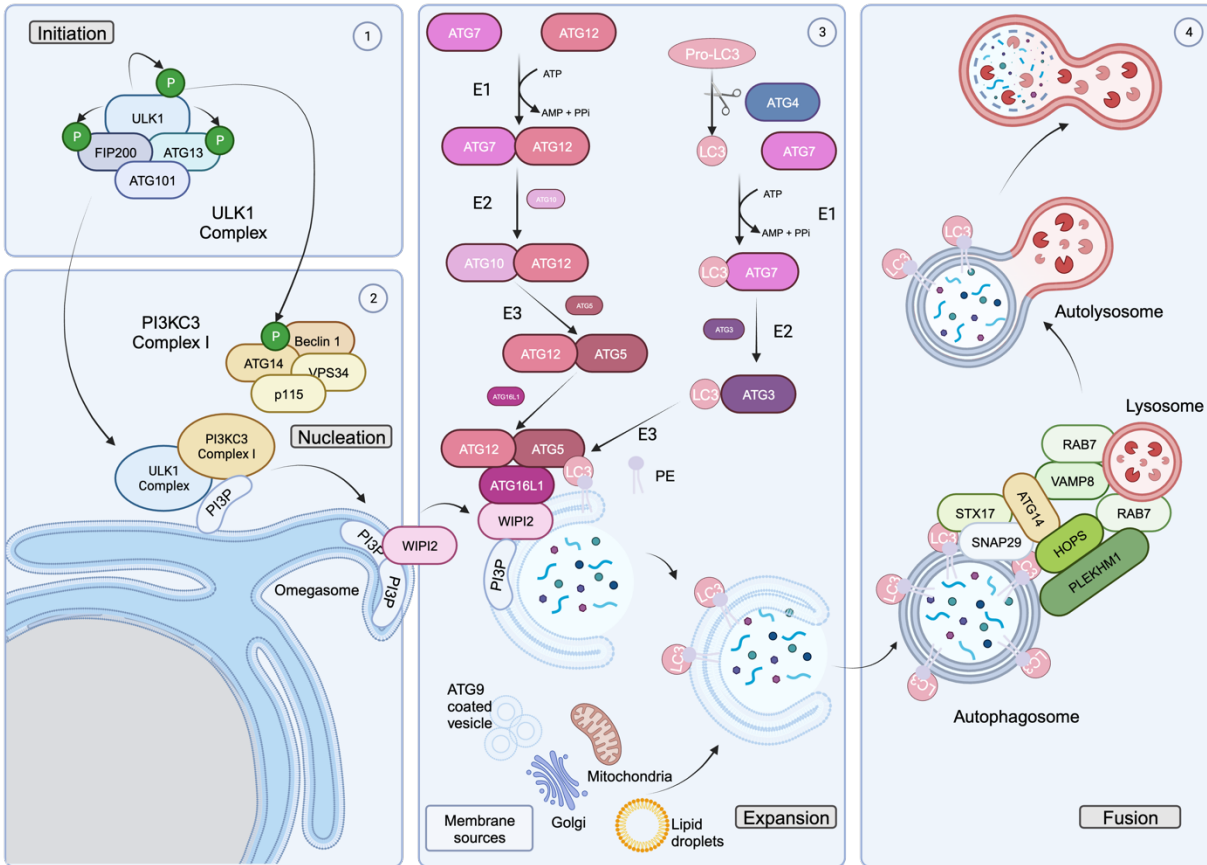


Figure 1-5: Mechanism of Macroautophagy

Schematic of autophagosome formation during macroautophagy. Figure created with BioRender.

1.3.1.2 Bulk or Selective Autophagy

To add to the complexity of this process, autophagy can occur in two different scenarios. The first being nonselective, or bulk autophagy and the second being selective autophagy. During bulk autophagy, the autophagosome forms in close proximity to the ER and the content of the autophagosome is nonselective containing random cytoplasmic content. Bulk autophagy is induced by cellular stressors such as starvation^{173,179}. On the other hand, selective autophagy occurs when a specific cargo is enriched within the autophagosome for degradation. Thus far, selective autophagy has been shown to

promote the degradation of damaged organelles, bacteria, viruses, proteins, inflammasome, glycogen and ferritin^{174,222,227-230}.

1.3.1.2.1 mTOR Signaling

Central to the regulation of bulk autophagy is mTOR. Functionally, mTOR is a serine/threonine kinase that is part of a complex which includes one of two effector proteins, RAPTOR or RICTOR, allowing for the formation of mTORC1 or mTORC2 respectively²³¹. mTOR sit at the nexus of metabolism controlling the switch between anabolism and catabolism¹⁸⁴.

Because of its high sensitivity to rapamycin, mTORC1 is the most extensively characterized of the mTOR complexes²³².¹⁸⁴ Activated mTORC1 promotes anabolic processes such as lipid and protein synthesis, ribosome biogenesis, anaerobic glycolysis while inhibiting catabolic processes including autophagy and lipid oxidation²³³. Additionally, active mTORC1 can promote proliferation. As a kinase, mTOR phosphorylates proteins involved in the activation of autophagy such as the ULK1 complex and TFEB preventing their activity. Under cellular stressors such as ER stress, hypoxia, low levels of amino acids, ATP or growth factors, mTORC1 activity is inhibited allowing for the activation of autophagy and the repression of anabolic processes^{184,231}.

Due to its central role in regulating metabolism in the cell, regulation of mTORC1 activity is quite complex. To name a few, mTORC1 can be activated via the phosphoinositide-3-kinase (PI3K) pathway and AKT upon growth factor stimulation. Low ratios of ATP:ADP or hypoxia can promote AMPK activation which in turns phosphorylates mTORC1 to inactivate it²³⁴. Lastly, amino acid levels can directly act on the complex itself through Rag

GTPase to influence its cellular sub-localization; high concentration of amino acid will cause mTORC1 to localize to the lysosome²³⁵. Activated mTORC1 can be found at the surface of the lysosome²³⁶.

Like its companion protein indicates, mTORC2 is insensitive to rapamycin inhibition and therefore its cellular functions are poorly described. Controlling mTORC2 activation is the insulin and PI3K pathway. Unlike mTORC1, mTORC2 does not localize at the lysosome. Instead, mTORC2 localizes at the ER and the mitochondrial membranes and their contact sites²³⁶. Active mTORC2 promotes cytoskeletal reorganization and cell migration along with survival through in part by activating ATK.

1.3.1.2.2 Selective Autophagy Mechanisms

Unlike bulk autophagy, selective autophagy can occur independently of mTORC1 inhibition where the cargo receptor themselves can activate the ULK complex to initiate autophagy at the cargo's location²²⁹. What triggers selective autophagy is dependent on the cargo to be degraded and can range from organelle damage to anti-inflammatory stimulus²²⁷⁻²²⁹.

The specificity of selective autophagy lies with the binding of selective autophagy receptors (SAR) to the cargo to be degraded. In order for a protein to be considered a SAR, it has to contain a LC3-interacting region (LIR) motif (Trp/Phe/Tyr-x-x-Leu/Ile/Val)²²⁷. This LIR motif can bind to LC3 at the surface of the nascent autophagosome providing a bridge between the autophagosome and the specific cargo to be engulfed²²⁹.

The tagging of the cargo to be degraded can be ubiquitin dependent or independent. In the case of ubiquitin dependent selective autophagy, SARs will typically, in addition to their LIR motif, contain a ubiquitin-binding domain able to recognize mono or polyubiquitin chains. Usually, K63 polyubiquitin chain targets cargo for autophagy while K48 polyubiquitin classically target the protein for proteasomal degradation instead^{228,237,238}. The most frequent SARs which binds ubiquitinated cargoes are comprised of the family of soluble SAR named the Sequestome-1-like receptors. This family of receptors include SQSTM1/p62, NBR1, CALCOCO2, TAX1BP1, TOLLIP and OPTN. Other, less known SARs include the tripartite motif (TRIM) family²²⁹.

Ubiquitin independent SARs can be soluble, or membrane bound. The soluble SARs are the receptors for ferritinophagy, glycophagy and ribophagy while membrane bound SARs are for mitophagy and ribophagy²²⁹.

Furthermore, autophagosome initiation will not occur at the ER like with bulk autophagy, but where the cargo is located²³⁰. Some SARs have the ability to not only bind LC3, but also components of the initiation complex of the autophagosome. The recruitment of the ULK1 complex at the cargo can be mediated through interactions between FIP200 and CALCOCO2^{230,239}, p62²⁴⁰⁻²⁴², OPTN^{230,243}, NBR1²⁴² or TAX1BP1²⁴². The source of the membranes for the phagophore for nucleation and elongation during selective autophagy is unclear. During the early steps of mitophagy, ATG9 structures were found to be recruited at the mitochondria suggesting that ATG9 vesicles may serve as a membrane source for the PAS in selective autophagy²⁴⁴.

1.3.2 Microautophagy

During microautophagy, the cargo is directly engulfed by the lysosome via lysosomal protrusions or invaginations. Due to the nature of the yeast single vacuole as compared to the multitude of lysosomes in mammalian cells, the majority of microautophagy mechanisms which have been elucidated so far are in yeast where the mammalian homologs have not always been observed. To complicate the matter, several of key macroautophagy proteins including ATG, ESCRT and SNARE protein complexes are required microautophagy^{245,246}. In addition, chaperones used in the recognition of the cargo during chaperone mediated autophagy are also required during microautophagy²⁴⁷. Together, due to the complexity of mammalian microautophagy, the process is poorly characterized.

1.3.2.1 Non-selective Microautophagy

Similarly, to macroautophagy, amino acid starvation can trigger the activation of microautophagy in a non-selective manner. Starvation rapidly induces and promotes the degradation of the sequestrome-1-like SARs. It is thought that this facilitates the switch from basal selective autophagy to non-selective autophagy during starvation thereby preventing excessive selective autophagy to occur²⁴⁸.

1.3.2.2 Selective Microautophagy

In mammalian cells, selective microautophagy has been observed for the degradation of mitochondria, ER, DNA, RNA, lysosomes, lipid droplets and proteins²⁴⁶. Of interest,

microautophagy may regulate the degradation of SCAP through interactions with p62 independently of macroautophagy thereby preventing SREBP nuclear translocation²⁴⁹.

1.3.3 Chaperone-Mediated Autophagy

Unlike macroautophagy and microautophagy, chaperone-mediated autophagy can only selectively promote the degradation of proteins. Chaperone-mediated autophagy substrates must contain a specific pentapeptide amino acid sequence, KFERQ¹⁷⁷, in order to be recognized by the chaperone mediated autophagy machinery. The properties of the amino acid present within the pentapeptide sequence are what mediates the interaction with the chaperone HSC70 more than the specific amino acid themselves. Therefore, post-translational modification of an amino acid sequence act as a KFERQ-like sequence while others may prevent or promotes the binding of HSC70²⁵⁰⁻²⁵³. Although HSC70 is the only chaperone known so far to bind the KFERQ sequence, the translocation of protein to the lysosome requires the assembly of other chaperones, referred to as cochaperones, for the process to occur. These cochaperones include CHIP, HSP40 and HOP²⁵⁴. Once the targeted protein is bound by HSC70, the complex can bind to the lysosome via LAMP2A. The unfolded protein is translocated into the lysosome following the assembly of the multimeric complex of LAMP2A²⁵⁵. Proteins which cannot unfold are not able to be translocated by LAMP2A²⁵⁶. The rate of chaperone-mediated autophagy is directly linked to the levels of LAMP2A at the surface of the lysosomal membrane²⁵⁵.

1.3.4 Autophagy in Disease

The presence of autophagosomes in the cells of the atherosclerotic plaque was first described as far back as 1961. The first study describing the role of autophagy in atherosclerosis was in smooth muscle cells where autophagic vacuoles were found in high abundance in the regions of fibrous cap. It was thought that the activation of autophagy in the cap promoted cap stability by enhancing cell survival⁶³. It wasn't until 2012 that the first two studies using macrophage-specific deletion of ATG5, a key protein required for autophagosome elongation, demonstrated that autophagy became defective during atherosclerosis progression^{257,258}. Razani et al. demonstrated that autophagy deficient macrophages had hyperactive NLRP3 inflammasome activation leading to an increase in inflammation and worse atherosclerotic burden²⁵⁷. Simultaneously, Liao et al. showed that autophagy deficiency in macrophages led to increase cell death concomitant with a reduction in efferocytosis leading to an increased atherosclerosis burden²⁵⁸. These two seminal studies clearly demonstrated the importance of functional autophagy in maintaining cellular homeostasis during disease. Moreover, these studies highlighted the multiple key cellular functions which can be altered by autophagy.

In human plaques, LC3 along with ATG16L1 expression has been linked to asymptomatic plaques while LC3 and ATG16L1 expression is remarkably decreased in symptomatic plaques^{259,260}. Moreover, an accumulation of LC3 and p62 in the plaque is correlated with a decrease in ATG14 which mediates autophagosome and lysosome fusion in CD68+ cells. This suggests that the autophagy dysfunction seen in plaque may be due to a

decrease in autophagosome and lysosome fusion leading to autophagosome accumulation.²⁶¹

1.3.4.1 Regulation of Autophagy in Foam Cells

Sirtuins (SIRT) are a protein family of deacetylase which removes the acetylation found on histones, transcription factors and cytoplasmic proteins regulating their transcription, activation and function.²⁶² SIRT1 and SIRT6 are down-regulated in atherosclerotic plaques^{29,263-265}, oxLDL treated macrophages^{263,264} and endothelial cells²⁶⁶. Furthermore, SIRT1 and SIRT6 have been demonstrated to regulate autophagy²⁶³⁻²⁶⁶. SIRT1 can deacetylate ATG5 increasing ATG5 activity²⁶⁵ while SIRT6 deacetylate HMGB1 and BECN1 promoting autophagy^{267,268}. Additionally, SIRT3 is downregulated by oxLDL treatment and can promote mitophagy²⁶⁹. SIRT3 can promote lipophagy through the activation of AMPK²⁷⁰.

TFEB is central to the regulation of autophagy. TFEB controls approximately two thirds of the transcription of genes involved in lysosomal biogenesis and autophagosome formation^{271,272}. Although initially, treatment with atherogenic lipids can activate autophagy through the activation of TFEB, longer exposure desensitizes the cells and TFEB's activity decreases and cells start to exhibit autophagy deficiency²⁷³. In macrophages, overexpression of TFEB or activation of TFEB using trehalose resulted in a significant reduction in atherosclerosis development by the means of autophagy activation as knockout of ATG5 abrogated the beneficial effect of TFEB²⁷⁴. The expression of endothelial TFEB is higher in areas of laminar shear stress and in the atherosclerotic resistant area of the aorta. Moreover, TFEB overexpression in endothelial cells reduced

plaque burden in ApoE^{-/-} mice due to a reduction in monocyte rolling and adhesion to the endothelium²⁷⁵. Lastly, in smooth muscle cells, TFEB controls cell fate. Inhibition of TFEB promoted smooth muscle cells dedifferentiation while overexpression inhibited cell death proliferation and migration, thereby reducing neointima formation and abdominal aneurysms²⁷⁶⁻²⁷⁹.

The modulation of mTORC1 signalling has been used now in the clinic to promote autophagy locally with drug eluting stents²⁸⁰. These stents are coated with rapamycin derivatives, called rapalogs, which works by potently inhibiting mTORC1²⁸¹. The effect of rapamycin on smooth muscle cells is to prevent proliferation²⁸². There is a debate on whether or not this effect is in part due to autophagy or solely relies on an interruption in cell cycle²⁸³. Macrophages isolated from mice fed a high-fat diet had higher levels of mTORC1 activity than macrophages isolated from chow fed mice²⁸⁴. Genetic deletion of RAPTOR in macrophages decreased plaque size and macrophage foam cell accumulation in the LDLR^{-/-}²⁸⁵ and ApoE^{-/-}²⁸⁶ mice. Furthermore, mTORC1 inhibition decreased circulating the chemokine CCL2 thereby reducing the recruitment of monocyte to the plaque²⁸⁵. Conversely, silencing of mTORC2 signalling in macrophages via the ablation of RICTOR promotes atherosclerosis development in ApoE^{-/-} mice. Interestingly, deletion of both RAPTOR and RICTOR leading to complete mTORC1 and mTORC2 inhibition respectively did not change atherosclerosis highlighting their complete opposite role when it comes to the development of atherosclerosis²⁸⁷. Overall, in macrophages, active mTORC1 signalling reduces autophagy and more particularly mitophagy, enhances apoptosis²⁸⁶ and increases monocyte recruitment²⁸⁵ leading to atherosclerosis

development whereas active mTORC2 signalling reduces inflammation and the secretion of IL-1 β and reduces atherosclerosis burden²⁸⁷.

1.3.4.2 Macrophage Autophagy

Macrophages play a critical role in the pathogenesis of atherosclerosis. Although macrophages are not the most abundant cell type of the plaque^{28,29,140}, the prevention of their recruitment abolished plaque development by 90% highlighting their importance during atherogenesis²⁸⁸. Therefore, any impairment in their function can have a great impact in the disease. In macrophages, autophagy sits at the nexus of multiple pathways including inflammation, cholesterol efflux and efferocytosis which, when they go awry, can promote atherogenesis^{128,258,274}.

Treatment of macrophages with modLDL, more notably oxLDL promotes autophagy impairment. The incubation of macrophages with oxLDL leads to an accumulation of polyubiquitinated protein and p62 aggregates while decreasing the autophagosome marker LC3-II^{289,290}. Additionally, oxLDL can induce severe lysosomal damage²⁷³. Moreover, treatment of human macrophages with agLDL promotes the activation of the MAPK pathway thereby reducing ULK1 and inhibiting autophagy²⁹¹.

The autophagy deficiency caused by oxLDL could be attributed to a remarked decreased in the autophagosome-lysosome fusion event. In oxLDL treated macrophages, LC3 and p62 accumulate and co-localize together indicative of autophagosome formation around the cargo, but LC3 and lysosomal marker LAMP2 colocalization does not increase. Additionally, key proteins involved in autophagosome/-lysosome fusion, ATG14 and STX17 are significantly decreased. Overexpression of ATG14 rescues the fusion event

and restores autophagy flux and reduces plaque burden suggesting that atherogenic lipids in macrophages disrupt autophagy flux by preventing fusions event²⁶¹.

1.3.4.2.1 Smooth Muscle Cell Autophagy

Stressors such as excess free cholesterol promotes autophagy in smooth muscle cells and protects against apoptotic cell death via the activation of mitophagy²⁹². Similarly, smooth muscle cells specific knockout of ATG7 in an ApoE^{-/-} (SMC-ATG7^{-/-}:ApoE^{-/-}) display an increase in mitochondrial ROS and apoptosis suggesting that functional mitophagy in smooth muscle cells protects against excessive cell death²⁹³. SMC-ATG7^{-/-}:ApoE^{-/-} fed a Western Diet displays bigger and more unstable plaques. Autophagy deficiency reduced cellular proliferation, increased cell death and induced cellular senescence^{283,293,294}. In addition, SMC-ATG7^{-/-}:ApoE^{-/-} spontaneously develop aortic aneurysms when fed a Western Diet²⁹⁴. This was due to an increase in lamina breaks. In these mice, the authors noted an increase in the MMP9 causing a disruption in the elastin integrity found within the lamina. Moreover, following stenosis surgery, SMC-ATG7^{-/-}:ApoE^{-/-} mice had significantly more intraplaque hemorrhage than autophagy competent mice²⁹⁵. These results highlight the importance of smooth muscle cells autophagy in preserving plaque stability and vessel wall integrity.

During the transition between a contractile to a synthetic phenotype, autophagy is activated to promote the degradation of contractile proteins. This switch can be induced by platelet-derived growth factor. Additionally, the activation of autophagy in the synthetic smooth muscle cells confers a protective effect towards oxidated lipids species and prevents cell death²⁹⁶.

1.3.4.2.2 Lysosomal Dysfunction

Contributing to foam cell formation and autophagy impairment is the lipid induced lysosomal dysfunction. The internalization of modLDL can also impact endocytic trafficking, especially oxLDL. Uptake of oxLDL is known to impact lysosomal function. First, oxLDL in lysosomes inhibits the proton pumps required for the acidification of the lysosomal lumen and induces lysosomal membrane damage. Together, this leads to the pH of the lysosome to become less acidic, rendering the hydrolytic enzymes inside less effective^{297,298}. These defective lysosomes are unable to fully digest the oxLDL particles and therefore, the cholesterol found inside these particles is trapped inside the lysosomal compartment²⁹⁹⁻³⁰². Overtime, the excess of cholesterol in the lysosomes will start to crystallize causing further lysosomal impairment including lysosomal membrane damage and increased permeability²⁷³. The lysosomal membrane permeability can increase the leakage of lysosomal enzymes²⁹⁹ which can act as damage associated molecular patterns (DAMP) and ultimately cause cell death.

1.3.4.2.3 Lipid Metabolism and Cholesterol Efflux

Excess lipids in foam cells are stored in lipid droplets. As mentioned above, lipid droplets can be selectively degraded via autophagy or lipophagy where LAL will hydrolyze cholesterol esters into free cholesterol. Moreover, selective autophagy of lipid droplets or lipophagy, contributes to 50% of the cholesterol efflux to delipidated apoA-I¹²⁸. Enhancing autophagy via TFEB overexpression promotes lipophagy mediated cholesterol efflux to apoA-I²⁷³.

Notably, a study by Jeong et al. demonstrated the important role of lipophagy in the contribution of atherogenesis. PRDX1, an antioxidant enzyme, was demonstrated help promote lipophagy in macrophage foam cells leading an increase in cholesterol efflux by reducing reactive oxygen species (ROS). Additionally, ApoE^{-/-} mice transplanted with PRDX1^{-/-} bone marrow had a significant increase in plaque size and lipid content as compared to the mice transplanted with wildtype bone marrow. Interestingly, PRDX1^{-/-} in the non-hematopoietic compartment did not alter suggesting an atheroprotective role of macrophage lipophagy during atherosclerosis progression.³⁰³

1.4 Lipid Droplets

Though to be inert storage of excess triglycerides and cholesterol, lipid droplets are now appreciated as being dynamic organelles capable mediating organelle communication and inflammation³⁰⁴. Originating from the ER membrane, lipid droplets are comprised of a monolayer of phospholipids with a neutral lipid core with a myriad of surface proteins allowing for membrane trafficking, structure, and metabolism³⁰⁵. Of these proteins, the most abundant are the perilipin (PLIN) family. While PLIN1 is exclusively expressed on adipocytes, PLIN2 and PLIN3 are expressed ubiquitously. The expression of PLIN4 is restricted to the white adipose tissue and skeletal muscle and PLIN5 can be found in the brown adipose tissue, cardiac and skeletal muscle, pancreatic beta cells and hepatic stellate cells³⁰⁶.

1.4.1 Lipid Droplet Biogenesis

The origins of lipid droplets were, for a long time, a controversial topic due to their uncommon monolayer outer membrane. The biogenesis of lipid droplets occurs in a

stepwise fashion starting with synthesis, nucleation, budding and detachment³⁰⁴. During synthesis phase, triacylglycerol and cholesterol esters are synthesized via the action of DGAT and ACAT respectively. These neutral lipids will accumulate in the ER phospholipid bilayer. As their synthesis increases and start to accumulate between the bilayer, the neutral lipids will merge together, creating what is known as an oil lens^{307,308}. The formation of the lens does not require proteins but is rather a demixing phenomenon which is a more favourable lower energy state³⁰⁹. As triglycerides and cholesterol esters are being produced and enters the lens, a nascent lipid droplet starts to bud from the ER membrane. Lipid droplet budding occurs in the ER membrane facing the cytosol and not the ER lumen. Controlling the site of budding is fat storage-inducing transmembrane (FIT) proteins and seipin proteins. FIT2 can bind neutral lipids and is thought to promote the localized accumulation of neutral lipids during the synthesis phase to facilitate the lens formation. In addition, FIT2 can interact with seipins during lipid droplet budding to stabilize the membrane curvature of the nascent lipid droplet³¹⁰. Additionally, it is thought that PLIN3 is recruited at the budding lipid droplet to help stabilize the membrane curvature^{311,312}. To compensate for this increase in membrane genesis, phospholipid synthesis is upregulated. More particularly, the production of phosphatidylcholine is upregulated³¹³. Upon phosphatidylcholine shortage, PLIN4 can promote lipid droplet genesis by binding directly triglycerides and acting as the phospholipid monolayer. During the growth of the lipid droplet, a seipin oligomer promotes the incorporation of neutral lipids into the lipid droplet core^{314,315}. In mammalian cells, lipid droplets can detach from the ER³¹⁶.

1.4.2 Lipid Droplet Degradation

Lipid droplet catabolism ensues through neutral and acid lipolysis. Lipid droplet size and localization determine, in part, which type of lipolysis is used. Present at the surface of lipid droplets are a myriad of lipases which allow for the hydrolysis of the neutral core comprised of triglyceride and cholesterol esters. The cytosolic hydrolysis of triglycerides is mediated by the sequential action of ATGL, HSL and MAGL to generate three free fatty acids and a glycerol backbone. On the other hand, hydrolysis of cholesterol esters is facilitated by the action of NCEH1¹²¹⁻¹²³, LIPE^{124,125} and CEH¹²⁶ into free fatty acid and cholesterol.

The degradation of lipid droplets via lipophagy is more complex. Lipid droplets have been demonstrated to undergo both macrolipophagy and microlipophagy. Moreover, chaperone-mediated autophagy of PLIN2 and PLIN3 allows for the access for neutral lipase ATGL to access the neutral lipid core³¹⁷. Interestingly, both ATGL and LIPE contain a LIR motif³¹⁸ and have been suggested as being non-ubiquitin SARs. Overall, the removal of PLIN2 and PLIN3 by chaperone-mediated autophagy indirectly promotes macrolipophagy by allowing the recruitment of non-ubiquitin dependent SARs ATGL at the surface of the lipid droplet³¹⁷.

The study from Schott et al. demonstrates the preferential targeting of lipid droplets larger than 1 μm in diameter for neutral lipolysis by ATGL in hepatocytes. Notably, the study suggests a cooperative role for neutral and acidic lipolysis where neutral lipolysis generates a pool of small lipid droplets which is available for lipophagy³¹⁹. In addition to

size, in macrophage foam cells, lipid droplets tend to cluster together minimizing access to both neutral lipases and autophagy machinery³²⁰.

1.5 Open Questions in the Role of Autophagy in Atherosclerosis

As mentioned previously, the rate-limiting step in reverse cholesterol transport is the hydrolysis of cholesterol esters into free cholesterol. Although Ouimet et al. highlighted the importance of lipophagy in promoting cholesterol efflux in macrophage foam cells, the mechanism by which lipophagy occurs was poorly described.

Since smooth muscle cells express markers typically used to identify macrophages, studies which have looked at autophagy flux in the plaque have studied the flux coming from a pool of cells of different origins and not only of macrophages. As such, the functionality of autophagy in the various foam cell populations was not known. Gaining a better understanding of how autophagy is active and controlled in each cell type of the foam cell population is key in order to better target therapeutic in order to target plaque resolution.

Although studies have shown that activation of autophagy reduces atherosclerosis upon hypercholesterolemia, this has little relevance to patients who are in the clinic. Patients who are at risk of developing cardiovascular disease or have cardiovascular diseases are treated with statins and therefore have low circulating cholesterol. As such, how modulating autophagy of established plaque under normal cholesterol levels is unknown.

1.6 Objectives

The first part of this thesis aimed to characterize the lipophagy machinery in macrophage foam cells and how this influence macrophage cholesterol efflux.

The second objective was to assess the functionality of autophagy in macrophage and vascular smooth muscle cell foam cells during atherogenesis and to assess the contribution of lipophagy mediated cholesterol efflux in each foam cell population. We hypothesized that both foam cell population will have a defective autophagy flux with impaired lipophagy mediated cholesterol efflux.

The final objective was to investigate the role of autophagy activation on atherosclerosis regression. We hypothesized that promoting autophagy activation using TFEB activation will promote plaque regression by restoring autophagy flux and lysosomal health.

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2 Manuscript 1: Identification of Novel Lipid Droplet Factors that Regulate Lipophagy and Cholesterol Efflux in Macrophage Foam Cells

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Identification of Novel Lipid Droplet Factors that Regulate Lipophagy and Cholesterol Efflux in Macrophage Foam Cells

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2.1 Abstract

Macrophage autophagy is a highly anti-atherogenic process that promotes the catabolism of cytosolic lipid droplets (LDs) to maintain cellular lipid homeostasis. Selective autophagy relies on tags such as ubiquitin and a set of selectivity factors including selective autophagy receptors (SARs) to label specific cargo for degradation. Originally described in yeast cells, ‘lipophagy’ refers to the degradation of LDs by autophagy. Yet, how LDs are targeted for autophagy is poorly defined. Here, we employed mass spectrometry to identify lipophagy factors within the macrophage foam cell LD proteome. In addition to structural proteins (e.g., PLIN2), metabolic enzymes (e.g., ACSL) and neutral lipases (e.g., PNPLA2), we found the association of proteins related to the ubiquitination machinery (e.g., AUP1) and autophagy (e.g., HMGB, YWHA/14-3-3 proteins). The functional role of candidate lipophagy factors (a total of 91) was tested using a custom siRNA array combined with high-content cholesterol efflux assays. We observed that

knocking down several of these genes, including *Hmgb1*, *Hmgb2*, *Hspa5*, and *Scarb2*, significantly reduced cholesterol efflux, and SARs SQSTM1/p62, NBR1 and OPTN localized to LDs, suggesting a role for these in lipophagy. Using yeast lipophagy assays, we established a genetic requirement for several candidate lipophagy factors in lipophagy, including HSPA5, UBE2G2 and AUP1. Our study is the first to systematically identify several LD-associated proteins of the lipophagy machinery, a finding with important biological and therapeutic implications. Targeting these to selectively enhance lipophagy to promote cholesterol efflux in foam cells may represent a novel strategy to treat atherosclerosis.

2.2 Introduction

Lipid droplets (LDs) are ubiquitous organelles specialized in neutral lipid storage and hydrolysis. Originating in the endoplasmic reticulum, LDs are comprised of a core of neutral lipids (cholesterol esters, triglycerides) surrounded by a phospholipid monolayer and several LD-associated proteins. The perilipin (PLIN) family are the most abundant structural proteins present on the surface of LDs. While PLIN1 (perilipin 1) is primarily expressed in adipocytes, PLIN2 (Perilipin 2) and PLIN3 (Perilipin 3) are ubiquitously expressed ¹. LDs also acquire a host of enzymes that regulate LD metabolism. Among these are neutral lipases such as LIPE (lipase E, hormone sensitive type) and CES1 (carboxylesterase 1) that mediate the hydrolysis of LD-associated neutral lipid, acting on the LD surface *in situ*. More recently, degradation of LDs by autophagy (lipophagy) was shown to play a vital role in the mobilization of fatty acids and cholesterol for energy production and cholesterol efflux, respectively ^{2,3}.

Autophagy is the cellular process by which cytoplasmic organelles are degraded by lysosomes ⁴. Conserved among eukaryotes, the fundamental mechanism of autophagy involves several proteins encoded by **AuTophagy**-related (*ATG*) genes discovered in the early 1990s in yeast ⁵. Autophagy in mammalian cells can be subdivided into three distinct pathways: macroautophagy, microautophagy, and chaperone-mediated autophagy ⁶. During macroautophagy/autophagy, portions of the cytoplasm are sequestered within double-membrane vesicles called autophagosomes, which fuse with the lysosome for cargo degradation and recycling. In microautophagy, cytoplasmic components are directly engulfed by the lysosome (in mammals) or the vacuole (in plant and fungi). Although traditionally thought of as a non-selective process, various forms of selective autophagy are now documented. The heart of this selectivity lies in the MAP1LC3/LC3 (microtubule associated protein 1 light chain 3)-interacting region (LIR) motif of specific autophagy receptors that target cargo for autophagic degradation ⁷. Our work has documented a role for autophagy in LD catabolism in lipid-laden macrophages (foam cells) ², yet the autophagy receptors that selectively target LDs for lipophagy are unknown.

Here, we employed mass spectrometry to characterize the human macrophage foam cell LD proteome to identify lipophagy selectivity factors. In addition to identifying PLIN2 as the major foam cell LD coat protein and other known LD proteins, we found several proteins related to the ubiquitination machinery and autophagy on LDs. The functional role of these candidate lipophagy selectivity factors (total of 91) in macrophage foam cells was tested using a custom siRNA array combined with high-content cholesterol efflux assays. We observed that knocking down several of these genes, including *Map1lc3b* (encoding a central protein in the autophagy pathway), *Tfeb* (transcription factor EB; a

regulator of autophagy and lysosome biogenesis) and its downstream lysosomal and autophagy genes *Mcoln1* (mucolipin 1; which is involved in lysosome exocytosis) and *Uvrag* (UV radiation resistance associated gene; involved in initiating autophagy) along with *Hmgb1* and *Hmgb2* (high-mobility group box 1/2; autophagy activators), and *Scarb2* (scavenger receptor class B, member 2), significantly reduced cholesterol efflux, suggesting a role for these proteins in lipophagy-mediated LD catabolism. Finally, determination of the genetic requirements for vacuolar entry of LDs and LD degradation in yeast reveals an aggrephagy-like mechanism for lipophagy involving Lhs1 (molecular chaperone of the ER lumen) a homolog of mammalian HSPA5 (heat shock protein family A member 5), Ubc7 (ubiquitin-conjugating enzyme), a homolog of mammalian UBE2G2 (ubiquitin conjugating enzyme E2 G2) and Cue1 (ubiquitin binding protein), a homolog of mammalian AUP1 (AUP1 lipid droplet regulating VLDL assembly factor; a lipid droplet regulating VLDL assembly factor). However, we observed that Cue5 (ubiquitin binding protein), the homolog of TOLLIP (toll interacting protein) in mammals that is typically required for the selective aggrephagy of protein aggregates ⁸, was not required.

2.3 Results

2.3.1 Lipid droplet ubiquitination occurs during lipophagy.

Autophagy is required for LD breakdown in lipid-loaded cells, such as those generated from culture with oleic acid ³. Similarly, we have previously observed that in contrast to native low density lipoprotein, pathophysiological forms of LDL such as oxidized LDL or aggregated low density lipoprotein (agLDL) trigger macrophage autophagy ². As expected, incubation of human THP-1 macrophages with agLDL led to the accumulation

of BODIPY-positive LDs (**Figure 2.1 A-C**). Under these conditions, lipophagy was active and was observed by association of the autophagosome marker LC3 at the LD surface (**Figure 2.1 A**), along with association of the classical autophagy receptor SQSTM1 (sequestosome 1) that functions in selective autophagy of several organelles (mitochondria in *mitophagy*, protein aggregates in *aggrephagy*, peroxisomes in *pexophagy*), on the surface of these LDs (**Figure 2.1 B**). Furthermore, LDs were frequently ubiquitinated (**Figure 2.1 C**), and we observed colocalization of ubiquitin and LC3 or colocalization of SQSTM1 and LC3 on the surface of ~2-3% cytoplasmic BODIPY⁺ LDs, which increased by 2-3-fold following inhibition of autophagy flux using chloroquine (**Figure 2.1 D**). We also observed a similar recruitment of LC3, SQSTM1 and ubiquitin on LDs that was further increased by inhibiting autophagy flux using chloroquine in oleic acid-loaded THP-1 macrophages (**Fig. 6.1**). Collectively, these results show active lipophagy in agLDL-loaded and oleic acid-loaded THP-1 macrophage foam cells, whereby ubiquitin, SQSTM1 and LC3 are recruited to the surface of LDs.

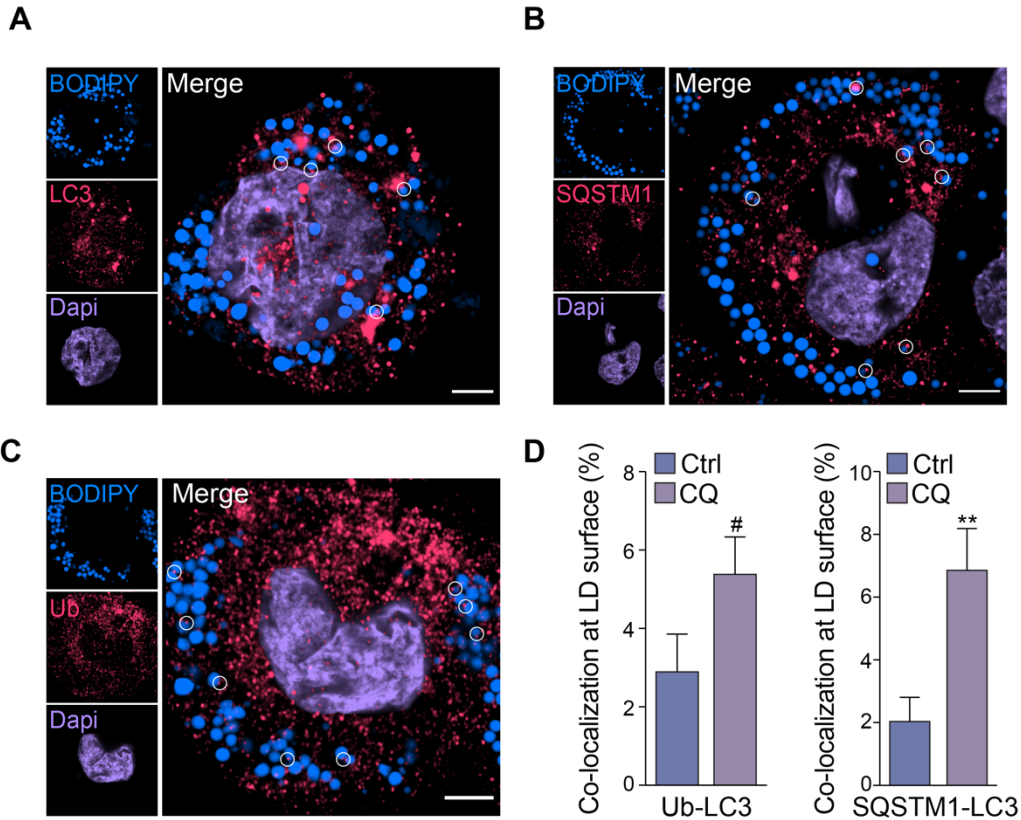


Figure 2-1: LC3, SQSTM1 and Ubiquitin Localize to Lipid Droplets in Macrophage
Human THP-1 macrophages were loaded with agLDL (50 $\mu\text{g/mL}$) for 30 h and equilibrated in BSA (2 mg/mL) overnight. Cells were then fixed and stained for LC3 (A), SQSTM1 (B), ubiquitin (Ub) (C) and BODIPY 493/503 to label neutral lipids. Lipid droplets (LDs) that were colocalized with LC3, SQSTM1 or Ub are circled. At right, quantification of the percent of cellular LDs tagged with Ub and LC3 or Ub and SQSTM1 colocalized at their surface in chloroquine (CQ)-treated cells as compared to control (Ctrl) is shown. Data are expressed as fold-change for the chloroquine treatment relative to untreated from one experiment representative of 3 independent experiments with similar results (mean $\pm$ s.e.m). # $P < 0.1$, ** $P < 0.005$. Representative images are from untreated cells. Scale bar: 5 μm .

2.3.2 Identification of putative lipophagy factors on the surface of lipid droplets.

To identify putative lipophagy factors, we performed mass spectrometry on isolated LDs from macrophage foam cells. Differentiated human THP-1 macrophages were incubated with agLDL to stimulate LD biogenesis and foam cell formation, and these cells were subsequently treated (or not) with chloroquine to inhibit autophagy (**Figure 2.2 A**). LDs

were isolated by sucrose gradient ultracentrifugation and de-lipidated using cold acetone precipitation and sonication ⁹ for subsequent mass spectrometry or western blotting, or directly plated onto coated coverslips for microscopy analysis (**Figure 2.2 B-D**). The purity of LDs was confirmed by enrichment of PLIN2 on LDs relative to the cytoplasm (**Figure 2.2 B**). Conversely, the cytoplasm was enriched in GAPDH (glyceraldehyde-3-phosphate dehydrogenase), CANX (calnexin) and LAMP1 (lysosomal associated membrane protein 1) as compared to LDs (**Figure 2.2 B**). The presence of LC3-I on LDs suggests that LC3 was recruited via interactions with a protein containing an LIR motif and that its lipidation to LC3-II occurs *in situ* on LDs (**Figure 2.2 B**), as previously suggested ^{2,3}. Localization of ubiquitin, SQSTM1 and LC3 to the surface of purified LDs was confirmed by immunofluorescence (**Figure 2.2 C,D**).

Consistent with other studies aimed at defining the LD proteome ¹⁰⁻¹⁸, we observed several structural LD proteins (PLIN2, PLIN3, VIM [vimentin]) along with LD proteins specialized in processes such as lipid transport (FABP4, FABP5 [fatty acid binding proteins 4 and 5]), lipid metabolism (ACSL3, ACSL4 [acyl-CoA synthetase long chain family member 3 and 4]), lipid hydrolysis (LDAH [lipid droplet associated hydrolase], PNPLA2 (patatin like phospholipase domain containing 2), FAF2 (Fas associated factor family member 2), protein quality control (molecular chaperones), vesicular transport (proteins), lysosomal function (CTSB [cathepsin B], NPC2 [NPC intracellular cholesterol transporter 2; an intracellular cholesterol transporter]), ubiquitination (AUP1) and autophagy regulation (CORO1A [coronin 1A], HMGB1) on LDs of both treatment groups (**Table 2.1**). Novel LD proteins of agLDL-loaded macrophage foam cells included the autophagy regulators: regulator complex protein LAMTOR5 (late endosomal/lysosomal

adaptor, MAPK and MTOR activator 5) and DAP (death associated protein), the cholesterol efflux regulator (desmocollin 1), and SCARB2. These were previously implicated in autophagy, cholesterol efflux and lysosomal cholesterol export ¹⁹⁻²² but not localized to LDs.

We computationally investigated the proteome of chloroquine-treated versus untreated cells to discover protein sequence motifs enriched among proteins detected in cells in which lipophagy was inhibited by blockage of lysosome function. Unbiased sequence motif enrichment analysis revealed the following motifs as being enriched in proteins present on the surface of foam cell LDs following autophagy inhibition: RAB protein motif, ubiquitin motif and 14-3-3 family of proteins motif (**Fig. 6.2**). Of note, the ubiquitin motif, being highly conserved among multiple ubiquitin proteins, could have originated from peptides of a single ubiquitin protein. We also investigated the active biological pathways in cells with inhibited lipophagy. Gene ontology ²³ enrichment analysis of the LD proteins enriched following chloroquine treatment showed enrichment of pathways that control cellular response to lipid, vesicular trafficking and regulation of autophagy (**Table 6.2**).

Table 2.1: Proteins on Human Macrophage Foam Cell Lipid Droplets

LDs were purified from agLDL-loaded THP-1 macrophages by density gradient centrifugation and subjected to mass spectrometry. Names and cell function of identified proteins are shown.

Protein	Function	Protein	Function
Autophagy regulators		Cholesterol Efflux	
CORO1A	Phagolysosome assembly and maturation	DSC1	Regulates apoAI-mediated cholesterol efflux
LAMTOR5	Lysosomal Ragulator complex / metabolic signaling	PSAP	Regulates cathepsin B/D activity, lipid trafficking and autophagy
PARK7	Positively regulates autophagy	Lipid transport	
VDAC2	Macroautophagy suppressor	FABP4	Fatty acid transport protein
YWHAZ	14-3-3 protein zeta/delta	FABP5	Fatty acid transport protein
YWHAG	14-3-3 protein gamma	PPIA	Regulates lipid droplet size
YWHAE	14-3-3 protein epsilon	GOT2	Facilitates cellular uptake of long-chain free fatty acids
YWHAQ	14-3-3 protein theta	Lysosomal function	
DAP	Negatively regulates autophagy	CTSB,D,L,S	Lysosomal cysteine proteases with roles in autophagy vesicle trafficking
HMGB1	Novel BECN1 interacting partner, promotes autophagy	SLC25A5	Lysosomal solute carrier transporters implicated in autophagy / phagocytosis
Chaperone proteins		SLC25A6	Lysosomal solute carrier transporters implicated in autophagy / phagocytosis
TBCs	Autophagy and protein aggregation	MYL6	Myosin proteins linking autophagy receptors to autophagosomes
EEF1A1	Regulation of chaperone-mediated autophagy	MYH6,7,9	Myosins
GRPEL1	Co-chaperone	MYL12A,B	Light chain myosin
HSP90AA1	Chaperone-mediated autophagy	NPC2	Autophagy-lysosomal activity, mitophagy and lipid efflux from lysosomes
HSP90AB1	Molecular chaperone	SCARB2	Lysosomal membrane protein, autophagy-lysosome pathway
HSP90B1	HSP90 paralogue, regulates innate and adaptive immunity	Neutral lipases	
HSPA8	Chaperone-mediated autophagy, autophagy and exocytosis	CES1	Cholesterol ester hydrolase
HSPA9	Mitochondrial chaperone	PNPLA2	Patatin-like phospholipase domain-containing protein 2
HSPB1	Regulation of autophagy	LDAH	Lipid droplet-associated hydrolase
HSPD1	Chaperonin	FAF2	Inhibits PLPL2
HSPE1	Chaperonin	Rab proteins	
HSPH1	Co-regulator of chaperone function	RAB1A	Regulation of autophagosome assembly
HYOU1	Hypoxia-regulated chaperone	RAB2A	Promotes autophagy
P4HB	ER chaperone	RAB5C	Member of the Ras-related protein family
PDIA3	ER chaperone	RAB6A	Regulates autophagy, binds to Atg8 family members
PDIA4	ER chaperone	RAB6B	Regulates autophagy, binds to Atg8 family members
TCP1	Chaperonin	RAB7A	Regulates lipophagy by mediating recruitment of lysosome to the LD
HSPA5	N-end rule proteolytic pathway, autophagy degradation	RAB10	Required for LC3 recruitment of autophagosomes
Histones		RAB11A	Regulates endosomal cholesterol movement to lipid droplets
H2A	Core histone	RAB11B	Regulates endosomal cholesterol movement to lipid droplets
H2B	Core histone	RAB14	Phagolysosome assembly
H3	Core histone	RAB18	Promotes LD growth and autophagosome formation
H4	Core histone	RAB21	Promotes lipid droplet growth and autophagosome formation
Lipid droplet proteins		Stress granule regulation	
PLIN2	Lipid droplet coat protein, intermediate-sized lipid droplets	FUS	Stress granule assembly factor
PLIN3	Lipid droplet coat protein, small lipid droplets	PABPN1,C4	mRNA translation regulators
VIM	Cytoskeletal protein	Cellular Trafficking	
Lipid metabolism		COPB2	Golgi coatamer complex, delivery of PLIN2 to lipid droplets
ACADM	Fatty acid oxidation	CLTC	Vesicle coat protein, intracellular trafficking and endocytosis
ACADVL	Fatty acid oxidation	ANXA2	Calcium dependent membrane protein involved in exocytosis
ACSL3	Fatty acid oxidation	CHMP4B	Part of the ESCRT machinery
ACSL4	Fatty acid oxidation	Ubiquitination machinery	
ALDH9A1	Aldehyde dehydrogenase	AUP1	Ubiquitin binding, UBE2G2
ASAH1	Lysosomal ceramidase	RPS27A	Monoubiquitin
CS	Citrate synthase	UBB, UBC	Polyubiquitin
CYB5R3	Desaturation and elongation of fatty acids, cholesterol synthesis	UBA52	Monoubiquitin
DLD	Lipoamide dehydrogenase	VCP	Aggresome assembly, autophagosome maturation
DLST	Mitochondrial succinyltransferase	Others	
GANAB	Catalytic subunit of glucosidase II	ALDOA	Fructose-biphosphate aldolase
HSD17B11	Short-chain alcohol dehydrogenase	ARHGDIA	Rho GDP-dissociation inhibitor
LSS	Lanosterol synthase	ARHGDIB	Rho GDP-dissociation inhibitor
MAN2B1	Alpha-mannosidase, lysosomal oligosaccharide degradation	CDC42	GTPase
MDH2	Malate dehydrogenase	GAPDH	Converts D-glyceraldehyde 3-phosphate to 3-phospho-D-glyceroyl phosphate
METTL7A	Methyltransferase	GNB1	GTPase
PLA2G4C	Phospholipase involved in lipid droplet biogenesis	LDHA	Converts pyruvate into lactate
RHOA,C	Regulation of lipases	RAC2	GTPase
SDHA	Mitochondrial electron transport chain, Complex II	RAP1A	GTPase
SUCLG1	Hydrolyses succinyl-CoA	TPD52L2	Promotes neutral lipid storage, contains a 14-3-3 binding motif
UQCRC1	Component of the ubiquinol-cytochrome c reductase complex	VAPA	Localized to membrane contact sites

Proteins selectively enriched on LDs of chloroquine-treated cells included HMGB2

(autophagy activator ²⁴), RAB8 ([RAB8A, member RAS oncogene family] autophagy-

dependent exocytosis ²⁵), APOD (apolipoprotein D; lysosome function-enhancing antioxidant ^{24,26}) and cold-shock protein CIRBP (cold inducible RNA binding protein; an autophagy activator ²⁷) with known roles in autophagy, along with MACROH2A1 (macroH2A.1 histone, core histone), RBP7 (retinol binding protein 7; retinol metabolism), ADGRL3 (adhesion G protein-coupled receptor L3; neuron guidance) and COLGALT1 (collagen beta(1-O)galactosyltransferase 1) with unknown roles in autophagy (**Figure 2.2 E**). We queried which of these contained putative LIR motifs, or other domains commonly found in selective autophagy cargo receptors known to date ²⁸, such as ubiquitin-associated (UBA) domains or ubiquitin-interacting motifs (UIM) ²⁹. Of the 37 proteins enriched on LDs of chloroquine-treated foam cells, none contained a UIM, while several contained predicted LIR motifs and only two proteins, ADGRL3 and COLGALT1, contained predicted UBAs (**Figure 6.2 E**). With this list in hand, we next asked which of these candidate lipophagy factors may be relevant to LD catabolism in macrophage foam cells.

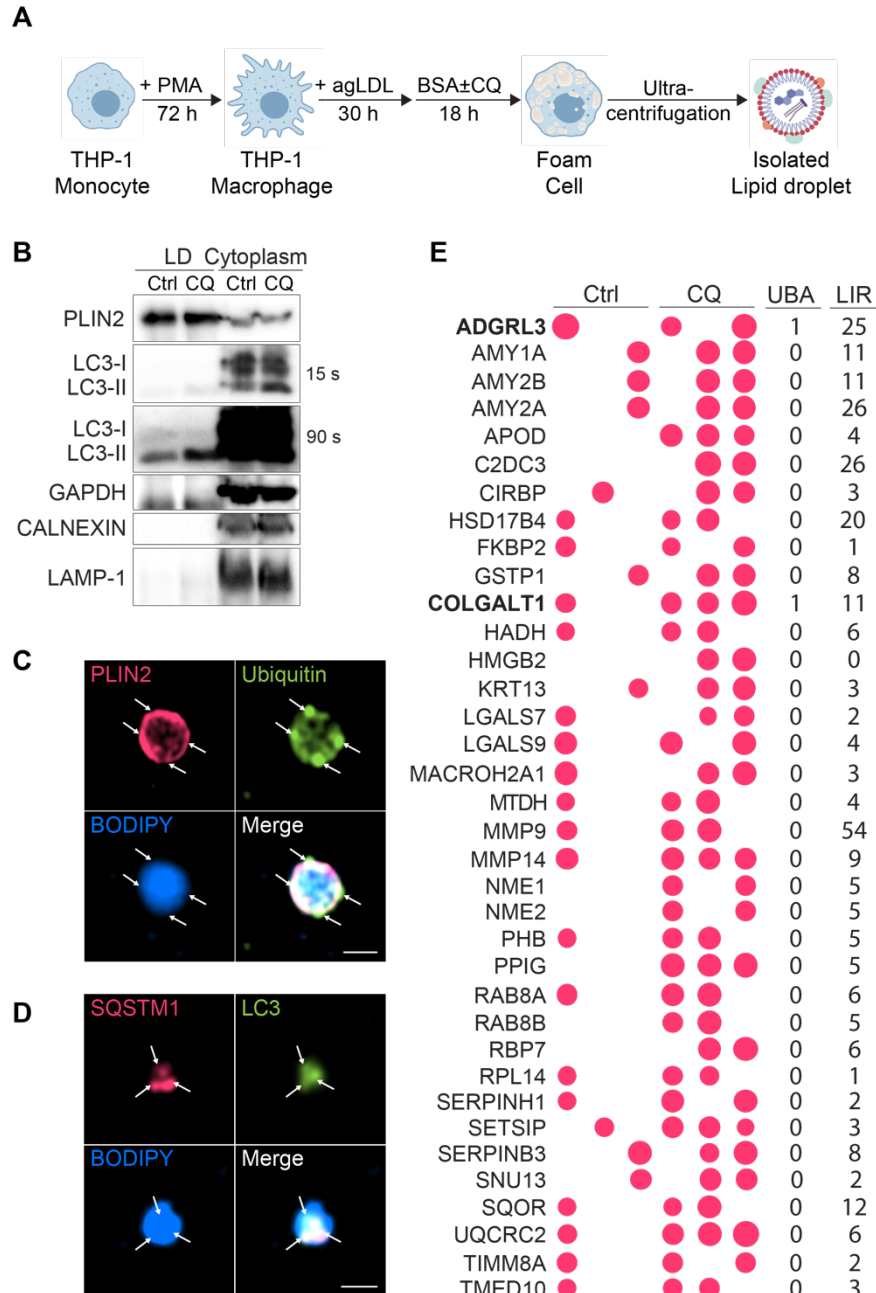


Figure 2-2: Proteins Enriched on Lipid Droplets Following Lipophagy Inhibition

(A) Schematic for human THP-1 macrophage lipid droplet (LD) isolation. (B) Western Blot analysis of LD and cytoplasmic fractions obtained by sucrose gradient ultracentrifugation. (C, D) Immunostaining of isolated LDs for PLIN2 and Ubiquitin (C), SQSTM1 and LC3 (D), and BODIPY 493/503 to label neutral lipids. (E) Enrichment of proteins at the surface of LDs in foam cells treated with chloroquine (CQ) relative to untreated (Ctrl), with the size of bubbles reflecting relative protein abundance. The number of putative ubiquitin-associated (UBA) and LC3-interacting (LIR) motifs identified in each protein is shown to the right. Scale bar, 1 μ m.

2.3.3 Knockdown of candidate selective lipophagy factors reduces cholesterol efflux.

Lipid-laden macrophages, or foam cells, are a hallmark of atherosclerosis, a chronic sterile inflammatory disease driven by the build-up of cholesterol-rich plaques in the artery wall. Within macrophage foam cells, LD catabolism via lipophagy releases free cholesterol for efflux, a process dependent on ABCA1 (ATP-binding cassette, sub-family A (ABC1), member 1) that pumps cellular cholesterol out to lipid-poor apolipoprotein A1 (APOA1), an extracellular cholesterol acceptor ². Bulk RNA-seq data comparing aortic intimal cells from *apolipoprotein E*^{-/-} mice fed a high-fat diet for 28 weeks and sorted into foamy or non-foamy macrophages ³⁰ was queried for log2FoldChange of our candidate selective lipophagy genes. In addition to the distinctive expression of genes related to cholesterol and fatty acid transport (*Abca1*, *Lipa* [lysosomal acid lipase A], *Npc1* [*NPC intracellular cholesterol transporter 1*], *Fabp4*, *Fabp5* [fatty acid binding protein 4 and 5]) previously reported in these cell populations ³⁰, transcriptome profiling revealed altered expression of candidate lipophagy regulators (**Figure 2.3 A**). Notably, *Mmp14* (matrix metalloproteinase 14 (membrane-inserted)), *Npc2*, *Rab31*, *Bnip3l* (BCL2/adenovirus E1B interacting protein 3-like), *Plin2* and *Psap* (prosaposin) were significantly elevated in foamy macrophages as compared to non-foamy macrophages, while *Mmp9* (matrix metalloproteinase 9), *Hmgb2*, *Fus* (fused in sarcoma) and *Hsp90aa1* (heat shock protein 90, alpha (cytosolic), class A member 1) were reduced (**Figure 2.3 A**). We also noted reduced expression of *Rab8*, *Ube2g2*, *Mmp9* (matrix metalloproteinase 9), *Tpd52l2* (tumor protein D52-like 2), *Phb* (prohibitin), *Hspa8* (heat shock protein 8), *Hspa5*, *Rab18*, *Rbbp7* (retinoblastoma binding protein 7, chromatin remodeling factor), *Coro1a*, *Hspb1* (heat shock protein 1), *Hmgb1*, *Ppp1cc* (protein phosphatase 1 catalytic subunit gamma),

Uvrag, *Sqstm1*, *Macroh2a1* and several 14-3-3 family of genes in foamy macrophages concomitant with elevated expression of *Slc25a5* (solute carrier family 25 (mitochondrial carrier, adenine nucleotide translocator), member 5), *Scarb2*, *Park7* (Parkinson disease (autosomal recessive, early onset) 7), *Atg5* (autophagy related 5), *Hsph1* (heat shock 105kDa/110kDa protein 1), *Hsd17b4* (hydroxysteroid (17-beta) dehydrogenase 4), *Nme1* (NME/NM23 nucleoside diphosphate kinase 1) and *Tmed10* (transmembrane p24 trafficking protein 10) (**Figure 2.3 A**). Collectively, bulk RNA-seq of foam cells from mice with advanced atherosclerosis revealed two clusters of candidate lipophagy genes: one with elevated expression in foam cells and another with elevated expression in non-foam cells. This suggests a physiological role for LD-associated candidate lipophagy factors in atherosclerotic foam cells.

To directly test the functional role of candidate lipophagy regulators and autophagy receptors (a total of 91) in macrophage foam cells, we employed a custom siRNA array to perform high-throughput cholesterol efflux assays (**Figure 2.3 B**). Murine thioglycolate-elicited peritoneal macrophages were plated onto siRNA complexes within a 96-well plate (reverse transfection) and incubated with agLDL and ³H-cholesterol to label LD-cholesterol. Seventy-two hours post-transfection, cholesterol efflux to apoA-I was quantified for 24 h. Upon knockdown, the gene that most significantly downregulated cholesterol efflux was *Map1lc3a*, a central protein in the autophagy pathway included as a positive control (**Figure 2.3 C**). Other known autophagy regulators in the array included the autophagy transcription factor *Tfeb* and its downstream genes *Uvrag* and *Mcoln1*, and *Atg5*, all of which also reduced cholesterol efflux following gene knockdown (**Figure 2.3 C**). Additionally, knockdown of the identified LD-associated proteins HMGB1 and

HMGB2, molecular chaperones HSP90AA1, HSPB1, HSPA5 and HSPH1, lysosomal proteins NPC2 and SCARB2, neutral lipases LDAH and PNPLA2, and Rab proteins RAB2, RAB5, RAB6, RAB10 and RAB13 all reduced cholesterol efflux (**Figure 2.3 C**). We also observed that knockdown of CIRBP and the stress granule-associated RNA binding protein FUS, general stress-response proteins ^{31,32}, resulted in impaired cholesterol efflux (**Figure 2.3 C**). Just below significance were the AUP1 and UBE2G2 genes that both reduced cholesterol efflux in 3 out of 4 experiments (**Figure 2.3 C**).

Although autophagy was initially described as a bulk degradation process, it is now recognized that diverse cargoes, including LDs, can undergo selective degradation. While selective autophagy employs a common mechanism, including the 'core autophagy machinery', it also relies on a set of selectivity factors including selective autophagy receptors (SARs) that label specific cargo for selective autophagic degradation and initiate autophagy ⁸. In mammalian cells, tags such as ubiquitination prevail in tagging organelles for degradation, as shown above for LDs (**Figure 2.1**). Several ubiquitin-dependent SARs have been identified, including SQSTM1, OPTN (optineurin), NBR1 (NBR1, autophagy cargo receptor), CALCOCO2 (calcium binding and coiled-coil domain 2), BNIP3L, and TAX1BP1 (Tax1 binding protein 1). Of these, we found that silencing OPTN or NBR1, but not BNIP3L or TAX1BP1, significantly reduced cholesterol efflux (**Figure 3C**). Silencing CALCOCO2 and SQSTM1 also reduced cholesterol efflux, although this did not reach significance (**Figure 2.3 C**). Given that knockdown of SARs OPTN, NBR1, CALCOCO2 and SQSTM1 reduced cholesterol efflux, we determined which of these colocalize with LDs in foam cells. Immunostaining in bone marrow-derived macrophages (BMDMs) lipid loaded with agLDL revealed the presence of OPTN, NBR1

and SQSTM1 on LDs, while CALCOCO2 was present on small BODIPY-positive puncta devoid of ubiquitin (**Figure 2.4**). Comparison of the number of LDs tagged with SARs in chloroquine-treated cells relative to control revealed a marked increase in OPTN, SQSTM1 and NBR1, but not CALCOCO2, at the surface of LDs upon autophagy inhibition (**Figure 2.4**). Colocalization of ubiquitin and OPTN (Ub-OPTN), ubiquitin and LC3 (Ub-LC3), LC3 and SQSTM1 (LC3-SQSTM1), and ubiquitin and NBR1 (Ub-NBR1) at the surface of LDs, but not ubiquitin and CALCOCO2, specifically increased upon chloroquine treatment (**Fig. 6.3**). Collectively, these experiments identify several molecular chaperones (HSP90AA1, HSPB1, HSPA5, HSPH1), HMGB proteins 1 and 2, lysosomal proteins (NPC2, SCARB2), Rab proteins (RAB2, RAB5, RAB6, RAB10, RAB13), stress proteins (CIRBP, FUS), ubiquitination factors (UBE2G2, AUP1) and SARs (OPTN, NBR1, SQSTM1) as direct and/or indirect regulators of lipophagy.

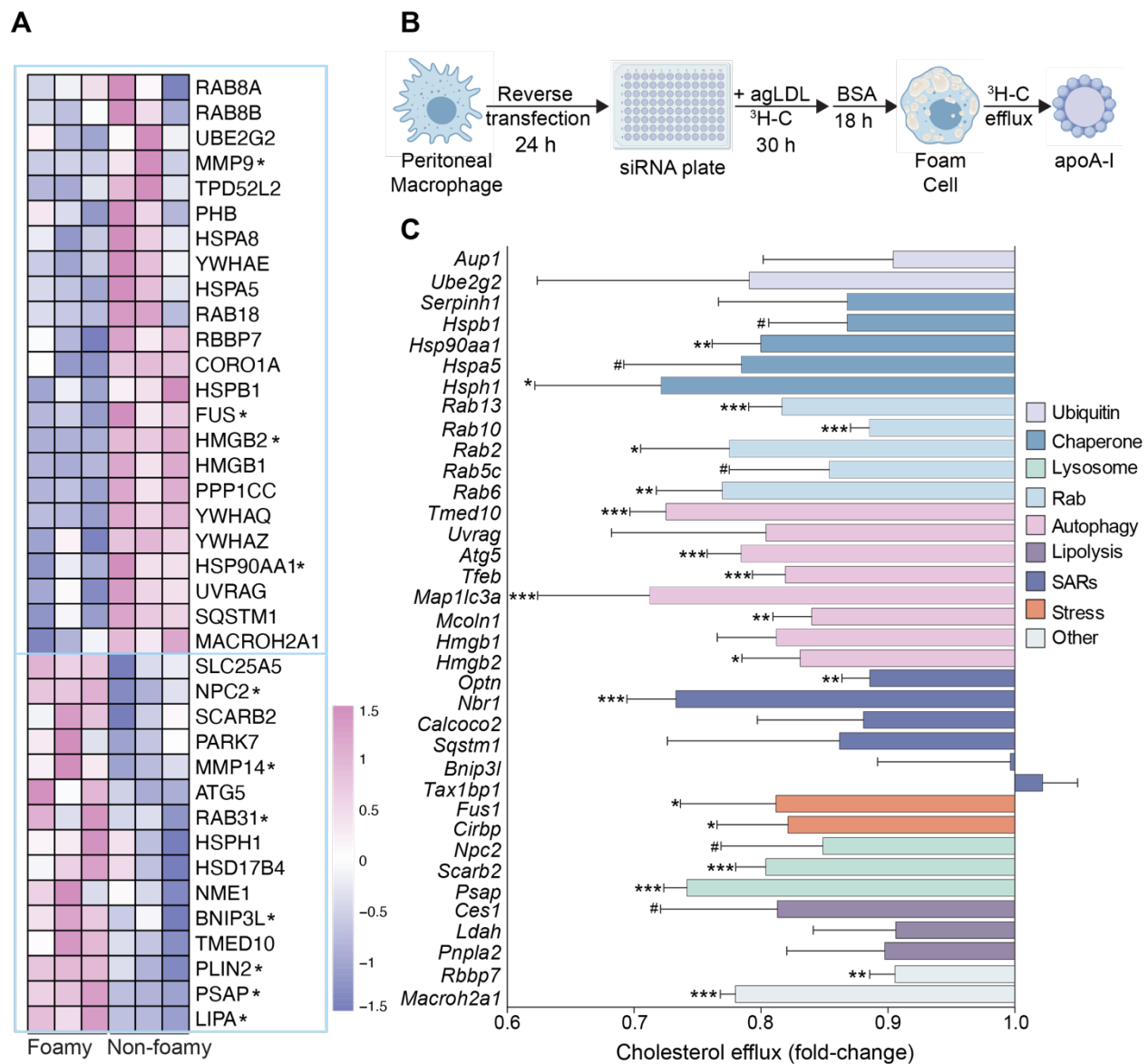


Figure 2-3: Lipophagy Candidate Genes are Dysregulated in Atherosclerosis and Regulate Foam Cell Cholesterol Efflux

(A) Bulk RNA-seq of atherosclerotic foam cells from *Apoe*^{-/-} mice fed a Western diet for 28 weeks. Genes denoted by an asterisk are significantly different between macrophage populations ($P < 0.05$) and heatmap color values correspond to the relative expression (Z-score-transformed RPKM values) of each gene across the six samples. Data was acquired from Kim *et al.*³⁰ (B) Schematic for reverse transfection of mouse peritoneal macrophages. (C) Efflux of ³H-cholesterol (x-axis) from agLDL-loaded mouse peritoneal macrophages transfected with siRNAs against indicated target mouse genes (y-axis) grouped under shared functional annotations: ubiquitination machinery (Ubiquitin), molecular chaperones (Chaperone), lysosome function (Lysosome), Rab proteins (Rab), Autophagy regulators (Autophagy), regulators of neutral lipolysis (Lipolysis), selective autophagy receptors (SARs), response to cell stress (Stress) and other. Data are the

mean $\pm$ s.e.m. of four independent experiments. # $P < 0.1$, * $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$.

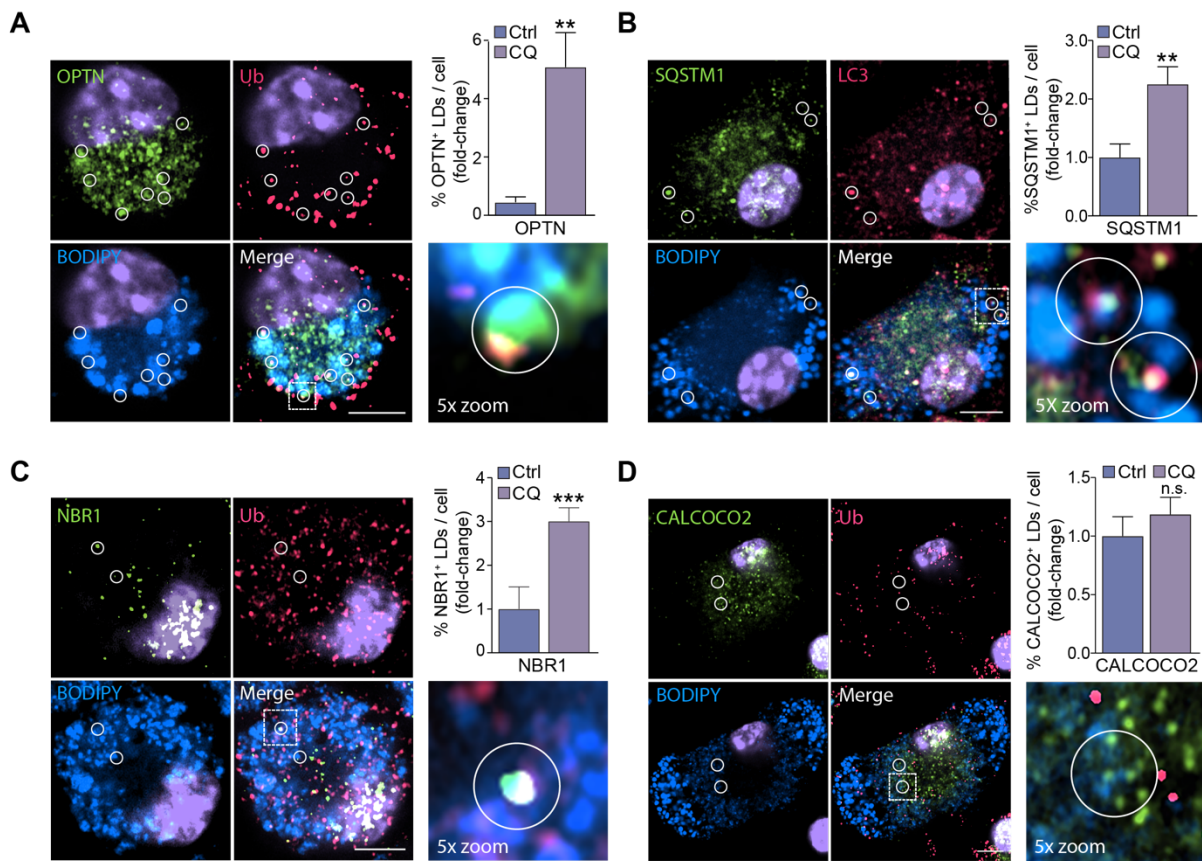


Figure 2-4: Selective Autophagy Receptors (SARs) OPTN, NBR1 and SQSTM1 Selectively Localize to Foam Cell Lipid Droplets

(A-D) Immunostaining for OPTN (A), SQSTM1 (B), NBR1 (C) or CALCOCO2 (D) in agLDL-loaded mouse bone marrow-derived macrophage foam cells stained with BODIPY 493/503 to label LD neutral lipids, with areas of interest circled. At right, quantification of the percent of cellular LDs tagged with SARs in chloroquine-treated cells (CQ) as compared to control (Ctrl) is shown. Data are expressed as fold-change for the chloroquine treatment relative to untreated from one experiment representative of 3 independent experiments with similar results (mean $\pm$ s.e.m). ** $P < 0.005$, *** $P < 0.0005$. Representative images are from CQ-treated cells. Scale bar, 5 μ m.

2.3.4 Genetic ablation of selective lipophagy factors in *Saccharomyces cerevisiae* impairs lipophagy

Microlipophagy studies in yeast have revealed a requirement for the core autophagy machinery (Atg12–Atg5, Atg8, etc.), yet few lipophagy-specific genes have been

identified to date ³³. Given that ~90% of core autophagy genes are conserved across eukaryotes, we employed synthetic genetic array (SGA) methodology to incorporate the well-established yeast LD marker Faa4 (long chain fatty acyl-CoA synthetase)-GFP (green fluorescent protein) and Vph1 (V-type proton ATPase subunit a)-mRuby2 into the yeast non-essential deletion mutant array (DMA) library, as previously described ^{34,35}. The resulting haploid deletion strains were grown in synthetic complete (SC) medium to stationary phase (**Figure 2.5 A**), a condition that stimulates the incorporation of LDs into the vacuole via microautophagy ³⁶. Fluorescence microscopy of LDs in wild-type (WT) and *atg8Δ* cells lacking a core *atg* gene required for autophagy showed that while LD number and distribution were comparable after 24 h of growth in SC medium after reaching early stationary phase (1 d), the number of vacuolar LDs was significantly reduced in *atg8Δ* cells after 5 days (5 d) in comparison to WT cells (**Figure 2.5 B**). These results are consistent with previous findings of the functional impact of *ATG8* deletion on lipophagy ^{33,36-38}.

To determine the role of our identified putative selective lipophagy factors in LD autophagy, we first compared the proteolytic processing of Faa4-GFP in the vacuole, as previously described ³³, where the appearance of one or two bands at ~27 kDa tracks vacuolar cleavage of Faa4. Cleavage of Faa4-GFP was readily observed in wild-type cells cultured to stationary phase, while this was largely impaired in *atg8Δ*, *atg32Δ* and *atg9Δ* strains, as previously described ³⁹ (**Figure 2.5 C**). No inhibition of Faa4-GFP degradation was observed in the *atg21Δ* strain lacking Atg21, a protein required for the cytoplasm-to-vacuole targeting pathway ⁴⁰ (**Figure 2.5 C**). Comparison of Faa4-GFP cleavage in numerous yeast strains lacking the *S. cerevisiae* homologs or close functional equivalents

of putative selective lipophagy factors identified in macrophages in this study or hypothesized to regulate lipophagy, identified several strains with reduced Faa4-GFP cleavage in ≥ 3 out of 4 independent experiments (**Figure 2.5 D** and **Fig. 6.4**). Among these were Ssa3, Ssb2, Sse1 and Sse2 (heat shock protein 70 family members), Bmh1 (brain modulosignalin homolog; a YWHA/14-3-3 protein), Ypt31/Ypt8, Ypt6, and Ypt11 (Rab family GTPases), Tgl4 (triacylglycerol lipase 4), Msi1 (subunit of chromatin assembly factor I) and Hat2 (histone acetyltransferase) identified as homologs of RBBP7, Hta1 (histone H2A) identified as a homolog of mammalian MACROH2A1, Ubc12 (Rub1-conjugating enzyme), CUE domain-containing protein Cue4 (ubiquitin binding protein), Rtg1 (ReTroGrade regulation protein 1), Vps38 (member of Vps34 ptdIns3k complex), Yvc1 (vacuolar calcium channel), along with proteins previously reported to regulate autophagy (Atg8, Vps34 [ptdIns3K] and Ego2 (exit from rapamycin-induced growth arrest), which is part of the yeast equivalent of the mammalian Ragulator complex, to which late endosomal/lysosomal adaptor, MAPK and MTOR activator 5 [LAMTOR5] belongs) (**Figure 2.5 D**). Consistent with a reported role for the energy sensor AMP-activated protein kinase (AMPK) in lipophagy ⁴¹, we observed that several strains with deletions in identified genes sharing functional overlap to mammalian AMPK (*hal5* Δ [*Hal5* is an Snf1p-related nutrient-responsive protein kinase], *kkq8* Δ [*Kkq8* is a serine/threonine protein kinase], *npr1* Δ [*Npr1* is a protein kinase], *gal83* Δ [*Gal83* is a beta-subunit of the Snf1 kinase complex]) had reduced Faa4-GFP cleavage (**Figure 2.5 D**). Of the known negative autophagy regulators found to localize on macrophage foam cell LDs (CORO1A, VDAC2 [voltage dependent anion channel 2] and death associated protein [DAP]), we found enhanced Faa4-GFP cleavage in *cm1* Δ (yeast homolog of CORO1A) and *por2* Δ

(yeast homolog of VDAC2) yeast strains, consistent with a role for CORO1A and VDAC2 in blocking phagosome-lysosome fusion ⁴² and suppressing autophagy ⁴³, respectively.

Next, we quantified LD entry into vacuoles in a subset of these strains: *atg8Δ*, *lhs1Δ* (*Lhs1* is a homolog of HSPA5), *rnp1Δ* (*Rnp1* is a ribonucleoprotein that shares function with mammalian CIRBP⁴⁴), *ubc7Δ*, *ubc12Δ*, *atg8Δ*, *ypt6Δ*, *ypt31Δ*, *atg15Δ* (Autophagy related protein 15, a phospholipase), *hsp82Δ* (Heat shock protein 82 is an ATP-dependent molecular chaperone), *ssa3Δ* (*Ssa3* is a homolog of HSPA8) and *npc2Δ* (Niemann-Pick Type C protein involved in sterol transport; is a homolog of NPC2) during stationary phase lipophagy. We also tested *cue1Δ* and *cue5Δ* strains; *Cue1* and *Cue5* are functional homologs of AUP1 and the aggrephagy receptor TOLLIP, respectively. Lipophagy was classified as the average number of cells containing 0 LDs in the vacuole (white bars), 1-2 LDs in the vacuole (grey bars) or more than 3 LDs in the vacuole (>3, black bars) (Figure 5e). By following Faa4-GFP LD entry into Vph1-mRuby2 vacuoles, we observed that LD entry was significantly impaired in the *atg8Δ*, *lhs1Δ*, *rnp1Δ*, *ubc7Δ*, *cue1Δ*, *ypt31Δ* and *npc2Δ* strains (**Figure 2.5 E**). In contrast, LD entry was intact in *atg15Δ* cells, whereas loss of the vacuolar putative lipase *Atg15*, a functional ortholog of LIPA that is required for the degradation of autophagic bodies, resulted in accumulation of vacuolar LDs (**Figure 2.5 E**), as previously reported ³³. Similarly, *ypt6Δ* and *ssa3Δ* exhibited impaired LD catabolism, while *hsp82Δ* and *cue5Δ* had no impact on lipophagy (**Figure 2.5 E**). Quantification of vacuolar entry of LDs in these strains confirmed our visual observations (**Figure 2.5 F**). The possibility that lipophagy or LD catabolism might be altered because of differences in the rates of LD biogenesis were ruled out, given that we noted equivalent amounts of cellular LDs in all strains at 1d of growth (**Fig. 6.5**).

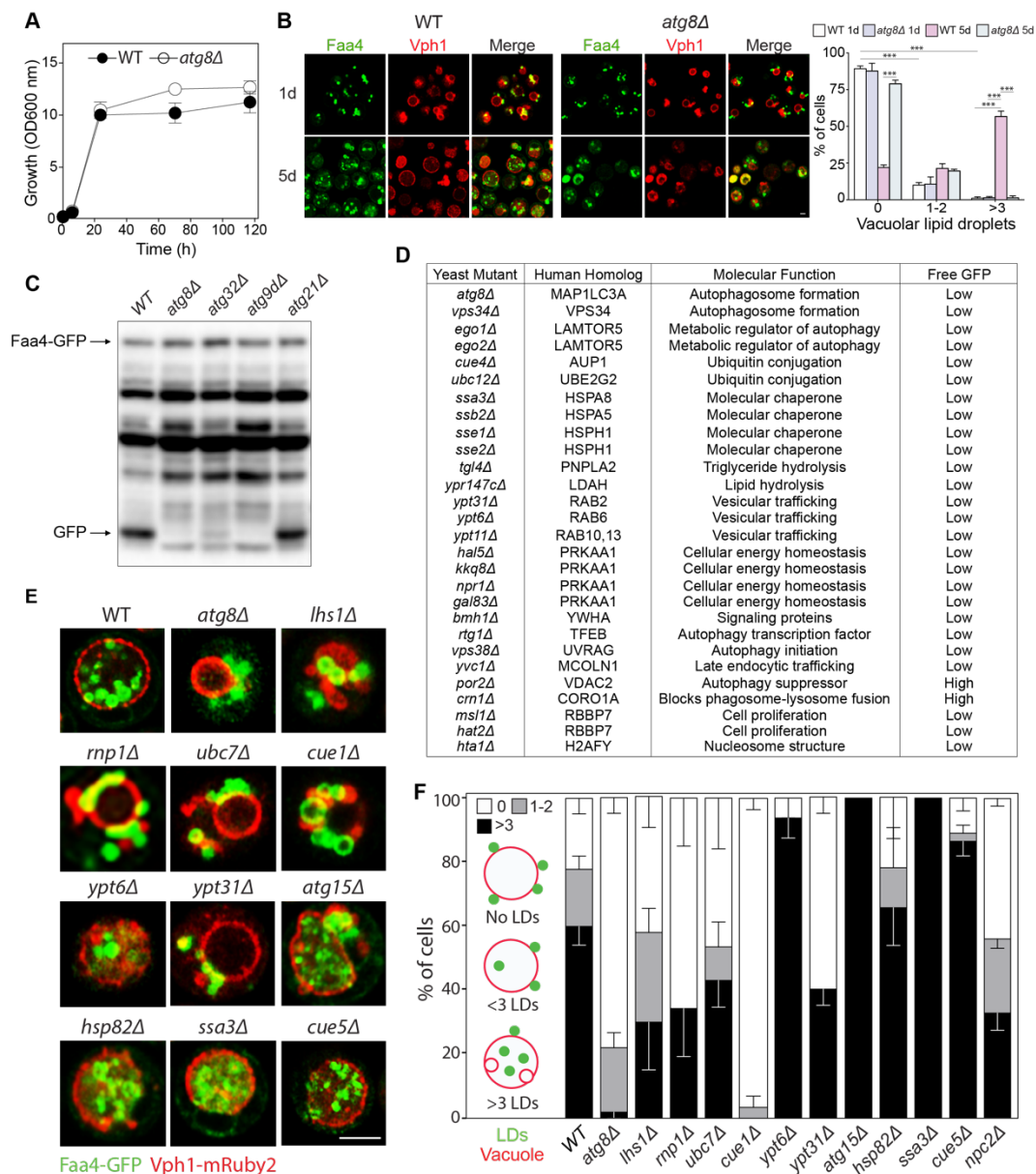


Figure 2-5: Genetic Ablation of Selective Lipophagy Factors Impairs Lipophagy

(A) Representative growth curves of WT and *atg8Δ* strains in SC medium. (B) Activation of lipophagy during stationary phase shown by confocal fluorescence microscopy of Faa4-GFP lipid droplets (LDs) within Vph1-mRuby2 vacuoles. (C) Appearance of the GFP fragment of the Faa4-GFP fusion protein, indicating uptake and degradation of Faa4-GFP in the vacuole. (D) Faa4-GFP cleavage in various yeast stains. (E) LDs internalization into the vacuole at day 5 of stationary phase. (F) Lipophagy quantification. Scale bar, 5 μ m.

2.3.5 Single LDs and clusters of LDs are tagged for autophagic degradation.

Because several mediators of aggrephagy, including OPTN, HSPA5, VCP (valosin containing protein) and 14-3-3 proteins were identified on LDs in our study and the LD protein AUP1 was previously shown to induce LD cluster formation⁴⁵, we wondered whether LDs targeted for lipophagy in macrophage foam cells were single or LD clusters. Interestingly, we observed several single or cluster-less LDs tagged with LC3 in THP-1 foam cells, as expected based on previous observations made by us and others ^{2,3}, but we also noted several clusters or aggregates of LDs that were tagged with LC3 (**Figure 2.6 A**). Quantification of LC3⁺ LDs in agLDL-loaded THP-1 cells treated (or not) with chloroquine to inhibit autophagy showed that the number of LC3⁺ single LDs and LC3⁺ LD clusters similarly increased upon autophagy inhibition (**Figure 2.6 B**), while the proportion of single LDs and LD clusters relative to total LDs tagged with LC3 remained constant in all conditions (**Figure 2.6 C**). We were surprised to find that LC3⁺ LDs in clusters represented a significant proportion (~45%) of the total LC3⁺ LD pool, while most LDs consisted of single or cluster-less LDs (**Figure 2.6 C**). These observations raised the possibility that single LDs and LD clusters may undergo selective lipophagy, potentially by distinct mechanisms.

The diameter of autophagosomes is typically 0.5-1.5 μm in size in mammals ⁴⁶, raising the question of how LD clusters of $> 2 \mu\text{m}$ in size might undergo conventional macroautophagy. We employed electron microscopy to further define the mechanisms of LD sequestration by autophagic vesicles in these foam cells. LDs were clearly identified as circular electron-transparent organelles not surrounded by double membranes that are

characteristic of other organelles (**Figure 2.6 D**). In addition to single LDs surrounded by single membranes and LDs encircled by a double membrane likely depicting engulfment in autophagosomes, we observed several LD clusters in foam cells (**Figure 2.6 D**). Single LDs as well as LD clusters could be found in association with electron-dense matter surrounding or appearing to engulf the LDs (**Figure 2.6 D**). These structures surrounding LDs and LD clusters were reminiscent of lysosome ‘arm-like’ extensions previously found to directly engulf autophagy cargo during mammalian microautophagy of mitochondria ⁴⁷. To further investigate the potential mechanisms for engulfment LD clusters by lysosomes, we performed live-cell imaging in primary BMDMs isolated from GFP-LC3 transgenic mice, allowing for the visualization of the association of single LDs or LD clusters with GFP-LC3 autophagosomes (macroautophagy) or directly with LysoTracker-positive lysosomes (microautophagy) in real-time. We found that LD clusters surrounded by faint and diffuse GFP-LC3 interacted with LysoTracker Red-positive lysosomes (**Figure 2.6 E ii.** and **Video S1**). The diffuse GFP-LC3 staining at the surface of LDs was distinct from the punctate GFP-LC3 staining of autophagosomes, which were frequently observed juxtaposed to or fusing with lysosomes in this cell (**Figure 2.6 E i.** and **Video S1**), suggesting that LC3-tagged LD clusters may directly be engulfed by lysosomes independent of autophagosome sequestration. Collectively, these data show that single LDs as well as LD clusters are tagged for autophagic degradation and suggest that microlipophagy, in addition to macrolipophagy, may represent an autophagic mechanism to degrade larger single LDs or LD clusters in macrophage foam cells. The proposed

Zoom of the regions outlined in the main panel. i) Co-localization of a LD, lysosome and GFP-LC3 puncta. ii) Co-localization of a LD and lysosome. Scale bar, 5 μ m. (F) Model of lipophagy mechanisms in mammalian macrophage foam cells, where single LDs or LD clusters are tagged for autophagic degradation, possibly beginning with a AUP1-UBE2G2 complex to initiate ubiquitin tagging of LDs for autophagic degradation. Single LDs may be engulfed by classical macroautophagy beginning with formation of autophagosomes at the phagophores assembly site (PAS) at the periphery or surface of LDs. In this scenario, phagophore maturation may be facilitated by RAB2 and RAB6. Alternatively, clusters of LDs exceeding the size of autophagosomes may be degraded through an aggrephagy-like mechanism mediated by SARs OPTN, NBR1 and SQSTM1 and lipophagy factors HSPA5 along with aggrephagy factors HDAC6 and 14-3-3 proteins to facilitate microlipophagy.

2.4 Discussion

Conventionally thought of as inert fat depots, LDs are now recognized as metabolically active organelles vital to lipid homeostasis and signaling. Comprised of a core of neutral lipids, such as triacylglycerols and sterol esters, LDs are delimited by a phospholipid monolayer surrounded by structural and functional proteins coordinating dynamic interactions of LDs with a variety of cellular organelles ⁴⁸. Degradation of LDs can result from the action of cytoplasmic lipases localized to LDs through a process known as neutral lipolysis. In addition, LDs can be degraded by the autophagy pathway. Originally observed in yeast cells in 1979 ⁴⁹, 'lipophagy' refers to the degradation of LDs by autophagy ³; yet, how LDs are targeted for autophagy remains poorly defined.

Pioneering genetic studies in yeast have identified upwards of 35 *ATG* genes, among which 17 are part of the core machinery used by all autophagy pathways ^{7,50}. Additionally, targeted forms of autophagy coordinate the selective recycling of organelles. Selective autophagy relies on tags such as ubiquitin and a set of selectivity factors including SARs to label specific cargo for degradation ⁸. Ubiquitin tagging of selective autophagy substrates prevails in mammals, a process that is mediated by E1 ubiquitin-activating and

E2 ubiquitin-conjugating enzymes coupled to E3 ubiquitin ligases. SARs that bind to ubiquitin and an Atg8-family protein member (LC3A, LC3B, LC3B2, LC3C, GABARAP [GABA type A receptor-associated protein], GABARAPL1 or GABARAPL2 GABA type A receptor associated protein like 1 and 2]) bridge target cargo to nascent autophagosomes. Here, we observed that treatment of macrophages with agLDL or oleic acid, which stimulates LD biogenesis and activates autophagy ^{2,3}, was associated with recruitment of ubiquitin, SQSTM1 and LC3 to LDs (**Figure 2.1** and **Fig. 6.1**). This observation is in accordance with a study that found that SQSTM1 was required to bridge LC3 to ubiquitinated LDs in hepatocytes ⁵¹, and others localizing SQSTM1 to LDs ^{52,53}, suggesting the selectivity of LD autophagy.

To identify selective lipophagy factors, we employed mass spectrometry and compared the LD proteome of macrophage foam cells cultured under basal conditions or upon autophagy inhibition. Because agLDL is the main physiological pro-atherogenic form of modified LDL in vivo ⁵⁴, characterization of agLDL-loaded macrophage foam cells was warranted to identify potential lipophagy factors that contribute to autophagy-mediated cholesterol efflux from these cells ². As anticipated, the proteome of agLDL-loaded human THP-1 macrophages contained several LD structural proteins and metabolic enzymes, including PLIN2, PLIN3, acyl-CoA synthetase long chain family member 3 and acyl-CoA synthetase long chain family member 4 (**Table 2.1**). PLIN2 is the major structural protein of macrophage foam cell LDs ² and its role in lipolysis is two-fold. First, PLIN2 protects LDs against autophagy-mediated catabolism, and conversely loss of PLIN enhances lipophagy ⁵⁵. Second, AMPK-dependent phosphorylation of PLIN2 allows its dissociation from LDs, preceding LD catabolism by cytosolic lipases or macroautophagy effectors ⁵⁶.

The human agLDL-loaded macrophage foam cell LD proteome described herein (**Table 2.1** and **Supplementary data**) closely resembles that recently described for THP-1 macrophages loaded with acetylated LDL, another model modified LDL used to induce LD biogenesis ⁵⁷.

Intriguingly, we identified several aggresome components on LDs. Aggresomes are inclusion bodies enriched with molecular chaperones, components of the ubiquitin-proteasome system, along with intermediate filaments such as VIM, all of which were identified on foam cell LDs in this study ⁵⁸. Specifically, we noted the presence of VIM, numerous molecular chaperones including tubulin-specific chaperones TBCB, TBCD, TBCE, TBCH, TBCQ, TBCZ (TBCs) previously shown to regulate autophagy and protein aggregation, HSPA5 that promotes aggrephagy ⁵⁹, VCP that promotes substrate degradation via the aggresome-autophagy pathway ⁶⁰, and finally 14-3-3 proteins YWHAЕ, YWHAG, YWHAQ and YWHAZ (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon, gamma, theta and zeta) that regulate autophagy ⁶¹ and were found to be indispensable for aggresome formation ⁶². Recently, Yan *et al.* reported that HDAC6 (histone deacetylase 6) could regulate LD turnover through SQSTM1-mediated aggresome formation, supporting the notion that selective autophagy may degrade LDs ⁵² and raising the possibility that HDAC6 (histone deacetylase 6) may regulate the formation of functional aggresomes for LD catabolism in foam cells.

To elucidate the functional role of identified lipophagy selective factors in LD catabolism and cholesterol efflux, we used siRNA to silence the expression of lipophagy candidate

genes in foam cells. Knockdown of several autophagy genes (*Map1lc3a*, *Tfeb*, *Uvrag*, *Mcoln1* and *Atg5*) resulted in reduced cholesterol efflux from murine macrophage foam cells (**Figure 2.3 C**). Additionally, knockdown of *Hmgb1* and *Hmgb2* encoding the autophagy activators HMGB1 and HGMB2, molecular chaperones HSP90AA1, HSPB1, HSPA5 and HSPH1, lysosomal proteins NPC2 and SCARB2, neutral lipases LDAH and PNPLA2, Rab proteins RAB2, RAB5, RAB6, RAB10 and RAB13, stress response proteins FUS and CIRBP, all found on foam cell LDs in this study, resulted in impaired cholesterol efflux (**Figure 2.3 C**). HMGB2, CIRBP, RBBP7, SERPINH1 (serine (or cysteine) peptidase inhibitor, clade H, member) and MACROH2A1 that were specifically enriched on LDs following autophagy inhibition (**Figure 2.2 E**) all reduced cholesterol efflux following gene silencing (**Figure 2.3 C**), although ADGRL3 and COLGALT1 that were predicted to contain both ubiquitin-binding and LIR motifs were not found to regulate cholesterol efflux.

Of the lipophagy candidates observed to regulate cholesterol efflux (**Figure 2.3 C**), some may be regulating efflux by directly regulating lipophagy (i.e. targeting of LDs to lysosomes) or alternatively these may control other aspects of cholesterol mobilization from autolysosomes (for macroautophagy) and/or lysosomes (for macro- and micro-lipophagy). Each of these scenarios, not mutually exclusive, would affect the availability of LD-derived cholesterol for efflux. One example is NPC2 and SCARB2. First, SCARB2 was shown to operate in parallel with NPC proteins to promote lysosome cholesterol export ²⁰. Second, in addition to its known function in lysosomal cholesterol removal, NPC2's sterol trafficking functions were shown to be vital for the insertion of ergosterol into the yeast vacuolar membrane, a process required for the formation of vacuolar

microdomains that enable microlipophagy in yeast³⁸. Presently, it is unclear if mammalian lipophagy can also operate through a microautophagic mechanism. However, other putative lipophagy proteins with known roles in lipid metabolism/trafficking may help facilitate lipophagy through similar means as NPC2.

In our study, numerous small GTPases of the RAB family were found in association with foam cell LDs, including RAB18. Excitement surrounding the discovery of Rab18 as a LD-associated protein that may regulate LD catabolism was dampened by more recent findings that Rab18 is not necessary for LD biogenesis or turnover^{63,64}. In our study, silencing Rab18 reduced cholesterol efflux, although this did not reach significance (**Table 6.3**). Consistent with others¹⁰, another protein that we identified in association with LDs was AUP1 that regulates polyubiquitylation via its CUE domain and binding to UBE2G2⁶⁵. Gene silencing of both *Aup1* and *Ube2g2* reduced cholesterol efflux in 3 out of 4 experiments, despite not reaching statistical significance (**Figure 2.3 C**). Moreover, we observed that the yeast homologs of AUP1 and UBE2G2, Cue1 and Ubc7 respectively, were required for vacuolar entry of Faa4-GFP (**Figure 2.5**). Interestingly, monoubiquitination of AUP1 was reported to promote LD clustering⁴⁵, a phenomenon readily observed in macrophage foam cells by fluorescence microscopy (**Figures 2.1 and 2.4**) and electron microscopy (**Figure 2.6 D**). LD clusters were also readily observed during time-lapse imaging (**Figure 2.6 E and Video S1**), indicating that clusters observed during fluorescence and electron microscopy of fixed cells are unlikely to be an artifact of static images. Moreover, LD clusters were readily observed in real-time employing non-invasive label-free optical nanoscopy in mouse pre-adipocytes and in oleic acid-loaded human HeLa cells⁶⁶, further negating the possibility that LD clusters are an artifact of

fixed cell microscopy and denoting their presence in other cell types in addition to macrophage foam cells. Based on our findings, it is tempting to speculate that AUP1-mediated clustering of LDs into aggresomes could activate an aggrephagy-like mechanism to promote lipophagy and cholesterol efflux (**Figure 2.6 F**).

Aggrephagy, mitophagy, xenophagy, along with other types of selective autophagy described to date ⁸ employ SQSTM1 to target ubiquitin-tagged substrate, as observed here for lipophagy (**Figure 2.1** and **Fig. 6.1**). For aggrephagy, additional SARs include OPTN, NBR1 and Cue5/TOLLIP, while mitophagy additionally requires Atg32, BNIP3L, OPTN, CALCOCO2, TAX1BP1, BNIP3 (, FUNDC1 (FUN14 domain containing protein 1), BCL2L13 (BCL2-like 13) and xenophagy additionally requires LGALS8 (galectin 8), OPTN, CALCOCO2, TAX1BP1 and TECPR1 (tectonin beta-propeller repeat containing protein 1) ⁸. Amongst SARs included in our siRNA array (*Optn*, *Nbr1*, *Calcoco2*, *Sqstm1*, *Bnip3l*, *Tax1bp1*), silencing OPTN, NBR1, CALCOCO2 and SQSTM1, but not BNIP3L or TAX1BP1, reduced cholesterol efflux (**Figure 2.3 C**). Amongst SARs that regulated cholesterol efflux, OPTN, NBR1 and SQSTM1 but not CALCOCO2 appear specific to lipophagy (**Figure 2.4** and **Fig. 6.3**), while Cue5 was not required for yeast lipophagy (**Figure 2.5 F**). However, whether TOLLIP loss of function in macrophage foam cells impairs lipophagy and cholesterol efflux remains to be tested. Collectively, our study identified LD-associated proteins, including molecular chaperones HSP90AA1, HSPB1, HSPA5, HSPH1, HMGB proteins, lysosomal proteins NPC2 and SCARB2, Rab proteins RAB2, RAB5, RAB6, RAB10, RAB13, ubiquitination factors UBE2G2 and AUP1, SARs OPTN, NBR1 and SQSTM1, that regulate cholesterol efflux in macrophage foam cells.

Our current understanding of microautophagy comes almost exclusively from studies in yeast, but limited evidence for microautophagy in mammalian cells suggests a particular lysosomal wrapping mechanism with similar features to that of yeast autophagy, where ‘wrapping lysosomes’ with an elongated morphology (arm-like extensions or invaginations) engulf cytoplasmic cargo ⁴⁷. While we observed ubiquitin tagging of LDs along with binding of SQSTM1, OPTN, NBR1 and LC3, pointing to active selective macro-lipophagy in macrophage foam cells (**Figure 2.4** and **Fig. 6.3**), we cannot exclude a possible contribution of microlipophagy to LD catabolism and cholesterol efflux. Indeed, electron microscopy revealed microscopic evidence for microlipophagy in lipid-loaded macrophages, where LDs were observed in close apposition to lysosomes that appear to be engulfing the LDs (**Figure 2.6 D**). Multispectral live-cell imaging previously showed that, under basal conditions, 10% of cellular LDs in COS-7 cells made contact sites with lysosomes ⁶⁷. These LD-lysosome contacts increased nearly twofold following nutrient starvation or oleic acid loading, likely reflecting increased lysosomal digestion of LDs under these autophagy-activating conditions ⁶⁷. Diffuse GFP-LC3 on LD clusters distinct from punctate GFP-LC3 on autophagosomes frequently observed fusing with lysosomes suggests that LC3-tagged LD clusters may be directly engulfed by lysosomes free of autophagosomes (**Figure 2.6 E**). The respective contributions of micro- versus macro-lipophagy to LD catabolism in mammalian cells remain to be determined (**Figure 2.6 F**).

The role of lipophagy in LD catabolism remains controversial. Both lipophagy and canonical autophagy have been suggested to contribute to lipid mobilization during starvation. In the first report of mammalian lipophagy, Singh *et al.* convincingly showed the tagging of hepatic LDs by LC3 and enrichment of LDs within autophagosomes,

highlighting the selectivity of autophagy-mediated LD catabolism under two metabolic extremes: starvation and high-fat diet feeding ³. Subsequently, we showed that all forms of atherogenic lipoproteins trigger autophagy in macrophages, leading to autophagy-mediated LD catabolism that was dependent on LIPA ². However, autophagy was also shown to sustain classical neutral lipolysis pathways during starvation, independent of lipophagy ⁶⁸. A reciprocal relationship between autophagy and LDs also exists, where rather than serving as substrates for lipophagy, LDs themselves control autophagosome biogenesis ^{69,70}. Other examples of indirect regulation of lipid mobilization by autophagy independent of lipophagy include a role for autophagy in the selective clearance of NCOR1 (nuclear receptor co-repressor 1), which suppresses PPAR α (peroxisome proliferator activated receptor alpha) activation and thus impairs lipid oxidation ⁷¹.

The genetic ease of single-cell microorganisms, development of powerful systems biology methods and the remarkable conservation of protein function across eukaryotes have made budding yeast *Saccharomyces cerevisiae* an ideal model to perform screens to identify regulatory and structural components of autophagy ⁴⁰. When yeast cells enter stationary phase lipophagy becomes active and promotes the mobilization of fatty acids from LDs. Directed studies investigating the genetic requirement for yeast lipophagy have identified several genes involved in this process ^{33,37,39}, including *ATG8*, *ATG32*, *ATG9* and *ATG15*. Here, we identify several additional genes that control LD turnover in yeast. First, we observed impaired cleavage of Faa4-GFP, a LD marker that undergoes proteolytic processing in the vacuole upon lipophagy, in *atg8 Δ* , *atg32 Δ* , *atg9 Δ* , *ypt6 Δ* , *ypt31 Δ* and *ssa3 Δ* strains while cells lacking the autophagy suppressors Por2 and Crn1 conversely exhibited increased Faa4-GFP cleavage (**Figure 2.5 D**). Quantification of LD

entry into the vacuole revealed reduced entry for *atg8Δ* (LC3), *rnp1Δ* (CIRBP), *ubc7Δ* (UBE2G2), *cue1Δ* (AUP1) and *ypt31Δ* (RAB2A), while entry was intact but vacuolar LD catabolism was impaired for *ypt6Δ* (RAB6) and *ssa3Δ* (HSPA8) (**Figure 2.5 E**). However, yeast lipophagy did not show a requirement for Cue5 (TOLLIP), a receptor typically required for aggrephagy ⁸. Although future studies are needed to further understand the role of candidate lipophagy proteins, particularly in macrophage foam cells, our study shows a unique requirement for several yeast genes including *CUE1*, *UBC7*, *CRN1*, *LHS1*, *RNP1* and *NPC2* in lipophagy. These genes were previously shown to not be required for mitophagy ⁷², highlighting a non-overlapping role for them in regulating lipophagy but not mitophagy.

Macroautophagy is a complex cellular recycling mechanism involving 6 sequential steps: *initiation* (activation of the ULK1/2 [unc-51 like autophagy activating kinase 1 and 2] complex), *nucleation* (requiring Beclin-1-Vps34-Vps35 and others), *elongation* (requiring two ubiquitination-like systems to conjugate PE to LC3), *closure* of the phagophores (requires Rab5), *maturation* of autophagosomes (driven by Beclin-1, lysosomal membrane proteins (e.g. LAMP-1, LAMP-2), GTP-binding proteins (e.g. Rab7), the cytoskeleton and lysosomal pH), and finally *degradation* within the lysosome lumen. The entire process also depends on an autophagic lysosome reformation cycle, whereby functional lysosomes are regenerated from autolysosomes to maintain lysosome homeostasis ⁷³. Lipid hydrolysis within vacuoles or lysosomes critical for the degradation and recycling of autophagic bodies, with defects in lysosomal hydrolysis and efflux having devastating consequences in humans ⁷⁴. Here, we identified factors regulating LD catabolism at various steps of this process: HMGB1 and 2 proteins likely working with

Beclin-1 initiate lipophagy, RAB2A likely regulating lipophagy at the maturation stages along with other Rab proteins such as RAB5 and RAB6A, and HSPA8 likely required at the degradation stage, similarly to LIPA. Further mechanistic studies are needed to understand how our identified lipophagy factors function in relation to each other and with other known players of the macro-/micro-autophagy pathways.

Whether lipolysis and lipophagy are independent or tandem pathways in macrophage foam cells is unknown. Given that LD composition can differ in various cell types, the importance of each pathway may also vary in different tissues. In macrophages loaded with atherogenic lipid, we showed that neutral and acid lipolysis equally contribute to LD catabolism and cholesterol efflux, while this was not the case in non-foamy macrophages that relied entirely on neutral lipolysis ². New insight into lipophagy mechanisms reveals that larger hepatic LDs preferentially depend on neutral lipolysis while small LDs undergo lipophagy, whereby PNPLA2 and lipophagy act sequentially ⁷⁵. Notably, macrophage foam cell LDs are significantly smaller in size as compared to those found in other cell types such as hepatocytes and adipocytes, likely due to a reduced capacity to expand because of limited fluidity caused by significant sterol enrichment. How distinct forms of modified LDL or lipid loading activate or regulate lipophagy is not well understood. Several studies have shown that lipoprotein-derived cholesterol can undergo intracellular oxidation within lysosomes, leading to increased reactive oxygen species production ^{76,77}. The later could in turn lead to autophagy activation through increased proapoptotic oxidative or endoplasmic reticulum stress to trigger autophagy in macrophages ⁷⁸. Alternatively, lysosomal stress itself could trigger autophagy. Lysosomal stress by treatment of macrophages with oxidized low density lipoprotein or cholesterol crystals

was shown to rapidly induce nuclear translocation of TFEB and transcriptional activation of lysosomal-autophagy genes ⁷⁹; a similar mechanism could account for agLDL-mediated autophagy activation.

Finally, PLIN2 turnover appears to have distinct roles in the biogenesis and catabolism of LDs. For example, chaperone-mediated autophagy degradation of PLIN2 was suggested to be a pre-requisite before the onset of lipolysis, allowing for access of cytoplasmic neutral lipases such as PNPLA2 to the LD neutral lipid core ⁵⁶. On the other hand, a ubiquitin-proteasome pathway that promotes the degradation of soluble/non-LD-bound PLIN2 could limit LD biogenesis ⁸⁰. We propose that AUP1/UBE2G2-mediated ubiquitination of PLIN2 or other protein substrates on the LD initiates the recruitment of a lipophagy complex comprised of SQSTM1, OPTN and NBR1 enabled by lipophagy factors HSPA5, RAB2A, RAB6A, CIRBP and NPC2 (**Figure 2.6 F**). An aggrephagy-like mechanism allowing for LD clustering may facilitate lipophagy, possibly through microautophagy, although this remains to be confirmed. That the expression of several lipophagy factors identified herein is dysregulated in macrophages during atherosclerosis development (**Figure 2.3 A**) suggests that alterations in lipophagy flux may be a mechanism contributing to atherogenesis. Whether the activity of lipophagy factors in macrophages can be modulated to enhance lipophagy in metabolic diseases remains to be tested. Stimulating lipophagy to promote reverse cholesterol transport and LD catabolism represents an attractive therapeutic strategy to treat heart disease and other metabolic diseases.

2.5 Materials and Methods

Cell culture.

Human THP-1 monocytes (ATCC, TIB-202™) were cultured in RPMI 1640 medium (Gibco, 11875093) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (P/S) (Gibco, 15140122), 2 mM L-glutamine (Gibco, 25030081) and 1 mM pyruvate (Gibco, 11360070). Monocytes were differentiated using 100 nM phorbol 12-myristate 13-acetate (Sigma, P1585-1MG) treatment for 72 h. Foam cells were generated by incubation with agLDL (50 µg/mL) for 30 h followed by an equilibration period of 24 h with or without 10 µM chloroquine (Sigma, C6628) in RPMI 1640 medium supplemented with 2 mg/mL fatty acid-free bovine serum albumin (BSA; Sigma, A6003). BMDMs were flushed from the femurs of C57BL/6N (Charles River Laboratories, 027) or GFP-LC3 transgenic mice (Riken, RBRC00806) and differentiated to macrophages by incubation in DMEM medium (Corning, 10013CV) supplemented with 10% FBS, 1% P/S, and 20% L929-conditioned medium for 7 days. Peritoneal macrophages were harvested 4 days after intra-peritoneal injection of 3% thioglycolate (BD Difco, B243010), as previously described⁸¹ and cultured in DMEM media supplemented with 10% FBS and 1% P/S.

Lipoprotein and APOAI preparation. LDL was isolated by sequential density ultracentrifugation⁸² and LDL was aggregated by vortexing, as previously described⁸³. Human recombinant APOAI was synthesized and purified as previously described⁸⁴, and confirmed endotoxin free using the Chromogenic Endotoxin Quant Kit (Pierce, A39552).

LD isolation. LDs were isolated from agLDL-loaded THP-1 macrophages by density ultracentrifugation similar to previously described⁹. Briefly, cells from 7-8 15-cm dishes

(20×10^7 cells / dish) were washed with ice cold 1X PBS, re-suspended in 2 mL of hypotonic lysis buffer and incubated on ice for 10min. Cells were lysed by repetitive passing through a 25-G needle, and the lysates were mixed with one third volume of 60% sucrose solution for a final sucrose concentration of 20% and transferred to a Beckmann polyallomer tube, overlaid with 5 mL of 5% sucrose solution and 5 mL of hypotonic lysis buffer, then spun at $28,000 \times g$ with a SW40 rotor for 30 minutes. LDs were recovered by aspirating the top buoyant fraction using an 18-G needle attached to a 3-mL syringe.

Tryptic digestion, mass spectrometry and data analysis. LD fractions were de-lipidated as previously ⁹, and proteins were dried using SpeedVac and re-suspended in 8 M urea. Sample protein concentrations were determined using the colorimetric Bio-Rad DC Protein Assay. Equal amounts of protein were reduced with 10 mM dithiothreitol for 30 min at 56°C with agitation. Proteins were alkylated by incubation at room temperature for 30 min with iodoacetamine with agitation. Urea within the samples was diluted to 2 M using 50 mM ammonium bicarbonate (pH 8). Protein samples were then digested using 0.125 g of trypsin for 5 µg of protein overnight at 37°C with agitation. Prior to desalting, peptides were diluted in 0.1% (v/v) formic acid and the pH was adjusted to 3.0 by addition of trifluoroacetic acid. Peptide desalting was completed using in-house made C18 desalting cartridges and samples were dried using a SpeedVac (SAVANT SpeedVac Concentrator).

Peptide mixtures were run using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS). An automated

Ekspert NanoLC 400 system (Eksigent) coupled to a LTQ Velos Pro Orbitrap Elite mass spectrometer (ThermoFisher Scientific) equipped with a nanoelectrospray interface operated in positive ion mode. Peptide samples were reconstituted in 0.5% (v/v) formic acid and loaded on a silica column packed with reverse phase Magic C18AQ resins (5 μm ; 200 Å pore size; Dr. Maisch GmbH). Peptide separation was done using an analytical column (75 μm $\times$ 10 cm) packed with reverse phase beads (3 μm ; 120 Å pore size; Dr. Maisch GmbH). Peptides were eluted with a solution of 5-25% acetonitrile (v/v) containing 0.1% formic acid (v/v) (JT Baker) for 120 min at a flow rate of 300 nL/min.

Bioinformatics analyses. MSConvert ⁸⁵ from the ProteoWizard package was used to convert Thermo RAW files to mzML files ^{86,87}. The resulting mass spectrometry data files were processed using the Trans-Proteomics Pipeline (TPP version 5.0) ⁸⁸. A database search was performed on mzML files to match MS/MS spectra to peptide sequences using the Comet algorithm against the UniProt Swiss-Prot database (downloaded in January 2017; number of protein entries = 20,130) ^{89,90}. The database search space considered tryptic peptides with a maximum of 2 miscleavages. Carbamidomethylation on cysteine was set as a static modification and methionine oxidation as a variable modification. Mass spectra were searched using a mass tolerance of 20 ppm for precursor ions and a fragment ion bin tolerance of 0.05. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium ⁹¹ via the PRIDE ⁹² partner repository with the dataset identifier PXD017511. The confidence of peptide-spectrum matches was then assessed using PeptideProphet ⁹³ and ProteinProphet ⁹⁴. Each experiment was filtered to a protein identification false discovery rate < 1% and only proteins identified with two or more peptides were considered for

downstream analyses. For sequence motif discovery analysis, protein sequences were analyzed using the MEME suite (MEME version 4.12.0), a motif-based sequence analysis tool ⁹⁵. MEME was used to identify sequence motifs that were overrepresented amongst proteins that were abundant in the chloroquine treated samples versus all proteins identified by mass spectrometry. MEME was set to assume motifs could occur at most one time in a protein sequence and to consider motif length ranging from 6 to 50. All reported motifs obtained an E-value $< 10^{-8}$ as estimated by MEME. Gene Ontology enrichment analysis of the LD proteins enriched following chloroquine treatment was performed using Ontologizer ⁹⁶, using the entire set of proteins identified in the treated samples as background. For the bubble chart presented in Figure 2, protein quantification was performed using normalized spectral counts over the entire experiment. Proteins were defined as abundant on LDs of chloroquine-treated cells if present in 2 or 3 replicates of the LD fraction of chloroquine-treated cells, but in 0 or 1 replicate in the LD fraction of untreated cells.

Western blot. For western blotting of LD and cytoplasmic fractions, LD proteins were precipitated with acetone prior to SDS-PAGE. The cytosolic fraction was re-suspended in 2x Laemmli sample buffer (Bio-Rad, 1610737) and denatured by heating at 95°C for 5 min. The LD fraction was re-suspended in Laemmli sample buffer and sonicated at 60°C for 5 h with vortexing every 10 min. For yeast cell extracts, cells were washed twice with ice-cold PBS, and lysed directly in 2x Laemmli sample buffer (Bio-Rad, 1610737) and boiled at 95°C for 15 min. Samples were electrophoresed on 12% TGX Stain-Free™ FastCast™ Acrylamide gels (Bio-Rad, 1610185) with All Blue Precision marker (Bio-Rad, 1610393). Gels were activated and transferred to 0.22 µm or 0.45 µm PVDF membranes

for 10 min using the Trans-Blot Turbo Transfer System (Bio-Rad). Stain-free blots were visualized and imaged to be used for protein normalization. Membranes were then blocked in 5% milk in TBST or PBST for 1 h, washed thrice with TBST or PBST. Membranes were anti-LC3 (1:1000, MBL PD014), anti-PLIN2 (1:2000, Fitzgerald 20R-2604), anti-lysosomal associated membrane protein 1 (1:1000, Novus Biologicals NBP2-25155), anti-calnexin (1:1000, Abcam ab10286), anti-GFP (1:1000, Novus Biologicals NB600-308) or anti-glyceraldehyde-3-phosphate dehydrogenase (1:5000, Life Technologies AM4300). Proteins were detected using an enhanced HRP-based chemiluminescence detection system (HRP-conjugated secondary antibodies from Amersham Biosciences and Clarity or Clarity Max Chemiluminescence Reagent from Bio-Rad) using a ChemiDoc XRS+ System (Bio-Rad).

Reverse transfection and cholesterol efflux. Peritoneal macrophages were reverse-transfected in a 96-well plate containing a custom siRNA library (High Throughput Biology Laboratory Services, NYU School of Medicine). 1×10^5 cells / well of thioglycolate-elicited peritoneal macrophages were seeded onto wells of a 96-well plate containing Silencer Pre-designed siRNA (Invitrogen) at a final concentration of 30 nM siRNA. Briefly, siRNA plate containing 450 nmol per well of siRNA were thawed from -80°C and centrifuged at $100 \times g$ for 1 min. 0.5 μL of RNAiMax (Invitrogen, 13778150) was diluted in 19.5 μL of OPTI-MEM (Gibco, 31985062), and 20 μL /well was added and allowed to complex with siRNA for 20 min at room temperature. 120 μL of cells (1×10^5 cells / well) were added in OPTI-MEM, plates were centrifuged at $100 \times g$ for 1 min and incubated at 37°C . 24 h post-transfection, 140 μL of 20% FBS media containing ^3H -cholesterol (Perkin Elmer, NET139001MC) and agLDL was added into each well (2.5 $\mu\text{Ci}/\text{mL}$ ^3H -cholesterol; 50

µg/mL agLDL). 30 h post-loading and labeling, media was replaced with 2 mg/mL fatty acid-free BSA DMEM and cells were equilibrated overnight. Cholesterol efflux to apoA-I (50 µg/mL) was carried out for 24 h, following which supernatants were removed and cells were dissolved in 0.5 N NaOH. Aliquots of supernatants and cell lysates were transferred onto 96-well Lumaplates (Perkin Elmer, 6006633), dried overnight and radioactivity within samples was quantified using the Hydex Sense plate reader (Gamble). Cholesterol efflux was calculated as a percentage of ^3H -cholesterol in the supernatant/ $(^3\text{H}$ -cholesterol in the supernatant + ^3H -cholesterol in cells) $\times 100\%$. For each experiment (n=4), fold-change efflux for each siRNA treatment was calculated relative to the average of control wells (scrambled siRNA).

Fluorescence microscopy. Isolated LDs were seeded on 8-well removable chamber slides (Ibidi) coated with poly-L-lysine (Sigma, P8920) overnight at 4°C and fixed with 4% paraformaldehyde (Alfa Aesar, 433689M) for 10 min at room temperature. For immunofluorescence (IF) of macrophage foam cells, cells were washed 3X in 1X PBS and fixed in 4% paraformaldehyde for 10 min at 37°C. Cells were blocked/permeabilized for 30 min at room temperature in 5% BSA/0.01% TritonX-100. Cells were incubated with anti-PLIN2 (1:200, Fitzgerald 20R-AP002), anti-LC3 (1:750, MBL PM036), anti-SQSTM1 (1:500, Abnova H00008878-M01), anti-ubiquitin (1:250, Novus Biologicals NB300-130), anti-NBR1 (1:250, Cell Signaling 9891), anti-CALCOCO2 GeneTex GTX115378 (1:1250), anti-OPTN (1:250, ThermoFisher Scientific 711879) for 2 h at 37°C or overnight at 4°C in wash buffer containing 1% BSA/0.025% Saponin (Sigma, S7900). Alexa Fluor or Alexa Fluor Plus antibody conjugates (1:500-1:1000, Invitrogen) were incubated for 1 h at 37°C in wash buffer. Cells were incubated with BODIPY 493/503 (10 µg/mL,

Invitrogen, D3922) for 30 min at room temperature where indicated to stain neutral lipids. Cells were counterstained with DAPI (Invitrogen, D1306) for 5 minutes at room temperature. Slides were mounted on #1,5 coverslips (Ibidi, 10811) with DAKO mounting media (Agilent, S302380-2). Images were captured on a Zeiss LSM880 confocal microscope with a 63X objective (NA 1.4) using the appropriate lasers and the Airyscan module. Proteins at the surface of LDs were quantified using Fiji's Multi-point counter tool from raw images or from Tiff image renderings generated in IMARIS (Bitplane) for quantification of LC3 punctae at the surface of single LDs or LD clusters. For yeast imaging, live strains were imaged on microscope slides in the same SC media that they were grown to stationary phase in. Images were captured on a Zeiss LSM880 confocal microscope with a 63X objective (NA 1.4) using the appropriate lasers and the Airyscan module. For fixed cell imaging, yeast strains were fixed according to the Koshland Lab protocol⁹⁷ and stained with 1.0 µg/mL BODIPY 493/503 at 30°C for 30 min for subsequent imaging using the CellVoyager CV1000 Disk Confocal Microscope (Olympus Life Science) with a 100X objective using the appropriate lasers. LD entry into vacuoles in wild-type or mutant strains was quantified using Fiji's Multi-point counter tool.

Bulk RNA-sequencing. The bulk RNA-seq dataset published by Kim et al.³⁰ was queried for log2FoldChange of our candidate selective lipophagy genes. Briefly, aortic intimal cells from apolipoprotein E^{-/-} mice fed a high-fat diet for 28 weeks were sorted into foamy and non-foamy macrophages and libraries were prepared from cell lysates using the SMARTer Ultra Low RNA Kit for Illumina Sequencing for subsequent amplification and sequencing on an Illumina HiSeq-3000 using single reads extending 50 bases³⁰.

Yeast experiments. SGA methodology was employed to incorporate the well-established yeast LD marker Faa4-GFP and vacuole marker Vph1-mRuby2 into the yeast non-essential DMA library (Horizon Discovery, YSC1053). A PCR-mediated gene insertion technique was used to add LD (Faa4-GFP) and vacuolar fluorescent tags (Vph1-mRuby2) into the SGA query strain, as previously described ⁹⁸. Query strain mutations were confirmed by PCR analysis. Manipulation of the DMA was performed using a Singer RoToR HDA (Singer Instruments). For high-throughput strain construction, the MAT α query strain was mated to the MAT α deletion mutant array, and SGA methodology was used with specific chemical selection steps on Nourseothricin (MJS Biolynx Inc., JBAB102XL) (Faa4-GFP), Hygromycin (Wisent Bioproducts, 450-141-XL) (Vph1-mRuby2) and G418 (Wisent Bioproducts, 400-130-IG) (DMA deletion) to select for final haploid strains with each mutation, as previously described ^{34,35}. The resulting Faa4-GFP/Vph1-mRuby2 deletion strains were grown in SC medium containing 0.17% yeast nitrogen base with 0.5% ammonium sulfate (Wisent Bioproducts, 800-152-LG), 2% dextrose (Wisent Bioproducts, 600-350-DG) and 0.206% complete amino acid mix (Sigma) at 30°C on a tube rotator at 7 x g to stationary phase to stimulate lipophagy, similarly to as previously described ³⁸. Day 1 and Day 5 time points were defined as the duration of growth following initial inoculation at OD₆₀₀ = 0.1. Yeast equivalents of mammalian genes were identified using the Human gene to yeast homolog on the YeastMine program found within the Saccharomyces Genome Database and using the OrthoMCL 2.0b6 program. In cases where no yeast equivalent genes were identified by OrthoMCL 2.0b6 and YeastMine, the literature was used to identify close gene equivalents based on structure, function, and protein motifs.

Electron microscopy. Cholesterol-loaded murine BMDMs were fixed in 1.6% glutaraldehyde prior to postfixation in osmium tetroxide and uranyl acetate en bloc staining as previously ². Samples were processed and embedded in Spurr epoxy resin, sectioned and counterstained with lead citrate, and digital images were obtained using a JEOL 1230 TEM at 60 kV adapted with a 2000 x 2000 pixel bottom mount CCD digital camera and AMT software.

Live cell microscopy. GFP-LC3 BMDMs were incubated with agLDL(50 µg/mL) for 30 h and equilibrated in fatty acid-free BSA DMEM overnight. Cells were stained with Monodansylpentane (Abcepta, SM1000a) and LysoTracker (Invitrogen, L7528) for 30 min at 37°C. Cells were imaged in fatty acid-free BSA FluoroBright DMEM media (Gibco, A1896701) at 37°C and 5% CO₂ on a Zeiss LSM880 confocal microscope with a 63X objective (NA 1.4) using the appropriate lasers and the Airyscan module.

Statistical analyses. Experiments were run in triplicates (or n=4 in some instances), and all presented values are mean ± SEM. The statistical significance of the differences between groups was determined using the two-tailed unpaired Student's t-test of the means with Prism V8.3.0 software (GraphPad Software Inc).

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2.7 Conflict of Interest

There are no conflicts of interest to report.

2.8 References

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3 Manuscript 2: Autophagy is Differentially Regulated in Leukocyte and Nonleukocyte Foam Cells during Atherosclerosis

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SR, AR, AP and MO conceptualized the study. AR, SR and MO wrote the manuscript. SR, AR, AP, ADK, DMB, CE, VV, LG, GF, EM, MAN, MG, RW, conducted the experiments and/or data analysis. All the authors reviewed the manuscript.

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Autophagy is Differentially Regulated in Leukocyte and Nonleukocyte Foam Cells during Atherosclerosis

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3.1 Abstract

Rationale: Atherosclerosis is characterized by an accumulation of foam cells within the arterial wall, resulting from excess cholesterol uptake and buildup of cytosolic lipid droplets (LDs). Autophagy promotes LD clearance by freeing stored cholesterol for efflux, a process that has been shown to be atheroprotective. While the role of autophagy in LD catabolism has been studied in macrophage (M ϕ)-derived foam cells, this has remained unexplored in vascular smooth muscle cell (VSMC)-derived foam cells that constitute a large fraction of foam cells within atherosclerotic lesions.

Objective: We performed a comparative analysis of autophagy flux in lipid-rich aortic intimal populations to determine whether VSMC-derived foam cells metabolize LDs similarly to their M ϕ counterparts.

Methods and Results: Atherosclerosis was induced in GFP-LC3 transgenic mice by PCSK9-AAV injection and Western diet feeding. Using flow cytometry of aortic digests,

we observed a significant increase in dysfunctional autophagy of VSMC-derived foam cells during atherogenesis relative to M ϕ -derived foam cells. Using cell culture models of lipid-loaded VSMC and M ϕ , we show that autophagy-mediated cholesterol efflux from VSMC foam cells was poor relative to M ϕ foam cells, and largely occurs when HDL is used as a cholesterol acceptor, as opposed to apoA-1. This was associated with the predominant expression of ABCG1 in VSMC foam cells. Using metformin, an autophagy activator, cholesterol efflux to HDL was significantly increased in VSMC, but not in M ϕ , foam cells.

Conclusions: These data demonstrate that VSMC and M ϕ foam cells perform cholesterol efflux by distinct mechanisms and that autophagy flux is highly impaired in VSMC foam cells, but can be induced by pharmacologic means. Further investigation is warranted into targeting autophagy specifically in VSMC foam cells, the predominant foam cell subtype of advanced atherosclerotic plaques, to promote reverse cholesterol transport and resolution of the atherosclerotic plaque.

3.2 Introduction

Reverse cholesterol transport (RCT) is an atheroprotective process by which cholesterol moves from foam cells in the vessel wall into the circulation for excretion in the feces. The first step of RCT is cholesterol efflux out of peripheral cells, and ATP binding cassette (ABC) transporters ABCA1/G1 are critical for this process¹. To achieve efflux, cholesterol esters within cytosolic lipid droplets (LDs) must first be hydrolyzed to generate free cholesterol². Because efflux is the rate-limiting step in RCT, mechanisms of LD hydrolysis including lipolysis by cytosolic lipases, have been investigated as key regulators of

cholesterol homeostasis². More recently, autophagy, a ubiquitous cellular process allowing for the orderly degradation and recycling of cellular components, was shown in macrophage (M ϕ) foam cells to promote LD catabolism and RCT³.

LDs have emerged as central regulators of metabolism and immunity⁴. Beyond the traditional view of LDs as simple lipid storage organelles, LDs fuel critical metabolic processes and produce bioactive lipids, thereby coordinating immune, metabolic and inflammatory responses⁴. Degradation of LDs occurs by a process called *lipophagy*, a selective autophagy pathway that has been described in M ϕ foam cells, which we have recently shown is dependent upon a host of specific selectivity factors dysregulated in atherosclerosis⁵. These findings are consistent with previous reports demonstrating that ablation of autophagy in M ϕ impairs RCT and promotes LD accumulation, inflammasome activation, apoptosis and overall accelerates atherosclerosis^{3, 6,7}. During atherogenesis, a progressive impairment of autophagy was observed in these plaques; however, as is typically done in atherosclerosis studies, these analyses relied on classical markers of monocyte-derived M ϕ to assess foam cell biology⁷.

Historically, atherosclerosis research has focused on defining cholesterol efflux mechanisms from M ϕ due to their central role in driving plaque development and, more recently, as targets for plaque regression⁸⁻¹⁰. However, this view was challenged by pioneering work in arterial foam cell biology which showed that vascular smooth muscle cells (VSMCs), previously viewed simply as structural components of the medial arterial layer, also comprise a significant proportion of the developing atheroma¹¹⁻¹⁶. Lipid-laden VSMCs undergo clonal expansion and migrate to the intima, where they take up lipids

and acquire a foam cell phenotype¹²⁻¹⁵, ultimately comprising 30-70% of foam cells within mouse and human atherosclerotic plaques^{12,13,16}. Therefore, understanding the mechanisms surrounding cholesterol efflux in VSMC-derived foam cells is equally important as studying these pathways in M ϕ foam cells; however, these have thus far remained largely uncharacterized in comparison.

To determine if autophagy becomes impaired similarly in both M ϕ - and VSMC-derived foam cells during the progression of atherosclerosis, we employed a flow cytometry-based method¹⁶ to measure autophagy flux in individual foam cell subtypes of the aorta in early- and late-stage atherosclerosis. GFP-LC3 transgenic mice, in which autophagy can be directly monitored in tissues¹⁷, were injected with an adeno-associated viral (AAV) vector encoding gain-of-function proprotein convertase subtilisin/kexin type 9 (PCSK9) and fed a Western diet to promote hypercholesterolemia for 6 or 25 weeks (early and late, respectively) post-AAV-PCSK9 injection. Mapping the cellular autophagy profile of arterial foam cell populations revealed an increase in autophagy-deficient leukocyte foam cells in late-stage relative to early-stage atherosclerosis. However, we observed a greater increase in this autophagy deficiency in the nonleukocyte foam cell population. These nonleukocyte foam cells showed intrinsic defects in autophagy *in vivo*, which was confirmed by direct comparison of autophagy flux *in vitro* using M ϕ and VSMCs foam cell models. While autophagy-mediated cholesterol efflux constituted an important LD clearance mechanism in M ϕ , this was limited in VSMC lipid-loaded cells. However, pharmacological activation of autophagy in VSMC foam cells significantly increased ABCG1-mediated cholesterol efflux to high density lipoprotein (HDL). Collectively, our findings reveal that cholesterol efflux operates via distinct mechanisms in M ϕ and VSMC

foam cells and that autophagy in these VSMCs endogenously operates at a submaximal level during lipid-loaded conditions. These data support plaque-targeting RCT therapies that should be specifically tailored to each arterial foam cell subtype to maximize treatment efficacy in atherosclerosis.

3.3 Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request. Detailed methods can be found in the Data Supplement.

Mice. All animal procedures were approved by the University of Ottawa Animal Care and Use Committee. Male and female C57Bl/6N (Charles River) or GFP-LC3 mice (gift from Dr. Peter Liu) were used for primary cell culture experiments. GFP-LC3 transgenic were used to monitor autophagy flux¹⁷ in foam cells during atherosclerosis development. Male and female mice aged 8-10 weeks were injected with 5×10^{11} particles of AAV-PCSK9 (University of Pennsylvania) in the peritoneum as previously¹⁸ and fed a Western Diet (Envigo, TD.88137) for 6 weeks (early timepoint) or 25 weeks (late timepoint). The mice were randomly assigned to each of the cohorts as described. The mice were anesthetized by isoflurane inhalation and sacrificed by exsanguination.

Aortic Digest and Flow Cytometry Staining. A full description of the aortic digest protocol can be found in the Data Supplement. Where applicable, aortic digests were incubated with 10nM of bafilomycin (Sigma) or DMSO at 37°C for 2 hours on a rotator. The cells were stained with LipidTOX Deep Red (Invitrogen, 1:500 dilution) and 50nM of LysoTracker Red (Invitrogen) during the final 30 minutes. The cells were washed twice in ice cold HBSS and incubated with Fixable Viability Stain 510 (BD Biosciences) for 30

minutes at 4°C. Two more washes were performed and the cells were then incubated with 0.5µg of Purified Rat Anti-Mouse CD16/CD32 (BD Bioscience) for 10 minutes at 4°C. Antibody cocktail containing CD45 APC/Fire 750 and MHC II BV785 (Biolegend) was added, and staining was performed for an additional 30 minutes at 4°C. Cells were washed twice prior to acquisition using a BD FACS Aria IIIu (Becton Dickinson). All flow cytometry analysis was performed using FlowJo (Becton Dickinson). Populations were gated as indicated in **Figure S1**. Positive staining for each of the antibodies was confirmed by the use of fluorescence minus one controls.

Statistical analyses. All data are presented as mean $\pm$ standard error of the mean (SEM). The normal distribution of data was determined by Shapiro-Wilk test. The statistical significance of the differences between groups was determined Prism V9.2.0 software (GraphPad Software Inc) using the two-tailed unpaired Student's t-test of the means, one-way or two-way ANOVA using a Holm-Sidak multiple comparison correction for the relevant comparisons as indicated in the figure legends. Power calculations for the animal studies were performed (using <http://powerandsamplesize.com/>) to attain 90% power with a type I (α) and type II (β) error rate of 5% and 0.10, respectively. These calculations were based on previous studies of autophagy induction in macrophage foam cells, which determined the sample size to be 6 mice per group.

3.4 Results

3.4.1 Autophagy becomes impaired in leukocyte and nonleukocyte foam cells during atherogenesis

Macroautophagy (hereby referred to as autophagy) is the main autophagy pathway used to rid cells of damaged organelles or proteins. Initiated by the engulfment of substrates in autophagosomes, the subsequent fusion of autophagosomes with lysosomes releases autophagosomal content into lysosomes for degradation by lysosomal hydrolases and proteases. During autophagy, microtubule-associated proteins 1A/1B light chain 3 (hereafter referred to LC3-I) is conjugated to phosphatidylethanolamine (becoming LC3-II) and localizes to autophagosome membranes, where LC3-II is degraded upon autophagosome-lysosome fusion. The degradation of LC3 by a functional autophagic pathway presents a method of tracking autophagic flux, particularly in GFP-LC3 transgenic mice¹⁷. In this model, autophagy leads to the formation and rapid degradation of GFP-LC3⁺ autophagosomes, resulting in an overall low GFP-LC3 signal. Conversely, impaired autophagy (due to lysosomal dysfunction or impaired autophagosome-lysosome fusion) leads to an accumulation of undegraded autophagosomes and thus an increase in GFP-LC3 signal.

To track autophagy in foam cells during early- and late-stage atherosclerosis, female and male GFP-LC3 mice were administered AAV-PCSK9 and fed a Western diet to promote hypercholesterolemia and plaque development as previously described^{19,20} (**Figure 3.1 a**). A single injection of AAV-PCSK9 was sufficient to induce and sustain hypercholesterolemia (**Figure 3.1 b**). As expected, we observed increased lipid

deposition by oil red O staining and an increase in overall plaque area in late-stage (25 weeks) compared to early-stage (6 weeks) atherosclerosis (**Figure 3.1 c**). No differences in lesion area were detected between male and female mice at the early stage; however, at the late stage there was a significant increase in the plaque area of female versus male mice as previously reported²¹. Aortic arches along with the brachiocephalic, right subclavian, right and left common carotid and left subclavian arteries were surgically dissected, and cells were dissociated by enzymatic and mechanical digestion. We employed a previously described flow cytometry-based foam cell quantification technique to perform a comparative analysis of autophagy flux in distinct foam cell populations from mouse atheromas¹⁶. Lipid-rich foam cells (Lipid^{hi}) were gated without removal of lower side scatter cells to avoid excluding potential VSMC foam cells, which exhibit lower granularity than M ϕ foam cells despite having similarly high neutral lipid content¹⁶. Within this lipid-enriched population, CD45 and MHC-II were used to distinguish leukocyte-derived (CD45⁺) and nonleukocyte-derived (CD45⁻MCH-II⁻) foam cells¹⁶ (**Figure 2.1 d & Figure 6.6**). This nonleukocyte fraction of Lipid^{hi} aortic foam cells was confirmed to be of VSMC origin as per this previously published protocol¹⁶. When foam cells were divided into nonleukocyte and leukocyte origin, nonleukocyte foam cells were the predominant foam cell subtype found to populate aortic arches even at early stages of atherosclerosis ($60 \pm 8\%$ versus $40 \pm 8\%$, respectively) (**Figure 3.1 e**). In late-stage lesions, this proportion further increased nonleukocyte-derived foam cells ($76 \pm 1\%$) with a concomitant reduction in leukocyte derived foam cells ($24 \pm 1\%$) (**Figure 3.1 e**). As expected, the absolute number of foam cells increased during the progression of atherosclerosis (**Figure 3.1 f**), although only the increase in nonleukocyte foam cells

reached significance, consistent with the increased skewing of the proportion of aortic foam cell populations toward nonleukocyte foam cells in late-stage atherosclerosis (**Figure 3.1e**).

To examine the changes in autophagy-associated lysosomal activity in foam cells during the progression of atherosclerosis, we quantified LysoTracker Red, which stains acidic vesicles such as functional lysosomes, in combination with GFP-LC3 by flow cytometry (**Figure 3.1 g**). During autophagy, activation of transcription factor EB (TFEB) increases lysosome numbers and catabolic activity to enhance the fusion with, and consequently breakdown of LC3-positive autophagosomes²² (**Figure 3.2 a**). As such, in cells with functional autophagy, there is an increase in lysosome numbers and function (Lyso^{hi}) paired with efficient degradation of GFP-LC3-positive autophagosomes (LC3^{lo}). However, when autophagy is impaired, autophagosomes accumulate (LC3^{hi}) despite having functional lysosomes (Lyso^{hi}). Cells exhibiting low expression of both markers (Lyso^{lo}LC3^{lo}) display low autophagy flux and represent a metabolically basal or quiescent state. The final population, Lyso^{lo}LC3^{hi}, represents a population with either active autophagosome biogenesis or low lysosomal capacity causing buildup of non-degraded autophagosomes. Interestingly, we observed a reduction in the proportion of Lyso^{lo}LC3^{lo} cells concomitant with an increase in both the metabolically active fractions (Lyso^{hi}LC3^{lo} and Lyso^{hi}LC3^{hi}) in late-stage relative to early-stage atherosclerosis for both leukocyte- and nonleukocyte-derived foam cells, demonstrating the need to induce autophagy during atherogenesis, presumably as a mechanism to handle excess lipids deposited in the arterial wall. The proportion of Lyso^{lo}LC3^{hi} cells, those exhibiting lysosomal dysfunction not associated with autophagy, was reduced in leukocyte but not nonleukocyte-derived

foam cells during the late stage plaque development (**Figure 3.1 g, 3.1 h**). Remarkably among the most striking observations made during the progression of atherosclerosis was a 4.7-fold induction of the autophagy-deficient population (Lyso^{hi}LC3^{hi}) in the nonleukocyte fraction between the early and late stage plaques (**Figure 3.1 g, 3.1 h**), indicating a greater degree of autophagy dysfunction in these foam cells during atherogenesis.

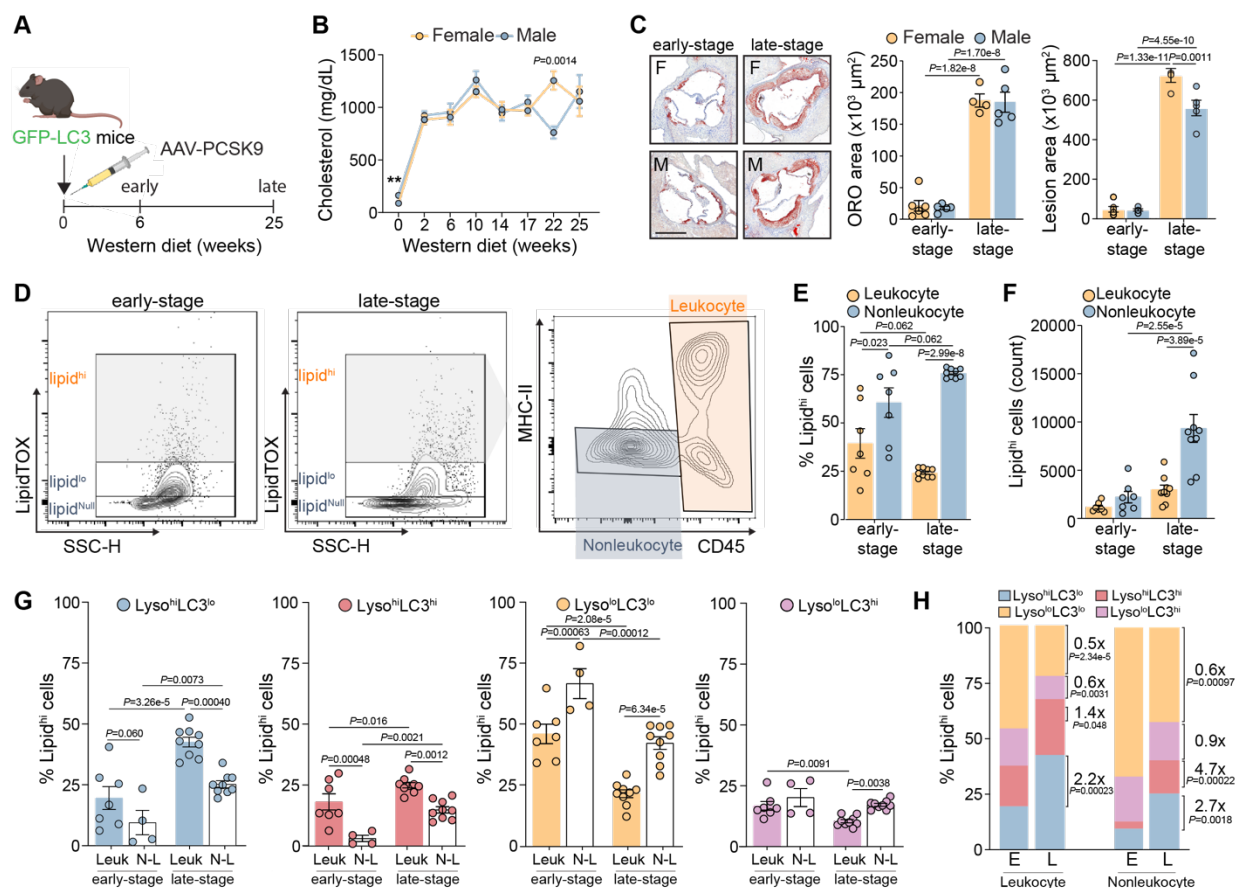


Figure 3-1: Impaired Autophagy in Foam Cells During Atherosclerosis

A. Schematic showing the experimental paradigm in GFP-LC3 mice following AAV-PCSK9 injection and Western diet feeding for 6 weeks (early, E) or 25 weeks (late, L). **B.** Plasma cholesterol levels during Western diet feeding (n=4-5 male, n=5 female). **C.** Representative images and quantification of lipid content and lesion area in the aortic root as identified by oil red O staining in male (M, n=5 early & late) and female (F, n=6 early, n=4 late) mice. Scale bar=700μm. **D.** Identification of Lipid^{hi} foam cells in aortic arches and their separation into leukocytes (Leuk) and non-leukocytes (N-L) populations (full gating scheme found in **Figure S1**). **E-F.** Percentage (E) and total cell numbers (F) of

Lipid^{hi} leukocyte and non-leukocyte cells in early- and late-stage atherosclerosis (n=7 early, n=9 late). **G-H**, Autophagy flux as determined by flow cytometric detection of GFP-LC3 content and LysoTracker staining (n=7 early leukocyte, n=4 early non-leukocyte, n=9 late leukocyte & non-leukocyte). For comparisons between 2 groups an unpaired t-test was used (A-B & H) or for comparisons between more than 2 groups a one-way ANOVA with a Holm-Sidak's post hoc test for multiple comparisons was used (C & E-G; corrected for 4 pre-selected comparisons) to determine statistical significance.

3.4.2 Autophagy flux is primarily defective in atherosclerotic nonleukocyte foam cells

To determine if the shift from Lyso^{lo}LC3^{lo} to Lyso^{hi}LC3^{lo} or Lyso^{hi}LC3^{hi} observed during atherosclerosis progression (**Figure 3.1 h**) represents a transition from a basal (Lyso^{lo}LC3^{lo}) population to one with a high metabolic (Lyso^{hi}) and functional or dysfunctional autophagy (LC3^{lo} or LC3^{hi} respectively), we quantified the shift in these markers using established *in vitro* cholesterol loading protocols for macrophages and VSMCs. Incubation of bone marrow-derived macrophages (BMDMs) with atherogenic lipoproteins such as aggregated LDL (agLDL) was previously shown to activate functional autophagy³. Consistent with this, we observed a shift from Lyso^{lo} to Lyso^{hi} BMDM upon lipid loading with agLDL (**Figure 3.2 b**). Addition of bafilomycin, a lysomotrophic drug that inhibits the vacuolar type H⁺-ATPase (v-ATPase) complex necessary for lysosomal acidification and disrupts autophagic flux, markedly reduced LysoTracker fluorescence (**Figure 3.2 b**), highlighting the functionality of the lysosomes in BMDMs upon lipid loading. We observed increased GFP-LC3 fluorescence in BMDMs upon lipid loading, indicative of increased autophagic flux in lipid-loaded BMDMs. We observed a further increase in this GFP-LC3 signal upon bafilomycin treatment, indicative of active autophagic degradation downstream of autophagosome formation upon lipid loading, which was blocked by bafilomycin (**Figure 3.2 b**). Conversely, while autophagy was basally functional in non-lipid-loaded primary mouse VSMC, as demonstrated by shifts in

LysoTracker and GFP-LC3 upon bafilomycin treatment, bafilomycin did not alter LysoTracker fluorescence and only slightly altered GFP-LC3 fluorescence upon lipid loading with cholesterol complexes, suggesting impaired autophagic flux in VSMC foam cells (**Figure 3.2 c**).

To determine whether lipid-rich nonleukocytes of the plaque demonstrated this diminished autophagic flux as compared to the leukocytes *in vivo*, we treated foam cells from the aorta of atherosclerotic mice with bafilomycin for 2 hours immediately after aortic digestion. As expected, bafilomycin treatment shifted the proportion of the autophagy high/functional (Lyso^{hi}LC3^{lo}) population in the leukocyte fraction (79% and 59% reduction, early vs. late stage respectively) (**Figure 3.2 d**). However, the magnitude of the shift in the autophagy high/functional (Lyso^{hi}LC3^{lo}) population was lower in nonleukocyte Lipid^{hi} cells at late stage (33%, $P=0.00015$) atherogenesis relative to leukocytes and absent at the earlier stages of atherosclerosis (**Figure 3.2 e**). This suggests that leukocyte-derived foam cells continue to possess functional autophagy in advanced atherosclerosis, whereas nonleukocyte-derived foam cells basally exhibit defective autophagy early in atherogenesis that persists over time. Indeed, the major proportion of nonleukocyte Lipid^{hi} cells localized to the autophagy quiescent (Lyso^{lo}LC3^{lo}) quadrant that showed no or minimal fluctuations with bafilomycin treatment (**Figure 3.2 e**). In contrast, the greatest shifts in the leukocyte fraction with bafilomycin treatment were decreases in the autophagy functional (Lyso^{hi}LC3^{lo}) and increases in the Lyso^{lo}LC3^{hi} populations (**Figure 3.2 d**) at the early stages when we observed the greatest autophagy flux (**Figure 3.1 g**), while both of these populations were unresponsive to bafilomycin treatment in nonleukocytes (**Figure 3.2 e**). Taken together, this demonstrates a greater autophagic

capacity in the leukocyte fraction at earlier stages of atherosclerosis, while the nonleukocytes show minimal changes suggesting a baseline defect in autophagy even at the initial stages of atherosclerosis.

Next, we quantified autophagic flux in primary mouse VSMCs, using a well-described cholesterol loading protocol previously shown to cause mouse aortic smooth muscle cells to acquire a foam cell phenotype^{14,15}. As expected, incubation of mouse primary VSMCs with methyl-beta-cyclodextrin (m β -CD)-cholesterol complexes for 72 hours led to increased expression of the conventional M ϕ marker CD68 and reduced expression of the smooth muscle protein ACTA2 (**Figure 3.3 a**). We also demonstrate that the cholesterol loading by m β -CD-cholesterol led to the accumulation of lipids in LDs and not within lysosomes, as shown by the accumulation of BODIPY-positive cytosolic LDs coated with the LD protein perilipin 2 (PLIN2) and devoid of LAMP-1 (**Figure 3.3 b**). These phenotypic changes were accompanied by dysfunctional autophagy flux, as evidenced by an accumulation of LC3 puncta that was unresponsive to bafilomycin treatment (**Figure 3.3 c**). Along with accumulation of LC3 puncta, we observed increased detection of the chaperone protein p62/SQSTM1, which is itself degraded during functional autophagy, further supporting that autophagy is defective in VSMC foam cells (**Figure 3.3 c**).

During autophagy, the addition of phosphatidylethanolamine to LC3-I to generate LC3-II requires an LC3 conjugation system comprised of ATG16L1/ATG5-12²³ (**Figure 3.2 a**). ATG16L1-containing complexes are essential for autophagy, and recently phospho-ATG16L1 (pATG16L1) levels were shown to directly correspond to autophagy rates²⁴.

Protein levels of LC3-I and LC3-II also serve as autophagy markers, with a functional autophagy flux resulting in further increase of LC3-II upon lysosomal inhibition that prevents autophagosome degradation²⁵. We observed increased protein levels of LC3-I, LC3-II and p62 in lipid-loaded VSMCs relative to non-lipid-loaded VSMCs by immunoblot, but importantly these autophagy markers were not further increased upon lysosomal inhibition using bafilomycin (**Figure 3.3 d**), together demonstrating a stark defect in the ability to initiate the required autophagic flux in these VSMCs in response to an increase in cellular lipid content. Consistent with this, both total ATG16L1 and pATG16L1 protein levels were markedly lower in VSMCs as compared to BMDMs, and while pATG16L1 was increased in lipid-loaded BMDMs relative to non-loaded BMDMs, lipid loading failed to increase pATG16L1 in VSMCs (**Figure 3d**). Taken together these data demonstrate that VSMC foam cells possess a comparatively low autophagic flux compared to Mφs.

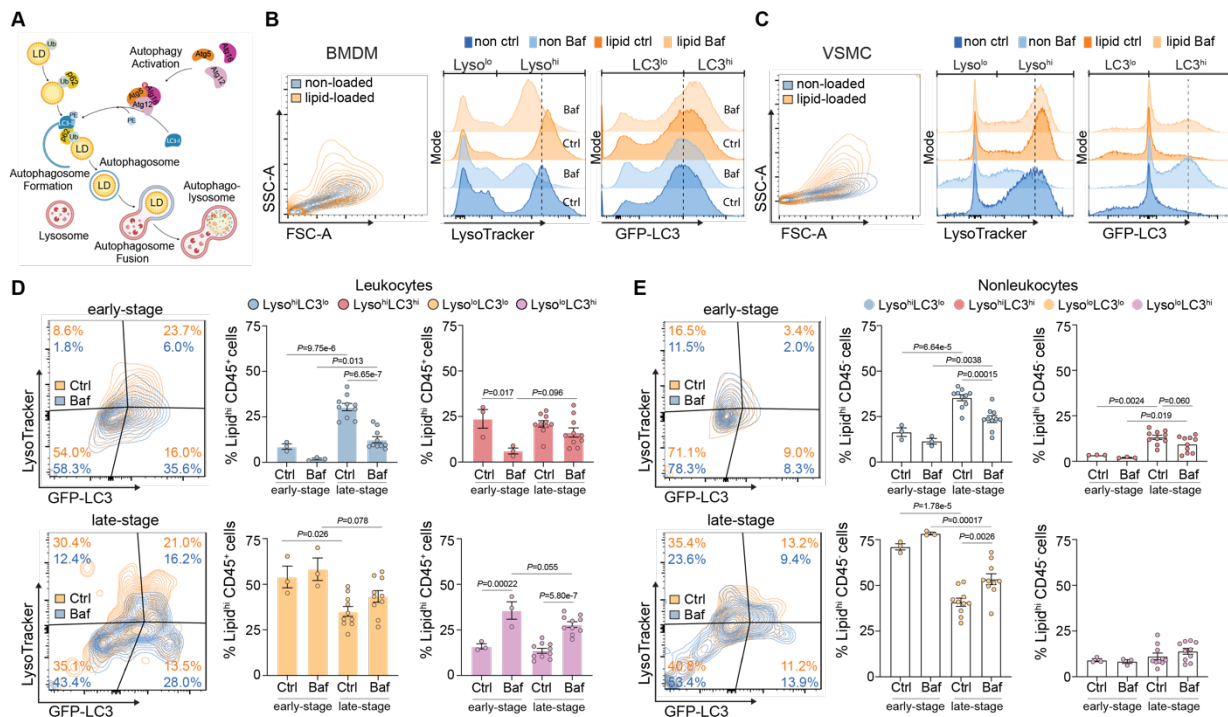


Figure 3-2: Autophagy Flux is Impaired in Nonleukocyte Foam Cells During Atherogenesis

A, Diagram of autophagy flux. **B-C**, Flow cytometric determination of LysoTracker and GFP-LC3 levels in primary bone marrow derived macrophages (BMDM) (**B**) and vascular smooth muscle cells (VSMC) (**C**) upon lipid loading using aggregated LDL and m β -CD-cholesterol respectively, alongside treatment with the autophagy inhibitor bafilomycin (Baf) or vehicle control (Ctrl). Plots were from representative experiments repeated 3 times for BMDMs and 2 times for the VSMCs. **D-E**, Autophagy flux of Lipid^{hi} leukocyte (**D**) or non-leukocyte (**E**) cells from aortic digests treated *ex vivo* with bafilomycin (n=3 early, n=10 late). A one-way ANOVA with a Holm-Sidak's post hoc test for multiple comparisons was used (D-E; corrected for 4 pre-selected comparisons) to determine significance for these data.

To rule out the possibility that the differences in autophagy flux observed in M ϕ s as compared to VSMCs might be attributable to the distinct cholesterol loading methods used for these two cell types, we sought to adopt a similar cholesterol loading protocol for M ϕ s and VSMCs to more directly compare autophagy flux. While treatment of M ϕ s with 80 μ g/mL of m β -CD-cholesterol complexes resulted in significant cell death (data not shown), treatment of M ϕ s and VSMCs with 35 μ g/mL of m β -CD-cholesterol complexes for 48 hours was sufficient to induce cytosolic LD accumulation in both cell types (**Figure 3.3 e**) and to convert VSMCs to macrophage-like cells, as evidenced by reduced levels of *Acta2* and *Cnn1* mRNA (**Figure 3.3 f**) concomitant with increased expression of the M ϕ markers Mac-2 (*Lgals3*) and CD68 (*Cd68*) (**Figure 3.3 g**) similar to previously observed²⁶. Like agLDL (**Figure 3.3 d**), cholesterol loading in M ϕ s using m β -CD-cholesterol complexes activated autophagy flux (**Figure 3.3 h**), while cholesterol loading in VSMCs employing the identical treatment protocol resulted in impaired autophagy flux (**Figure 3.3 h**). Finally, we also confirmed our observations in a more physiologically relevant VSMC foam model by agLDL loading. Similar to the m β -CD-cholesterol-loading conditions, agLDL loading of VSMCs failed to induce pATG16L1 in response to lipid loading as compared to macrophages (**Figure 3.3 i**).

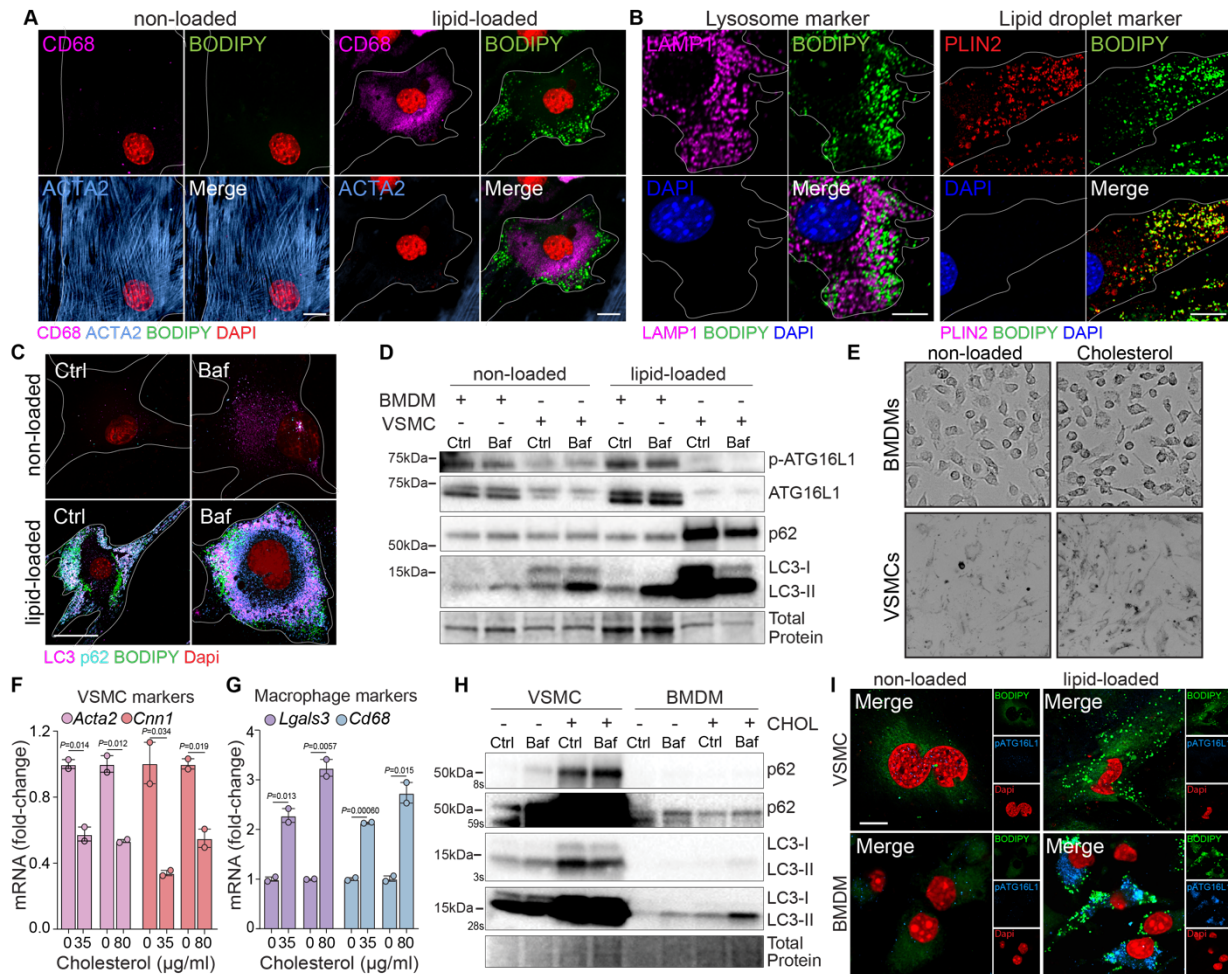


Figure 3-3: VSMC-Derived Foam Cells Fail to Initiate Autophagy Upon Lipid Loading

A-C, Immunofluorescence images of transdifferentiated primary VSMCs for macrophage and VSMC lineage markers (A), lipid (B) and autophagic markers (C). Images are from representative experiments repeated 3 times. **D**, Immunoblotting of non-lipid loaded as compared to agLDL-loaded BMDMs and 80 μg/ml mβ-CD cholesterol-loaded VSMCs. **E**, Brightfield images of BMDMs and VSMCs incubated with 35 μg/ml mβ-CD cholesterol for 48 hours. **F-G**, Gene expression analysis of VSMCs (F) and Mφ (G) markers in VSMCs loaded with the indicated concentrations of mβ-CD-cholesterol (n=2 technical replicates per group, representative of 2-4 independent experiments). Fold changes are reported relative to 0 μg/ml mβ-CD cholesterol for each gene. **H**, Immunoblotting of BMDMs and VSMCs incubated with 35 μg/ml mβ-CD-cholesterol. **I**, Immunofluorescence images of primary VSMCs and BMDMs lipid loaded with agLDL and stained for BODIPY and the active autophagy marker pATG16L1 (representative images of 3 independent experiments). Western blots presented in D and H are representative of 3 experiments. An unpaired t-test was used to determine statistical significance (F & G). Scale bar=20 μm for all images.

To evaluate markers of autophagy *in vivo*, fluorescence microscopy of the neutral lipid stain monodansylpentane (MDH) and leukocyte marker CD45 was used to distinguish between leukocyte- and non-leukocyte derived foam cell populations in the aortic root (**Figure 3.4 a**). Immunofluorescence staining was performed for the autophagy markers p62 and pATG16L1. We observed higher levels of p62 and pATG16L1 protein in leukocyte-derived foam cells in late-stage atherosclerosis as compared to early-stage atherosclerosis (**Figure 3.4 b, c**), together demonstrating the functional capacity for autophagy to occur in these leukocyte foam cells during the progression of atherosclerosis. While p62 and pATG16L1 protein levels were low in nonleukocyte foam cells in the earlier stages of atherosclerosis, p62 but not pATG16L1 staining was elevated in nonleukocyte foam cells in late-stage atherosclerosis (**Figure 3.4 b, c**), corroborating that autophagy flux is defective and fails to initiate in this cell population in advanced atherosclerotic plaque. In line with this, foam cells with elevated LC3 and pATG16L1 were primarily detected in leukocyte, but not nonleukocyte foam cells in late-stage atherosclerosis (**Figure 3.4 b, c**), correlating with active nascent autophagosome formation, further supporting that autophagy flux is superior in leukocyte foam cells even in late atherosclerosis. Quantification of p62, pATG16L1 and LC3 in the leukocyte and nonleukocyte fractions of the atherosclerotic plaques (**Figure 3.4 e-g**) was performed as described in the Online Supplement (**Figure 6.7**).

To further investigate the autophagic profile of atherogenic arteries, by expression of its components, we explored transcriptomic analyses of autophagy and lysosomal genes in Mφs and VSMCs undergoing phenotypic modulation in *Apoe*^{-/-} mice fed an atherogenic for 8 and 16 weeks using a publicly available dataset²⁷. We observed an increase in the

lipid droplet marker *Plin2* in these modulated SMCs (**Figure 6.8**, like **Figure 3.2 f**, suggesting that this population is comprised in part of VSMC-derived foam cells. We uncovered elevated expression of autophagy activators *Uvrag* (UV radiation resistance-associated gene), *Hmgb2* (high-mobility group box 2), *Coro1a* (coronin 1A), *Rab8b* (member RAS oncogene family) and lysosomal genes *Npc2* (NPC intracellular cholesterol transporter 2), *Psap* (prosaposin), *Lipa* (lysosomal acid lipase A), *Lamp1* (lysosomal associated membrane protein 1), *Ctsb* (cathepsin B) and *Ctsd* (cathepsin D) in Mφs as compared to modulated VSMCs, concomitant with increased expression of the autophagy suppressor *Dap1* (death associated protein) in these modulated VSMCs (**Figure 6.8**). The expression of these genes in Mφs and modulated VSMCs in the aortic root was consistent at baseline, 8 weeks and 16 weeks of diet, except for *Plin2* expression, which increased in modulated VSMCs during atherosclerosis progression and indicative of LD accumulation in later stages of atherosclerosis (**Figure 6.8**). Collectively, these results demonstrate a deficiency in the expression of autophagy-related components and autophagic flux in lipid-enriched VSMCs as compared to Mφ-derived cells in atherosclerotic plaques.

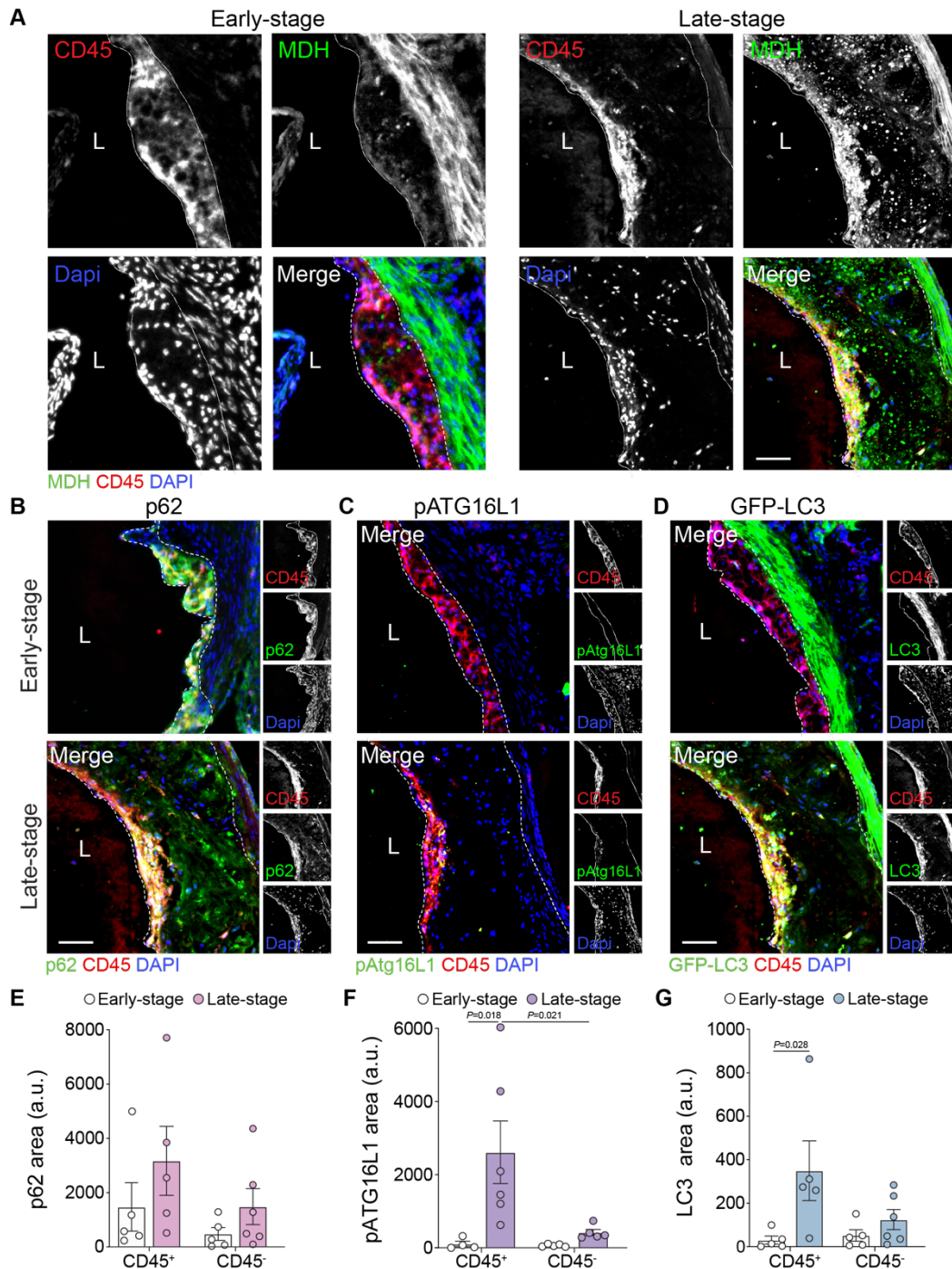


Figure 3-4: Autophagy Components are Differentially Expressed Between Leukocyte and Nonleukocyte Foam Cells in Atherosclerotic Plaque

A, Representative immunofluorescence staining of CD45 (red) and monodansylpentane (MDH) in aortic sinus sections in early-stage or late-stage atherosclerosis. **B-G**, Representative images (**B-D**) and quantification (**E-G**) of CD45 (red) and monodansylpentane (MDH) (green) with p62 (green), pATG16L1 (green) or GFP-LC3 (green) and their colocalization (yellow) in aortic sinus sections (n=4-5 early-stage, n=5-

6 late-stage). Dashed lines indicate plaque borders. L=lumen. Scale bar=50 μ m. A two-way ANOVA with a Holm-Sidak's post hoc test for multiple comparisons was used (E-G; corrected for comparisons between all experimental groups) to determine significance for these data.

3.4.3 Autophagy activation in VSMC, but not macrophage, foam cells promotes cholesterol efflux

The above cell culture studies were performed in primary macrophages and VSMCs, as the changes the autophagic capacity in these cells upon lipid loading would be the most representative as occurs *in vivo*. However, given that BMDMs and VSMCs are derived from separate tissues and protocols, a direct comparison of these cell types is limited using these approaches. As our goal was to perform a direct comparative analysis of the role of autophagy in cholesterol efflux from foam cells, we generated VSMCs in parallel with M ϕ from immortalized E14 mouse embryonic stem cells (mESCs), using previously established protocols for differentiation of mESCs into M ϕ ²⁸ and VSMCs^{29,30}. Acquisition of VSMC and M ϕ markers by this differentiation protocol was confirmed by gene expression (**Figure 6.9 and Figure 3.5 a**). Incubation of mESC-derived VSMCs with m β -CD-cholesterol complexes for 72 hours converted them into the previously described²⁶ VSMC-derived foam cells, as evidenced by the formation of LDs, increased expression of CD68 and reduced expression ACTA2 by microscopy (**Figure 3.5 a**). These phenotypic changes were accompanied by decreased mRNA expression of VSMC-related genes ACTA2 (*Acta2*) and α -tropomyosin (*Tpm1*) (**Figure 3.5 b**), while M ϕ markers Mac-2 and CD68 were increased (**Figure 3.5 c**), as previously observed²⁶.

We next assessed the role of autophagy in regulating cholesterol efflux from these mESC-derived foam cells. This was achieved by adding ³H-cholesterol to the cholesterol-loading

media during foam cell genesis (M β CD-cholesterol complexes for VSMCs and agLDL for M ϕ), prior to addition of cholesterol acceptors apoA-I or HDL and in the presence or absence of autophagy inhibitors (chloroquine) or activators (metformin). In line with previous observations³, autophagy inhibition using chloroquine led to a ~70% reduction in cholesterol efflux to apoA-I and a modest but significant reduction in efflux to HDL (~25%) in mESC-derived M ϕ foam cells (**Figure 3.5 d**). In contrast, chloroquine treatment had no effect on cholesterol efflux to apoA-I in mESC-derived VSMC foam cells, but there was a trend to a reduction in cholesterol efflux to HDL by ~70% ($P=0.23$; **Figure 3.5 e**). We next tested if autophagy activation by metformin could enhance cholesterol efflux from mESC-derived M ϕ or VSMC foam cells. We observed no increase in mESC-derived M ϕ foam cell cholesterol efflux upon metformin treatment (**Figure 3.5 d**), whereas metformin increased cholesterol efflux to HDL two-fold in mESC-derived VSMC foam cells (**Figure 3.5 e**). Modified LDL loading in macrophages has been previously shown to induce autophagy flux^{3,31}. Therefore, we surmised that the failure of metformin to further induce cholesterol efflux from agLDL-loaded M ϕ might be explained by the fact that autophagy was already maximally induced by lipid-loading in these cells. Indeed, we observed a significant induction of cholesterol efflux in BMDMs when incubated with radiolabeled cholesterol incorporated into modified LDL (acetylated LDL) as compared to that delivered in a free form (non-loaded) (**Figure 3.5 f**). While metformin treatment did show a trend towards an increase in cholesterol efflux in the BMDMs incubated with free cholesterol ($P=0.077$), this did not occur in the acLDL-treated conditions due to autophagy already having been activated in these cells by pro-atherogenic lipid loading.

Consistent with reduced autophagy-mediated cholesterol efflux observed in VSMC foam cells, immunoblotting revealed increased p62 protein levels in lipid-loaded mESC-derived VSMCs (**Figure 3.5 g**). Accumulation of p62 in mESC-derived VSMC foam cells was associated with increased LC3-II protein levels that were not further increased following lysosomal inhibition by treatment with chloroquine, indicating that elevated LC3-II in these cells was attributable to defective autophagy (**Figure 3.5 g**). Conversely, treatment with metformin restored autophagy flux, as evidenced by the further increase in LC3-II protein upon addition of chloroquine in metformin-treated mESC-derived VSMC foam cells (**Figure 3.5 g**). Collectively, these results indicate that basal autophagy function and cholesterol efflux in VSMC foam cells is poor. Furthermore, VSMC foam cells appear to primarily efflux to HDL but not lipid-poor apoA-I, which suggests that autophagy-mediated cholesterol efflux can occur in VSMC-derived foam cells, and it does so by different mechanisms as compared to their more commonly studied M ϕ counterparts. Altogether, we demonstrate that despite a defect in the induction of autophagy upon lipid loading (**Figures 3.2-3.4**), autophagy in VSMC foam cells and subsequent cholesterol efflux to HDL can be induced by pharmacologic means through the use of the clinically relevant compound metformin.

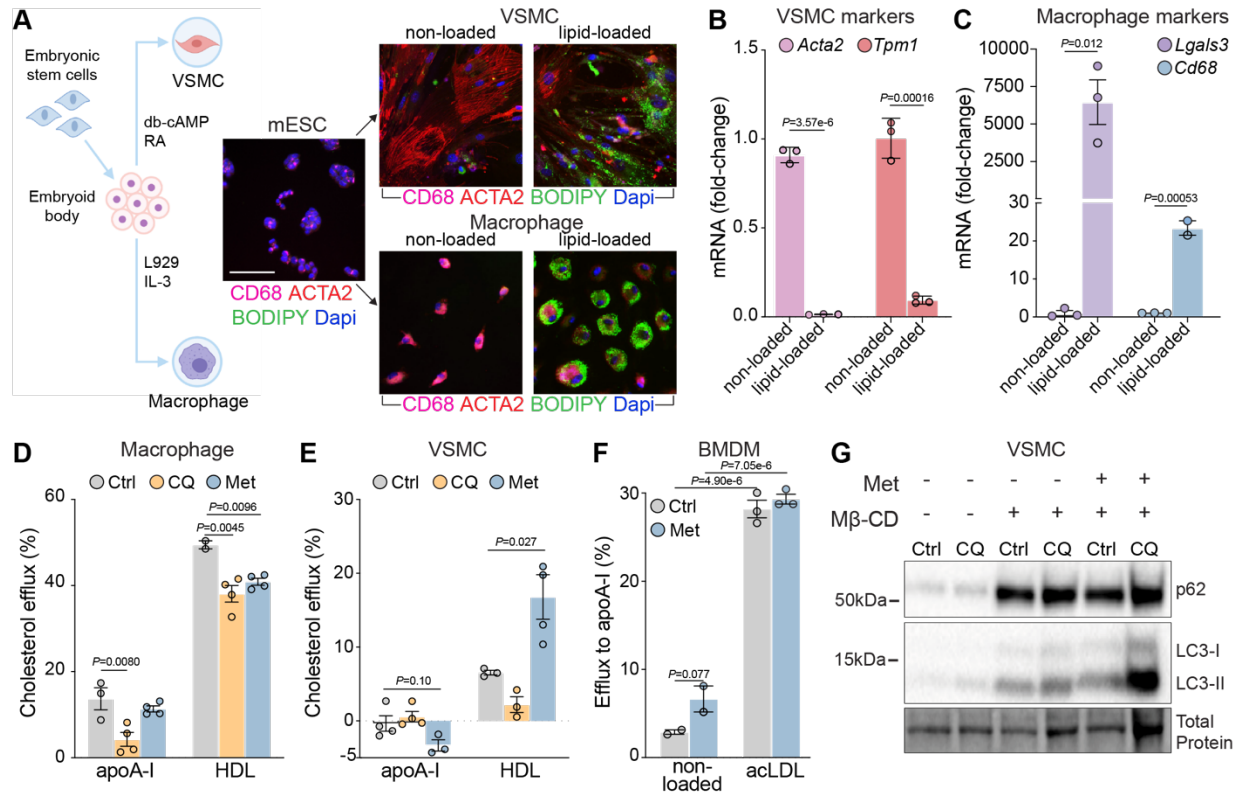


Figure 3-5: Autophagy-Mediated Cholesterol Efflux is Impaired in VSMC Foam Cells

A, Schematic and representative fluorescence microscopy images of lipid-loaded VSMC and Mφs differentiated from embryonic stem cells (ESC). Scale bar=100µm. **B-C**, Gene expression analysis of smooth muscle (B) and Mφ (C) markers in ESC-derived VSMC foam cells (n=3 technical replicates per group, representative of 3 independent experiments). Fold changes are reported relative to the non-lipid loaded treatment for each gene. **D-F**, Efflux of radiolabelled cholesterol from Mφs (D & F) and transdifferentiated smooth muscle cells (E) treated with chloroquine (CQ) or metformin (Met) relative to no treatment (Ctrl) (n=2-4 control, n=3-4 CQ & Met, technical replicates per group, representative of 3 independent experiments). **G**, Immunoblotting of transdifferentiated VSMCs treated with chloroquine for autophagy markers p62 and LC3. Western blots are representative images of 3 independent experiments. For comparisons between 2 groups an unpaired t-test was used (B-C) or for comparisons between more than 2 groups a one-way ANOVA with a Holm-Sidak's post hoc test for multiple comparisons was used (D-F; D-E were corrected for 2 pre-selected comparisons and F was corrected for 4 pre-selected comparisons) to determine statistical significance.

3.4.4 Elevated expression of ABCG1 as compared to ABCA1 in nonleukocyte foam cells

We observed that several autophagy-related and lysosomal genes that were differentially regulated between plaque Mφs and modulated VSMCs, such as *Uvrags*, *Ctsb*, *Ctsd*, *Lamp1* and *Lipa*, are targets of the autophagy transcription factor TFEB (**Figure 6.8**). We therefore quantified *Tcf7l1* expression in lipid-loaded primary VSMCs lacking *Atg5*, which is required for autophagy²³. We observed a marked reduction in *Tcf7l1* mRNA in VSMCs upon lipid loading (**Figure 3.6 b**), suggesting that reduced expression of autophagy and lysosome genes in modulated VSMCs is attributable, in part, to reduced *Tcf7l1* expression in PLIN2-lipid-enriched VSMCs (**Figure 3.2 f-h**). In Mφ foam cells, it has been proposed that autophagy regulates the transcriptional activity of liver X receptor (LXR)³², which in turn promotes the expression of the ABCA1 and ABCG1 cholesterol efflux transporters. Consistent with this, upon VSMC lipid loading, *Atg5* knockdown partially reduced lipid-induced expression of *Abca1* (**Figure 3.6 c**). Interestingly, inhibiting autophagy by *Atg5* knockdown did not impair induction of *Abcg1* in lipid-loaded VSMCs (**Figure 3.6 d**). In addition, the magnitude of *Abcg1* upregulation upon VSMC lipid loading was greater in magnitude than that of *Abca1* upregulation (**Figure 3.6 c, d**). These results suggest that impaired autophagy initiation in lipid-loaded VSMCs leads to reduced lysosome functionality via reduced TFEB-mediated lysosomal gene expression, along with impaired autophagy-associated regulation of ABCA1, upon lipid accumulation.

Cholesterol efflux to HDL is primarily dependent on ABCG1, as opposed to cholesterol efflux to apo-A1 which is primarily dependent on ABCA1^{1,33}. Given the appreciable HDL-mediated cholesterol efflux from ESC-derived lipid-loaded VSMCs that we observed after

metformin treatment (**Figure 3.5 e**), we hypothesized that VSMC-derived foam cells rely more on ABCG1 expression than ABCA1 for cholesterol efflux. Quantification of ABCA1 and ABCG1 expression at the protein level by immunoblot showed that cholesterol loading induced upregulation of ABCA1 to a greater extent than ABCG1 in Mφs (**Figure 3.6 e**). Conversely, ABCG1 protein expression in VSMCs was basally higher than that of ABCA1 (**Figure 3.6 e**), although ABCG1 expression was unaltered upon lipid loading. Finally, we sought to determine relative ABCA1 and ABCG1 expression in leukocyte and non-leukocyte derived cells of the aortic root during atherosclerosis. Immunofluorescence staining revealed that ABCA1 expression was significantly upregulated only in leukocyte foam cells during the progression to late-stage atherosclerosis and was greater than that of the nonleukocyte fraction, while ABCG1 expression was unchanged in leukocyte and nonleukocyte foam cells in late-stage atherosclerosis (**Figure 3.6 f-h**). Together, these *in vitro* and *in vivo* studies suggest the reliance of VSMC-derived foam cells on ABCG1 for autophagy-mediated cholesterol efflux that remains physiologically defective in the atherosclerotic plaque.

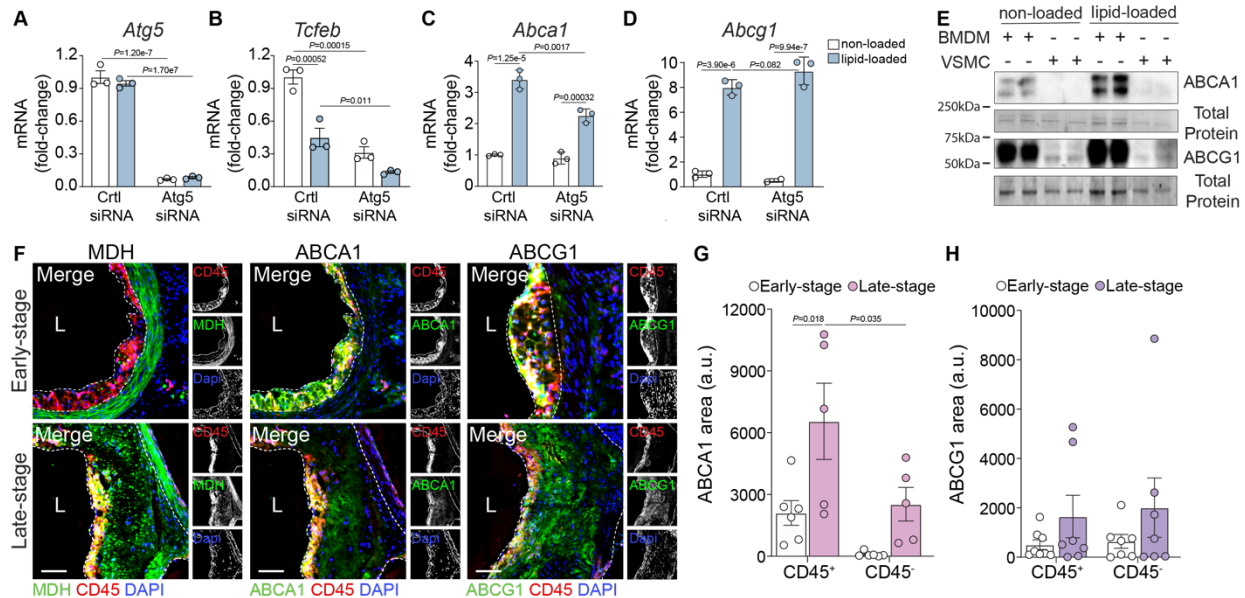


Figure 3-6: VSMC Derived Foam Cells Predominantly Express ABCG1

A-D, Gene expression analysis of *Atg5*, *Tcfel*, *Abca1* and *Abcg1* in lipid-loaded primary mouse VSMCs. Representative of 3 experiments using 3 technical replicates. Fold changes are reported relative to the non-lipid loaded control siRNA treatment group. **E**, Immunoblotting of lipid-loaded BMDMs or VSMCs for the cholesterol efflux transporters ABCA1 and ABCG1. Western blots are representative of 3 experiments. **F-H**, Representative immunofluorescence images and quantification of leukocyte (CD45⁺) and nonleukocyte (CD45⁻) foam cells with ABCA1 (n=6 early-stage, n=5 late-stage) or ABCG1 (n=7-8 early- & late-stage) and their colocalization in the aortic sinus atherosclerotic plaques. Dashed lines indicate plaque borders. L=lumen. Scale bar=50 μm. For the data in this figure a one-way ANOVA (A-D; corrected for 4 pre-selected comparisons) or two-way ANOVA (G-H; corrected for comparisons between all experimental groups) with a Holm-Sidak's post hoc test for multiple comparisons was used to determine statistical significance.

3.5 Discussion

Since it was first reported by the Framingham Heart Study, the inverse association between HDL concentration and cardiovascular disease (CVD) has been the target of intense investigation. Since then, study of the mechanisms by which HDL protects against atherosclerosis has led to the identification of the RCT pathway³⁴. A prevailing notion of the revised HDL hypothesis, based on the strong experimental and emerging clinical evidence that a major function of HDL particles is to mediate RCT³⁵⁻³⁸, is that HDL function

- rather than its concentration in the plasma - is the key attribute for CVD risk reduction. Therefore, advances in RCT-promoting strategies present an opportunity to halt or reverse atherosclerosis³⁹. Because the rate-limiting step in RCT is the hydrolysis of LDs that accumulate within foam cells to generate cholesterol for efflux², we herein sought to better understand this process in VSMC foam cells and, more specifically, the role of autophagy in VSMC foam cell cholesterol efflux.

Cholesterol stored within LDs can be accessed via lipolysis mediated by cytosolic neutral lipases², or LDs can be targeted for autophagic degradation by *lipophagy*^{2,3,40}. In macrophage foam cells, autophagy-mediated LD degradation accounts for the generation of >50% of free cholesterol for efflux upstream of ABCA1³. Although it has been known for some time that VSMCs in the intima can also accumulate cholesterol through a variety of pathways, conventional histological markers such as CD68 used to identify M ϕ in plaques also include foam cells of the VSMC lineage, confounding the contribution of VSMC foam cells in atherosclerosis³⁹. The development of lineage tracing mice^{13,15,26} and assays for human plaque¹² have identified a substantial proportion of plaque M ϕ -marker positive foam cells as originating from VSMCs, ranging from 30-70% and increasing with the stage of atherosclerosis^{11,15,16}. While cholesterol accumulation plays a critical role in VSMC foam cell pathogenesis, the role of autophagy in regulating LD catabolism and cholesterol efflux in VSMC foam cells is unknown.

To quantify autophagic flux in distinct foam cell populations during atherosclerosis progression, we used PCSK9-AAV to phenocopy the *Ldlr*-knockout mouse^{18,20} in combination with GFP-LC3 transgenic mice to monitor autophagy¹⁷. Using flow cytometry

gating schemes recently described to distinguish M ϕ (leukocyte) and VSMC (nonleukocyte) foam cells in the aorta¹⁶, we observed an increase in the absolute number of foam cells of only nonleukocyte origin in late-stage lesions (25 weeks) as compared to early-stage lesions (6 weeks) (**Figure 3.1 f**). This was accompanied by an increase in the proportion of nonleukocyte foam cells as atherosclerosis progressed, from 60% to 76% of aortic arch foam cells in early versus late atherosclerosis, respectively (**Figure 3.1 e**), as previously reported¹⁶. By simultaneously using LysoTracker, a pH sensitive dye, we quantified functional lysosomal content in parallel with the autophagosome marker GFP-LC3 in foam cells from atherosclerotic aortic digests at early and late stages of atherogenesis, or *in vitro* in lipid-loaded M ϕ s or VSMCs.

In GFP-LC3-expressing cells, autophagosome biogenesis causes a spike in GFP fluorescence. As autophagy ensues, autophagosomes mature into autophago-lysosomes and lysosomes, and autophagosome degradation is accompanied by loss of the GFP signal. Conversely, impaired (or inhibition of) autophagy leads to the accumulation of autophagosomes and higher GFP signal using this model. In turn, LysoTracker stains cellular acidic compartments including lysosomes to detect autophagy-associated lysosomal activity. Using these parameters, we found that the proportion of foam cells defective in autophagy, where LC3 accumulates despite high lysosomal activity (Lyso^{hi}LC3^{hi}), increased in late-stage atherosclerosis in leukocyte (1.4-fold) and nonleukocyte (4.7-fold) foam cells (**Figure 3.1 g-h**). This was most dramatic in nonleukocyte foam cells, suggesting a more pronounced defect in autophagy in nonleukocyte foam cells during atherogenesis.

To determine the extent of this autophagy defect, aortic digests were treated *ex vivo* with bafilomycin, a compound used to inhibit lysosomal acidification and autophagy flux (**Figure 3.2d-e**). Interestingly, the autophagy quiescent (Lyso^{lo}LC3^{lo}) quadrant, where the bulk of nonleukocyte foam cells localized to, showed minimal fluctuation with bafilomycin treatment. In contrast, amongst the greatest shifts in the leukocyte fraction with bafilomycin treatment were decreases in the autophagy functional (Lyso^{hi}LC3^{lo}) and increases in the Lyso^{lo}LC3^{hi} population. The latter could represent either a population with active autophagosome biogenesis or low lysosomal capacity causing buildup of non-degraded autophagosomes, both of which result in high GFP signal. Because the increase in the Lyso^{Lo}LC3^{hi} leukocyte foam cell population upon treatment with bafilomycin, which inhibits lysosomal degradation and thus promotes an accumulation of autophagosomes, this indicates that leukocyte foam cells efficiently induced autophagosome biogenesis in early atherosclerosis and continue to do so even in late-stage atherosclerosis. However, the Lyso^{Lo}LC3^{hi} population was unresponsive to bafilomycin treatment in nonleukocyte foam cells, suggesting that nonleukocyte foam cells possess low autophagosome biogenesis. Together, our data demonstrate a lower capacity of nonleukocyte foam cells to initiate autophagy in atherosclerosis. While leukocyte foam cells continue to exhibit functional autophagy even in late-stage atherosclerosis, nonleukocyte foam cells possess low basal autophagy flux in early atherogenesis that persists as the disease progresses. The time points we chose for our studies of autophagy, early versus late, represent two extremes of the atherosclerotic spectrum, where lipid loading begins as compared to when foam cells might become overwhelmed by their excess lipid content. However, during atherogenesis, several

maladaptive processes progressively occur, including dysfunctional cellular turnover and recruitment. Future studies of autophagy at intermediate time points could therefore further elucidate dynamics of atherosclerotic plaques and contribute to our understanding of cholesterol metabolism in atherosclerosis.

Quantification of autophagy markers in the aortic root of atherosclerotic mice corroborated that leukocyte foam cells exhibit higher autophagy flux as compared to nonleukocyte foam cells (**Figure 3.4**). First, protein levels of phosphorylated ATG16L1 that are directly correlated with active autophagosome formation were elevated in leukocyte foam cells of established late-stage atherosclerotic plaques, but not in nonleukocyte foam cells. This is consistent with human carotid atherosclerotic plaques, where ATG16L1 is primarily expressed in macrophage foam cells but not in SMCs⁴¹. Second, leukocyte foam cells had elevated levels of the autophagy markers p62 and GFP-LC3 in late-stage atherosclerosis in overlapping pATG16L1-positive areas, whereas nonleukocyte foam cells displayed an accumulation of p62 and GFP-LC3 in areas devoid of pATG16L1, implying that M ϕ foam cells *in vivo* can perform functional autophagy whereas VSMC foam cells cannot. Together with our observation that nonleukocyte foam cells had increased proportions of Lyso^{lo}LC3^{hi} cells that possess high autophagosome content but low lysosome function in late stages, this suggests that nonleukocyte foam cells have impaired autophagosome biogenesis in combination with impaired lysosome functionality. Consistent with this, we found reduced expression of autophagy initiators (*Uvrag*, *Hmgb2*, *Coro1a* and *Rab8b*) and lysosomal genes (*Npc2*, *Psap*, *Lipa*, *Lamp1*, *Ctsb* and *Ctsd*) and increased expression of the autophagy suppressor *Dap1* in VSMCs undergoing phenotypic modulation during atherogenesis as compared to M ϕ s from the same mice

(**Figure 6.8**)²⁷. These observations are consistent with a recent study showing that lysosomal capacity is reduced in VSMCs as compared to macrophages⁴² and demonstrate an additional defect in the initiation of autophagy.

To test if autophagy regulates LD catabolism and cholesterol efflux in VSMC and M ϕ foam cells alike, we turned to established foam cell culture models. *In vitro* models of M ϕ foam cells formed by loading with atherogenic lipoproteins such as acetylated, oxidized or aggregated LDL have been well described^{3,43-50}. In atherosclerotic plaques, most cholesterol-rich LDL is aggregated and tightly bound to the subendothelial matrix⁴⁴. Thus, agLDL is a major pathophysiological form of modified LDL that converts M ϕ s to foam cells in the arterial wall, and the concentration used in our studies to generate M ϕ foam cells is believed to be analogous to the process *in vivo*^{43,44}, with the majority of agLDL-associated cholesterol being stored in cytoplasmic LDs^{3,43-45}. In contrast, agLDL loading of VSMCs results in lysosomal trapping of agLDL-associated cholesterol, with these cells remaining devoid of established LDs and failing to upregulate ABCA1^{12,42}. Interestingly, several studies have shown VSMC conversion to foam cells following incubation with m β -CD-cholesterol complexes, with m β -CD-cholesterol-derived cholesterol bypassing initial lysosome lipoprotein processing and allowing for their conversion to foam cells^{14,15,26}.

VSMC conversion to foam cells is accompanied by loss of expression of VSMC markers and acquisition of expression of M ϕ markers¹³. In our study, following VSMC lipid-loading with m β -CD-cholesterol complexes as previously²⁶, we observed a loss of prototypical SMC markers and the acquisition of M ϕ -associated markers (**Figures 3.3 & 3.5**). Similar to agLDL-loaded M ϕ s, where the majority of agLDL-associated cholesterol is stored in

cytoplasmic LDs^{3,43-45}, excess m β -CD-cholesterol derived cholesterol in VSMCs was primarily localized to PLIN2-positive LDs, as shown by the accumulation of numerous cytoplasmic BODIPY-positive vesicles that co-localized with the *bona fide* LD marker PLIN2. This shows that VSMC foam cells are laden with PLIN2-positive LDs, similarly to M ϕ foam cells^{3,5}, allowing us to study autophagy in these foam cells that exhibit an accumulation of cytoplasmic cholesterol-enriched LDs irrespective of the protocols used to induce LD formation. We found that autophagy flux was deficient in both mESC-derived and primary mouse VSMC foam cells relative to M ϕ s, as evidenced by low levels of expression of ATG16L1, a key regulator of autophagosome formation (**Figure 3.5**). We also observed an increase in the autophagy cargo receptor p62 and autophagosome marker LC3 upon lipid-loading of VSMCs as compared to M ϕ s. As this increase was not further exacerbated upon autophagy inhibition using bafilomycin provides further evidence that the autophagic capacity of VSMC-derived foam cells is limited compared to that of M ϕ s.

A recent study shows that the expression profiles of m β -CD-cholesterol-loaded VSMCs do not mirror those of VSMCs in mouse atherosclerotic lesions⁵¹. To further confirm that VSMCs exhibit impaired autophagy induction upon cholesterol loading using a more physiological model of VSMC loading, we examined pATG16L1 as a marker of active autophagy in agLDL-loaded VSMCs and M ϕ s relative to their non-loaded counterparts (**Figure 3.3**). pATG16L1 was specifically induced in M ϕ s upon agLDL loading, but not in VSMCs, thus again pointing to active autophagy in M ϕ foam cells but not VSMC foam cells. Interestingly, while agLDL alone or in the presence of conditioned media from M ϕ s did not consistently lipid load VSMCs, the incubation of VSMCs in the presence of agLDL

in conditioned media from agLDL-loaded M ϕ consistently resulted in a marked increase in VSMC neutral lipids. Mounting evidence suggests that lysosomal acid lipase (LAL) is secreted from macrophages and participates in extracellular hydrolysis of agLDL⁵²⁻⁵⁵. This process requires macrophage lysosome exocytosis⁴⁴ that involves the secretion of LAL. In parallel, it was shown that LAL can be endocytosed by VSMCs to promote cholesterol metabolism subsequent to lysosomal lipolysis in these cells⁴². Altogether, it is tempting to speculate that VSMC atherogenic lipoprotein loading with M ϕ foam cell-derived conditioned media best mimics *in vivo* VSMC foam cell conversion. In atherogenic plaques, macrophage foam cell-derived LAL might be endocytosed by VSMCs along with agLDL to generate VSMC foam cells or alternatively agLDL in contact with M ϕ could lead to local increases in free cholesterol levels as previously shown⁵². The relative contributions of these processes to the *in vivo* generation of VSMC foam cells in the plaque require further investigation.

Despite induction of M ϕ markers after cholesterol loading, the phagocytic capacity of VSMC foam cells is weak and distinct from M ϕ s²⁶. Given that M ϕ autophagy and TFEB activation promote efferocytosis and lysosomal catabolism in atherosclerotic plaques^{6,56-58}, a potential explanation for why VSMC foam cells exhibit poor M ϕ -like function²⁶ is their limited autophagic capacity. Amongst known autophagy transcription factors, TFEB is induced in M ϕ s by atherogenic lipids⁵⁹ and exerts global control over the autophagy gene network, thereby linking autophagy to both lysosomal function and lipid utilization⁶⁰. Because several autophagy and lysosomal genes that were elevated in M ϕ s as compared to modulated VSMCs are known TFEB targets, we performed gene expression analysis for *Tcfef* in primary mouse VSMC foam cells (**Figure 3.6 b**). We observed reduced *Tcfef*

mRNA levels in VSMCs upon lipid-loading. In parallel, we observed reduced TFEB nuclear translocation in lipid-loaded relative to non-lipid-loaded VSMCs (data not shown), further supporting the notion that autophagosome and lysosome biogenesis is defective in VSMC foam cells.

Cholesterol efflux to apoA-I and HDL is primarily mediated via the ABCA1 and ABCG1 cholesterol transporters, respectively². We previously established that M ϕ foam cell autophagy was closely linked to ABCA1^{3,58}, and this was corroborated in mESC-derived M ϕ foam cells, where chloroquine inhibited most of apoA-I-mediated cholesterol efflux but not efflux to HDL. While VSMCs in culture models and human atherosclerotic lesions express less ABCA1 and are impaired in their capacity for cholesterol efflux to apoA-I⁶¹, we observed that ABCG1-mediated efflux to HDL in VSMC foam cells could be stimulated by autophagy activation using metformin. Intriguingly, *Abcg1* expression was independent of autophagy, while *Abca1* expression was at least partially dependent on functional autophagy in these VSMC foam cells (**Figure 3.6 c-d**). Gain- and loss-of-function studies will be required to establish the respective contributions of ABCA1, ABCG1, or other cholesterol transporters to autophagy-mediated cholesterol efflux from VSMC foam cells. Interestingly, metformin could only enhance cholesterol efflux in macrophages loaded with free cholesterol, but not cholesterol incorporated in lipoproteins (acLDL) which is known to stimulate autophagy (**Figure 3.5 f**), suggesting that further increasing autophagy in M ϕ foam cells is not a viable strategy to promote RCT. However, our results indicate that in advanced atherosclerosis, nonleukocyte foam cells, which comprise the bulk of autophagy-deficient foam cells, may benefit from autophagy activation to promote HDL-mediated cholesterol efflux and overall promote RCT. In turn, M ϕ foam cells that

exhibit more functional autophagy may further benefit from increasing the specific targeting and degradation of LDs, as opposed to stimulation of bulk autophagy *per se*, to enhance autophagy-dependant ABCA1-mediated M ϕ foam cell RCT.

Simultaneously increasing macrophage autophagy and lysosomal biogenesis by M ϕ -specific TFEB overexpression reduces atherosclerosis⁵⁹. Moreover, activation of TFEB in plaque macrophages by administration of the natural sugar trehalose in mice slows the progression of atherosclerosis in a macrophage-dependent manner⁶². Similar to macrophage autophagy, VSMC autophagy is atheroprotective, where autophagy-defective VSMCs become prematurely senescent and promote atherogenesis⁶³. Our data here demonstrate that while the initiation of autophagy in lipid-loaded VSMCs is defective, it can be pharmacologically induced using metformin. Metformin is the oldest and most widely used antidiabetic glucose-lowering drug, and emerging evidence from clinical trials and real world registry point to a role for metformin in protecting against both coronary events and progression to heart failure⁶⁴. Even in patients with diabetes and reduced kidney function or a history of coronary artery disease (CAD), treatment with metformin is associated with lower risk of major adverse cardiovascular events (MACE)^{65,66}.

Beyond the cardiovascular protection conferred by anti-hyperglycemic agents in diabetic patients⁶⁷ and that metformin is associated with lower risk of MACE and lower all-cause mortality compared to other anti-hyperglycemic drugs⁶⁸, metformin is the only anti-diabetic drug with proven reduction of micro- and macrovascular complications⁶⁹. For example, inhibition of the mechanistic target of rapamycin (mTOR) by rapalogs prevents the development of atherosclerotic plaques in several animal models, and indirect

inhibition of mTOR by metformin reduces neointimal formation through inhibition of SMC proliferation and migration, and activation of autophagy^{69,70}. In addition, activation of adenosine monophosphate-activated protein kinase (AMPK), the target of metformin, attenuates atherosclerosis in several models of atherogenesis^{11,71}. Further research is needed to establish whether targeted delivery of metformin to VSMC foam cells within atherosclerotic plaques holds therapeutic promise for attenuating atherosclerosis in atherogenic mouse models or MACE in humans. Our data therefore support adjusting therapeutic strategies to regulate metabolic and inflammatory cell processes in atherosclerotic plaques, whereby targeting nonleukocyte foam cells, in addition to traditional leukocyte foam cells, will be key to more effectively treat CVD.

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3.8 Disclosures

None

3.9 References

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4 Manuscript 3: Trehalose Promotes Atherosclerosis Regression in Female Mice

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Trehalose Promotes Atherosclerosis Regression in Female Mice

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4.1 Abstract

Introduction: Atherosclerosis is a chronic inflammatory disease caused by the deposition of lipids within the artery wall. During atherogenesis, efficient autophagy is needed to facilitate efferocytosis and cholesterol efflux, limit inflammation and lipid droplet buildup, and eliminate defective mitochondria and protein aggregates. Central to the regulation of autophagy is the transcription factor EB (TFEB), which coordinates the expression of lysosomal biogenesis and autophagy genes. In recent years, trehalose has been shown to promote TFEB activation and protect against atherogenesis. Here, we sought to investigate the role of autophagy activation during atherosclerosis regression.

Methods and Results: Atherosclerosis was established in C57BL/6N mice by injecting AAV-PCSK9 and 16 weeks of Western diet feeding, followed by switching to a chow diet to induce atherosclerosis regression. During the regression period, mice were either injected with trehalose concomitant with trehalose supplementation in their drinking water or injected with saline for 6 weeks. Female mice receiving trehalose had reduced atherosclerosis burden, as evidenced by reduced plaque lipid content, macrophage numbers and IL-1 β content in parallel with increased plaque collagen deposition, which was not observed in their male counterparts. In addition, trehalose-treated female mice

had lower levels of circulating leukocytes, including inflammatory monocytes and CD4⁺ T cells. Lastly, we found that autophagy flux in male mice was basally higher than in female mice during atherosclerosis progression.

Conclusions: Our data demonstrate a sex-specific effect of trehalose in atherosclerosis regression, whereby trehalose reduced lipid content, inflammation, and increased collagen content in female mice but not in male mice. Furthermore, we discovered inherent differences in the autophagy flux capacities between the sexes: female mice exhibited lower plaque autophagy than males, which rendered the female mice more responsive to atherosclerosis regression. Our work highlights the importance of understanding sex differences in atherosclerosis to personalize the development of future therapies to treat cardiovascular diseases.

4.2 Introduction

Atherosclerosis is characterized by the deposition of lipid-laden cells, known as foam cells, within the intimal layer of medium and large arteries ¹. During the progression of atherosclerosis, high levels of cholesterol-rich low-density lipoprotein (LDL) particles enter the intimal layer of the arteries, where they become modified and activate the overlying endothelium. In turn, activated endothelial cells secrete cytokines and chemokines that recruit circulating monocytes. Upon entry, monocytes differentiate into macrophages that engulf the modified LDL ^{2,3}. The excess intracellular cholesterol undergoes esterification for storage within lipid droplets to prevent cellular lipotoxicity, resulting in macrophage foam cell formation ⁴.

In recent years, several mouse studies have reported that atherosclerotic plaque growth is halted upon the reversal of hypercholesterolemia, and these plaques undergo remodelling. This process was coined atherosclerosis "*regression*." A regressing atherosclerotic plaque exhibits one or more of the following: reduced size, reduced cholesterol content, dampened inflammation, reduced foam cell numbers, increased collagen content and/or increased fibrous cap size ^{5,6}. However, our understanding of the mechanisms that promote atherosclerosis regression is incomplete. Nonetheless, therapies that reduce circulating cholesterol levels in humans have provided clinical evidence that human atherosclerotic plaques can undergo regression, including remodelling and a reduction of overall plaque area ^{7,8}. Still, the amelioration of atherosclerosis in humans following hypercholesterolemia reversal is modest and more effective therapies to reverse this disease are needed. Here, we tested if autophagy activation promotes atherosclerosis regression.

Autophagy is a constitutive process that promotes cell homeostasis by degrading intracellular components to their constituent building blocks (i.e. amino acids, fatty acids) ^{9,10}. Autophagy can facilitate the removal of dysfunctional mitochondria ¹¹, protein aggregates ¹², inflammasomes ¹³ and lipid droplets ^{14,15}, all of which accumulate during atherogenesis ^{16,17}. We and others have shown that during atherosclerosis development, plaque foam cell autophagy becomes dysfunctional ^{12,13,18,19}. One possibility is that functional autophagy cannot ensue due to decreased lysosomal degradative capacity during atherosclerosis ²⁰⁻²². Central to the regulation of autophagy and lysosome function is the transcription factor EB (TFEB) ²³. Nuclear translocation of TFEB leads to the expression of genes involved in autophagosome and lysosome biogenesis, increasing

processes such as autophagy, phagocytosis, lipid catabolism, exocytosis, and endocytosis²³⁻²⁵. Furthermore, activation of TFEB using the disaccharide trehalose reduced atherosclerosis progression^{26,27}, highlighting the therapeutic potential of autophagy activation for atherosclerosis treatment.

Human patients diagnosed with cardiovascular disease already have established atherosclerotic plaques and are typically prescribed a lipid-lowering therapy, such as statins. As such, herein, we sought to determine whether autophagy activation subsequent to cholesterol lowering could promote the regression of pre-existing atherosclerotic plaques in a pre-clinical model of atherosclerosis. First, we induced atherogenesis in C57BL/6N mice by overexpression of the gain-of-function mutant PCSK9 followed by 16 weeks of Western diet feeding. Following the development of atherosclerosis, atherosclerosis regression was initiated by a switch to a chow diet concurrent with the administration of trehalose for 6 weeks. We found that trehalose reduced plaque lipid content, inflammation, and increased collagen content in female mice but not in male mice. Interestingly, we observed lower basal autophagy in the plaques of female mice compared to males, which rendered the female mice more responsive to both the diet switch and trehalose treatment. Our data suggests that differential autophagy flux between sexes underlies their distinct capacities to respond to therapeutic interventions, highlighting the need for adapted therapies for better efficiency in the treatment of atherosclerosis.

4.3 Materials and Methods

Animals

Male and female C57BL/6N (Charles River) mice 6 weeks of age were injected intraperitoneally with 1×10^{11} particles of AAV-PSCK9 (University of Pennsylvania), and 7 days post-injection, the mice were started on a Western Diet (Envigo, TD88137) for 16 weeks. Mice were randomized to the baseline, regression saline or regression trehalose groups. The baseline group was sacrificed following the 16 weeks of Western Diet. To induce atherosclerosis regression, the regression saline and trehalose groups were switched to a standard laboratory chow diet for 6 weeks, as previously described ⁵ to induce atherosclerosis regression. During the chow diet feeding, mice received bi-weekly subcutaneous injections of either saline or trehalose (Fisher, 2g/kg). Mice receiving the trehalose injections received 3% trehalose water *ad libitum*. The animals were fasted for 4 hours before sacrifice or blood collection. All animal procedures were approved by the University of Ottawa Animal Care and Use Committee.

Plaque histology

Aortic sinuses were immediately frozen at sacrifice in OCT (Fisher) and stored at -80°C until ready to be sectioned. Embedded sinuses were cut at 10µm of thickness, and serial sections were collected. Neutral lipid content was assessed using Oil Red O staining as previously described ¹⁹. Masson Trichrome (Sigma) staining was performed according to the the manufacturer's instructions. Atherosclerotic plaques were imaged using an Aperio Versa 8 (Leica) microscope at 20x (NA 0.8). Plaque area was quantified as previously described ²⁸ using FIJI (NIH) and was blinded according to sex and treatment groups.

Liver Histology

Livers were fixed in 10% formalin at sacrifice and cryopreserved in 30% sucrose before embedding in OCT. Livers were cryosectioned at a thickness of 10µm. Neutral lipid content was assessed using Oil Red O as above. Liver sections were imaged using an Aperio Versa 8 (Leica) microscope at 40x (NA 0.8). Neutral lipid quantification was performed using FIJI (NIH) and was blinded with respect to sex and treatment groups.

Plasma Cholesterol Measurement

Blood was collected following a 4-hour fast by saphenous vein bleed or cardiac puncture at the time of sacrifice in EDTA-coated microtubes (Sarstedt). Blood was centrifuged at 900xg for 10 minutes at 4°C, and the plasma was collected. Total plasma cholesterol was measured using the Infinity Cholesterol Liquid Stable Reagent (Thermo Scientific) as per the manufacturer's recommendation.

Immune Phenotyping

Whole blood was collected following 16 weeks of Western Diet feeding and at 5 weeks of chow diet feeding by saphenous vein bleeds. Blood was lysed for 5 minutes at room temperature using BD Pharmlyse (BD Biosciences). Leukocytes were blocked for 5 minutes at room temperature using FcBlock (BD Biosciences) in FACS buffer (3% FBS in PBS), followed by incubation with primary conjugated antibodies for 20 minutes on ice. The antibodies used are listed in Supplementary Table 1. Cells were incubated on ice with the viability marker Fixable Viability Stain 700 (BD Biosciences) for 30 minutes. Cells were fixed on ice with BD Cytofix (BD Biosciences) for 15 minutes. Samples were

acquired using BD FACS Aria IIIu (BD Biosciences). Sample analysis was performed using FlowJo 10.8.2 (BD Biosciences).

Immunofluorescence

Aortic sinuses were fixed in 4% paraformaldehyde (Fisher) for 10 minutes at room temperature and washed in PBS. The sections were blocked and permeabilized in 10% normal horse serum (Vector) and 0.1% Triton X-100 (Fisher) for 30 minutes at room temperature. Primary antibodies were diluted in 1% normal horse serum and incubated for 2 hours at room temperature or overnight at 4°C. Secondary antibodies were incubated at room temperature for 1 hour 30 minutes in 1% normal horse serum. Nuclei were stained using DAPI (Invitrogen) for 20 minutes at room temperature. Slides were mounted using Prolong Glass (Invitrogen) and #1.5 coverslips (Ibidi). The aortic sinuses were imaged using a Zeiss AxioObserver Z1 at 20x (NA 0.8). Images were quantified using FIJI (NIH) and blinded with respect to sex and treatment groups. The antibodies used are listed in Supplementary Table 1.

Aortic Digests and Autophagy Flux Quantification

Thoracic aortas were surgically excised and digested as previously described¹⁹. Single cell suspensions were treated ex vivo for two hours with either DMSO (Fisher BioReagent) or Bafilomycin (Sigma) in DMEM (Gibco) supplemented with 10% FBS (Gibco). Cells were stained for 30 minutes with the CYTO-ID Green Detection Reagent from the CytolD Autophagy Detection Kit 2.0 (Enzo Life Sciences) and LysoTracker Red DND-99 (Invitrogen). The aortic digest was incubated with FcBlock (BD Biosciences) followed by incubation with primary conjugated antibodies for 30 minutes on ice. Cells

were stained for neutral lipids using LipidTOX Deep Red (Invitrogen) ¹⁹. Samples were acquired using BD FACS Aria IIIu (BD Biosciences). Sample analysis was performed using FlowJo 10.8.2 (BD Biosciences).

Cell Culture

Peritoneal macrophages were harvested from 8-week old male and female C57BL/6N mice (Charles River) 3 days after intraperitoneal injection of 1mL 3% thioglycolate (BD Difco), and cultured in DMEM media supplemented with 10% FBS and 1% penicillin-streptomycin (Gibco). Macrophages were lipid-loaded by incubation with 50µg/mL of aggregated LDLs (agLDL) for 24h, followed by an overnight equilibration in DMEM supplemented with 2 mg/mL of fatty acid-free BSA (Sigma), as previously ^{14,15}. For autophagy flux analyses, cells were treated with DMSO or Bafilomycin 2h prior harvest.

Western Blotting

Peritoneal macrophages were lysed in 2X Laemmli Sample Buffer (Bio-Rad) containing β-mercaptoethanol and boiled at 95°C for 5 minutes. Samples were run on 8-16% Criterion TGX Stain Free Pre-cast Gels (BioRad) and UV-activated for total protein quantification using the ChemiDoc XRS+ System (Bio-Rad). Proteins were transferred onto 0.22µm PVDF Membranes (Bio-Rad) using the Trans-Blot Turbo Transfer System (Bio-Rad). Immunoblotting for indicated proteins was performed with primary antibodies overnight at 4°C followed by horseradish peroxidase conjugated secondary antibodies for 1 hour at room temperature. Proteins were developed using either Clarity (Bio-Rad) or Clarity Max (Bio-Rad) ECL Substrates and imaged on the ChemiDoc XRS+ system (Bio-Rad).

Statistical Analysis

The data is presented as mean $\pm$ SEM. Statistical analyses were performed using Prism Graphpad V 10.0.2 (Graphpad Software Inc). All samples were analyzed for outliers using the ROUT test. The normal distribution of the data points was assessed using the Shapiro-Wilk test. Statistical significance was determined using a 2-tailed Student T test, 1-way ANOVA or 2-way ANOVA using Holm-Sidak multiple comparison correction. A *P*-value of <0.05 was considered as statistically significant.

4.4 Results

4.4.1 The administration of trehalose did not alter body composition nor circulating cholesterol in female mice

Male and female C57BL/6N mice were injected with an adeno-associated virus (AAV) encoding gain-of-function in proprotein convertase subtilisin/kexin type 9 (PCSK9) to induce hypercholesterolemia. One week post-AAV injection, mice were fed a Western diet rich in fat and cholesterol to increase circulating cholesterol further and promote atherosclerotic plaque development. After 16 weeks of Western diet feeding, a randomized group of mice was sacrificed for baseline measurements, while the remaining mice were randomized for the regression treatment groups. The mice were switched to a chow (no added cholesterol and fat) diet for 6 weeks, as previously described²⁹, to initiate atherosclerosis regression. During regression, the mice received bi-weekly injections of trehalose or saline for 6 weeks. The mice in the trehalose treatment group also had water supplemented with 3% trehalose (Figure 1A).

The female regression groups exhibited weight loss during the 6 weeks of chow diet feeding. Male mice treated with trehalose did not lose weight, whereas the saline regression group exhibited weight loss (Figure 1B). Circulating plasma cholesterol at sacrifice also did not differ between saline and trehalose groups (Figure 1C). Interestingly, regressing male mice had higher levels of circulating plasma cholesterol (429 ± 43 mg/dL for saline and 518 ± 51 mg/dL for trehalose) as compared to females (218 ± 13 mg/dL for saline and 209 ± 16 mg/dL for trehalose) regardless of treatment groups (Figure 1C). Following the atherosclerosis regression paradigm, circulating plasma cholesterol returned to baseline following the diet switch from Western diet to chow in female but not male mice (Figure 1C). Unlike their female counterparts, post-regression plasma cholesterol levels of male mice were not restored to pre-Western diet levels and were twice that of pre-Western diet levels (Figure 1C).

During Western diet feeding, mice develop fatty livers, whereas upon atherosclerosis regression, hepatic steatosis resolves²⁹. Because fatty livers are associated with higher levels of circulating cholesterol³⁰ and we observed persistent elevated cholesterol concentrations in male mice even following the diet switch to chow (Figure 1C), we next assessed liver steatosis by Oil Red O staining and quantification. In female mice, as expected, Oil Red O staining was reduced following 6 weeks of chow diet feeding with no difference between the saline and trehalose groups (Figure 1D-E). Despite a significant reduction in hepatic lipid content in male mice following the diet switch to chow, liver

steatosis was not fully resolved following atherosclerosis regression (Figure 1D-E). Although lipid droplet size decreased in regressing male mice, a significant amount of lipids remained in both saline and trehalose groups (Figure 1D-E). Overall, our data reveals a sexual dimorphism in whole-body lipid metabolism in mice undergoing atherosclerosis regression in the AAV-PCSK9 diet switch mouse model.

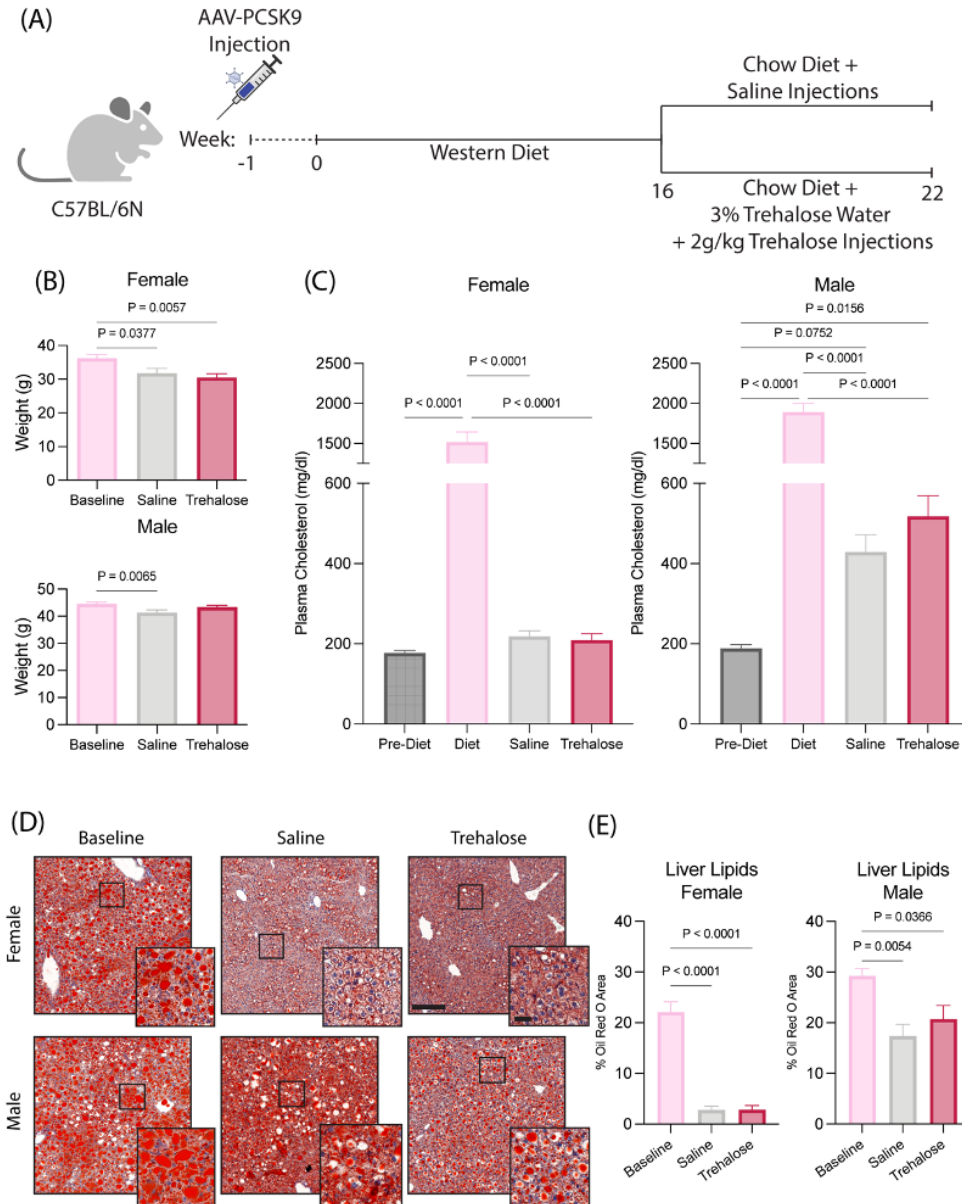


Figure 4-1: Trehalose Does Not Alter Body Composition or Circulating Cholesterol

(A), Schematic of the experimental design. (B) Body weight of female and male mice during the six weeks of chow diet feeding (n=10-11 males; n=12 females). (C) Plasma cholesterol taken one week post AAV-PCSK9 injections, during Western Diet feeding and after six weeks of chow diet feeding (n=10 males; n=12 females). (D-E) Representative images and quantification of livers stained by Oil Red O (n=4-5 males; n=4-6 females). Scale bar = 200µm and 50µm in zoom in images. Statistical significance was assessed with a one-way ANOVA with a Holm-Sidak's post hoc test for multiple comparisons correction when comparing more than two groups.

4.4.2 Trehalose promotes plaque regression in female mice

Regressing atherosclerotic plaques are characterized by one or more of the following: reduced size, reduced cholesterol content, dampened inflammation, reduced foam cell numbers, increased collagen content and/or increased fibrous cap size ^{5,6}. In female mice, atherosclerotic plaque growth was halted upon the reversal of hypercholesterolemia, as evidenced by comparable total plaque area in cross-sections of aortic roots in female mice across the groups (Figure 2A, 2B). In male mice, plaque area increased significantly during atherosclerosis regression by 45% and 40% in the saline and trehalose groups, respectively (Figure 2B).

Given that collagen content is positively correlated with plaque stability during atherosclerosis regression ⁵, we next quantified plaque collagen content using Masson Trichrome staining. We observed increased collagen content in the mice fed a chow diet for 6 weeks regardless of treatment and sex (Figure 2C). In female mice, trehalose further increased plaque collagen content by 17% compared to saline, whereas in male mice, trehalose decreased collagen content by 13% compared to saline (Figure 2C).

Finally, to quantify plaque neutral lipids, we stained aortic roots with Oil Red O (Figure 2D). In female mice, trehalose treatment significantly reduced lipid content as compared to saline-treated mice (Figure 2E). The chow diet reduced lipid content in the male mice treated with saline but not trehalose (Figure 2E). Collectively, these data demonstrate that plaque growth is halted upon the reversal of hypercholesterolemia in female mice as compared to male mice and that trehalose reduces the neutral lipid content while increasing collagen deposition in female but not male mice.

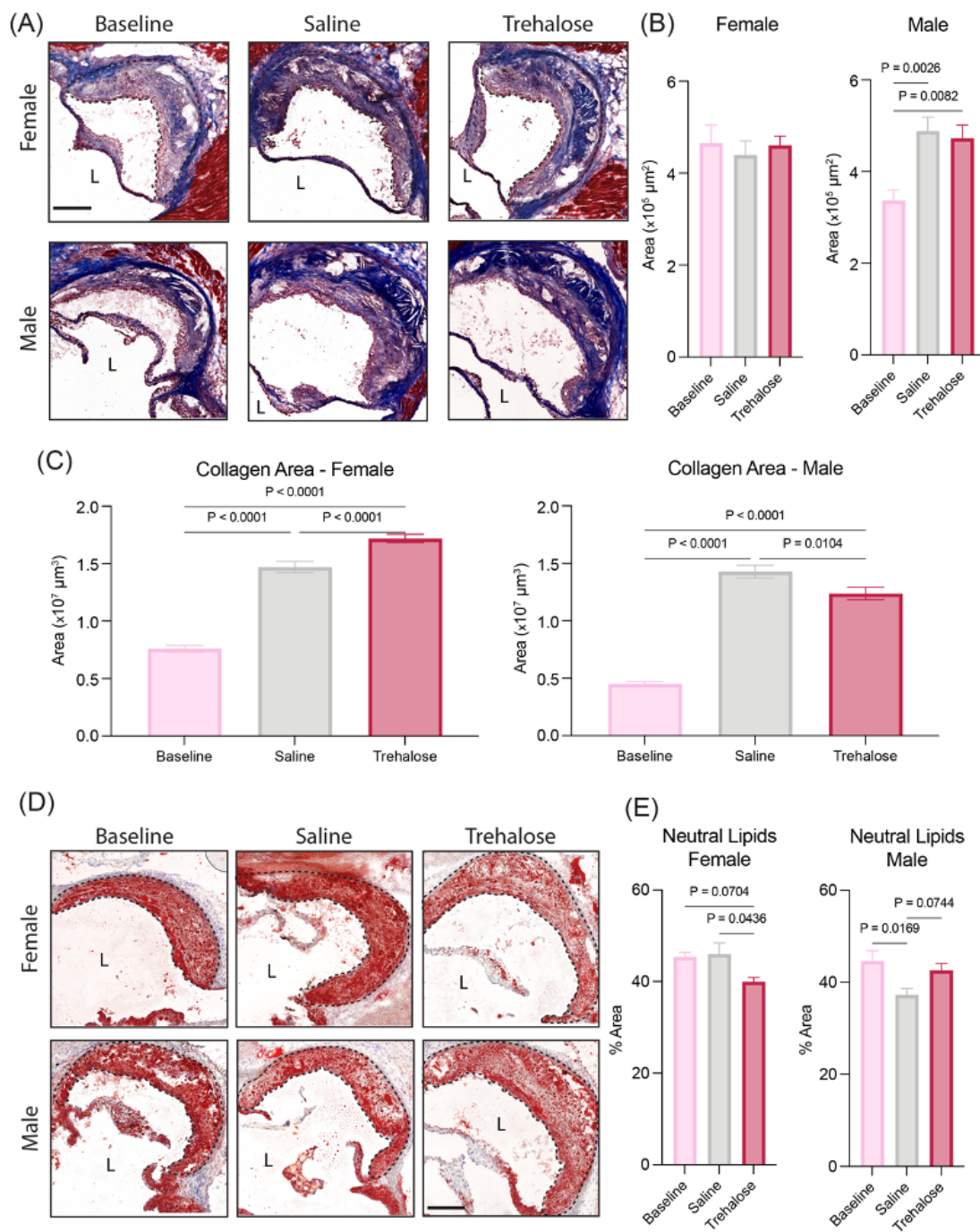


Figure 4-2: Trehalose Promotes Plaque Size Stabilization in Female Mice

(A) Representative micrographs of aortic root stained with Masson Trichrome. (n=8-11 males; n=10-12 females). Scale bar=200μm. L=Lumen (B) Quantification of atherosclerotic lesion area in the aortic root. (C) Quantification of the total collagen area throughout the aortic root (area under the curve) as quantified by the blue stain of Masson Trichrome. (D) Representative micrographs of aortic root stained with Oil Red O. Scale

bar=200µm. L=Lumen (E) Quantification of the percentage of the neutral lipid content of the total plaque area (n=9-10 males; n=10-12 females). Statistical significance was assessed using a one-way ANOVA with Holm-Sidak's post hoc test for multiple comparison correction when comparing more than two groups.

4.4.3 Differential regulation of autophagy in male and female mice

To determine if atherosclerosis regression and/or trehalose activate autophagy, we performed immunostaining of aortic roots with the autophagosome marker microtubule-associated protein 1A/1B-light chain 3 (LC3). LC3 is a crucial component of autophagy that gets incorporated into the membranes of autophagosomes during their biogenesis and, as such, is a specific marker of autophagy ³¹. Atherosclerosis regression did not increase LC3 expression in the plaques of male mice, while we observed increased LC3 expression in those of female mice treated with either saline (25%) or trehalose (30%) (Figure 3A-B).

Autophagy is a dynamic process best quantified as the flux of autophagosomes to lysosomes ³². During autophagy, the cargo to be degraded is engulfed by autophagosomes that fuse with nearby lysosomes. However, in the presence of bafilomycin that inhibits lysosomal acidification, lysosome function is inhibited, leading to a buildup of autophagosomes in bafilomycin-treated cells. Therefore, the accumulation of autophagosomes upon bafilomycin treatment reflects a high cellular autophagy flux. Aortic arches were digested and treated with bafilomycin *ex vivo* to quantify autophagy flux during atherosclerosis regression, as we previously did for atherosclerosis progression studies ¹⁹. Aortic digests were stained with the autophagosome dye CytoID, which labels autophagic compartments. In parallel, lipid-rich (Lipid^{hi}) arterial foam cells

were identified using the lipid dye BODIPY, with leukocyte-derived foam cells identified as CD45⁺ and MHC-II⁺ ¹⁹.

Using flow cytometry of aortic digests, we observed that Lipid^{hi}CD45⁺MHC-II⁺ leukocyte foam cells from male aortas had significantly higher autophagy flux at baseline than the females (Figure 3C-D). Because CytoID accumulates in lysosomes upon autophagy inhibition by bafilomycin treatment, the dye is quenched, decreasing the fluorescence intensity. As seen in Figure 3C, the reduction in CytoID fluorescence upon bafilomycin treatment was most striking in Lipid^{hi}CD45⁺MHC-II⁺ leukocyte foam cells from male as compared to female mice, which was quantified in Figure 3D. These results highlight sex differences in basal autophagy flux, whereby macrophage foam cells from female plaques have a markedly lower basal autophagy flux than males. Interestingly, the autophagy flux was similar *in vitro* in mouse peritoneal macrophages isolated from male compared to female mice in both unloaded and lipid-loaded conditions (Supplementary Figure 2), suggesting that the sex differences observed in plaque macrophage autophagy flux are not attributable to cell-intrinsic sex differences, but rather to more complex physiological sex differences between male and female atherosclerotic mice.

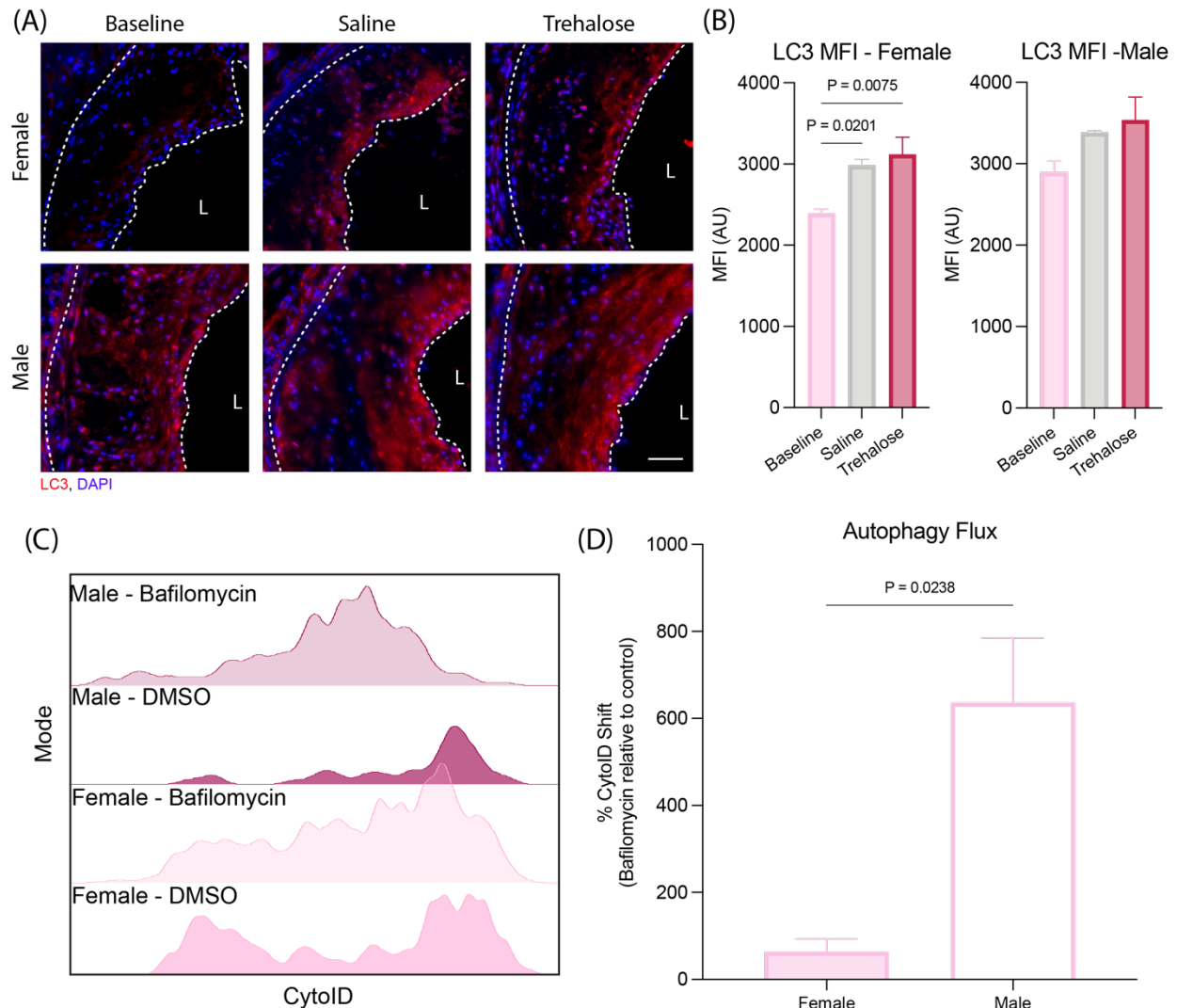


Figure 4-3: Autophagy is Differentially Regulated Between Female and Male Mice

(A) Representative immunofluorescence of the aortic root stained with the autophagosome marker LC3 (red) and nucleus (blue). Scale bar 50 μ m. (B) Quantification of the mean fluorescence intensity (MFI) of the autophagosome marker LC3 within the total plaque area (n=6 males and females). (C) Representative histogram of CytolD fluorescence intensity (n=3 males and females) (D) Fold change of the CytolD MFI to Bafilomycin (n=3 males and females). Statistical significance was assessed with an unpaired t-test when comparing two groups or one-way ANOVA with a Holm-Sidak's post hoc test for multiple comparisons correction when comparing more than two groups.

4.4.4 Trehalose reduces inflammation in female mice

Quantification of circulating immune cells at baseline and 5 weeks of atherosclerosis regression revealed a significant reduction in the total number of leukocytes after chow

diet feeding in the male saline and trehalose groups and in the female trehalose group (Supplemental Figure 1A). However, during atherosclerosis regression in female mice, trehalose only significantly reduced circulating immune cells and leukocytes (Figure 4A). Monocytes can be further subcategorized into two different subsets: Ly6C^{hi} and Ly6C^{lo}. Ly6C^{hi} monocytes are elevated in hypercholesterolemia and are the major subtype recruited to atherosclerotic plaques ³³. In our study, female mice treated with trehalose had significantly fewer Ly6C^{hi} monocytes, with Ly6C^{lo} monocytes trending toward a decrease (Figure 4B-C). In addition to monocytes, T cells, particularly CD4⁺ T cells, were found to be pro-atherogenic ³⁴. Interestingly, CD4⁺ T cells were also significantly reduced in trehalose-treated female mice compared to saline controls (Figure 4D), while CD8⁺ T cells were unchanged (Supplemental Figure 1B). Although total leukocyte numbers were reduced in male mice upon atherosclerosis regression (Supplemental Figure 1A), leukocytes, monocytes and T numbers were unchanged in the trehalose group compared to the saline group (Figure 4A-D).

To quantify the macrophage content of atherosclerotic plaques, we performed immunofluorescence staining of the aortic root with the macrophage marker CD68. In line with reduced levels of circulating Ly6C^{hi} monocytes observed in the regressing mice (Figure 4B), we observed a significant decrease in plaque macrophage content in both male and female mice during atherosclerosis regression in saline and trehalose groups (Figure 4E-F). To evaluate the inflammatory status of plaque macrophages, we performed immunostaining of aortic roots with the pro-inflammatory macrophage marker CD68 and the pro-inflammatory marker CD38. The latter is described as a cell surface marker expressed by pro-inflammatory macrophages³⁵. In both male and female mice,

atherosclerosis regression markedly reduced CD68⁺CD38⁺ plaque area in the saline and trehalose groups (Figure 4G-H). Trehalose treatment further reduced CD68⁺CD38⁺ plaque area in female mice but not male mice (Figure 4H), indicating increased inflammation resolution in trehalose-treated female mice.

Finally, because macrophage autophagy promotes the clearance of the NLRP3 inflammasome to limit the production of pro-inflammatory interleukin (IL)-1 β in atherosclerosis ¹³, we next sought to quantify plaque IL-1 β levels in our study groups. Surprisingly, IL-1 β levels were upregulated by 32% in saline-treated female mice relative to baseline, while trehalose treatment mitigated this increase back to baseline levels (Figure 4I-J). IL-1 β levels were not significantly different between the groups in male mice (Figure 4I-J). Overall, trehalose treatment in female mice reduced circulating inflammatory immune cells, plaque inflammatory macrophages and plaque IL-1 β .

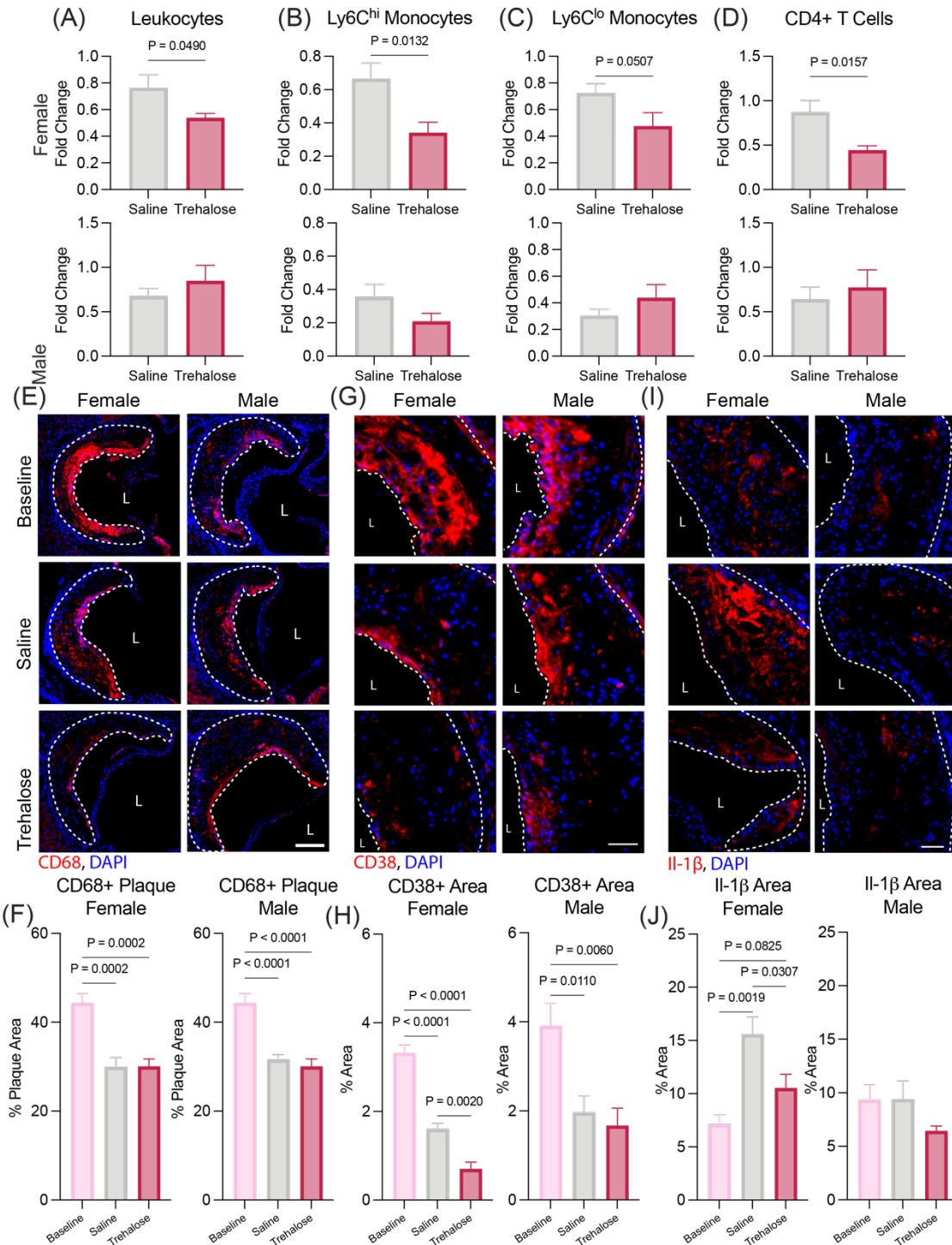


Figure 4-4: Trehalose Reduces Inflammation in Female Mice

(A) Fold change of the total circulating (A) leukocytes, (B), Ly6Chi monocytes, (C) Ly6Clo monocytes and (D) CD4⁺ T cells following five weeks of chow diet and treatment over the baseline total cells. (E) Representative immunohistochemistry images of the aortic roots stained with CD68 (red) and nucleus (blue). Scale bar 50μm. L=Lumen (D) Quantification of the percentage of CD68 positive area of the total plaque (n=6 males and females). (G)

Representative immunohistochemistry of aortic roots stained with inflammatory marker CD38 (red) and nucleus (blue). Scale bar 50µm. (H) Quantification of the percentage of CD38 positive, CD68 positive area found in the total plaque area (n=6 males and females). (I) Representative immunohistochemistry of aortic roots stained with inflammatory marker Il-1b (red) and nucleus (blue). Scale bar 50µm. (J) Quantification of the percentage of Il-1b positive area found in the total plaque area (n=6 males and females). Statistical significance was assessed with an unpaired t-test when comparing two groups or one-way ANOVA with a Holm-Sidak's post hoc test for multiple comparisons correction when comparing more than two groups.

4.5 Discussion

Even with the development of cholesterol-lowering drugs, cardiovascular diseases significantly burden the healthcare system ³⁶. Indeed, cholesterol-lowering drugs may slow disease progression and result in mild plaque shrinkage ³⁷, but they have yet to promote the complete reversal of atherosclerosis. Rapamycin and its derivatives (rapalogs) have been used in drug-eluting stents to promote autophagy via mTORC1 inactivation. These studies have produced small but significant reductions in atherosclerosis ³⁸⁻⁴⁰, suggesting that plaque autophagy activation is an exciting target to promote atherosclerosis regression. However, the systemic inhibition of mTORC1 by rapamycin and rapalogs has detrimental side effects, including immunosuppression, hyperglycemia, insulin resistance and dyslipidemia ⁴¹, thereby making it unsuitable for the reversal of atherosclerosis.

In recent years, TFEB activation using the disaccharide trehalose has gained attention as a potential therapeutic approach to promote autophagy. By causing low-grade lysosomal stress to activate TFEB, trehalose drives the transcription of genes involved in lysosome and autophagosome biogenesis, thereby increasing autophagy flux ^{23,24}. In pre-clinical models of cardiovascular disease, trehalose reduces disease burden ^{26,42,43}. Moreover, recent clinical trials have demonstrated that trehalose is safe for use and improves arterial

dilation in middle and old-aged adults ^{44,45}. Trehalose is currently under investigation for the treatment of amyotrophic lateral sclerosis ⁴⁶, Parkinson's disease ⁴⁷ and spinocerebellar ataxia ⁴⁸. Excitingly, TFEB activation by trehalose occurs in a mTORC1-independent manner ^{49,50}, making the use of trehalose to reverse atherosclerosis a promising therapeutic approach.

Here, we tested whether trehalose could ameliorate atherosclerosis in the AAV-PCSK9 diet switch mouse model. This model employs overexpression of a gain-of-function PCSK9 mutant in C57BL/6N mice and 16 weeks of Western diet feeding to establish atherosclerosis (baseline). Then, atherosclerosis regression was initiated by a diet switch to chow, and the mice were treated with trehalose or saline for 6 weeks. As previously demonstrated ^{43,51}, the administration of trehalose did not alter body composition or plasma cholesterol levels in mice in our study. Circulating plasma cholesterol returned to baseline following the diet switch to chow in female mice. In contrast, the circulating plasma cholesterol of male mice was not restored to baseline but was lowered to double that of pre-Western diet levels. In female mice undergoing atherosclerosis regression, we observed positive remodelling of atherosclerotic plaques, including the halting of plaque growth, reduced plaque neutral lipid content and inflammatory macrophages concomitant with increased plaque collagen and autophagosomes. Moreover, trehalose further enhanced the positive remodeling of atherosclerotic plaques in regressing female mice, further reducing plaque neutral lipid and inflammatory macrophages and potentiating plaque collagen deposition and autophagy as compared to controls. In contrast, plaque growth was not halted in male mice undergoing atherosclerosis regression. Rather, we observed increased plaque area in regressing male mice, potentially due to a moderate

hypercholesterolemia that persisted following the switch to chow diet in both trehalose- and saline-treated groups.

In addition to reductions in plasma cholesterol, the mitigation of inflammation is critical to atherosclerosis resolution⁸. Upon hypercholesterolemia, the production of immune cells is dysregulated and skewed towards myelopoiesis, leading to an increase in the circulating monocyte pool⁵². This increase in circulating monocytes perpetuates monocyte recruitment to the arterial intimal space, promoting atherosclerosis progression. Conversely, in atherosclerosis regression, the recruitment of circulating monocytes is reduced, resulting in reduced plaque macrophage numbers⁵³. In conjunction with enhancing autophagy, several studies show that TFEB activation alleviates inflammation⁵⁴⁻⁵⁶. Also, the activation of TFEB attenuates endothelial inflammation by decreasing the expression of adhesion molecules and chemokines, thereby reducing monocyte adhesion and infiltration^{55,57}. In our study, we observed a reduction in the total number of circulating leukocytes after chow diet feeding in the saline and trehalose regression groups compared to baseline in both sexes. Meanwhile, trehalose only further reduced circulating leukocytes relative to the saline group in females. Moreover, trehalose further reduced pro-inflammatory Ly6C^{hi} monocytes and CD4⁺ T cells relative to saline in female mice, indicative of increased inflammation resolution in trehalose-treated female mice.

In addition to employing circulating immune cell phenotyping to assess inflammation in atherosclerosis, levels of the pro-inflammatory cytokine IL-1 β can be used as a local marker of inflammation. Plaque IL-1 β production is highest in patients with more complex,

less stable plaques ⁵⁸ and conversely, the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) demonstrated that resolution of residual inflammation using a monoclonal antibody targeting IL-1 β antibody reduces major adverse cardiovascular events ⁵⁹. Interestingly, in our study, we observed that a switch to a chow diet significantly increased IL-1 β levels in plaques of the female mice as compared to baseline. While this observation seems counterintuitive because inflammation resolution is critical to atherosclerosis regression ⁸, IL-1 β is required for the maintenance of a fibrous cap rich in smooth muscle cells and collagen ⁶⁰. In regressing female mice, trehalose mitigated the IL-1 β increase back to baseline levels, while plaque IL-1 β levels were not significantly different between the groups in male mice, highlighting sex differences in inflammation resolution during atherosclerosis regression. Further studies are needed to understand the mechanisms by which IL-1 β levels are altered in the different mouse cohorts, and whether this is occurring via autophagy- and/or inflammasome-dependent mechanisms.

Interestingly, autophagy flux comparisons revealed lower basal autophagy in the plaques of female mice than in male mice. This is consistent with previous observations demonstrating that male mice have a higher autophagy flux ^{61,62} and require higher doses of rapamycin than female mice to activate autophagy ⁶³. This elevated autophagic flux in males can be beneficial during Alzheimer's disease by preventing the accumulation of cytotoxic amyloid beta plaques ⁶¹ or detrimental in the case of myocardial infarction, where excessive autophagy promotes cardiomyocyte death ⁵⁶. Here, the higher autophagy flux observed in male mice may have had a protective effect by reducing atherogenesis, as observed by the smaller plaque sizes at baseline in males compared to females. The heightened ability of females to undergo atherosclerosis regression may

lie in their inherent higher capacity to perform cholesterol efflux ⁶⁴ given higher circulating HDL levels in female mice ⁶⁵, as well as increased hepatic lipolysis in female mice relative to males ^{66,67}. Lastly, treatment with statins leads to more significant regression in females than males ⁶⁸, highlighting the differential capacity of plaque regression between sexes. To conclude, our study demonstrates key disparities in autophagy flux between sexes, leading to significant differences in atherosclerosis resolution. Elucidating the regulation of autophagy in both sexes is critical to harnessing autophagy activation to promote plaque regression as a therapeutic approach for the treatment of atherosclerosis.

4.6 Conflict of Interest

The authors have no conflict of interest to declare.

4.7 Author Contributions

S.R. and M.O. conceptualized the study. S.R., V.R., C.E. and T.L. conducted the experiments, and S.R. performed the data analysis. S.R. and M.O. wrote the manuscript.

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5 Discussion

There is currently an urgent need for new therapeutics to treat atherosclerosis and promote atherosclerosis regression. Standard of care for patients with pre-clinical or clinical manifestations of atherosclerosis is still statin therapies^{8,10}. Although statins are effective at reducing MACE^{7,12,107}, cardiovascular disease remains the leading cause of death worldwide². Central to the modulation of inflammation and lipid metabolism lies autophagy. Although the atheroprotective role of autophagy is greatly appreciated, the mechanisms by which autophagy becomes defective during atherosclerosis is not known. Moreover, the mechanisms by which selective autophagy of lipid droplets, one of the rate-limiting steps of reverse cholesterol transport is poorly characterized. The overarching aim of this thesis was to uncover the mechanism by which lipophagy was regulated in the foam cells of the plaque and whether promoting autophagy in a pre-established plaque would promote atherosclerosis regression.

5.1 Lipophagy in Macrophage Foam Cells

Although lipophagy was discovered in 2009 by Singh et al. in mammalian cells¹²⁷, the regulation of lipophagy to this day remains elusive. Prior to the beginning of my doctorate, the identification of SARs implicated in lipophagy was not well described. The only SARs to date which had been identified at the surface of the lipid droplet had been p62³²¹ as a ubiquitin dependent SAR and potentially ATGL as a non-ubiquitin dependent SAR³¹⁷, but no systematic analysis of lipophagy specific SARs had been conducted³²². In chapter 2, we first demonstrated the presence of ubiquitin at the surface of lipid droplets in macrophage foam cells. Furthermore, we showed the colocalization of LC3 with either

ubiquitin or p62 demonstrating active lipophagy in macrophage foam cells (Figure 2.1). Using an unbiased proteomics approach, we characterized the proteome of human macrophage foam cell lipid droplets (Table 2.1). By performing a comparative analysis of the lipid droplets isolated from cells where autophagy was inhibited or not, we uncovered a unique protein signature of the lipophagy machinery. We identified 37 proteins which were enriched at the surface of the lipid droplet upon autophagy inhibition (Figure 2.2.). Moreover, when we cross-referenced the proteins found at the surface of macrophage foam cell lipid droplets with RNA sequencing data obtained from foamy macrophages of atherosclerotic plaque digests of ApoE^{-/-} mice fed a Western diet for 28 weeks, we observed that several of our targets were dysregulated in foamy macrophages. The down-regulation of UBE2G2, RAB18, RAB8, HSC70, HMGB1, UVRAG and p62 and the upregulation of BNIP3L, PARK7 and ATG5 may suggest that lipophagy is dysfunctional in macrophage foam cells, but that other forms of autophagy such as mitophagy may still be active (Figure 2.3). Our study was the first to systematically identify p62, OPTN and NBR1 as being lipophagy SARs (Figure 2.4). Due to the ease of genetic deletion and autophagy characterization in yeast, we employed a synthetic genetic array to screen a larger amount of potential lipophagy modulators. Similarly, to the small interfering RNA screen, we found that proteins related to molecular chaperones, ubiquitination, autophagosome formation, AMPK significantly disrupted lipophagy (Figure 2.5). Notably, we observed that within the same cell, lipid droplets could be observed as single lipid droplets or in clusters. Logically, lipid droplets found in clusters would make the association of the rather larger autophagy machinery quite difficult due to the space constraints. Surprisingly, both lipid droplets in clusters were tagged with the

autophagosome marker LC3. With closer observation, we noticed that single lipid droplets were surrounded by a bilayer membrane or an LC3-coated vesicle while clusters were surrounded by structures resembling wrapping lysosomes. This led us to conclude that single lipid droplets could undergo microlipophagy while clusters may undergo macrolipophagy (Figure 2.6). Although microautophagy is not impacted by genetic deletion of ATG5 and LAMP2A to inhibit macroautophagy and chaperone-mediated autophagy respectively³²³, it is still possible that other autophagy components such as LC3 could promote microautophagy by promoting the interactions between cargo and lysosome. Since then, only one other potential lipophagy receptor has been identified. During lipophagy, ORP8 localizes to the lipid droplet and is phosphorylated by AMPK enhancing the binding of ORP8 to LC3/GABARAP³²⁴.

Although our study identified three SARs (p62, OPTN and NBR1), these SARs are not unique to lipophagy²²⁷⁻²²⁹. Out of the 37 enriched proteins upon autophagy inhibition, only two contained a ubiquitin domain along with potential LIR motifs. Unfortunately, genetic knockdown of ADGRL3 and COLGALT1 did not reduce cholesterol efflux suggesting that, under these conditions, they do not play a role in lipophagy. Although these proteins were promising, it is important to note that the LIR motif is quite broad and was detected in high abundance in the majority of the 37 proteins. The lipid droplet isolation was done using ultracentrifugation according to buoyancy³²⁵. Although we did see an enrichment of the lipid droplet coat protein PLIN2 in the fraction collected in addition to the autophagosome marker LC3-II indicative that we collected events of lipophagy, it is possible that smaller lipid droplets or lipid droplet clusters were not included in the fraction collected. A study from Schott and al. demonstrated that smaller lipid droplets did not readily float to the

surface of their gradient and needed an additional step to be purified. These smaller lipid droplets had a higher abundance of LC3-II than their bigger counterpart³¹⁹. Additionally, we cannot ignore the fact that lipid droplets interact with various organelles. As such, it is possible that some proteins detected are in fact not associated directly with the lipid droplet, but through these contact sites. Since then, other groups have validated a proximity-labelling technique which allows for the isolation of proteins found at the surface and proximity of the lipid droplet and significantly decreased the isolation of non-specific proteins^{326,327}. Using this new proximity labelling technique combined with a genetic deletion of ATG5 could provide a more stringent list of potential lipophagy regulators. The identification of specific SARs and lipophagy modulators remains an active area of investigation which could have a great impact on the treatment of lipid storage disorders such as atherosclerosis.

5.2 Defective Autophagy During Atherosclerosis Progression

Defective autophagy flux in the atherosclerotic plaque has been proven time and time again^{257,258,273,274,328}. As previously mentioned, the use of Mac2 and CD68 as macrophages markers led to the misidentification of the cellular origins of foam cells therefore, there was a need for a better systematic approach to assess the functionality of autophagy in the foam cells populations. In chapter 3, we sought to investigate the functionality of autophagy and autophagy mediated cholesterol efflux in macrophage and vascular smooth muscle foam cells of the atherosclerotic plaque. We first observed that upon autophagy inhibition *ex vivo* that macrophage foam cells had functional autophagy flux and lysosomes. Conversely, smooth muscle cell foam cells had very little autophagy

flux and poorly acidified lysosomes (Figure 3.1, 3.2, 3.3). Using immunofluorescence, we confirmed that macrophages of the plaque expressed p62 and LC3 to similar levels while smooth muscle cells had high levels of p62 with lower expression of LC3. Additionally, we demonstrated that the marker of active autophagosome formation p-ATG16L1 was present within the macrophage fraction of the plaque and not the smooth muscle cell foam cells. Overall, these data demonstrated a functional autophagy flux in macrophages. In smooth muscle cells, although they seem to be capable of cargo tagging, autophagosome formation is not occurring resulting in an accumulation of p62 (Figure 3.4). Lastly, we investigated the ability of the foam cells to perform cholesterol efflux. As expected, macrophages foam cells have a high capacity to perform cholesterol efflux where the majority of autophagy mediated cholesterol efflux occurs to apoA-I¹²⁸. Smooth muscle cells cholesterol efflux to apoA-I was non-existent while efflux to HDL was poor. Unlike macrophages, autophagy mediated cholesterol efflux in smooth muscle cells is to HDL and not apoA-I (Figure 3.5). It has been previously suggested that there might be a synergistic relationship between cholesterol efflux between macrophages and smooth muscle cells. Indeed, macrophages are capable of generating HDL particles by lipidating lipid poor apoA-I. These newly generated HDL particles can then be further lipidated by the smooth muscle cells^{141-143,145}. Similarly, when we assessed the expression of ABCA1 and ABCG1 responsible for the efflux of cholesterol to apoA-I and HDL respectively, we observed that smooth muscle cells expressed more ABCG1 as compared to ABCA1 while macrophages expressed both similarly (Figure 3.6). Lastly, promoting autophagy activation using a widely used drug metformin, we were able to rescue the defect in the autophagy flux in smooth muscle cells. Metformin did not have any effect on

macrophages. We suspect that the inhibition of mTORC1 via the AMPK pathway³²⁹⁻³³¹ is already in effect and therefore metformin's effect is null or that the autophagy activation has already reached maximal capacity and cannot be further enhanced. Overall, our study demonstrated key differences in the regulation of autophagy between smooth muscle and macrophage foam cells.

The interpretation of the autophagy flux using the GFP-LC3 reporter is one of the limitations of the study. One could argue that high levels of GFP-LC3 could both indicate autophagosome accumulation or high autophagy flux. To clarify these ambiguities, we used an *ex vivo* treatment with the autophagy inhibitor bafilomycin to prevent autophagosome degradation therefore allowing us to measure flux. Since then, a new autophagy reporter mouse, GFP-RFP-LC3 has become commercially available³³². The GFP in this reporter is pH sensitive and therefore is quenched upon lysosomal entry allowing to track the formation of cytosolic autophagosome (GFP-RFP) and autophagosome which have been engulfed by the lysosome (RFP). With this model, a high autophagy flux results in an increase in RFP signal while autophagy defects can be seen as an accumulation of the GFP-RFP signal.

Smooth muscle cell foam cells have been discovered for well over sixty years now, but they remain very poorly characterized. There is still an enormous amount of work which needs to be done in the field of smooth muscle cell foam cells in order to fully begin to understand their role in the pathogenesis of atherosclerosis. One major obstacle in this field is the inconsistencies in the methods which are used to generate the foam cells and what are the definition of a smooth muscle cell foam cells. Often, authors fail to

demonstrate that treatment with lipids induces significant accumulation of intracellular lipid droplets or quantitative accumulation of cholesterol over non-loaded cells or demonstrate the switch in the phenotype. The development of a more standardized method to induce smooth muscle cell foam cells which resembles the one in the plaque would be beneficial in characterizing these cells in vitro.

5.3 Autophagy During Atherosclerosis Regression

Currently, patients who present in the clinic and receive treatment for atherosclerosis, whether being preventative or acute, have pre-existing plaques^{6,88}. These patients are prescribed potent cholesterol lowering agent^{8,10}. The vast majority of animal studies who investigate atherosclerosis and potential treatment options do so during the atherosclerosis progression phase where plasma cholesterol is remarkably high. As such, the results from these studies, although important mechanistically, are not representative of the condition this disease presents and is treated in the clinic. In chapter 4, we aimed to uncover the potential role of autophagy activation in promoting atherosclerosis regression in pre-established plaques. We demonstrated that males had persistent high levels of circulating cholesterol and liver steatosis following six weeks of standard chow diet unlike female mice (Figure 4.1). The activation of TFEB with the use of trehalose in order to promote both autophagy and lysosomal biogenesis further enhanced the atherosclerosis regression in female mice, but not in male mice (Figure 4.2). Upon further investigation, we observed a reduced in autophagy flux in aortic digest from female mice following 16 weeks on Western diet as opposed to male mice. In line with this observation, female mice had higher levels of autophagosome marker LC3 upon trehalose treatment

while male demonstrated no differences (Figure 4.3). This demonstrates potential differential regulation in the activation and levels of autophagy which may be dependent on sex hormones. Lastly, activation of autophagy in female reduced significantly the amount of pro-inflammatory macrophages and pro-inflammatory cytokine IL-1 β (Figure 4.4). Collectively, we demonstrated clear differences in atherosclerosis regression and autophagy flux between males and females.

The differential effect of trehalose on autophagy activation mimics the results of another study where higher doses of rapamycin was required in order to obtain an effect in male mice³³³. Future studies should include higher doses or more frequent injections to potentially see if a similar effect as to the rapamycin occurs with trehalose in male mice.

5.4 Concluding Remarks

Although there is still much to uncover, the modulation of autophagy continues an interesting target to promote plaque regression. Through the work highlighted in these three studies, we have demonstrated a critical role of autophagy, more particularly, macrophage autophagy in the pathogenesis of atherosclerosis. Additional studies are still required to further characterize lipophagy mechanisms and regulators. As highlighted by the systemic use of rapamycin, activation of bulk autophagy is not a good viable target to prevent atherosclerosis. With the discovery of the regulatory pathways which mediates the activation of selective autophagy, these may offer us better therapeutics to treat atherosclerosis. Ideally, these drugs would be able to localize to the plaque and be able to promote lipophagy, mitophagy and aggrephagy to remove excess lipids, defective

mitochondria and protein aggregates respectively without activating bulk non-selective autophagy; all of which greatly accumulates during atherosclerosis.

A question that remains is the fate of smooth muscle cells foam cells. Are they able to egress out of the atherosclerotic plaque, undergo apoptosis and be effectively cleared by macrophages or do they simply revert to a more classical contractile smooth muscle cell phenotype and stay in the vessel wall albeit in a less pathogenic way? There is evidence *in vitro* that suggest that cholesterol unloading of smooth muscle cells reduces the expression of macrophages markers whereas smooth muscle cells markers are increased thereby retuning to a contractile phenotype³³⁴, but this remains to be proven *in vivo*.

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6 Appendix

6.1 Supplemental Figures Manuscript #1

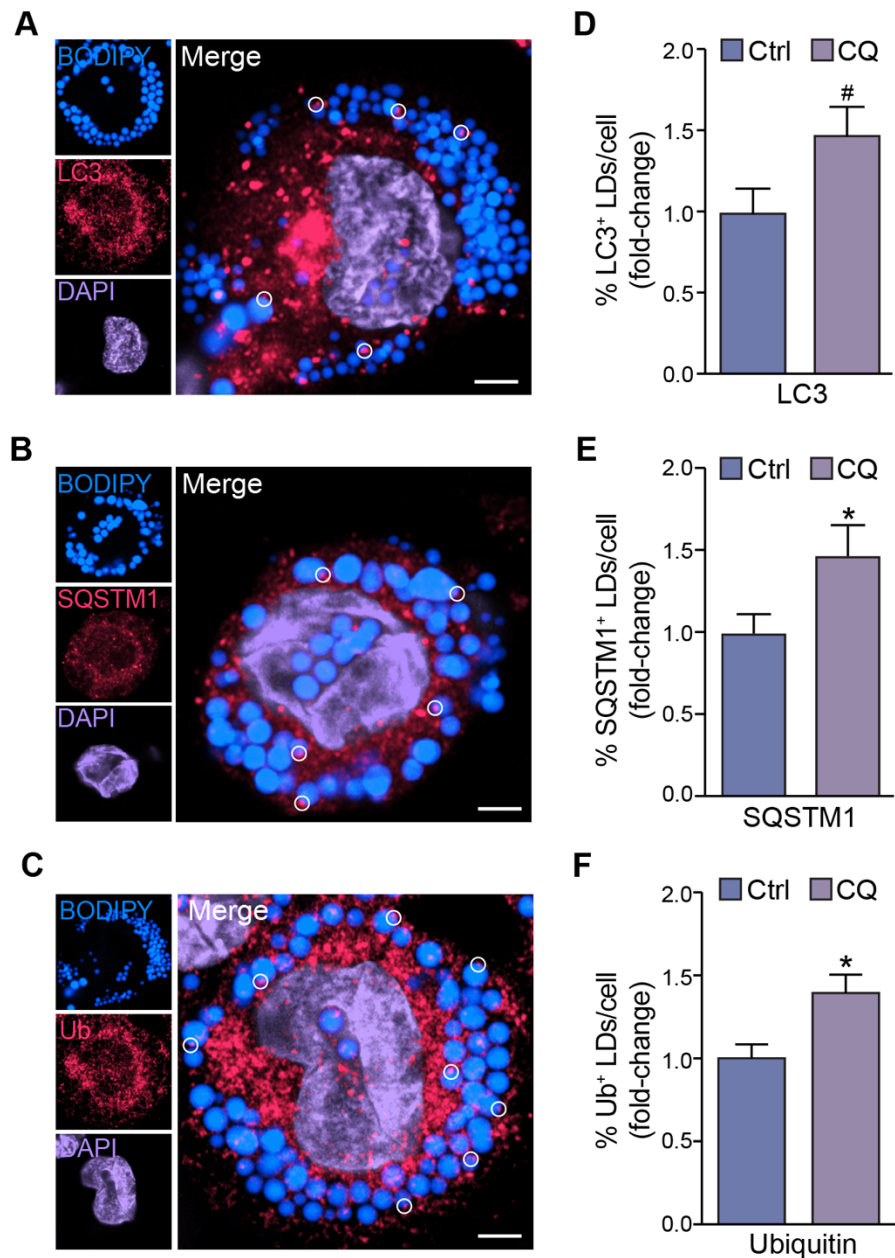


Figure 6-1: LC3, SQSTM1 and Ubiquitin Localize to Lipid Droplets in Oleic Acid-Loaded Macrophage Foam Cells

Human THP-1 macrophages were loaded with oleic acid (400 μ M) for 30 h and equilibrated in BSA (2 mg/mL) overnight. Cells were then fixed and stained for LC3 (A), SQSTM1 (B), ubiquitin (Ub) (C) and BODIPY 493/503 to label neutral lipids, and lipid droplets (LDs) that were colocalized with LC3, SQSTM1 or Ub are circled. At right, quantification of the percent of cellular LDs tagged with LC3, SQSTM1 or Ub in

chloroquine (CQ)-treated cells as compared to control (Ctrl) is shown. Data are expressed as fold-change for CQ treatment relative to untreated from one experiment representative of 3 independent experiments with similar results (mean $\pm$ s.e.m). # $P < 0.1$, * $P < 0.05$. Representative images are from untreated cells. Scale bar: 5 μ m.

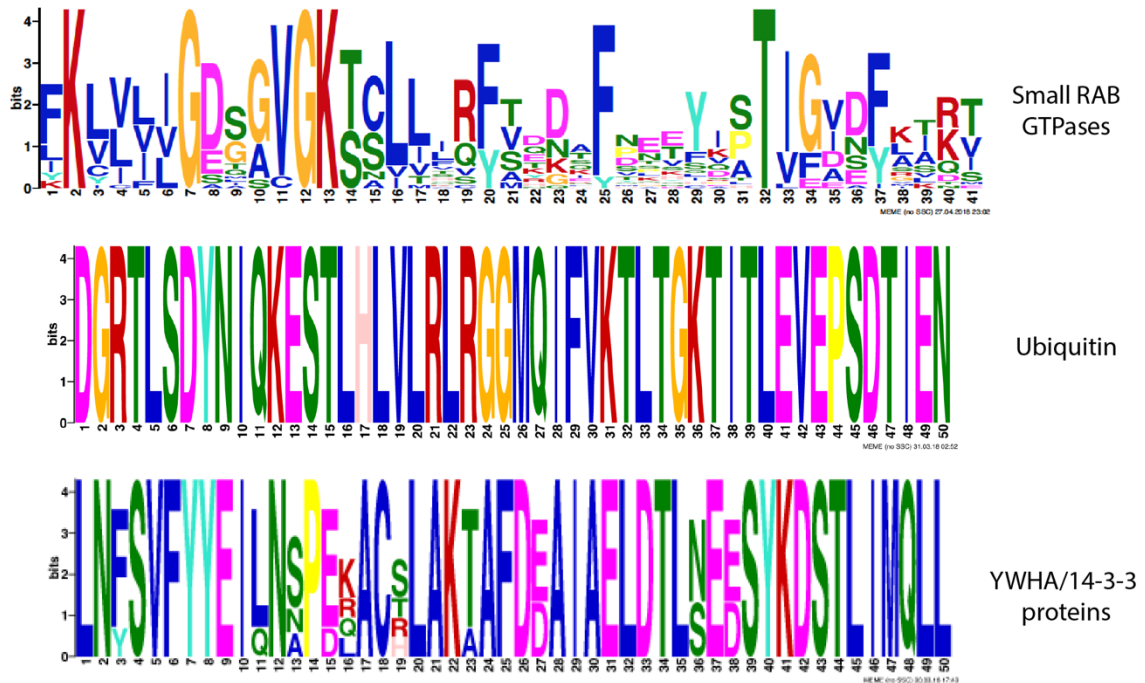


Figure 6-2. Motifs Enriched in Foam Cells Following Autophagy Inhibition

Motif enrichment analysis for motifs enriched in chloroquine-treated macrophage agLDL-loaded THP-1 foam cells reveals enrichment of motifs found in RAB proteins, ubiquitin and YWHA/14-3-3 proteins.

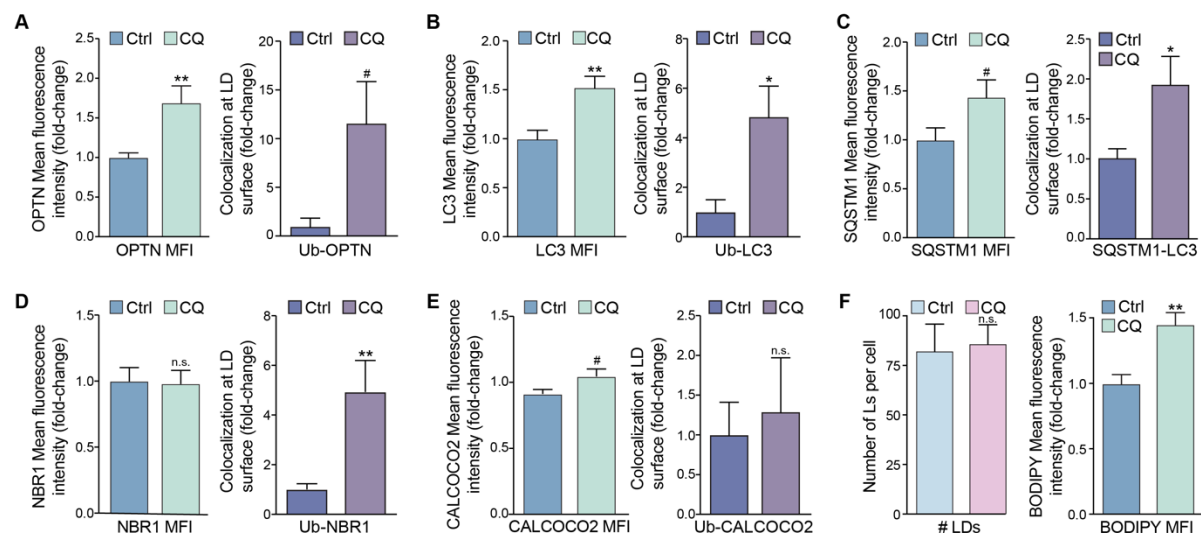


Figure 6-3. Ubiquitin and SARs Colocalize on Lipid Droplets in agLDL-Loaded Macrophage Foam Cells

Quantification of colocalization of Ub-OPTN (**A**), Ub-LC3 (**B**), LC3-SQSTM1 (**C**), Ub-NBR1 (**D**) and Ub-CALCOCO2 (**E**) on the surface of lipid droplets (LDs), along with the number of cellular LDs (**F**) and mean fluorescence intensity (MFI) of each SAR, LC3 and BODIPY (**A-F**) in chloroquine-treated cells (CQ) agLDL-loaded mouse BMDM foam cells as compared to control (Ctrl). Data are expressed as fold-change for CQ treatment relative to untreated from one experiment representative of 3 independent experiments with similar results (mean $\pm$ s.e.m). # $P < 0.1$, * $P < 0.05$, ** $P < 0.005$.

Yeast Mutant	Human Homolog	Molecular Function	Faa4-GFP Cleavage			
			Ex1	Ex2	Ex3	Ex4
<i>atg8Δ</i>	MAP1LC3A	Autophagosome formation	Low	Low	Low	High
<i>vps34Δ</i>	PIK3C3	Autophagosome formation	Low	Low	Low	High
<i>ego1Δ</i>	LAMTOR5	Metabolic regulator of autophagy	Low	Low	n/a	Low
<i>ego2Δ</i>	LAMTOR5	Metabolic regulator of autophagy	Low	Low	Low	Low
<i>cue4Δ</i>	AUP1	Ubiquitin conjugation	High	Low	Low	Low
<i>ubc12Δ</i>	UBE2G2	Ubiquitin conjugation	Low	Low	Low	High
<i>ssa3Δ</i>	HSPA5	Molecular chaperone	Low	Low	n/a	Low
<i>ssb2Δ</i>	HSPA5	Molecular chaperone	Low	High	Low	Low
<i>sse1Δ</i>	HSPH1	Molecular chaperone	Low	Low	High	Low
<i>sse2Δ</i>	HSPH1	Molecular chaperone	Low	Low	High	Low
<i>tgl4Δ</i>	PNPLA2	Triglyceride hydrolysis	Low	Low	Low	Low
<i>ypr147cΔ</i>	LDAH	Lipid hydrolysis	Low	Low	n/a	Low
<i>ypt31Δ</i>	RAB2	Vesicular trafficking	Low	Low	n/a	Low
<i>ypt6Δ</i>	RAB6	Vesicular trafficking	Low	Low	Low	High
<i>ypt11Δ</i>	RAB10,13	Vesicular trafficking	Low	High	Low	Low
<i>hal5Δ</i>	PRKAA1	Cellular energy homeostasis	Low	Low	Low	Low
<i>kkq8Δ</i>	PRKAA1	Cellular energy homeostasis	Low	Low	Low	Low
<i>npr1Δ</i>	PRKAA1	Cellular energy homeostasis	Low	Low	Low	Low
<i>gal83Δ</i>	PRKAA1	Cellular energy homeostasis	Low	Low	Low	Low
<i>bmh1Δ</i>	YWHA	Signaling proteins	High	Low	Low	Low
<i>rtg1Δ</i>	TFEB	Autophagy transcription factor	Low	Low	Low	High
<i>vps38Δ</i>	UVRAG	Autophagy initiation	Low	Low	Low	High
<i>yvc1Δ</i>	MCOLN1	Late endocytic trafficking	Low	Low	Low	High
<i>por2Δ</i>	VDAC2	Autophagy suppressor	High	Low	High	High
<i>crn1Δ</i>	CORO1A	Blocks phagosome-lysosome fusion	High	High	n/a	High
<i>msi1Δ</i>	RBBP7	Cell proliferation	Low	High	Low	Low
<i>hat2Δ</i>	RBBP7	Cell proliferation	Low	High	Low	Low
<i>hta1Δ</i>	MACROH2A1	Nucleosome structure	Low	Low	Low	High

Figure 6-4. Faa4-GFP Cleavage in Various Yeast Strains

Appearance of the GFP fragment of the Faa4-GFP fusion protein, indicating uptake and degradation of Faa4-GFP in the vacuole, was assessed by western blot for the indicated yeast strains. Results are shown for n=4 independent experiments.

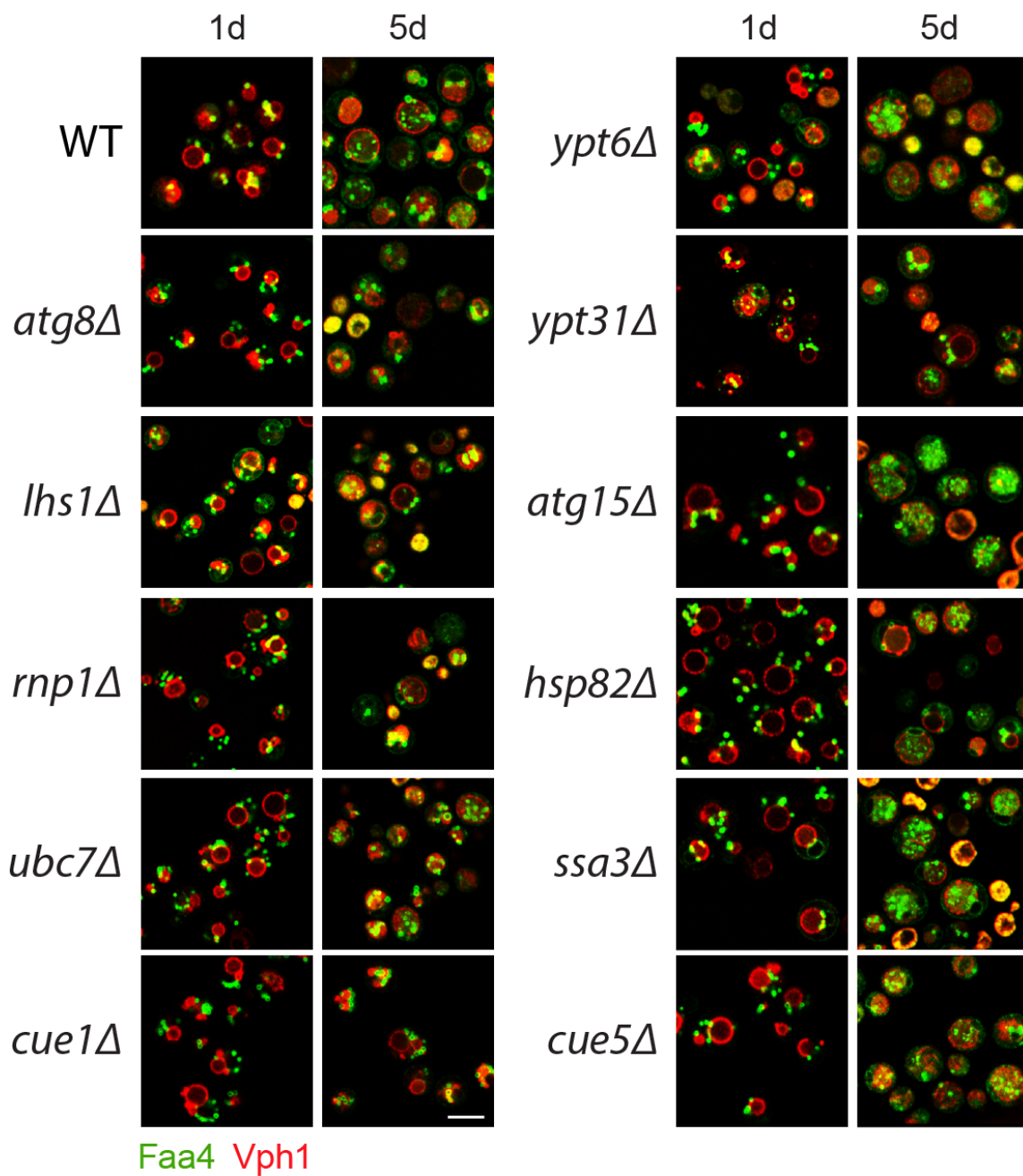


Figure 6-5. Faa4-GFP Entry Into Vacuoles

Yeast strains expressing Faa4-GFP and Vph1-mRuby2 were grown in SC media for 1 or 5 days (1 d versus 5 d) following inoculation. Scale bar: 10 μ m.

Table 6.1. Complete List of Proteins on Human Macrophage Foam Cell Lipid Droplets

LDs were purified from agLDL-loaded THP-1 macrophages by density gradient centrifugation and subjected to mass spectrometry. Names and cell function of identified proteins are shown. Table has been shorten to fit this format. Full table can be found in the supplemental data of the published manuscript.

#	UniProt ID	Gene Name	Description	Ex1	Ex2	Ex3	Ex1 CQ	Ex2 CQ	Ex3 CQ
1	P05090	<i>APOD</i>	Apolipoprotein D OS=Homo sapien	N/A	N/A	N/A	-22.35251	-22.521304	-20.810519
2	P36873	<i>PPP1CC</i>	Serine/threonine-protein phosphat	N/A	N/A	N/A	-23.845954	-23.543802	-24.102949
3	Q92930	<i>RAB8B</i>	Ras-related protein Rab-8A OS=Ho	N/A	N/A	N/A	-19.759274	-23.769992	N/A
4	P15531	<i>NME1</i>	Nucleoside diphosphate kinase A O	N/A	N/A	N/A	-20.016419	N/A	-20.254514
5	P22392	<i>NME2</i>	Nucleoside diphosphate kinase A O	0	0	0	-20.016419	0	-20.254514
6	P26583	<i>HMGB2</i>	High mobility group protein B2 OS=	N/A	N/A	N/A	N/A	-21.712599	-23.420908
7	Q4AC94	<i>C2CD3</i>	C2 domain-containing protein 3 OS	N/A	N/A	N/A	N/A	-25.268202	-24.083944
8	Q16576	<i>RBBP7</i>	Histone-binding protein RBBP4 OS=	N/A	N/A	N/A	N/A	-22.739634	-24.421507
9	P0DME0	<i>SETSI</i>	Protein SETSIP OS=Homo sapiens G	N/A	-19.6438	N/A	-21.444135	-21.39172	-17.608931
10	P50281	<i>MMP14</i>	Matrix metalloproteinase-14 OS=H	-22.27358	N/A	N/A	-22.61137	-21.51954	-21.939279
11	Q96P63	<i>SERPINB12</i>	Serpin B12 OS=Homo sapiens GN=S	N/A	-23.552063	N/A	-23.634931	-22.878272	-22.330513
12	Q8NBJ5	<i>COLGALT1</i>	Procollagen galactosyltransferase 1	-20.512819	N/A	N/A	-20.708905	-22.412892	-24.851544
13	P22695	<i>UQCRC2</i>	Cytochrome b-c1 complex subunit	-19.469787	N/A	N/A	-21.692878	-23.208584	-25.978918
14	P14780	<i>MMP9</i>	Matrix metalloproteinase-9 OS=Ho	-20.150218	N/A	N/A	-22.413913	-24.288367	N/A
15	P35232	<i>PHB</i>	Prohibitin OS=Homo sapiens GN=P	-18.972995	N/A	N/A	-20.56409	-22.831132	N/A
16	P49755	<i>TMED10</i>	Transmembrane emp24 domain-co	-18.723284	N/A	N/A	-19.433364	-20.590156	N/A
17	P50914	<i>RPL14</i>	60S ribosomal protein L14 OS=Hom	-19.33529	N/A	N/A	-19.99506	-19.622559	N/A
18	P51659	<i>HSD17B4</i>	Peroxisomal multifunctional enzym	-19.189192	N/A	N/A	-18.973605	-22.649687	N/A
19	P61006	<i>RAB8A</i>	Ras-related protein Rab-8A OS=Ho	-21.300804	N/A	N/A	-22.170181	-23.769992	N/A
20	Q16836	<i>HADH</i>	Hydroxyacyl-coenzyme A dehydrog	-18.099665	N/A	N/A	-19.281845	-21.845954	N/A
21	Q86UE4	<i>MTDH</i>	Protein LYRIC OS=Homo sapiens GN	-19.167725	N/A	N/A	-19.399971	-23.958987	N/A
22	Q9Y6N5	<i>SQOR</i>	Sulfide:quinone oxidoreductase, mi	-17.502993	N/A	N/A	-19.098029	-23.586364	N/A
23	O60220	<i>TIMM8A</i>	Mitochondrial import inner membr	-20.044493	N/A	N/A	-19.796131	N/A	-20.058514
24	P26885	<i>FKBP2</i>	Peptidyl-prolyl cis-trans isomerase	-19.569649	N/A	N/A	-18.856358	N/A	-21.136427
25	P50454	<i>SERPINH1</i>	Serpin H1 OS=Homo sapiens GN=SE	-18.290461	N/A	N/A	-22.271169	N/A	-23.388214
26	O00182	<i>LGALS9</i>	Galectin-9 OS=Homo sapiens GN=L	-22.220792	N/A	0	-22.791201	0	-23.948503
27	Q9HAR2	<i>ADGRL3</i>	Adhesion G protein-coupled recept	-26.150337	N/A	N/A	-19.758978	N/A	-25.259036
28	P47929	<i>LGALS7</i>	Galectin-7 OS=Homo sapiens GN=L	-20.617514	N/A	N/A	N/A	-17.992372	-20.182492
29	P55769	<i>SNU13</i>	NHP2-like protein 1 OS=Homo sapi	N/A	N/A	-20.878995	N/A	-22.29121	-21.269145
30	Q14011	<i>CIRBP</i>	Cold-inducible RNA-binding protein	N/A	-20.919496	N/A	N/A	-23.545815	-22.007085
31	P09211	<i>GSTP1</i>	Glutathione S-transferase P OS=Ho	N/A	N/A	-20.666306	N/A	-22.74174	-22.678608
32	P13646	<i>KRT13</i>	Keratin, type I cytoskeletal 13 OS=H	N/A	N/A	-19.596798	N/A	-21.605065	-22.918767
33	P04745	<i>AMY1A</i>	Alpha-amylase 1 OS=Homo sapiens	N/A	N/A	-22.581143	N/A	-23.649355	-23.0331
34	P04746	<i>AMY2A</i>	Alpha-amylase 1 OS=Homo sapiens	N/A	N/A	-22.581143	N/A	-23.649355	-23.0331
35	P19961	<i>AMY2B</i>	Alpha-amylase 1 OS=Homo sapiens	N/A	N/A	-22.581143	N/A	-23.649355	-23.0331
36	P29508	<i>SERPINB3</i>	Serpin B3 OS=Homo sapiens GN=SE	N/A	N/A	-23.262934	N/A	-19.081189	-23.800787
37	O75367	<i>MACROH2A1</i>	Core histone macro-H2A.1 OS=Hom	-23.162036	N/A	N/A	N/A	-21.550932	-24.243113
38	O00522	<i>KRIT1</i>	Krev interaction trapped protein 1	-24.216818	-22.882838	-22.986352	-22.302636	-20.597841	N/A
39	O75410	<i>TACC1</i>	Transforming acidic coiled-coil-cont	-18.781942	-23.291869	N/A	-17.839829	-21.951221	N/A
40	O75970	<i>MPDZ</i>	Multiple PDZ domain protein OS=H	-25.069828	N/A	-23.393666	-24.136849	-24.037465	N/A
41	P00387	<i>CYB5R3</i>	NADH-cytochrome b5 reductase 3	-16.985392	-19.08	N/A	-17.653003	-19.696901	N/A
42	P00505	<i>GOT2</i>	Aspartate aminotransferase, mitoc	-20.705279	-22.153447	N/A	-22.022896	-21.593659	N/A

43	P03952	<i>KLKB1</i>	Plasma kallikrein OS=Homo sapiens	-23.660259	-22.245931	-22.310384	-22.099314	-19.273009	N/A
44	P04843	<i>RPN1</i>	Dolichyl-diphosphooligosaccharide-	-17.102463	-21.021132	N/A	-17.434919	-22.107682	N/A
45	P07686	<i>HEXB</i>	Beta-hexosaminidase subunit beta	-20.049115	-20.856453	-20.877493	-20.366175	-24.583005	N/A
46	P12883	<i>MYH7</i>	Myosin-7 OS=Homo sapiens GN=M	N/A	-24.928271	-26.439398	-25.79255	-24.954793	N/A
47	P16070	<i>CD44</i>	CD44 antigen OS=Homo sapiens GN	-21.200671	-21.601927	-22.965066	-22.649356	-22.93568	N/A
48	P16615	<i>ATP2A2</i>	Sarcoplasmic/endoplasmic reticulu	-21.718592	-25.276999	N/A	-22.662152	-24.973401	N/A
49	P27824	<i>CANX</i>	Calnexin OS=Homo sapiens GN=CA	-17.344535	-21.426968	N/A	-17.299144	-21.469909	N/A
50	P39656	<i>DDOST</i>	Dolichyl-diphosphooligosaccharide-	-19.535625	-22.020183	N/A	-21.229919	-21.363924	N/A
51	P48047	<i>ATP5PO</i>	ATP synthase subunit O, mitochond	-17.994772	-21.458604	N/A	-18.944484	-20.6161	N/A
52	P48449	<i>LSS</i>	Lanosterol synthase OS=Homo sapi	-20.073049	-21.68763	N/A	-21.581745	-23.147534	N/A
53	P49748	<i>ACADVL</i>	Very long-chain specific acyl-CoA d	-20.917938	-22.868194	N/A	-21.376447	-23.226325	N/A
54	P50897	<i>PPT1</i>	Palmitoyl-protein thioesterase 1 OS	-17.840534	-20.365097	-19.487906	-21.504837	-22.078902	N/A
55	P61106	<i>RAB14</i>	Ras-related protein Rab-14 OS=Ho	-18.359538	-19.725015	N/A	-18.497504	-20.887529	N/A
56	Q13636	<i>RAB31</i>	Ras-related protein Rab-31 OS=Ho	-19.67539	-21.097897	N/A	-21.374114	-21.393373	N/A
57	Q96AG4	<i>LRRC59</i>	Leucine-rich repeat-containing prot	-16.791479	-19.97112	N/A	-18.554348	-19.681728	N/A
58	Q96CS3	<i>FAF2</i>	FAS-associated factor 2 OS=Homo s	-19.143245	-20.055186	N/A	-19.293246	-20.7579	N/A
59	Q9P0L0	<i>VAPA</i>	Vesicle-associated membrane prot	-18.535447	-21.596817	N/A	-19.43278	-23.221406	N/A
60	Q9Y679	<i>AUP1</i>	Ancient ubiquitous protein 1 OS=H	-19.917772	-20.375409	N/A	-20.453619	-21.30474	N/A
61	P04264	<i>KRT1</i>	Keratin, type II cytoskeletal 1 OS=H	-11.386389	-10.927123	-10.944595	-10.713019	-10.342396	-10.648605
62	P35527	<i>KRT9</i>	Keratin, type I cytoskeletal 9 OS=Ho	-12.945454	-12.054029	-12.666684	-12.682423	-13.075766	-11.699581
63	P06702	<i>S100A9</i>	Protein S100-A9 OS=Homo sapiens	-14.531783	-13.787137	-13.226493	-13.395429	-11.355484	-13.150471
64	P13645	<i>KRT10</i>	Keratin, type I cytoskeletal 10 OS=H	-13.111193	-12.74583	-13.169704	-13.744925	-12.273999	-13.96978
65	P62937	<i>PPIA</i>	Peptidyl-prolyl cis-trans isomerase	-16.349932	-16.198805	-13.855133	-18.162161	-16.703841	-14.044303
66	P05387	<i>RPLP2</i>	60S acidic ribosomal protein P2 OS	-16.783851	-16.010763	-14.318939	-16.312212	-18.12515	-14.321136
67	P81605	<i>DCD</i>	Dermcidin OS=Homo sapiens GN=D	-15.604323	-16.005766	-14.818168	-12.868004	-13.214689	-14.406945
68	P61604	<i>HSPE1</i>	10 kDa heat shock protein, mitoch	-12.983218	-13.272133	-13.888336	-12.094042	-15.194214	-14.533461
69	P84103	<i>SRSF3</i>	Serine/arginine-rich splicing factor	-19.04966	-17.891667	-16.981576	-19.43112	-18.839628	-14.720821
70	P35908	<i>KRT2</i>	Keratin, type II cytoskeletal 2 epide	-15.258251	-13.957554	-14.978999	-14.530084	-15.40963	-14.73751
71	P17096	<i>HMGA1</i>	High mobility group protein HMG-I	-16.542409	-15.074556	-14.603332	-15.454067	-14.288324	-14.845755
72	Q9NZT1	<i>CALML5</i>	Calmodulin-like protein 5 OS=Hom	-18.988548	-18.599328	-17.246904	-17.402476	-14.816966	-14.873468
73	Q01518	<i>CAP1</i>	Adenylyl cyclase-associated protein	-15.425415	-15.016364	-14.424878	-17.361762	-16.318544	-14.987418
74	P22626	<i>HNRNPA2B1</i>	Heterogeneous nuclear ribonucleo	-17.640688	-17.367995	-14.63909	-20.349152	-18.045237	-15.130098
75	P09382	<i>LGALS1</i>	Galectin-1 OS=Homo sapiens GN=L	-15.625151	-14.932647	-14.484238	-17.25512	-16.97011	-15.199948
76	P08670	<i>VIM</i>	Vimentin OS=Homo sapiens GN=VI	-13.915205	-14.530693	-14.95311	-15.309874	-14.910588	-15.296453
77	P05109	<i>S100A8</i>	Protein S100-A8 OS=Homo sapiens	-16.691876	-16.149111	-15.419726	-15.70617	-13.392363	-15.322438
78	P63313	<i>TMSB10</i>	Thymosin beta-10 OS=Homo sapien	-16.679899	-14.342883	-14.812576	-16.30168	-15.121224	-15.374507
79	P31949	<i>S100A11</i>	Protein S100-A11 OS=Homo sapien	-16.453401	-15.019234	-16.257047	-19.471381	-17.9831	-15.443186
80	P60174	<i>TPI1</i>	Triosephosphate isomerase OS=Ho	-17.594742	-15.11515	-14.831232	-17.712481	-16.25574	-15.463528
81	P12273	<i>PIP</i>	Prolactin-inducible protein OS=Hom	-17.682597	-17.468256	-15.287569	-15.634827	-15.330481	-15.471366
82	P40926	<i>MDH2</i>	Malate dehydrogenase, mitochond	-13.827775	-14.653334	-14.880661	-13.955789	-15.636671	-15.477743
83	P60709	<i>ACTB</i>	Actin, cytoplasmic 1 OS=Homo sapi	-16.226178	-15.085902	-14.805654	-19.684469	-19.481848	-15.492911
84	P63261	<i>ACTG1</i>	Actin, cytoplasmic 1 OS=Homo sapi	-16.226178	-15.085902	-14.805654	-19.684469	-19.481848	-15.492911
85	P61769	<i>B2M</i>	Beta-2-microglobulin OS=Homo sap	-17.115009	-18.356328	-18.549691	-16.292271	-16.83358	-15.562242
86	P23528	<i>CFL1</i>	Cofilin-1 OS=Homo sapiens GN=CFL	-14.806465	-12.891338	-12.630067	-15.939386	-14.90871	-15.584098
87	P62805	<i>H4-16</i>	Histone H4 OS=Homo sapiens GN=	-15.402854	-15.303457	-15.078633	-15.174176	-14.363736	-15.627349
88	P07237	<i>P4HB</i>	Protein disulfide-isomerase OS=Ho	-14.844926	-16.110118	-14.990089	-15.261345	-17.037635	-15.633095
89	P62158	<i>CALM1</i>	Calmodulin OS=Homo sapiens GN=	-16.812611	-15.448085	-15.589804	-16.292721	-16.975457	-15.67825
90	P30101	<i>PDIA3</i>	Protein disulfide-isomerase A3 OS=	-14.199449	-15.619456	-15.593288	-15.009922	-16.750289	-15.890741
91	P62328	<i>TMSB4X</i>	Thymosin beta-4 OS=Homo sapiens	-18.222878	-14.999269	-14.632972	-17.432952	-16.53071	-16.020346

92	Q01469	<i>FABP5</i>	Fatty acid-binding protein, epiderm	-17.083647	-17.034191	-15.878919	-17.653581	-17.121222	-16.299687
93	P11021	<i>HSPA5</i>	78 kDa glucose-regulated protein O	-14.407133	-16.298024	-15.918455	-15.035152	-16.730366	-16.331574
94	P02768	<i>ALB</i>	Serum albumin OS=Homo sapiens	-17.341535	-19.098968	-18.619001	-18.159744	-18.922347	-16.351919
95	P02533	<i>KRT14</i>	Keratin, type I cytoskeletal 14 OS=H	-17.497983	-17.653823	-16.352274	-18.074855	-16.554298	-16.429363
96	P06748	<i>NPM1</i>	Nucleophosmin OS=Homo sapiens	-19.580116	-16.841515	-15.90373	-19.792984	-19.618176	-16.525761
97	P27797	<i>CALR</i>	Calreticulin OS=Homo sapiens GN=	-14.950284	-15.741703	-16.201373	-14.800144	-16.296621	-16.625099
98	P61978	<i>HNRNPK</i>	Heterogeneous nuclear ribonucleo	-19.499922	-17.205706	-17.248006	-23.118565	-21.809327	-16.920574
99	P13647	<i>KRT5</i>	Keratin, type II cytoskeletal 5 OS=H	-17.961665	-17.419096	-17.403557	-18.043861	-17.012375	-17.000062
100	P16401	<i>H1-5</i>	Histone H1.5 OS=Homo sapiens GN	-19.369757	-17.816466	-16.570048	-19.186874	-18.534076	-17.003296
101	P05204	<i>HMG2</i>	Non-histone chromosomal protein	-17.752525	-16.919129	-16.579971	-17.331034	-15.745499	-17.029861
102	P62857	<i>RPS28</i>	40S ribosomal protein S28 OS=Hom	-19.151736	-18.45934	-18.079959	-16.687515	-19.420016	-17.115368
103	P63104	<i>YWHAZ</i>	14-3-3 protein zeta/delta OS=Hom	-18.740286	-17.367818	-16.421209	-20.198908	-20.197795	-17.155213
104	P00441	<i>SOD1</i>	Superoxide dismutase [Cu-Zn] OS=	-17.998029	-16.436017	-16.494129	-17.353671	-17.826817	-17.156508
105	P06454	<i>PTMA</i>	Prothymosin alpha OS=Homo sapie	-19.967985	-16.509595	-17.239712	-18.806492	-17.744756	-17.172808
106	P06576	<i>ATP5F1B</i>	ATP synthase subunit beta, mitoch	-14.814929	-17.918544	-17.076489	-15.885011	-18.802517	-17.52929
107	Q00839	<i>HNRNPU</i>	Heterogeneous nuclear ribonucleo	-20.332423	-18.957849	-18.330109	-23.053307	-20.657477	-17.535667
108	P19338	<i>NCL</i>	Nucleolin OS=Homo sapiens GN=N	-18.918443	-16.367818	-15.991746	-19.102305	-18.84298	-17.541609
109	P10809	<i>HSPD1</i>	60 kDa heat shock protein, mitoch	-15.148157	-17.561271	-16.615927	-15.58962	-18.479411	-17.546558
110	P62241	<i>RPS8</i>	40S ribosomal protein S8 OS=Homo	-18.588504	-18.636735	-16.867052	-19.700111	-19.967366	-17.552972
111	P31151	<i>S100A7</i>	Protein S100-A7 OS=Homo sapiens	-16.081641	-17.377772	-16.360834	-16.628672	-14.932502	-17.561721
112	Q01105	<i>SET</i>	Protein SETSIP OS=Homo sapiens G	N/A	-19.6438	-23.089722	-21.444135	-21.39172	-17.608931
113	Q9H299	<i>SH3BGL3</i>	SH3 domain-binding glutamic acid-	-19.402197	-16.947922	-16.03947	-19.558463	-18.510217	-17.641815
114	P04406	<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehyd	-18.220194	-17.609292	-17.227794	-19.093512	-17.407958	-17.684028
115	Q04695	<i>KRT17</i>	Keratin, type I cytoskeletal 17 OS=H	-17.829625	-19.612868	-18.741242	-20.92267	-17.56543	-17.702547
116	P04075	<i>ALDOA</i>	Fructose-bisphosphate aldolase A O	-18.027035	-17.436515	-16.103806	-19.635212	-17.894086	-17.715345
117	P06733	<i>ENO1</i>	Alpha-enolase OS=Homo sapiens G	-18.745253	-17.816964	-16.945557	-20.102887	-18.763256	-17.777161
118	P09651	<i>HNRNPA1</i>	Heterogeneous nuclear ribonucleo	-19.513676	-17.958597	-17.130943	-19.943409	-19.114695	-17.811828
119	Q07955	<i>SRSF1</i>	Serine/arginine-rich splicing factor	-20.767921	-17.422848	-16.740995	-20.193921	-21.088895	-17.832802
120	P61916	<i>NPC2</i>	Epididymal secretory protein E1 OS	-16.673928	-18.874343	-17.267879	-16.130785	-19.83633	-17.847241
121	P10412	<i>H1-4</i>	Histone H1.4 OS=Homo sapiens GN	-18.726292	-17.581447	-17.152367	-19.472289	-19.370492	-17.847356
122	P16402	<i>H1-3</i>	Histone H1.4 OS=Homo sapiens GN	-18.726292	-17.581447	-17.152367	-19.472289	-19.370492	-17.847356
123	P16403	<i>H1-2</i>	Histone H1.4 OS=Homo sapiens GN	-18.726292	-17.581447	-17.152367	-19.472289	-19.370492	-17.847356
124	P60660	<i>MYL6</i>	Myosin light chain 6B OS=Homo sa	-18.726262	-17.471331	-17.41064	-19.892002	-20.11923	-17.877504
125	P62861	<i>FAU</i>	40S ribosomal protein S30 OS=Hom	-20.004933	-17.716725	-17.157549	-18.231974	-18.274953	-17.994149
126	P07910	<i>HNRNPC</i>	Heterogeneous nuclear ribonucleo	-20.357266	-18.658508	-17.713027	-20.8131	-20.906165	-18.01046
127	P02545	<i>LMNA</i>	Prelamin-A/C OS=Homo sapiens GN	-18.414632	-17.025108	-17.667598	-18.276591	-16.24949	-18.045558
128	P07477	<i>PRSS1</i>	Trypsin-1 OS=Homo sapiens GN=PR	-18.605817	-16.085626	-17.141765	-20.017144	-18.987157	-18.048978
129	P04792	<i>HSPB1</i>	Heat shock protein beta-1 OS=Hom	-18.476979	-19.179365	-18.312684	-19.979033	-19.510093	-18.057247
130	Q00584	<i>RNASET2</i>	Ribonuclease T2 OS=Homo sapiens	-19.737117	-22.266863	-20.496316	-20.08922	-21.782217	-18.180461
131	P02042	<i>HBD</i>	Hemoglobin subunit delta OS=Hom	-16.917115	-18.871663	-19.68897	-19.488343	-18.589901	-18.183713
132	P02100	<i>HBE1</i>	Hemoglobin subunit delta OS=Hom	-16.917115	-18.871663	-19.68897	-19.488343	-18.589901	-18.183713
133	P68871	<i>HBB</i>	Hemoglobin subunit delta OS=Hom	-16.917115	-18.871663	-19.68897	-19.488343	-18.589901	-18.183713
134	P69891	<i>HBG1</i>	Hemoglobin subunit delta OS=Hom	-16.917115	-18.871663	-19.68897	-19.488343	-18.589901	-18.183713
135	P69892	<i>HBG2</i>	Hemoglobin subunit delta OS=Hom	-16.917115	-18.871663	-19.68897	-19.488343	-18.589901	-18.183713
136	P08779	<i>KRT16</i>	Keratin, type I cytoskeletal 16 OS=H	-16.208959	-17.76775	-16.887987	-19.542996	-17.580646	-18.19092
137	P07602	<i>PSAP</i>	Prosaposin OS=Homo sapiens GN=	-17.716793	-18.165203	-18.397778	-16.279691	-18.082166	-18.194045
138	Q06830	<i>PRDX1</i>	Peroxiredoxin-1 OS=Homo sapiens	-19.370241	-18.223777	-18.225939	-20.153969	-18.334394	-18.242865
139	P80723	<i>BASP1</i>	Brain acid soluble protein 1 OS=Ho	-17.161692	-17.335514	-18.060493	-16.908698	-17.291356	-18.287464
140	P11142	<i>HSPA8</i>	Heat shock cognate 71 kDa protein	-18.94494	-18.107169	-17.616792	-20.822197	-19.817901	-18.302926
141	P68104	<i>EEF1A1</i>	Elongation factor 1-alpha 1 OS=Ho	-18.216227	-20.250056	-20.615047	-22.160701	-20.100207	-18.320516
142	Q5VTE0	<i>EEF1A1P5</i>	Elongation factor 1-alpha 1 OS=Ho	-18.216227	-20.250056	-20.615047	-22.160701	-20.100207	-18.320516

143	Q8N1N4	KRT78	Keratin, type II cytoskeletal 78 OS=	-19.626073	-18.453747	-20.595778	-19.557563	-18.775454	-18.334515
144	Q02818	NUCB1	Nucleobindin-1 OS=Homo sapiens	-20.062925	-18.149817	-16.920248	-17.162479	-18.013834	-18.337172
145	P25311	AZGP1	Zinc-alpha-2-glycoprotein OS=Hom	-22.65711	N/A	-21.292824	-21.592227	-19.112957	-18.388241
146	P67936	TPM4	Tropomyosin alpha-4 chain OS=Ho	-22.325729	-18.496889	-18.707576	-22.562948	-22.291139	-18.438871
147	O43399	TPD52L2	Tumor protein D54 OS=Homo sapie	-16.099969	-18.104284	-16.319621	-15.763349	-18.26021	-18.480692
148	Q8NBQ5	HSD17B11	Estradiol 17-beta-dehydrogenase 1	-15.947458	-14.964559	-20.332373	-14.430655	-16.05488	-18.515428
149	Q99497	PARK7	Protein deglycase DJ-1 OS=Homo s	-19.103825	-18.336177	-17.879296	-19.149439	-19.485969	-18.575178
150	P07919	UQCRH	Cytochrome b-c1 complex subunit	-14.274197	-16.944954	-16.144677	-12.631628	-17.617965	-18.671908
151	P04179	SOD2	Superoxide dismutase [Mn], mitoch	-13.662975	-15.904428	-17.12006	-15.998363	-16.229142	-18.6836
152	Q5T749	KPRP	Keratinocyte proline-rich protein O	-18.842488	-18.343797	-18.72804	-19.155209	-18.822372	-18.72039
153	P07858	CTSB	Cathepsin B OS=Homo sapiens GN=	-16.94937	-18.733209	-18.271787	-17.540677	-19.966849	-18.750717
154	P14625	HSP90B1	Endoplasmic OS=Homo sapiens GN	-17.174531	-18.87011	-18.025064	-17.645614	-20.065719	-18.880893
155	Q02878	RPL6	60S ribosomal protein L6 OS=Homo	-20.637002	-19.671866	-18.633288	-19.500105	-21.029356	-18.925886
156	P10451	SPP1	Osteopontin OS=Homo sapiens GN	-22.978863	-20.02178	-18.84259	-20.692875	-22.278463	-18.939539
157	P23246	SFPQ	Splicing factor, proline- and glutam	-23.129273	-19.618103	-19.259812	-23.481925	-22.226868	-18.964742
158	O00754	MAN2B1	Lysosomal alpha-mannosidase OS=	-18.482541	-19.306325	-17.922546	-17.873092	-19.675051	-18.967136
159	P23284	PPIB	Peptidyl-prolyl cis-trans isomerase	-16.329025	-17.779469	-17.928534	-17.405577	-16.09519	-18.988885
160	Q14103	HNRNPD	Heterogeneous nuclear ribonucleo	-19.783765	-19.18559	-18.535718	-21.953911	-21.629887	-18.990885
161	P14314	PRKCSH	Glucosidase 2 subunit beta OS=Ho	-18.424244	-18.626245	-18.604247	-18.455466	-20.190175	-19.001301
162	P26373	RPL13	60S ribosomal protein L13 OS=Hom	-19.439597	-20.075455	-18.106845	-20.471956	-20.397965	-19.061171
163	Q04837	SSBP1	Single-stranded DNA-binding prote	-17.541706	-18.76739	-20.288931	-17.442557	-19.675553	-19.09988
164	O95881	TXNDC12	Thioredoxin domain-containing pro	-18.800678	-18.498405	-18.26385	-18.140991	-21.291488	-19.11101
165	P10606	COX5B	Cytochrome c oxidase subunit 5B,	-18.222995	-19.331453	-18.852917	-17.282704	-19.24235	-19.120966
166	P05114	HMG1	Non-histone chromosomal protein	-21.234031	-19.171582	-20.462993	-19.622507	-19.58604	-19.141732
167	P15924	DSP	Desmoplakin OS=Homo sapiens GN	-20.293868	-19.867468	-20.156797	-21.247975	-19.841618	-19.182616
168	P30405	PPIF	Peptidyl-prolyl cis-trans isomerase	-17.006182	-19.504904	-17.902205	-18.092257	-18.362275	-19.210288
169	P62750	RPL23A	60S ribosomal protein L23a OS=Ho	-19.936091	-19.538706	-17.937584	-21.059015	-20.528136	-19.221233
170	P14618	PKM	Pyruvate kinase PKM OS=Homo sap	-19.927599	-19.232252	-17.731356	-23.196569	-21.73295	-19.291243
171	P49207	RPL34	60S ribosomal protein L34 OS=Hom	-18.433693	-19.173859	-18.897905	-19.301958	-19.340995	-19.310121
172	P62917	RPL8	60S ribosomal protein L8 OS=Homo	-19.093439	-19.316036	-18.847619	-20.452713	-21.668489	-19.369354
173	P30040	ERP29	Endoplasmic reticulum resident pro	-17.457935	-20.237475	-19.345173	-18.274649	-21.501137	-19.446959
174	Q5T750	C1orf68	Skin-specific protein 32 OS=Homo s	-19.547067	-19.882508	-20.021457	-20.100572	-19.928098	-19.44716
175	P06396	GSN	Gelsolin OS=Homo sapiens GN=GSN	-20.464259	-19.373839	-17.904487	-22.580762	-20.919014	-19.451923
176	P0C0S8	H2AC11	Histone H2A type 1-B/E OS=Homo	-18.432353	-19.03918	-21.013721	-18.055414	-16.751339	-19.483702
177	P20671	H2AC7	Histone H2A type 1-B/E OS=Homo	-18.432353	-19.03918	-21.013721	-18.055414	-16.751339	-19.483702
178	Q16777	H2AC20	Histone H2A type 1-B/E OS=Homo	-18.432353	-19.03918	-21.013721	-18.055414	-16.751339	-19.483702
179	Q6FI13	H2AC18	Histone H2A type 1-B/E OS=Homo	-18.432353	-19.03918	-21.013721	-18.055414	-16.751339	-19.483702
180	Q96KK5	H2AC12	Histone H2A type 1-B/E OS=Homo	-18.432353	-19.03918	-21.013721	-18.055414	-16.751339	-19.483702
181	Q99878	H2AC14	Histone H2A type 1-B/E OS=Homo	-18.432353	-19.03918	-21.013721	-18.055414	-16.751339	-19.483702
182	Q9BTM1	H2AJ	Histone H2A type 1-B/E OS=Homo	-18.432353	-19.03918	-21.013721	-18.055414	-16.751339	-19.483702
183	P38159	RBMX	RNA-binding motif protein, X chrom	-20.979204	-19.166949	-19.161282	-21.702701	-21.159093	-19.497629
184	P51991	HNRNPA3	Heterogeneous nuclear ribonucleo	-20.776539	-18.646827	-18.059301	-21.942597	-20.543633	-19.501876
185	P67809	YBX1	Nuclease-sensitive element-binding	-20.176884	-18.538585	-18.953021	-22.359997	-21.798245	-19.507094
186	P14923	JUP	Junction plakoglobin OS=Homo sap	-18.119445	-17.706106	-18.052064	-19.93501	-17.668176	-19.564811
187	Q9Y2B0	CNPY2	Protein canopy homolog 2 OS=Hom	-18.113643	-18.819848	-18.657952	-17.641381	-22.237509	-19.590611
188	P39023	RPL3	60S ribosomal protein L3 OS=Homo	-19.743239	-20.046875	-19.153217	-22.186101	-20.076319	-19.594449
189	P24534	EEF1B2	Elongation factor 1-beta OS=Homo	-20.878892	-19.758303	-18.85218	-20.73344	-20.048922	-19.645716
190	P49006	MARCKSL1	MARCKS-related protein OS=Homo	-20.663257	-19.879726	-19.063805	-20.086858	-20.192009	-19.646727
191	P08238	HSP90AB1	Heat shock protein HSP 90-beta OS	-21.729907	-19.787006	-19.007725	-23.611292	-21.532432	-19.720843
192	P62753	RPS6	40S ribosomal protein S6 OS=Homo	-20.003756	-20.620441	-19.558545	-21.989921	-21.716814	-19.724553
193	P61981	YWHAQ	14-3-3 protein gamma OS=Homo s	-21.185125	-19.622797	-18.731896	-21.629725	-21.20913	-19.734682

194	P13796	LCP1	Plastin-2 OS=Homo sapiens GN=LC	-21.980477	-20.373066	-19.221007	-25.113802	-21.989377	-19.739865
195	P62851	RPS25	40S ribosomal protein S25 OS=Hom	-18.565098	-18.523291	-18.824862	-19.755552	-19.077628	-19.745152
196	P21796	VDAC1	Voltage-dependent anion-selective	-16.659943	-19.712291	-19.453822	-17.263908	-19.135523	-19.750702
197	P61626	LYZ	Lysozyme C OS=Homo sapiens GN=	-20.313209	-18.75994	-18.314197	-18.556577	-18.625731	-19.829556
198	P04040	CAT	Catalase OS=Homo sapiens GN=CA	-17.888308	-19.387527	-19.137283	-18.757801	-21.253621	-19.836347
199	Q15293	RCN1	Reticulocalbin-1 OS=Homo sapiens	-19.007046	-19.605764	-19.266308	-18.758041	-20.190031	-19.845165
200	P07339	CTSD	Cathepsin D OS=Homo sapiens GN=	-18.373871	-19.917428	-19.171334	-19.391632	-19.195713	-19.858934
201	P29692	EEF1D	Elongation factor 1-delta OS=Homo	-19.973018	-19.415022	-18.903948	-20.627708	-20.039402	-19.926838
202	Q9UKY7	CDV3	Protein CDV3 homolog OS=Homo s	N/A	-18.909614	-19.566163	-21.680865	-20.675889	-19.945426
203	P15880	RPS2	40S ribosomal protein S2 OS=Homo	-20.487026	-20.354619	-20.211998	-21.182192	-20.811319	-19.974206
204	P25774	CTSS	Cathepsin S OS=Homo sapiens GN=	-18.406706	-19.742351	-19.833312	-17.836907	-20.296685	-19.987467
205	Q86V81	ALYREF	THO complex subunit 4 OS=Homo s	-20.473397	-19.049367	-18.172489	-21.138068	-20.537409	-19.994893
206	P62424	RPL7A	60S ribosomal protein L7a OS=Hom	-19.382417	-19.268402	-18.638686	-20.279403	-19.801648	-19.998498
207	O43852	CALU	Calumenin OS=Homo sapiens GN=C	-19.396582	-18.667723	-18.284027	-18.838157	-19.56254	-20.023033
208	Q15084	PDIA6	Protein disulfide-isomerase A6 OS=	-15.682419	-18.376437	-18.273207	-18.086704	-18.77952	-20.03111
209	P26641	EEF1G	Elongation factor 1-gamma OS=Ho	-21.242169	-20.321292	-19.641317	-21.967756	-21.238851	-20.069248
210	P07900	HSP90AA1	Heat shock protein HSP 90-alpha O	-21.581373	-20.03114	-19.710248	-24.115832	-22.465562	-20.104049
211	P38646	HSPA9	Stress-70 protein, mitochondrial OS	-16.681644	-18.5943	-18.026905	-17.074892	-19.133491	-20.16079
212	P62491	RAB11A	Ras-related protein Rab-11A OS=Ho	-18.041011	-19.711222	-20.709359	-18.18974	-18.736705	-20.161789
213	Q15907	RAB11B	Ras-related protein Rab-11A OS=Ho	-18.041011	-19.711222	-20.709359	-18.18974	-18.736705	-20.161789
214	P18124	RPL7	60S ribosomal protein L7 OS=Homo	-19.661387	-20.080841	-19.525112	-20.621404	-21.095631	-20.182806
215	P09622	DLD	Dihydrolipoyl dehydrogenase, mito	-18.471292	-18.762957	-19.157364	-17.330027	-19.744728	-20.192362
216	P61247	RPS3A	40S ribosomal protein S3a OS=Hom	-19.748462	-19.907533	-19.024845	-22.018948	-20.720167	-20.199548
217	P42766	RPL35	60S ribosomal protein L35 OS=Hom	-19.462447	-19.528281	-18.684936	-20.490931	-20.342163	-20.261422
218	P62258	YWHAE	14-3-3 protein epsilon OS=Homo sa	-22.27074	-20.702565	-19.824718	-22.918839	-21.678858	-20.288356
219	P09496	CLTA	Clathrin light chain A OS=Homo sap	-20.836113	-19.847742	-19.300354	-20.539241	-20.846473	-20.332189
220	Q01130	SRSF2	Serine/arginine-rich splicing factor	-21.179301	-20.614471	-19.071667	-23.298568	-21.890901	-20.372498
221	P25705	ATP5F1A	ATP synthase subunit alpha, mitoch	-15.639812	-18.630974	-19.923923	-16.278569	-17.572558	-20.409015
222	P68431	H3C1	Histone H3.1 OS=Homo sapiens GN	N/A	-20.690422	-22.189642	-20.260935	-19.565956	-20.412282
223	P84243	H3-3A	Histone H3.1 OS=Homo sapiens GN	-20.576937	-20.690422	-22.189642	-20.260935	-19.565956	-20.412282
224	Q16695	H3-4	Histone H3.1 OS=Homo sapiens GN	N/A	-20.690422	-22.189642	-20.260935	-19.565956	-20.412282
225	Q71DI3	H3C13	Histone H3.1 OS=Homo sapiens GN	N/A	-20.690422	-22.189642	-20.260935	-19.565956	-20.412282
226	P08134	RHOC	Rho-related GTP-binding protein Rh	N/A	-20.145179	-20.440174	-20.048732	-21.461506	-20.421312
227	P61586	RHOA	Rho-related GTP-binding protein Rh	-24.722696	-20.145179	-20.440174	-20.048732	-21.461506	-20.421312
228	P12814	ACTN1	Alpha-actinin-1 OS=Homo sapiens	-22.285147	-21.129282	-20.87926	-23.48451	-23.953356	-20.522073
229	P07711	CTSL	Cathepsin L1 OS=Homo sapiens GN	-19.839869	-19.722821	-17.708809	-19.370199	-20.721899	-20.543582
230	P52907	CAPZA1	F-actin-capping protein subunit alp	-17.795429	-20.998704	-18.781788	-20.203261	-22.181655	-20.620662
231	P11310	ACADM	Medium-chain specific acyl-CoA de	-17.561443	-19.494936	-17.549114	-19.010679	-20.245041	-20.623292
232	Q6ZVX7	NCCRP1	F-box only protein 50 OS=Homo sa	-21.528429	-20.575581	-20.680368	-21.317848	-19.691798	-20.626348
233	Q86YZ3	HRNR	Homerin OS=Homo sapiens GN=HR	-20.843631	-20.826397	-20.963748	-21.378485	-21.705541	-20.654584
234	Q99541	PLIN2	Perilipin-2 OS=Homo sapiens GN=P	-15.652537	-19.176943	-20.169202	-15.752454	-18.515674	-20.66609
235	P31946	YWHAH	14-3-3 protein beta/alpha OS=Hom	-22.121038	-20.89807	-20.827473	-23.2383	-22.810216	-20.676952
236	P50552	VASP	Vasodilator-stimulated phosphopro	-20.92195	-21.860339	-20.189991	-22.91339	-21.791761	-20.71017
237	P51149	RAB7A	Ras-related protein Rab-7a OS=Ho	-15.632145	-17.817267	-20.249203	-14.746139	-18.306041	-20.721975
238	Q08554	DSC1	Desmocollin-1 OS=Homo sapiens G	-21.690049	-21.2254	-21.795934	-22.507627	-22.466663	-20.731393
239	Q15942	ZYX	Zyxin OS=Homo sapiens GN=ZYX PE	-22.209923	-21.204583	-19.060588	-24.08701	-23.368814	-20.73402
240	Q07020	RPL18	60S ribosomal protein L18 OS=Hom	-18.991338	-19.774661	-20.351452	-20.665363	-19.214752	-20.759868
241	P61026	RAB10	Ras-related protein Rab-10 OS=Ho	-16.273087	-17.582832	-20.745645	-18.859875	-21.381323	-20.778397
242	P99999	CYCS	Cytochrome c OS=Homo sapiens G	-16.707636	-18.984751	-17.650558	-17.413798	-19.918167	-20.785203
243	Q9BS26	ERP44	Endoplasmic reticulum resident pro	-19.675768	-21.149997	-20.132501	-20.534185	-21.118014	-20.810232
244	P55072	VCP	Transitional endoplasmic reticulum	-20.020125	-20.807195	-20.582709	-21.891744	-21.729179	-20.824178

245	Q5D862	FLG2	Filaggrin-2 OS=Homo sapiens GN=F	-21.010617	-19.862385	-21.163145	-20.2111	-18.532643	-20.858701
246	P47756	CAPZB	F-actin-capping protein subunit bet	-19.038538	-22.496127	-22.116575	-21.525074	-21.990954	-20.880596
247	P07305	H1-O	Histone H1.0 OS=Homo sapiens GN	-23.4985	-20.752869	-20.661447	-21.567429	-20.808066	-20.887345
248	Q14697	GANAB	Neutral alpha-glucosidase AB OS=H	-18.777769	-20.451521	-20.0734	-19.875536	-22.159426	-20.888249
249	P82979	SARNP	SAP domain-containing ribonucleo	-21.912084	-20.5243	-19.574651	-21.629095	-20.840493	-20.90947
250	Q02413	DSG1	Desmoglein-1 OS=Homo sapiens G	-21.779169	-20.981404	-22.032853	-21.577459	-20.441145	-20.972731
251	P11279	LAMP1	Lysosome-associated membrane gl	-19.50134	-20.363193	-20.637585	-18.525473	-20.227948	-21.136379
252	P52926	HMGA2	High mobility group protein HMGI-	N/A	-21.544115	-22.445117	-21.859327	-20.697047	-21.163667
253	P20700	LMNB1	Lamin-B1 OS=Homo sapiens GN=LM	-22.266843	-22.15177	-20.995702	-22.547951	-20.91709	-21.187906
254	P28799	GRN	Granulins OS=Homo sapiens GN=G	-20.403664	-20.783026	-21.076959	-19.325493	-21.383616	-21.206685
255	Q6UWP8	SBSN	Suprabasin OS=Homo sapiens GN=	-21.20039	-24.108084	-22.284422	-22.477752	-21.400945	-21.235593
256	P40925	MDH1	Malate dehydrogenase, cytoplasmic	-20.10308	-21.065358	-20.289866	-22.482729	-21.90223	-21.250624
257	Q16698	DECR1	2,4-dienoyl-CoA reductase, mitoch	-18.836728	-21.08583	-20.453198	-19.885018	-21.295847	-21.369043
258	P32119	PRDX2	Peroxiredoxin-2 OS=Homo sapiens	-21.921053	-21.474913	-21.264163	-22.460308	-20.076272	-21.465077
259	P26038	MSN	Moesin OS=Homo sapiens GN=MSN	-19.820789	-20.685655	-21.526329	-21.749023	-20.409412	-21.485718
260	Q8NBX0	SCCPDH	Saccharopine dehydrogenase-like o	-17.758069	-19.297852	-22.394578	-17.453523	-18.32544	-21.5211
261	O14773	TPP1	Tripeptidyl-peptidase 1 OS=Homo s	-18.728741	-22.960085	-19.983319	-20.12106	-20.784843	-21.523447
262	Q9H8H3	METTL7A	Methyltransferase-like protein 7A O	-17.093793	-16.722133	-22.158185	-16.086211	-16.31567	-21.531449
263	P18077	RPL35A	60S ribosomal protein L35a OS=Ho	-20.162195	-20.20286	-20.307636	-20.472527	-20.138474	-21.561812
264	P55145	MANF	Mesencephalic astrocyte-derived n	-18.375494	-20.766068	-20.573828	-18.055857	-20.322355	-21.598012
265	P15153	RAC2	Ras-related C3 botulinum toxin sub	-18.614545	-20.365326	-21.177746	-19.746547	-20.769012	-21.602342
266	P13473	LAMP2	Lysosome-associated membrane gl	-20.576809	-21.435098	-21.111262	-19.83741	-21.285748	-21.603189
267	Q9UHL4	DPP7	Dipeptidyl peptidase 2 OS=Homo s	-20.259922	-22.868665	-21.330211	-20.805925	-23.853718	-21.61775
268	Q6KB66	KRT80	Keratin, type II cytoskeletal 80 OS=	-22.093165	-21.669325	-23.055522	-23.080396	-21.594924	-21.761523
269	Q14534	SQLE	Squalene monooxygenase OS=Hom	-17.537695	-17.350125	N/A	-17.424659	-19.26501	-21.835331
270	P18859	ATP5PF	ATP synthase-coupling factor 6, mit	-18.909176	-20.874253	-20.858002	-18.069907	-20.88332	-21.849818
271	P51148	RAB5C	Ras-related protein Rab-5C OS=Ho	-18.404405	-20.310241	-22.047858	-19.924755	-20.135051	-21.859369
272	P05107	ITGB2	Integrin beta-2 OS=Homo sapiens G	-16.729473	-20.907182	-23.338346	-17.429502	-20.547842	-21.861119
273	O60664	PLIN3	Perilipin-3 OS=Homo sapiens GN=P	-18.496143	-20.892941	-20.171458	-19.728875	-20.620384	-22.032229
274	P15586	GNS	N-acetylglucosamine-6-sulfatase O	-20.902836	-23.16491	-22.161767	-20.631126	-22.770351	-22.142309
275	P13667	PDIA4	Protein disulfide-isomerase A4 OS=	-18.794401	-22.57713	-20.837276	-20.539444	-22.688459	-22.143422
276	Q9NP72	RAB18	Ras-related protein Rab-18 OS=Ho	-21.525661	-21.201874	N/A	-21.590393	-20.933374	-22.16071
277	P04259	KRT6B	Keratin, type II cytoskeletal 6B OS=	-21.024256	-21.664316	-21.262609	-24.706727	-20.08398	-22.293497
278	P61254	RPL26	60S ribosomal protein L26 OS=Hom	-21.072259	-20.487693	-19.759231	-21.127958	-20.867014	-22.31533
279	Q9NX63	CHCHD3	MICOS complex subunit MIC19 OS=	-18.309075	-22.889372	-21.914517	-19.068108	-24.03184	-22.316302
280	Q8NC51	SERBP1	Plasminogen activator inhibitor 1 R	-20.928341	-20.222701	-19.356848	-21.757589	-21.701634	-22.330547
281	P62269	RPS18	40S ribosomal protein S18 OS=Hom	-19.419272	-18.897967	-22.661417	-21.383084	-20.1295	-22.429081
282	Q15365	PCBP1	Poly(rC)-binding protein 1 OS=Hom	-22.178438	-22.208768	-22.297984	-23.557093	-21.958205	-22.495714
283	P62701	RPS4X	40S ribosomal protein S4, X isoform	-21.191857	-21.954257	-21.662998	-22.6921	-22.788448	-22.583201
284	Q6UX53	METTL7B	Methyltransferase-like protein 7B O	-18.450814	-20.138766	-23.792885	-18.870733	-19.725331	-22.583437
285	P07437	TUBB	Tubulin beta chain OS=Homo sapie	-19.949553	-18.696194	-18.283183	-24.075904	-23.781578	-22.639288
286	O75844	ZMPSTE24	CAAX prenyl protease 1 homolog O	-18.480159	-20.88171	N/A	-18.363114	-20.653665	-22.778626
287	P15144	ANPEP	Aminopeptidase N OS=Homo sapie	-20.38034	-22.634657	-22.945444	-21.825515	-22.367104	-22.818448
288	O75223	GGCT	Gamma-glutamylcyclotransferase O	N/A	-22.772709	-21.966621	-23.075556	-20.263864	-22.83908
289	P20930	FLG	Filaggrin OS=Homo sapiens GN=FLG	-23.989453	-23.981607	-23.487352	-23.624899	-21.801201	-22.883927
290	Q14956	GNPMB	Transmembrane glycoprotein NMB	-20.115448	-22.960509	N/A	-19.701197	-22.581921	-22.973481
291	P61019	RAB2A	Ras-related protein Rab-2A OS=Ho	-18.294926	-20.834627	N/A	-18.570994	-19.557747	-23.057027
292	P48735	IDH2	Isocitrate dehydrogenase [NADP],	-17.929317	-22.206524	-20.989454	-19.401612	-20.822821	-23.064256
293	P04908	H2AC4	Histone H2A type 1-B/E OS=Homo	-21.555017	-21.791567	-23.113518	-21.546352	-19.709463	-23.11202
294	Q7L7L0	H2AW	Histone H2A type 1-B/E OS=Homo	-21.555017	-21.791567	-23.113518	-21.546352	-19.709463	-23.11202
295	Q93077	H2AC6	Histone H2A type 1-B/E OS=Homo	-21.555017	-21.791567	-23.113518	-21.546352	-19.709463	-23.11202

296	P62873	<i>GNB1</i>	Guanine nucleotide-binding protein	-20.525678	-23.035561	-23.333956	-22.696141	-22.620826	-23.157117
297	Q9Y4L1	<i>HYOU1</i>	Hypoxia up-regulated protein 1 OS	-20.175055	-23.53763	-22.129837	-21.780624	-24.47117	-23.205769
298	P31930	<i>UQCRC1</i>	Cytochrome b-c1 complex subunit	-19.262279	-23.58998	-22.444453	-22.235722	-23.299689	-23.23843
299	P45880	<i>VDAC2</i>	Voltage-dependent anion-selective	-18.384916	-23.106054	-22.753471	-18.777315	-21.26419	-23.251299
300	P22307	<i>SCP2</i>	Non-specific lipid-transfer protein O	-18.463088	-22.014191	-22.567376	-20.236064	-22.288261	-23.262635
301	Q8WVV4	<i>POF1B</i>	Protein POF1B OS=Homo sapiens G	-25.022008	-25.92911	-24.405756	-24.443461	-22.074306	-23.27146
302	P36957	<i>DLST</i>	Dihydrolipoyllysine-residue succiny	-19.934159	-23.506038	-21.594573	-22.043803	-22.677889	-23.313472
303	P51571	<i>SSR4</i>	Translocon-associated protein subu	-18.885699	-21.972309	N/A	-19.555541	-21.541009	-23.317631
304	P05141	<i>SLC25A5</i>	ADP/ATP translocase 2 OS=Homo s	-19.62706	-21.051594	-23.893401	-21.229165	-21.959456	-23.46447
305	Q99536	<i>VAT1</i>	Synaptic vesicle membrane protein	-18.664131	-21.983775	-21.765246	-19.56534	-22.667402	-23.52111
306	Q9Y490	<i>TLN1</i>	Talin-1 OS=Homo sapiens GN=TLN1	-23.535599	-22.442641	-22.436491	-28.27602	-26.701908	-23.594869
307	O95573	<i>ACSL3</i>	Long-chain-fatty-acid--CoA ligase 3	-17.251859	-19.501973	0	-16.730511	-19.652541	-23.659719
308	O75396	<i>SEC22B</i>	Vesicle-trafficking protein SEC22b O	-20.123445	-21.898382	N/A	-19.77508	-22.170911	-23.971612
309	Q0VD83	<i>APOBR</i>	Apolipoprotein B receptor OS=Hom	-19.486291	-24.730063	-23.573894	-20.175529	-23.524718	-24.058182
310	Q7Z794	<i>KRT77</i>	Keratin, type II cytoskeletal 1b OS=	-21.726177	-19.626931	-19.08813	-19.615169	-22.080857	-24.244473
311	Q7KZF4	<i>SND1</i>	Staphylococcal nuclease domain-co	-19.812354	-24.199987	-24.133393	-21.362683	-22.237468	-24.386618
312	O60488	<i>ACSL4</i>	Long-chain-fatty-acid--CoA ligase 4	-19.729111	-21.974086	N/A	-21.990978	-22.081155	-24.485716
313	P00367	<i>GLUD1</i>	Glutamate dehydrogenase 1, mitoc	-20.269881	-22.711965	-24.75947	-21.082975	-23.404708	-25.214306
314	O94905	<i>ERLIN2</i>	Erlin-1 OS=Homo sapiens GN=ERLIN	-19.720871	-22.621994	-22.284574	-20.417435	-21.469504	-25.229161
315	Q9UG22	<i>GIMAP2</i>	GTPase IMAP family member 2 OS=	-20.734045	-20.715533	-24.334431	-20.230038	-20.229075	-25.324637
316	P13533	<i>MYH6</i>	Myosin-6 OS=Homo sapiens GN=M	N/A	-19.566296	-26.982367	-26.191073	-24.424742	-25.793323
317	O14966	<i>RAB29</i>	Ras-related protein Rab-7L1 OS=Ho	-23.972258	N/A	N/A	-22.129654	N/A	N/A
318	O15018	<i>PDZD2</i>	PDZ domain-containing protein 2 O	N/A	N/A	N/A	-26.638509	N/A	N/A
319	O15173	<i>PGRMC2</i>	Membrane-associated progesteron	-20.754991	N/A	N/A	-21.340902	N/A	N/A
320	O43504	<i>LAMTOR5</i>	Ragulator complex protein LAMTO	-19.655942	-20.037784	-20.651445	-18.052921	N/A	N/A
321	O43715	<i>TRIAP1</i>	TP53-regulated inhibitor of apopto	N/A	N/A	N/A	-21.038053	N/A	N/A
322	O43914	<i>TYROBP</i>	TYRO protein tyrosine kinase-bindi	-19.729462	N/A	N/A	-19.424156	N/A	N/A
323	O75380	<i>NDUFS6</i>	NADH dehydrogenase [ubiquinone]	-20.643864	N/A	-22.317216	-20.499665	N/A	N/A
324	O75911	<i>DHRS3</i>	Short-chain dehydrogenase/reduct	-21.051253	N/A	N/A	-19.785502	N/A	N/A
325	O75964	<i>ATP5MG</i>	ATP synthase subunit g, mitochond	-19.020641	N/A	N/A	-21.065065	N/A	N/A
326	O95202	<i>LETM1</i>	LETM1 and EF-hand domain-contai	-22.771687	N/A	N/A	-24.005724	N/A	N/A
327	O95571	<i>ETHE1</i>	Persulfide dioxygenase ETHE1, mito	-19.911514	N/A	-22.120257	-20.335987	N/A	N/A
328	O96000	<i>NDUFB10</i>	NADH dehydrogenase [ubiquinone]	-19.434352	N/A	N/A	-20.404013	N/A	N/A
329	O96011	<i>PEX11B</i>	Peroxisomal membrane protein 11	-21.663054	N/A	N/A	-22.280356	N/A	N/A
330	P02649	<i>APOE</i>	Apolipoprotein E OS=Homo sapiens	-20.256034	N/A	N/A	-21.044296	N/A	N/A
331	P02656	<i>APOC3</i>	Apolipoprotein C-III OS=Homo sapi	-17.909636	-20.577568	N/A	-17.100003	N/A	N/A
332	P02686	<i>MBP</i>	Myelin basic protein OS=Homo sap	-22.912707	-23.144075	-22.934757	-21.91476	0	N/A
333	P04839	<i>CYBB</i>	Cytochrome b-245 heavy chain OS=	-21.984726	N/A	N/A	-23.035238	N/A	N/A
334	P04844	<i>RPN2</i>	Dolichyl-diphosphooligosaccharide-	-18.709117	N/A	N/A	-20.142538	N/A	N/A
335	P04899	<i>GNAI2</i>	Guanine nucleotide-binding protein	-23.212001	N/A	N/A	-23.239642	N/A	N/A
336	P05023	<i>ATP1A1</i>	Sodium/potassium-transporting AT	-23.236482	N/A	N/A	-25.027039	N/A	N/A
337	P05388	<i>RPLP0</i>	60S acidic ribosomal protein P0 OS	-20.729802	-21.314172	N/A	-22.553947	N/A	N/A
338	P05556	<i>ITGB1</i>	Integrin beta-1 OS=Homo sapiens G	-22.78671	N/A	N/A	-24.133479	N/A	N/A
339	P07954	<i>FH</i>	Fumarate hydratase, mitochondrial	-21.061497	N/A	N/A	-22.418828	N/A	N/A
340	P08240	<i>SRPRA</i>	Signal recognition particle receptor	-20.83948	N/A	N/A	-24.190301	N/A	N/A
341	P08574	<i>CYC1</i>	Cytochrome c1, heme protein, mito	-19.753512	N/A	N/A	-21.373805	N/A	N/A
342	P08754	<i>GNAI3</i>	Guanine nucleotide-binding protein	N/A	N/A	N/A	-23.239642	N/A	N/A
343	P0C0S5	<i>H2AZ1</i>	Histone H2A.Z OS=Homo sapiens G	-20.118631	N/A	N/A	-21.036436	N/A	N/A
344	P0C7P4	<i>UQCRCFS1P1</i>	Putative cytochrome b-c1 complex	-20.963874	N/A	N/A	-19.714788	N/A	N/A
345	P0CG47	<i>UBB</i>	Polyubiquitin-B OS=Homo sapiens	-19.683724	-18.944634	-19.243065	-19.1287	0	N/A
346	P0CG48	<i>UBC</i>	Polyubiquitin-B OS=Homo sapiens	-19.683724	-18.944634	-19.243065	-19.1287	0	N/A

347	P12109	COL6A1	Collagen alpha-1(VI) chain OS=Hom	-22.152294	-25.560666	N/A	-23.218625	N/A	N/A
348	P13804	ETFA	Electron transfer flavoprotein subu	-18.040389	-19.126556	-23.241918	-20.912108	N/A	N/A
349	P13995	MTHFD2	Bifunctional methylenetetrahydrof	-20.710573	N/A	N/A	-22.149272	N/A	N/A
350	P14854	COX6B1	Cytochrome c oxidase subunit 6B1	-17.57128	-19.315862	-18.765491	-17.064203	N/A	N/A
351	P16435	POR	NADPH--cytochrome P450 reducta	-23.120878	N/A	N/A	-23.106514	N/A	N/A
352	P19367	HK1	Hexokinase-1 OS=Homo sapiens GN	-22.0176	N/A	N/A	-25.194928	N/A	N/A
353	P20292	ALOX5AP	Arachidonate 5-lipoxygenase-activa	-20.476377	N/A	N/A	-20.454072	N/A	N/A
354	P20340	RAB6A	Ras-related protein Rab-6A OS=Ho	-21.547331	-22.764375	N/A	-22.95086	0	N/A
355	P20674	COX5A	Cytochrome c oxidase subunit 5A,	-18.146412	-19.375576	-19.572084	-19.274765	N/A	N/A
356	P21964	COMT	Catechol O-methyltransferase OS=	-16.958443	-21.526898	N/A	-20.025279	N/A	N/A
357	P23219	PTGS1	Prostaglandin G/H synthase 1 OS=H	-19.776299	N/A	N/A	-20.76653	N/A	N/A
358	P23368	ME2	NAD-dependent malic enzyme, mit	-21.507047	N/A	N/A	-22.581673	N/A	N/A
359	P23786	CPT2	Carnitine O-palmitoyltransferase 2,	-21.830067	N/A	N/A	-23.447519	N/A	N/A
360	P24539	ATP5PB	ATP synthase F(0) complex subunit	-21.143081	N/A	N/A	-22.145403	N/A	N/A
361	P24752	ACAT1	Acetyl-CoA acetyltransferase, mitoc	-18.554517	N/A	N/A	-21.496628	N/A	N/A
362	P26010	ITGB7	Integrin beta-7 OS=Homo sapiens G	N/A	N/A	N/A	-25.914053	N/A	N/A
363	P27105	STOM	Erythrocyte band 7 integral membr	-21.807273	N/A	N/A	-22.782976	N/A	N/A
364	P28288	ABCD3	ATP-binding cassette sub-family D	N/A	N/A	N/A	-24.275988	N/A	N/A
365	P30049	ATP5F1D	ATP synthase subunit delta, mitoch	-19.435746	N/A	N/A	-18.474722	N/A	N/A
366	P30533	LRPAP1	Alpha-2-macroglobulin receptor-as	-19.581771	-24.039126	-23.43156	-19.95163	N/A	N/A
367	P33121	ACSL1	Long-chain-fatty-acid--CoA ligase 1	-20.139333	N/A	N/A	-21.230592	N/A	N/A
368	P34897	SHMT2	Serine hydroxymethyltransferase,	-19.532233	-21.777278	-21.215631	-21.489559	N/A	N/A
369	P35613	BSG	Basigin OS=Homo sapiens GN=BSG	N/A	N/A	N/A	-22.346228	N/A	N/A
370	P36542	ATP5F1C	ATP synthase subunit gamma, mito	-19.286521	N/A	N/A	-20.704418	N/A	N/A
371	P36776	LONP1	Lon protease homolog, mitochondr	-21.035219	N/A	N/A	-23.627257	N/A	N/A
372	P38117	ETFB	Electron transfer flavoprotein subu	-18.535443	-23.06246	N/A	-19.747058	N/A	N/A
373	P39019	RPS19	40S ribosomal protein S19 OS=Hom	-19.170855	N/A	-22.451917	-20.901761	N/A	N/A
374	P40939	HADHA	Trifunctional enzyme subunit alpha	-19.778592	0	N/A	-20.715713	N/A	N/A
375	P46459	NSF	Vesicle-fusing ATPase OS=Homo sa	-20.911552	-23.692659	N/A	-23.793464	N/A	N/A
376	P46778	RPL21	60S ribosomal protein L21 OS=Hom	-21.406757	-20.38177	-20.044685	-21.267059	N/A	N/A
377	P46977	STT3A	Dolichyl-diphosphooligosaccharide-	-22.131822	N/A	N/A	-23.675301	N/A	N/A
378	P47985	UQCRCF1	Putative cytochrome b-c1 complex	-20.963874	N/A	N/A	-19.714788	N/A	N/A
379	P50213	IDH3A	Isocitrate dehydrogenase [NAD] su	-20.89423	-22.615324	-21.734201	-22.129098	N/A	N/A
380	P51153	RAB13	Ras-related protein Rab-13 OS=Ho	-22.640601	N/A	N/A	-22.032905	N/A	N/A
381	P51572	BCAP31	B-cell receptor-associated protein 3	-18.191176	-20.282351	N/A	-18.4968	N/A	N/A
382	P52815	MRPL12	39S ribosomal protein L12, mitoch	-19.515875	N/A	N/A	-20.15816	N/A	N/A
383	P53007	SLC25A1	Tricarboxylate transport protein, m	-20.016789	N/A	N/A	-20.817185	N/A	N/A
384	P53597	SUCLG1	Succinate--CoA ligase [ADP/GDP-fo	-20.731138	-25.188882	-23.50104	-22.976985	N/A	N/A
385	P53634	CTSC	Dipeptidyl peptidase 1 OS=Homo s	-21.130181	N/A	N/A	-24.151261	N/A	N/A
386	P55084	HADHB	Trifunctional enzyme subunit beta,	-19.125443	N/A	N/A	-19.933696	N/A	N/A
387	P55327	TPD52	Tumor protein D52 OS=Homo sapie	-20.559482	N/A	N/A	-21.053243	N/A	N/A
388	P56385	ATP5ME	ATP synthase subunit e, mitochond	-17.048822	-19.905369	-19.724275	-16.916953	N/A	N/A
389	P60604	UBE2G2	Ubiquitin-conjugating enzyme E2 G	-19.87396	N/A	N/A	-20.84964	N/A	N/A
390	P61421	ATP6V0D1	V-type proton ATPase subunit d 1 O	-22.314862	N/A	N/A	-22.288847	N/A	N/A
391	P61619	SEC61A1	Protein transport protein Sec61 sub	-22.350087	N/A	N/A	-22.524213	N/A	N/A
392	P62879	GNB2	Guanine nucleotide-binding protein	-21.909005	-24.223956	N/A	-23.218133	0	N/A
393	P62987	UBA52	Polyubiquitin-B OS=Homo sapiens	-19.683724	-18.944634	-19.243065	-19.1287	N/A	N/A
394	P63096	GNAI1	Guanine nucleotide-binding protein	N/A	N/A	N/A	-23.239642	N/A	N/A
395	P63218	GNG5	Guanine nucleotide-binding protein	-19.47185	N/A	N/A	-19.732234	N/A	N/A
396	P84095	RHOG	Rho-related GTP-binding protein Rh	-19.699966	0	-22.677638	-21.679682	N/A	N/A
397	Q00325	SLC25A3	Phosphate carrier protein, mitoch	-18.797309	N/A	N/A	-19.669261	N/A	N/A

398	Q03519	TAP2	Antigen peptide transporter 2 OS=H-	-21.436319	N/A	N/A	-22.447063	N/A	N/A
399	Q07021	C1QBP	Complement component 1 Q subco	N/A	N/A	N/A	-18.720699	N/A	N/A
400	Q07065	CKAP4	Cytoskeleton-associated protein 4	-20.896503	N/A	N/A	-21.36551	N/A	N/A
401	Q12797	ASPH	Aspartyl/asparaginyl beta-hydroxyl	-21.46928	N/A	N/A	-24.119064	N/A	N/A
402	Q12907	LMAN2	Vesicular integral-membrane prote	-19.831649	N/A	N/A	-20.321736	N/A	N/A
403	Q12931	TRAP1	Heat shock protein 75 kDa, mitocho	-21.98203	N/A	N/A	-23.699785	N/A	N/A
404	Q13011	ECH1	Delta(3,5)-Delta(2,4)-dienoyl-CoA is	-19.467876	N/A	-20.960319	-20.141021	N/A	N/A
405	Q13438	OS9	Protein OS-9 OS=Homo sapiens GN	-22.983038	N/A	N/A	-23.139935	N/A	N/A
406	Q13488	TCIRG1	V-type proton ATPase 116 kDa sub	-23.781796	N/A	N/A	-23.709905	N/A	N/A
407	Q13510	ASAH1	Acid ceramidase OS=Homo sapiens	-20.569523	N/A	-22.559075	-22.158331	N/A	N/A
408	Q13586	STIM1	Stromal interaction molecule 1 OS=	-22.658068	N/A	N/A	-23.059584	N/A	N/A
409	Q13637	RAB32	Ras-related protein Rab-32 OS=Ho	-21.778657	N/A	N/A	-22.728635	N/A	N/A
410	Q14061	COX17	Cytochrome c oxidase copper chap	-20.704632	N/A	N/A	-20.094963	N/A	N/A
411	Q14108	SCARB2	Lysosome membrane protein 2 OS=	-19.41746	-24.046201	N/A	-19.044267	N/A	N/A
412	Q14165	MLEC	Malectin OS=Homo sapiens GN=ML	-21.007852	N/A	N/A	-21.538111	N/A	N/A
413	Q14696	MESD	LDLR chaperone MESD OS=Homo s	-21.838849	N/A	N/A	-21.238927	N/A	N/A
414	Q14739	LBR	Lamin-B receptor OS=Homo sapien	-21.603551	N/A	N/A	-22.324453	N/A	N/A
415	Q15738	NSDHL	Sterol-4-alpha-carboxylate 3-dehyd	-19.647765	-20.913778	N/A	-19.000712	N/A	N/A
416	Q16718	NDUFA5	NADH dehydrogenase [ubiquinone]	-20.69588	N/A	N/A	-20.867675	N/A	N/A
417	Q16891	IMMT	MICOS complex subunit MIC60 OS=	-20.453553	N/A	N/A	-22.138488	N/A	N/A
418	Q32P28	P3H1	Prolyl 3-hydroxylase 1 OS=Homo sa	-23.763908	N/A	N/A	-23.895618	N/A	N/A
419	Q53GQ0	HSD17B12	Very-long-chain 3-oxoacyl-CoA red	-20.343329	-22.704373	N/A	-21.380435	N/A	N/A
420	Q5JTV8	TOR1AIP1	Torsin-1A-interacting protein 1 OS=	-22.809558	N/A	N/A	-24.7107	N/A	N/A
421	Q5VUM1	SDHAF4	Succinate dehydrogenase assembly	-21.8424	N/A	N/A	-22.082511	N/A	N/A
422	Q5VYK3	ECPAS	Proteasome-associated protein EC	N/A	N/A	N/A	-21.704087	N/A	N/A
423	Q6IAA8	LAMTOR1	Ragulator complex protein LAMTO	-20.079441	N/A	N/A	-18.979956	N/A	N/A
424	Q6NUK1	SLC25A24	Calcium-binding mitochondrial carr	-20.411074	N/A	N/A	-21.974363	N/A	N/A
425	Q6PI48	DARS2	Aspartate--tRNA ligase, mitochondr	N/A	N/A	N/A	-24.969637	N/A	N/A
426	Q709C8	VPS13C	Vacuolar protein sorting-associated	-21.797871	N/A	N/A	-23.110686	N/A	N/A
427	Q71UI9	H2AZ2	Histone H2A.Z OS=Homo sapiens G	-20.118631	N/A	N/A	-21.036436	N/A	N/A
428	Q75N90	FBN3	Fibrillin-3 OS=Homo sapiens GN=FB	N/A	N/A	N/A	-24.431511	N/A	N/A
429	Q7L5N7	LPCAT2	Lysophosphatidylcholine acyltransf	-20.204679	-22.882005	N/A	-20.877649	N/A	N/A
430	Q7Z2K6	ERMP1	Endoplasmic reticulum metallolep	-22.637459	N/A	N/A	-24.485617	N/A	N/A
431	Q86UP2	KTN1	Kinectin OS=Homo sapiens GN=KTN	-22.748946	N/A	N/A	-24.682466	N/A	N/A
432	Q8IUE6	H2AC21	Histone H2A type 2-B OS=Homo sa	N/A	N/A	N/A	-21.430424	N/A	N/A
433	Q8IV08	PLD3	Phospholipase D3 OS=Homo sapien	-19.495124	N/A	N/A	-19.780178	N/A	N/A
434	Q8IZV5	RDH10	Retinol dehydrogenase 10 OS=Hom	-21.473549	N/A	N/A	-21.569847	N/A	N/A
435	Q8N4H5	TOMM5	Mitochondrial import receptor sub	-18.049798	N/A	N/A	-20.326872	N/A	N/A
436	Q8N5I4	DHRX	Dehydrogenase/reductase SDR fam	-21.895975	N/A	N/A	-20.111527	N/A	N/A
437	Q8NBS9	TXNDC5	Thioredoxin domain-containing pro	-22.712708	-24.546317	-22.364913	-22.106726	N/A	N/A
438	Q8NHW5	RPLP0P6	60S acidic ribosomal protein P0 OS	-20.729802	-21.314172	N/A	-22.553947	N/A	N/A
439	Q92504	SLC39A7	Zinc transporter SLC39A7 OS=Hom	-23.711803	N/A	N/A	-24.084381	N/A	N/A
440	Q92820	GGH	Gamma-glutamyl hydrolase OS=Ho	-22.708659	N/A	N/A	-20.398401	N/A	N/A
441	Q96AH8	RAB7B	Ras-related protein Rab-7b OS=Ho	-19.012652	N/A	N/A	-19.025121	N/A	N/A
442	Q96DR8	MUCL1	Mucin-like protein 1 OS=Homo sapi	N/A	N/A	N/A	-20.407438	N/A	N/A
443	Q96I99	SUCLG2	Succinate--CoA ligase [GDP-forming	-21.807028	N/A	N/A	-23.637639	N/A	N/A
444	Q96LJ7	DHRX1	Dehydrogenase/reductase SDR fam	-20.263937	N/A	N/A	-20.768914	N/A	N/A
445	Q99623	PHB2	Prohibitin-2 OS=Homo sapiens GN=	-19.666739	N/A	N/A	-21.470258	N/A	N/A
446	Q99653	CHP1	Calcineurin B homologous protein 1	-19.810829	N/A	N/A	-21.580276	N/A	N/A
447	Q99798	ACO2	Aconitate hydratase, mitochondrial	-21.395854	-21.087624	N/A	-23.131832	N/A	N/A
448	Q9BRR6	ADPGK	ADP-dependent glucokinase OS=Ho	-22.467452	N/A	N/A	-24.95293	N/A	N/A

449	Q9BSJ8	ESYT1	Extended synaptotagmin-1 OS=Ho	-21.921019	N/A	N/A	-23.367288	N/A	N/A
450	Q9BVC6	TMEM109	Transmembrane protein 109 OS=H	-21.267913	N/A	N/A	-22.869111	N/A	N/A
451	Q9GZT3	SLIRP	SRA stem-loop-interacting RNA-bin	-19.067943	N/A	N/A	-20.095946	N/A	N/A
452	Q9H3N1	TMX1	Thioredoxin-related transmembran	-20.581485	N/A	N/A	-21.2189	N/A	N/A
453	Q9H6V9	LDAH	Lipid droplet-associated hydrolase	-17.072693	-17.916371	N/A	-16.949344	N/A	N/A
454	Q9H9B4	SFXN1	Sideroflexin-1 OS=Homo sapiens G	-21.959091	N/A	N/A	-23.067373	N/A	N/A
455	Q9H9S3	SEC61A2	Protein transport protein Sec61 sub	-22.350087	N/A	N/A	-22.524213	N/A	N/A
456	Q9HAT2	SIAE	Sialate O-acetylesterase OS=Homo	-23.378563	N/A	N/A	-23.660595	N/A	N/A
457	Q9HCN8	SDF2L1	Stromal cell-derived factor 2-like pr	N/A	N/A	N/A	-20.625225	N/A	N/A
458	Q9HD20	ATP13A1	Manganese-transporting ATPase 13	-23.01618	N/A	N/A	-23.452286	N/A	N/A
459	Q9NNW7	TXNRD2	Thioredoxin reductase 2, mitochon	-21.961145	-24.168064	-22.726073	-22.838977	N/A	N/A
460	Q9NQC3	RTN4	Reticulon-4 OS=Homo sapiens GN=	-21.675486	-24.360668	N/A	-22.293728	N/A	N/A
461	Q9NRW1	RAB6B	Ras-related protein Rab-6A OS=Ho	-21.547331	-22.764375	N/A	-22.95086	0	N/A
462	Q9NTJ5	SACM1L	Phosphatidylinositide phosphatase	-22.001139	N/A	N/A	-23.117959	N/A	N/A
463	Q9NUU6	OTULINL	Inactive ubiquitin thioesterase FAM	-22.565966	N/A	N/A	-22.499965	N/A	N/A
464	Q9NX40	OCIAD1	OCIA domain-containing protein 1	-20.27561	N/A	N/A	-21.252875	N/A	N/A
465	Q9P0V8	SLAMF8	SLAM family member 8 OS=Homo s	-18.742405	-23.148951	N/A	-19.33108	N/A	N/A
466	Q9P270	SLAIN2	SLAIN motif-containing protein 2 O	-21.263242	N/A	N/A	-20.955311	N/A	N/A
467	Q9P2E9	RRBP1	Ribosome-binding protein 1 OS=Ho	-24.172862	N/A	N/A	-25.949193	N/A	N/A
468	Q9P2R7	SUCLA2	Succinate--CoA ligase [ADP-forming	-23.002324	N/A	N/A	-24.295003	N/A	N/A
469	Q9UBR2	CTSZ	Cathepsin Z OS=Homo sapiens GN=	-18.773014	N/A	N/A	-19.997797	N/A	N/A
470	Q9UGP8	SEC63	Translocation protein SEC63 homol	-22.841015	N/A	N/A	-25.412167	N/A	N/A
471	Q9UII2	ATP5IF1	ATPase inhibitor, mitochondrial OS	N/A	N/A	N/A	-19.620845	N/A	N/A
472	Q9UJZ1	STOML2	Stomatin-like protein 2, mitochond	-19.137883	N/A	N/A	-21.915582	N/A	N/A
473	Q9UN70	PCDHGC3	Protocadherin gamma-C3 OS=Hom	N/A	N/A	N/A	-20.419203	N/A	N/A
474	Q9UP65	PLA2G4C	Cytosolic phospholipase A2 gamma	-21.110842	-24.256227	N/A	-21.415463	N/A	N/A
475	Q9Y277	VDAC3	Voltage-dependent anion-selective	-19.456074	N/A	N/A	-20.227847	N/A	N/A
476	Q9Y5J7	TIMM9	Mitochondrial import inner membr	-19.385818	-21.331557	N/A	-19.080083	N/A	N/A
477	Q9Y5J9	TIMM8B	Mitochondrial import inner membr	-20.459729	N/A	N/A	-19.881722	N/A	N/A
478	Q9Y5M8	SRPRB	Signal recognition particle receptor	-20.135867	N/A	N/A	-21.981027	N/A	N/A
479	Q9Y6C9	MTCH2	Mitochondrial carrier homolog 2 O	-20.962852	N/A	N/A	-21.408721	N/A	N/A
480	P41219	PRPH	Peripherin OS=Homo sapiens GN=P	N/A	N/A	N/A	-23.71038	N/A	0
481	P62820	RAB1A	Ras-related protein Rab-1A OS=Ho	-18.127375	-21.211811	-23.08613	-19.397549	0	0
482	P10599	TXN	Thioredoxin OS=Homo sapiens GN=	-18.507299	-19.158125	-16.003643	-20.896896	N/A	-17.014979
483	P62899	RPL31	60S ribosomal protein L31 OS=Hom	N/A	-20.696306	-18.395698	-20.805178	N/A	-17.980365
484	P30048	PRDX3	Thioredoxin-dependent peroxide re	-16.098021	-17.620944	-17.062898	-16.055312	N/A	-18.372452
485	P62314	SNRPD1	Small nuclear ribonucleoprotein Sm	-19.776526	-16.300216	-17.204729	-21.31815	N/A	-18.383009
486	Q969H8	MYDGF	Myeloid-derived growth factor OS=	-18.338815	-19.726848	-18.9505	-18.769422	N/A	-18.644347
487	P02655	APOC2	Apolipoprotein C-II OS=Homo sapie	-14.955607	-19.464439	-19.238736	-13.946653	N/A	-18.716659
488	P07108	DBI	Acyl-CoA-binding protein OS=Homo	-20.694992	-17.567442	-17.583689	-19.894699	N/A	-18.913819
489	O75947	ATP5PD	ATP synthase subunit d, mitochond	-14.988745	-19.823709	-18.226696	-14.89422	N/A	-18.982768
490	P61353	RPL27	60S ribosomal protein L27 OS=Hom	-18.409178	-18.992433	-18.257184	-20.39828	N/A	-19.235382
491	Q14847	LASP1	LIM and SH3 domain protein 1 OS=	-21.56712	-20.730771	-20.55992	-20.878558	N/A	-19.315265
492	P07195	LDHB	L-lactate dehydrogenase B chain OS	-22.152534	-19.902941	-19.35173	-24.367243	N/A	-19.654527
493	P56381	ATP5F1E	ATP synthase subunit epsilon, mito	-17.647307	-19.264163	-19.386671	-17.736327	N/A	-19.675962
494	Q5VTU8	ATP5F1EP2	ATP synthase subunit epsilon, mito	-17.647307	-19.264163	-19.386671	-17.736327	N/A	-19.675962
495	Q13247	SRSF6	Serine/arginine-rich splicing factor	-23.850394	-21.220257	-20.666616	-24.686412	N/A	-19.816411
496	P62829	RPL23	60S ribosomal protein L23 OS=Hom	-19.598477	-20.871644	-21.828814	-21.14791	N/A	-19.823442
497	P46779	RPL28	60S ribosomal protein L28 OS=Hom	-19.19311	-19.878473	-19.430466	-21.115705	N/A	-19.903331
498	Q9BT09	CNPY3	Protein canopy homolog 3 OS=Hom	-19.188695	-20.399625	-20.259379	-18.835347	0	-19.90697
499	P62072	TIMM10	Mitochondrial import inner membr	-19.382698	-19.745991	-19.733501	-18.762385	N/A	-20.166672

500	P36578	RPL4	60S ribosomal protein L4 OS=Homo	-20.138225	-21.357662	-19.440556	-23.186473	N/A	-20.226028
501	P04080	CSTB	Cystatin-B OS=Homo sapiens GN=C	-18.225921	-19.302182	-17.399578	-19.70529	N/A	-20.24588
502	Q9Y6H1	CHCHD2	Putative coiled-coil-helix-coiled-coil	-20.534661	-19.931232	-19.549558	-19.682581	N/A	-20.627065
503	P98179	RBM3	RNA-binding protein 3 OS=Homo sa	-22.604987	-20.252772	-19.738438	-21.034144	N/A	-20.682832
504	P07203	GPX1	Glutathione peroxidase 1 OS=Hom	-19.134373	N/A	-19.658419	-19.812392	N/A	-20.872434
505	Q9UMX5	NENF	Neudesin OS=Homo sapiens GN=N	-20.772772	-20.750477	-20.94877	-20.200263	N/A	-21.348636
506	P54819	AK2	Adenylate kinase 2, mitochondrial	-17.512072	-20.442858	-20.772251	-20.022221	N/A	-21.418833
507	P36222	CHI3L1	Chitinase-3-like protein 1 OS=Homo	-21.908531	N/A	-20.816315	-20.967668	N/A	-21.765586
508	Q9HAV7	GRPEL1	GrpE protein homolog 1, mitochon	-18.711262	-20.805689	-20.171101	-18.59306	N/A	-21.776223
509	P00390	GSR	Glutathione reductase, mitochondr	-21.865239	-21.863516	-20.57487	-21.913476	N/A	-21.925766
510	P61158	ACTR3	Actin-related protein 3 OS=Homo s	-22.464792	-23.917941	-22.132712	-24.48636	0	-22.184412
511	P10253	GAA	Lysosomal alpha-glucosidase OS=H	-21.261954	-23.091469	-22.40364	-20.813378	N/A	-22.33165
512	P60953	CDC42	Cell division control protein 42 hom	-21.517286	-20.370786	0	-24.102599	0	-22.354547
513	P23141	CES1	Liver carboxylesterase 1 OS=Homo	-21.120808	-23.597068	-21.350379	-22.673509	N/A	-22.413246
514	P49411	TUFM	Elongation factor Tu, mitochondrial	-18.653786	-21.242119	-20.892381	-19.748727	N/A	-22.71207
515	P62979	RPS27A	Polyubiquitin-B OS=Homo sapiens	-19.683724	-18.944634	-19.243065	-19.1287	N/A	-22.811747
516	P35579	MYH9	Myosin-9 OS=Homo sapiens GN=M	-20.830489	-25.016184	-23.161966	-24.335894	N/A	-23.028966
517	P14927	UQCRB	Cytochrome b-c1 complex subunit	-19.126998	-21.376351	N/A	-18.301739	N/A	-23.325395
518	Q9BX68	HINT2	Histidine triad nucleotide-binding p	-20.4502	-22.587126	-20.852505	-20.203032	N/A	-23.571741
519	P26440	IVD	Isovaleryl-CoA dehydrogenase, mit	-20.864739	-21.713376	-20.130969	-21.122081	N/A	-23.942991
520	O75390	CS	Citrate synthase, mitochondrial OS	-19.381324	-24.188651	N/A	-20.702067	N/A	-23.99695
521	P29966	MARCKS	Myristoylated alanine-rich C-kinase	-19.797509	N/A	-22.125835	-21.141365	N/A	-24.043311
522	P31040	SDHA	Succinate dehydrogenase [ubiquino	-21.590766	-21.200402	-20.296071	-21.973105	N/A	-24.878866
523	Q08170	SRSF4	Serine/arginine-rich splicing factor	-25.985639	-25.8746	-24.059631	-24.686412	N/A	-24.933965
524	P78527	PRKDC	DNA-dependent protein kinase cat	-25.99592	-26.076967	0	-24.649085	N/A	-26.300269
525	O43566	RGS14	Regulator of G-protein signaling 14	N/A	N/A	N/A	N/A	-23.579182	N/A
526	O60493	SNX3	Sorting nexin-3 OS=Homo sapiens G	-20.753916	N/A	N/A	N/A	-21.752513	N/A
527	O60906	SMPD2	Sphingomyelin phosphodiesterase	N/A	N/A	N/A	N/A	-20.351885	N/A
528	O75342	ALOX12B	Arachidonate 12-lipoxygenase, 12R	N/A	N/A	N/A	N/A	-22.735867	N/A
529	O75477	ERLIN1	Erlin-1 OS=Homo sapiens GN=ERLIN	N/A	N/A	-22.284574	N/A	-21.469504	N/A
530	P02538	KRT6A	Keratin, type II cytoskeletal 6A OS=	-21.301074	0	-20.668562	0	-18.912481	N/A
531	P07196	NEFL	Neurofilament light polypeptide OS	N/A	N/A	N/A	N/A	-23.282882	N/A
532	P23490	LORICRIN	Loricrin OS=Homo sapiens GN=LOR	N/A	N/A	-21.793861	N/A	-20.761843	N/A
533	P23634	ATP2B4	Plasma membrane calcium-transpo	0	N/A	N/A	N/A	-23.68867	N/A
534	P27482	CALML3	Calmodulin-like protein 3 OS=Hom	N/A	N/A	N/A	N/A	-20.282487	N/A
535	P35268	RPL22	60S ribosomal protein L22 OS=Hom	-19.376437	N/A	-20.321118	N/A	-20.473237	N/A
536	P62834	RAP1A	Ras-related protein Rap-1A OS=Ho	-22.703196	-22.995969	-23.778519	N/A	-22.783286	N/A
537	Q13867	BLMH	Bleomycin hydrolase OS=Homo sap	-24.170156	N/A	-22.991152	N/A	-18.971126	N/A
538	Q15149	PLEC	Plectin OS=Homo sapiens GN=PLEC	-26.950309	-28.504448	N/A	N/A	-27.281173	N/A
539	Q71U36	TUBA1A	Tubulin alpha-1B chain OS=Homo s	N/A	N/A	N/A	N/A	-22.649078	N/A
540	Q8NHH9	ATL2	Atlastin-2 OS=Homo sapiens GN=A	N/A	N/A	N/A	N/A	-25.094818	N/A
541	Q9NSB2	KRT84	Keratin, type II cuticular Hb4 OS=H	N/A	N/A	N/A	N/A	-24.529792	N/A
542	Q9UL16	CFAP45	Cilia- and flagella-associated protei	N/A	N/A	N/A	N/A	-21.543067	N/A
543	Q9UNX3	RPL26L1	60S ribosomal protein L26 OS=Hom	N/A	N/A	N/A	N/A	-20.867014	N/A
544	Q9Y5L2	HILPDA	Hypoxia-inducible lipid droplet-asso	N/A	-20.584404	N/A	N/A	-19.554282	N/A
545	P68363	TUBA1B	Tubulin alpha-1B chain OS=Homo s	0	0	N/A	0	-22.649078	0
546	Q16352	INA	Alpha-internexin OS=Homo sapiens	-23.819929	-22.910457	0	N/A	-24.691017	0
547	Q9BQE3	TUBA1C	Tubulin alpha-1B chain OS=Homo s	0	0	0	0	-22.649078	0
548	P07737	PFN1	Profilin-1 OS=Homo sapiens GN=PF	-20.478536	-18.570283	-14.980968	N/A	-20.563937	-15.285779
549	P00558	PGK1	Phosphoglycerate kinase 1 OS=Hom	-18.703353	-17.26066	-16.260641	N/A	-20.656188	-16.886261
550	P28066	PSMA5	Proteasome subunit alpha type-5 O	-23.487221	-18.129762	-16.777614	N/A	-21.521007	-18.331397

551	O75347	TBCA	Tubulin-specific chaperone A OS=H	-20.388945	-18.371628	-17.774928	N/A	-19.731791	-19.004813
552	P35321	SPRR1A	Cornifin-B OS=Homo sapiens GN=S	-20.199055	-19.229756	-19.804603	0	-22.168021	-19.109367
553	P08758	ANXA5	Annexin A5 OS=Homo sapiens GN=	-21.869519	-18.291507	-18.355977	N/A	-20.746835	-19.342739
554	P69905	HBA1	Hemoglobin subunit alpha OS=Hom	-17.504374	-19.579911	-22.202221	N/A	-19.009438	-19.425959
555	O60814	H2BC12	Histone H2B type 1-K OS=Homo sa	-18.553196	-18.759763	-21.010101	0	-16.800639	-19.524674
556	P57053	H2BS1	Histone H2B type 1-K OS=Homo sa	-18.553196	-18.759763	-21.010101	N/A	-16.800639	-19.524674
557	P58876	H2BC5	Histone H2B type 1-K OS=Homo sa	-18.553196	-18.759763	-21.010101	0	-16.800639	-19.524674
558	P62807	H2BC10	Histone H2B type 1-K OS=Homo sa	-18.553196	-18.759763	-21.010101	0	-16.800639	-19.524674
559	Q5QNW6	H2BC18	Histone H2B type 1-K OS=Homo sa	-18.553196	-18.759763	-21.010101	0	-16.800639	-19.524674
560	Q93079	H2BC9	Histone H2B type 1-K OS=Homo sa	-18.553196	-18.759763	-21.010101	0	-16.800639	-19.524674
561	Q99877	H2BC15	Histone H2B type 1-K OS=Homo sa	-18.553196	-18.759763	-21.010101	0	-16.800639	-19.524674
562	Q99879	H2BC14	Histone H2B type 1-K OS=Homo sa	-18.553196	-18.759763	-21.010101	0	-16.800639	-19.524674
563	Q99880	H2BC13	Histone H2B type 1-K OS=Homo sa	-18.553196	-18.759763	-21.010101	0	-16.800639	-19.524674
564	P06753	TPM3	Tropomyosin alpha-3 chain OS=Ho	-24.150622	-20.765602	-18.696581	N/A	-20.806196	-19.56632
565	Q9H1E3	NUCKS1	Nuclear ubiquitous casein and cycli	-21.90382	-19.621647	-19.176218	N/A	-19.913923	-19.576436
566	P31146	CORO1A	Coronin-1A OS=Homo sapiens GN=	-20.776765	-20.286304	-19.041831	N/A	-22.241077	-19.652037
567	P62266	RPS23	40S ribosomal protein S23 OS=Hom	-19.06035	-20.418582	-19.588576	N/A	-19.83676	-19.69576
568	P00918	CA2	Carbonic anhydrase 2 OS=Homo sa	-20.780829	-19.18886	-19.310435	N/A	-20.626418	-19.720003
569	Q16658	FSCN1	Fascin OS=Homo sapiens GN=FSCN	-23.280307	-21.47636	-19.773955	N/A	-22.059317	-19.767439
570	P23396	RPS3	40S ribosomal protein S3 OS=Homo	-20.567935	-20.199867	-20.209006	N/A	-20.407183	-19.968604
571	O14979	HNRNPDL	Heterogeneous nuclear ribonucleo	-22.770271	-20.529473	-20.351483	N/A	-23.15692	-20.324116
572	P62244	RPS15A	40S ribosomal protein S15a OS=Ho	-19.386666	-19.552648	N/A	N/A	-19.095334	-20.416584
573	P53999	SUB1	Activated RNA polymerase II transc	N/A	-20.260547	-19.803873	N/A	-21.518178	-20.452127
574	P14678	SNRPB	Small nuclear ribonucleoprotein-as	N/A	-19.977251	-19.337454	N/A	-23.023191	-20.586392
575	P63162	SNRPN	Small nuclear ribonucleoprotein-as	N/A	-19.977251	-19.337454	N/A	-23.023191	-20.586392
576	Q03252	LMNB2	Lamin-B2 OS=Homo sapiens GN=LM	-23.11791	-22.95174	-20.992849	N/A	-21.0046	-20.67279
577	P00338	LDHA	L-lactate dehydrogenase A chain O	-20.659453	-20.978197	-20.403875	N/A	-22.10768	-20.747651
578	P13639	EEF2	Elongation factor 2 OS=Homo sapie	-25.557536	-21.380098	-20.365417	0	-23.0805	-20.748216
579	P25788	PSMA3	Proteasome subunit alpha type-3 O	-22.518947	-20.913019	-19.830462	N/A	-22.457343	-20.853993
580	Q15517	CDSN	Corneodesmosin OS=Homo sapiens	-22.540859	-22.883819	-22.775036	N/A	-23.734835	-20.90119
581	P59998	ARPC4	Actin-related protein 2/3 complex s	-20.530542	-20.754045	-21.234231	N/A	-21.062662	-21.054848
582	P31944	CASP14	Caspase-14 OS=Homo sapiens GN=	-21.627767	-23.413662	-20.121131	N/A	-18.329485	-21.076964
583	P40121	CAPG	Macrophage-capping protein OS=H	N/A	-20.201183	-20.432844	N/A	-21.586722	-21.194304
584	P61313	RPL15	60S ribosomal protein L15 OS=Hom	-20.332098	-21.074412	-18.865187	N/A	-21.352736	-21.241038
585	P27348	YWHAQ	14-3-3 protein theta OS=Homo sap	-22.947577	-20.890786	-18.574306	N/A	-23.801837	-21.341428
586	P62888	RPL30	60S ribosomal protein L30 OS=Hom	-19.439799	-20.081601	-19.703968	N/A	-20.30447	-21.40278
587	P62249	RPS16	40S ribosomal protein S16 OS=Hom	-20.111783	-19.502371	N/A	N/A	-20.926418	-21.431596
588	P04083	ANXA1	Annexin A1 OS=Homo sapiens GN=	-21.40967	-20.504818	-19.378899	N/A	-22.364298	-21.473843
589	P08621	SNRNP70	U1 small nuclear ribonucleoprotein	-24.951426	-22.019298	-20.755417	N/A	-21.860368	-21.532513
590	P46781	RPS9	40S ribosomal protein S9 OS=Homo	-23.429897	-20.186943	-21.650816	N/A	-20.676805	-21.708818
591	O15143	ARPC1B	Actin-related protein 2/3 complex s	-21.008271	-21.548047	-20.953896	N/A	-21.335538	-21.719455
592	P16949	STMN1	Stathmin OS=Homo sapiens GN=ST	N/A	-18.954405	-19.452566	N/A	-23.399992	-21.744072
593	P35637	FUS	RNA-binding protein FUS OS=Homo	-23.375029	-22.039563	-19.201685	N/A	-23.522701	-22.03363
594	P13010	XRCC5	X-ray repair cross-complementing p	-23.593074	-25.060332	-23.914755	N/A	-23.469734	-22.241074
595	P50990	CCT8	T-complex protein 1 subunit theta	-23.998585	-21.816881	-21.163427	N/A	-22.658894	-22.305162
596	P05089	ARG1	Arginase-1 OS=Homo sapiens GN=A	-19.636142	-21.81771	-21.740624	N/A	-20.604974	-22.307317
597	O15144	ARPC2	Actin-related protein 2/3 complex s	-22.238001	-22.302131	-21.707016	N/A	-22.016002	-22.341791
598	P28070	PSMB4	Proteasome subunit beta type-4 OS	-22.086327	-21.795803	-21.215527	N/A	-22.571821	-22.363274
599	P30041	PRDX6	Peroxiredoxin-6 OS=Homo sapiens	-20.64668	N/A	-22.372111	N/A	-21.362133	-22.388284
600	P68371	TUBB4B	Tubulin beta-4B chain OS=Homo sa	-21.890808	-21.877221	-21.892041	N/A	-23.303415	-22.462682
601	P52272	HNRNPM	Heterogeneous nuclear ribonucleo	-24.431554	-22.925851	-22.386042	N/A	-25.629777	-22.564588

602	P30050	<i>RPL12</i>	60S ribosomal protein L12 OS=Hom	-19.090128	-20.787994	N/A	N/A	-21.992685	-22.567063
603	P27695	<i>APEX1</i>	DNA-(apurinic or apyrimidinic site)	-24.763548	0	-23.934216	N/A	-20.41371	-22.681079
604	Q09028	<i>RBBP4</i>	Histone-binding protein RBBP4 OS=	N/A	-23.721074	-22.199548	N/A	-22.739634	-22.799167
605	P62277	<i>RPS13</i>	40S ribosomal protein S13 OS=Hom	-20.301107	-20.045356	-21.04249	N/A	-21.330783	-23.044374
606	Q15050	<i>RRS1</i>	Ribosome biogenesis regulatory pr	N/A	-23.014264	-21.45084	N/A	-24.285266	-23.121188
607	Q13835	<i>PKP1</i>	Plakophilin-1 OS=Homo sapiens GN	-23.3029	-23.976365	-23.877005	N/A	-23.47083	-23.179823
608	Q12906	<i>ILF3</i>	Interleukin enhancer-binding factor	-24.332497	-23.85564	-23.192733	N/A	-24.517011	-23.366593
609	E9PAV3	<i>NACA</i>	Nascent polypeptide-associated co	-24.966199	-23.378716	-23.943556	N/A	-26.070653	-23.944336
610	Q13765	<i>KRT79</i>	Nascent polypeptide-associated co	-24.966199	-23.378716	-23.943556	N/A	-26.070653	-23.944336
611	Q5XKE5	<i>EZR</i>	Keratin, type II cytoskeletal 79 OS=	N/A	-23.070622	-22.42388	N/A	-22.038978	-24.149324
612	P15311	<i>ESYT2</i>	Ezrin OS=Homo sapiens GN=EZR PE	N/A	-25.726059	-25.849927	N/A	-23.903623	-24.365968
613	A0FGR8	<i>ILVBL</i>	Extended synaptotagmin-2 OS=Ho	-24.20735	N/A	N/A	N/A	N/A	N/A
614	A1L0T0	<i>GGT3P</i>	Acetolactate synthase-like protein	-24.419349	N/A	N/A	N/A	N/A	N/A
615	A6NGU5	<i>DDTL</i>	Putative gamma-glutamyltranspept	N/A	N/A	N/A	0	N/A	N/A
616	A6NHG4	<i>TUBB8B</i>	D-dopachrome decarboxylase-like	N/A	N/A	-22.370206	N/A	N/A	N/A
617	A6NNZ2	<i>KAZN</i>	Tubulin beta-8 chain-like protein LO	N/A	N/A	N/A	N/A	N/A	N/A
618	B1AJZ1	<i>HMGB1P1</i>	Putative uncharacterized protein C	N/A	N/A	-21.085027	N/A	N/A	N/A
619	B2RPK0	<i>DNASE2</i>	Putative high mobility group protei	N/A	-22.18288	N/A	N/A	N/A	N/A
620	O00115	<i>AGPS</i>	Deoxyribonuclease-2-alpha OS=Ho	N/A	N/A	N/A	N/A	N/A	N/A
621	O00116	<i>PGRMC1</i>	Alkylldihydroxyacetonephosphate s	-22.994616	N/A	N/A	N/A	N/A	N/A
622	O00264	<i>PDHX</i>	Membrane-associated progesteron	N/A	N/A	N/A	N/A	N/A	N/A
623	O00330	<i>GOLIM4</i>	Pyruvate dehydrogenase protein X	-24.16614	N/A	N/A	N/A	N/A	N/A
624	O00461	<i>NDUFA4</i>	Golgi integral membrane protein 4	N/A	N/A	N/A	N/A	N/A	N/A
625	O00483	<i>DDX3X</i>	Cytochrome c oxidase subunit NDU	-19.579498	N/A	N/A	N/A	N/A	N/A
626	O00571	<i>PDXK</i>	ATP-dependent RNA helicase DDX3	N/A	N/A	N/A	N/A	N/A	N/A
627	O00764	<i>SCAMP3</i>	Pyridoxal kinase OS=Homo sapiens	N/A	-21.836099	-21.269662	N/A	N/A	N/A
628	O14828	<i>PPEF1</i>	Secretory carrier-associated memb	-23.79371	N/A	N/A	N/A	N/A	N/A
629	O14829	<i>MGST3</i>	Serine/threonine-protein phosphat	N/A	N/A	N/A	N/A	N/A	N/A
630	O14880	<i>UQCQRQ</i>	Microsomal glutathione S-transfera	-20.475708	N/A	N/A	N/A	N/A	N/A
631	O14949	<i>PLXNB2</i>	Cytochrome b-c1 complex subunit	-19.714816	N/A	N/A	N/A	N/A	N/A
632	O15031	<i>PFDN6</i>	Plexin-B2 OS=Homo sapiens GN=PL	-26.079705	N/A	N/A	N/A	N/A	N/A
633	O15212	<i>STX7</i>	Prefoldin subunit 6 OS=Homo sapie	N/A	N/A	-22.470273	N/A	N/A	N/A
634	O15400	<i>DDX3Y</i>	Syntaxin-7 OS=Homo sapiens GN=S	-23.585169	N/A	N/A	N/A	N/A	N/A
635	O15523	<i>TAPBP</i>	ATP-dependent RNA helicase DDX3	N/A	N/A	N/A	N/A	N/A	N/A
636	O15533	<i>PHGDH</i>	Tapasin OS=Homo sapiens GN=TAP	-22.521851	N/A	N/A	N/A	N/A	N/A
637	O43175	<i>MTSS1</i>	D-3-phosphoglycerate dehydrogen	N/A	N/A	N/A	N/A	N/A	N/A
638	O43312	<i>EMC8</i>	Metastasis suppressor protein 1 OS	-25.134863	N/A	N/A	N/A	N/A	N/A
639	O43402	<i>PPIH</i>	ER membrane protein complex sub	-22.702358	N/A	N/A	N/A	N/A	N/A
640	O43447	<i>TIMM44</i>	Peptidyl-prolyl cis-trans isomerase	N/A	N/A	-22.621876	N/A	N/A	N/A
641	O43615	<i>NDUFS5</i>	Mitochondrial import inner membr	-22.228301	N/A	N/A	N/A	N/A	N/A
642	O43920	<i>OPA1</i>	NADH dehydrogenase [ubiquinone]	-21.232126	N/A	N/A	N/A	N/A	N/A
643	O60313	<i>PLOD3</i>	Dynamin-like 120 kDa protein, mito	-24.287012	N/A	N/A	N/A	N/A	N/A
644	O60568	<i>SNAP91</i>	Procollagen-lysine,2-oxoglutarate 5	-21.955407	N/A	N/A	N/A	N/A	N/A
645	O60641	<i>SNX2</i>	Clathrin coat assembly protein AP1	N/A	N/A	N/A	N/A	N/A	N/A
646	O60749	<i>DPM1</i>	Sorting nexin-2 OS=Homo sapiens G	N/A	N/A	N/A	N/A	N/A	N/A
647	O60762	<i>DKC1</i>	Dolichol-phosphate mannosyltrans	-21.804698	N/A	N/A	N/A	N/A	N/A
648	O60832	<i>EDF1</i>	H/ACA ribonucleoprotein complex	N/A	-23.217943	-25.345899	N/A	N/A	N/A
649	O60869	<i>PFDN1</i>	Endothelial differentiation-related f	N/A	-20.890054	N/A	N/A	N/A	N/A
650	O60925	<i>ATP6V1G1</i>	Prefoldin subunit 1 OS=Homo sapie	N/A	-21.901233	N/A	N/A	N/A	N/A
651	O75348	<i>PRPF40A</i>	V-type proton ATPase subunit G 1	N/A	N/A	N/A	N/A	N/A	N/A
652	O75400	<i>NDUFB1</i>	Pre-mRNA-processing factor 40 ho	N/A	N/A	-24.650962	N/A	N/A	N/A

653	O75438	PMPCB	NADH dehydrogenase [ubiquinone]	-20.597063	N/A	N/A	N/A	N/A	N/A
654	O75439	IDH1	Mitochondrial-processing peptidas	-23.380037	N/A	N/A	N/A	N/A	N/A
655	O75874	ALDH1L1	Isocitrate dehydrogenase [NADP] c	-21.929041	N/A	N/A	N/A	N/A	N/A
656	O75891	TOMM70	Cytosolic 10-formyltetrahydrofolat	-24.625679	N/A	N/A	N/A	N/A	N/A
657	O94826	UFL1	Mitochondrial import receptor sub	-21.932287	N/A	N/A	N/A	N/A	N/A
658	O94874	GLS	E3 UFM1-protein ligase 1 OS=Hom	-24.043252	N/A	N/A	N/A	N/A	N/A
659	O94925	NDUFB4	Glutaminase kidney isoform, mitoc	-23.60428	N/A	N/A	N/A	N/A	N/A
660	O95168	RTN3	NADH dehydrogenase [ubiquinone]	N/A	N/A	N/A	N/A	N/A	N/A
661	O95197	VAPB	Reticulon-3 OS=Homo sapiens GN=	-24.134768	N/A	N/A	N/A	N/A	N/A
662	O95292	CELF2	Vesicle-associated membrane prot	-21.147257	N/A	N/A	N/A	N/A	N/A
663	O95319	FMNL1	CUGBP Elav-like family member 2 O	N/A	N/A	-24.221605	N/A	N/A	N/A
664	O95466	SGPL1	Formin-like protein 1 OS=Homo sap	N/A	N/A	N/A	N/A	N/A	N/A
665	O95470	KRT75	Sphingosine-1-phosphate lyase 1 O	-23.899481	N/A	N/A	N/A	N/A	N/A
666	O95678	STARD3NL	Keratin, type II cytoskeletal 75 OS=	N/A	N/A	N/A	N/A	N/A	N/A
667	O95772	LSM8	MLN64 N-terminal domain homolo	N/A	N/A	N/A	N/A	N/A	N/A
668	O95777	AIFM1	U6 snRNA-associated Sm-like prote	N/A	-18.99304	N/A	N/A	N/A	N/A
669	O95831	ALDH1A1	Apoptosis-inducing factor 1, mitoch	-22.13809	N/A	N/A	N/A	N/A	N/A
670	P00352	MT-CO2	Retinal dehydrogenase 1 OS=Homo	-23.83021	N/A	N/A	N/A	N/A	N/A
671	P00403	OTC	Cytochrome c oxidase subunit 2 OS	N/A	N/A	N/A	N/A	N/A	N/A
672	P00480	TGFB1	Ornithine carbamoyltransferase, m	-22.435868	N/A	N/A	N/A	N/A	N/A
673	P01137	IL1B	Transforming growth factor beta-1	-23.153418	N/A	N/A	N/A	N/A	N/A
674	P01584	PIGR	Interleukin-1 beta OS=Homo sapien	-23.298721	N/A	N/A	N/A	N/A	N/A
675	P01833	IGHA1	Polymeric immunoglobulin recepto	N/A	N/A	-23.180566	N/A	N/A	N/A
676	P01876	HLA-A	Ig alpha-1 chain C region OS=Homo	N/A	N/A	-19.87539	N/A	N/A	N/A
677	P01892	APOC1	HLA class I histocompatibility antige	0	N/A	N/A	N/A	N/A	N/A
678	P02654	FN1	Apolipoprotein C-I OS=Homo sapie	-21.009122	N/A	N/A	N/A	N/A	N/A
679	P02751	LTF	Fibronectin OS=Homo sapiens GN=	N/A	N/A	-26.712694	N/A	N/A	N/A
680	P02788	FTL	Lactotransferrin OS=Homo sapiens	N/A	N/A	-22.612622	N/A	N/A	N/A
681	P02792	FTH1	Ferritin light chain OS=Homo sapie	N/A	N/A	N/A	N/A	N/A	N/A
682	P02794	MT-ATP8	Ferritin heavy chain OS=Homo sapi	N/A	N/A	N/A	N/A	N/A	N/A
683	P03928	SLPI	ATP synthase protein 8 OS=Homo s	-19.35054	N/A	N/A	N/A	N/A	N/A
684	P03973	GBA	Antileukoproteinase OS=Homo sap	N/A	-20.878446	-19.471029	N/A	N/A	N/A
685	P04062	APOB	Glucosylceramidase OS=Homo sapi	-24.873805	N/A	N/A	N/A	N/A	N/A
686	P04114	OAT	Apolipoprotein B-100 OS=Homo sa	N/A	N/A	N/A	N/A	N/A	N/A
687	P04181	ALDOB	Ornithine aminotransferase, mitoch	-23.528173	N/A	N/A	N/A	N/A	N/A
688	P05062	ALDH2	Fructose-bisphosphate aldolase B O	-17.658868	-25.364719	N/A	N/A	N/A	N/A
689	P05091	ICAM1	Aldehyde dehydrogenase, mitoch	-21.488551	N/A	N/A	N/A	N/A	N/A
690	P05362	KRT8	Intercellular adhesion molecule 1 O	-21.743276	N/A	N/A	N/A	N/A	N/A
691	P05787	GLA	Keratin, type II cytoskeletal 8 OS=H	N/A	N/A	N/A	N/A	N/A	N/A
692	P06280	HEXA	Alpha-galactosidase A OS=Homo sa	-22.764555	N/A	N/A	N/A	N/A	N/A
693	P06865	EPHX1	Beta-hexosaminidase subunit alpha	N/A	N/A	-20.084619	N/A	N/A	N/A
694	P07099	CAPN1	Epoxide hydrolase 1 OS=Homo sapi	-22.994816	N/A	N/A	N/A	N/A	N/A
695	P07384	CA3	Calpain-1 catalytic subunit OS=Hom	N/A	N/A	-24.148678	N/A	N/A	N/A
696	P07451	LYN	Carbonic anhydrase 3 OS=Homo sa	-19.087508	N/A	N/A	N/A	N/A	N/A
697	P07948	SLC3A2	Tyrosine-protein kinase Lyn OS=Ho	-25.25827	N/A	N/A	N/A	N/A	N/A
698	P08195	PDHA1	4F2 cell-surface antigen heavy chai	-21.472276	N/A	N/A	N/A	N/A	N/A
699	P08559	PDHA1	Pyruvate dehydrogenase E1 compo	-21.285138	N/A	N/A	N/A	N/A	N/A
700	P08571	CD14	Monocyte differentiation antigen C	N/A	N/A	N/A	N/A	N/A	N/A
701	P08575	PTPRC	Receptor-type tyrosine-protein pho	-24.31236	N/A	N/A	N/A	N/A	N/A
702	P08631	HCK	Tyrosine-protein kinase Lyn OS=Ho	-25.25827	N/A	N/A	N/A	N/A	N/A
703	P08708	RPS17	40S ribosomal protein S17 OS=Hom	-20.212791	-20.437162	N/A	N/A	N/A	N/A

704	P08727	KRT19	Keratin, type I cytoskeletal 19 OS=H	N/A	N/A	N/A	N/A	N/A	N/A
705	P09234	SNRPC	U1 small nuclear ribonucleoprotein	N/A	N/A	-22.390281	N/A	N/A	N/A
706	P09467	FBP1	Fructose-1,6-bisphosphatase 1 OS=	-22.239682	N/A	N/A	N/A	N/A	N/A
707	P09669	COX6C	Cytochrome c oxidase subunit 6C O	-19.305958	N/A	N/A	N/A	N/A	N/A
708	P09874	PARP1	Poly [ADP-ribose] polymerase 1 OS	-23.870001	N/A	N/A	N/A	N/A	N/A
709	P0C7I6	CCDC159	Coiled-coil domain-containing prot	-23.617846	N/A	N/A	N/A	N/A	N/A
710	P0DMV8	HSPA1A	Heat shock 70 kDa protein 1A OS=H	N/A	0	N/A	N/A	0	N/A
711	P0DMV9	HSPA1B	Heat shock 70 kDa protein 1A OS=H	N/A	0	N/A	N/A	0	N/A
712	P10145	CXCL8	Interleukin-8 OS=Homo sapiens GN	-19.446438	N/A	N/A	0	N/A	N/A
713	P10319	HLA-B	HLA class I histocompatibility antige	0	N/A	N/A	0	N/A	N/A
714	P10515	DLAT	Dihydrolipoyllysine-residue acetyltr	-22.018761	N/A	N/A	N/A	N/A	N/A
715	P11177	PDHB	Pyruvate dehydrogenase E1 compo	-20.817739	N/A	N/A	N/A	N/A	N/A
716	P11215	ITGAM	Integrin alpha-M OS=Homo sapiens	-24.197657	N/A	N/A	N/A	N/A	N/A
717	P11233	RALA	Ras-related protein Ral-A OS=Homo	N/A	N/A	N/A	N/A	N/A	N/A
718	P11498	PC	Pyruvate carboxylase, mitochondri	-23.838102	N/A	N/A	N/A	N/A	N/A
719	P11717	IGF2R	Cation-independent mannose-6-ph	N/A	N/A	N/A	N/A	N/A	N/A
720	P12035	KRT3	Keratin, type II cytoskeletal 3 OS=H	N/A	N/A	-27.15112	N/A	N/A	N/A
721	P12236	SLC25A6	ADP/ATP translocase 2 OS=Homo s	-22.061678	0	-23.893401	0	N/A	N/A
722	P13073	COX4I1	Cytochrome c oxidase subunit 4 iso	-20.315902	N/A	N/A	N/A	N/A	N/A
723	P13284	IFI30	Gamma-interferon-inducible lysoso	-21.095088	N/A	N/A	N/A	N/A	N/A
724	P13501	CCL5	C-C motif chemokine 5 OS=Homo s	-20.609633	N/A	N/A	N/A	N/A	N/A
725	P13674	P4HA1	Prolyl 4-hydroxylase subunit alpha-	-23.888393	N/A	N/A	N/A	N/A	N/A
726	P13798	APEH	Acylamino-acid-releasing enzyme O	N/A	N/A	-23.371881	N/A	N/A	N/A
727	P14649	MYL6B	Myosin light chain 6B OS=Homo sa	N/A	N/A	N/A	N/A	N/A	N/A
728	P15090	FABP4	Fatty acid-binding protein, adipocyt	-20.744709	N/A	-21.103443	N/A	N/A	N/A
729	P15259	PGAM2	Phosphoglycerate mutase 2 OS=Ho	N/A	N/A	-21.292856	N/A	N/A	N/A
730	P16104	H2AX	Histone H2AX OS=Homo sapiens G	N/A	N/A	N/A	N/A	N/A	N/A
731	P17174	GOT1	Aspartate aminotransferase, cytopl	-23.859243	N/A	N/A	N/A	N/A	N/A
732	P17540	CKMT2	Creatine kinase S-type, mitochondr	N/A	-18.910224	N/A	N/A	N/A	N/A
733	P17661	DES	Desmin OS=Homo sapiens GN=DES	N/A	N/A	N/A	N/A	N/A	N/A
734	P18031	PTPN1	Tyrosine-protein phosphatase non-	-23.168484	N/A	N/A	N/A	N/A	N/A
735	P18206	VCL	Vinculin OS=Homo sapiens GN=VCL	-24.67756	N/A	N/A	N/A	N/A	N/A
736	P18464	HLA-B	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
737	P18465	HLA-B	HLA class I histocompatibility antige	0	N/A	N/A	0	N/A	N/A
738	P18583	SON	Protein SON OS=Homo sapiens GN	-26.041043	N/A	N/A	N/A	N/A	N/A
739	P18621	RPL17	60S ribosomal protein L17 OS=Hom	-20.41794	-20.087586	-19.235522	N/A	N/A	N/A
740	P18669	PGAM1	Phosphoglycerate mutase 2 OS=Ho	-22.646056	N/A	-21.292856	N/A	N/A	N/A
741	P19013	KRT4	Keratin, type II cytoskeletal 4 OS=H	N/A	N/A	-19.990176	N/A	0	N/A
742	P19440	GGT1	Putative gamma-glutamyltranspept	-26.002884	N/A	N/A	0	N/A	N/A
743	P20020	ATP2B1	Plasma membrane calcium-transpo	N/A	N/A	N/A	N/A	N/A	N/A
744	P20290	BTF3	Transcription factor BTF3 OS=Hom	N/A	N/A	N/A	N/A	N/A	N/A
745	P20338	RAB4A	Ras-related protein Rab-4A OS=Ho	-23.467326	N/A	N/A	N/A	N/A	N/A
746	P20339	RAB5A	Ras-related protein Rab-5A OS=Ho	-22.461229	N/A	N/A	N/A	N/A	N/A
747	P20645	M6PR	Cation-dependent mannose-6-phos	N/A	N/A	N/A	N/A	N/A	N/A
748	P20702	ITGAX	Integrin alpha-X OS=Homo sapiens	-26.163329	N/A	N/A	N/A	N/A	N/A
749	P20933	AGA	N(4)-(beta-N-acetylglucosaminy)-L	N/A	N/A	N/A	N/A	N/A	N/A
750	P21281	ATP6V1B2	V-type proton ATPase subunit B, br	-22.953086	N/A	N/A	N/A	N/A	N/A
751	P22087	FBL	rRNA 2'-O-methyltransferase fibrill	N/A	N/A	N/A	N/A	N/A	N/A
752	P25789	PSMA4	Proteasome subunit alpha type-4 O	N/A	N/A	-21.141155	N/A	N/A	N/A
753	P25942	CD40	Tumor necrosis factor receptor sup	N/A	N/A	N/A	N/A	N/A	N/A
754	P26599	PTBP1	Polypyrimidine tract-binding protei	N/A	-24.168484	-22.509782	N/A	0	N/A

755	P28062	PSMB8	Proteasome subunit beta type-8 OS	N/A	N/A	-21.389324	N/A	N/A	N/A
756	P28065	PSMB9	Proteasome subunit beta type-9 OS	N/A	N/A	-22.206746	N/A	N/A	N/A
757	P28331	NDUFS1	NADH-ubiquinone oxidoreductase	-24.444279	N/A	N/A	N/A	N/A	N/A
758	P28370	SMARCA1	Probable global transcription activa	N/A	-25.602932	N/A	N/A	N/A	N/A
759	P30042	GATD3A	ES1 protein homolog, mitochondria	-20.055261	N/A	-21.283066	N/A	N/A	N/A
760	P30044	PRDX5	Peroxiredoxin-5, mitochondrial OS=	-20.284446	N/A	N/A	N/A	N/A	N/A
761	P30046	DDT	D-dopachrome decarboxylase-like	N/A	N/A	-22.370206	N/A	N/A	N/A
762	P30084	ECHS1	Enoyl-CoA hydratase, mitochondria	-21.953382	N/A	N/A	N/A	N/A	N/A
763	P30273	FCER1G	High affinity immunoglobulin epsilo	-19.49244	N/A	N/A	N/A	N/A	N/A
764	P30464	HLA-B	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
765	P30484	HLA-B	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
766	P30490	HLA-B	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
767	P30491	HLA-B	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
768	P30492	HLA-B	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
769	P30493	HLA-B	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
770	P30495	HLA-B	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
771	P30498	HLA-B	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
772	P30519	HMOX2	Heme oxygenase 2 OS=Homo sapie	-24.423933	N/A	N/A	N/A	N/A	N/A
773	P30685	HLA-B	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
774	P30837	ALDH1B1	Aldehyde dehydrogenase X, mitoch	-24.567999	N/A	N/A	N/A	N/A	N/A
775	P31512	FMO4	Dimethylaniline monooxygenase [N	N/A	N/A	N/A	N/A	N/A	N/A
776	P31689	DNAJA1	DnaJ homolog subfamily A member	-21.906964	N/A	N/A	N/A	N/A	N/A
777	P32754	HPD	4-hydroxyphenylpyruvate dioxygen	-20.421862	N/A	N/A	N/A	N/A	N/A
778	P32969	RPL9	60S ribosomal protein L9 OS=Homo	N/A	N/A	N/A	N/A	N/A	N/A
779	P33897	ABCD1	ATP-binding cassette sub-family D	N/A	N/A	N/A	N/A	N/A	N/A
780	P34931	HSPA1L	Heat shock 70 kDa protein 1A OS=H	N/A	N/A	N/A	N/A	0	N/A
781	P35241	RDX	Radixin OS=Homo sapiens GN=RDX	N/A	N/A	N/A	N/A	N/A	N/A
782	P35580	MYH10	Myosin-10 OS=Homo sapiens GN=	N/A	N/A	N/A	N/A	N/A	N/A
783	P35606	COPB2	Coatomer subunit beta' OS=Homo	-27.00461	N/A	-24.502474	N/A	N/A	N/A
784	P35914	HMGCL	Hydroxymethylglutaryl-CoA lyase,	-21.617084	N/A	N/A	N/A	N/A	N/A
785	P35998	PSMC2	26S protease regulatory subunit 7 O	N/A	N/A	-23.898829	N/A	N/A	N/A
786	P36268	GGT2	Putative gamma-glutamyltranssept	N/A	N/A	N/A	N/A	N/A	N/A
787	P36871	PGM1	Phosphoglucomutase-1 OS=Homo	-22.832349	N/A	N/A	N/A	N/A	N/A
788	P38606	ATP6V1A	V-type proton ATPase catalytic sub	N/A	N/A	N/A	N/A	N/A	N/A
789	P42166	TMPO	Lamina-associated polypeptide 2, is	-24.374933	N/A	N/A	N/A	N/A	N/A
790	P42167	TMPO	Lamina-associated polypeptide 2, is	-24.374933	N/A	N/A	N/A	N/A	N/A
791	P42677	RPS27	40S ribosomal protein S27 OS=Hom	-20.003644	N/A	-21.141081	N/A	N/A	N/A
792	P42704	LRPPRC	Leucine-rich PPR motif-containing p	-22.129393	N/A	N/A	N/A	N/A	N/A
793	P42765	ACAA2	3-ketoacyl-CoA thiolase, mitochond	-22.275723	N/A	N/A	N/A	N/A	N/A
794	P43243	MATR3	Matrin-3 OS=Homo sapiens GN=M	N/A	N/A	N/A	N/A	N/A	N/A
795	P43304	GPD2	Glycerol-3-phosphate dehydrogena	-23.960231	N/A	N/A	N/A	N/A	N/A
796	P43307	SSR1	Translocon-associated protein subu	-21.122424	N/A	N/A	N/A	N/A	N/A
797	P46776	RPL27A	60S ribosomal protein L27a OS=Ho	N/A	N/A	-19.179078	N/A	N/A	N/A
798	P46783	RPS10	40S ribosomal protein S10 OS=Hom	-20.390349	N/A	N/A	N/A	N/A	N/A
799	P47755	CAPZA2	F-actin-capping protein subunit alp	-22.639314	N/A	N/A	N/A	N/A	N/A
800	P48163	ME1	NADP-dependent malic enzyme OS	-23.065563	N/A	N/A	N/A	N/A	N/A
801	P48444	ARCN1	Coatomer subunit delta OS=Homo	-23.404724	N/A	N/A	N/A	N/A	N/A
802	P48651	PTDSS1	Phosphatidylserine synthase 1 OS=	N/A	N/A	N/A	N/A	N/A	N/A
803	P48681	NES	Nestin OS=Homo sapiens GN=NES	N/A	N/A	-25.426908	N/A	N/A	N/A
804	P49257	LMAN1	Protein ERGIC-53 OS=Homo sapien	-21.576962	N/A	N/A	N/A	N/A	N/A
805	P49585	PCYT1A	Choline-phosphate cytidyltransfer	-24.498256	N/A	N/A	0	N/A	N/A
806	P49721	PSMB2	Proteasome subunit beta type-2 OS	N/A	N/A	-22.788169	N/A	N/A	N/A

807	P50053	KHK	Ketohexokinase OS=Homo sapiens	-23.029075	N/A	N/A	N/A	N/A	N/A
808	P50416	CPT1A	Carnitine O-palmitoyltransferase 1,	-24.465025	N/A	N/A	N/A	N/A	N/A
809	P51151	RAB9A	Ras-related protein Rab-9A OS=Ho	-22.890982	N/A	N/A	N/A	N/A	N/A
810	P51553	IDH3G	Isocitrate dehydrogenase [NAD] su	-23.19822	N/A	N/A	N/A	N/A	N/A
811	P51798	CLCN7	H(+)/Cl(-) exchange transporter 7 O	N/A	N/A	N/A	N/A	N/A	N/A
812	P51970	NDUFA8	NADH dehydrogenase [ubiquinone]	-21.073441	N/A	N/A	N/A	N/A	N/A
813	P53621	COPA	Coatomer subunit alpha OS=Homo	-24.49676	N/A	N/A	N/A	N/A	N/A
814	P54709	ATP1B3	Sodium/potassium-transporting AT	N/A	N/A	N/A	N/A	N/A	N/A
815	P54727	RAD23B	UV excision repair protein RAD23 h	-23.718796	N/A	-22.429417	N/A	N/A	N/A
816	P54920	NAPA	Alpha-soluble NSF attachment prot	-22.685316	N/A	N/A	N/A	N/A	N/A
817	P55036	PSMD4	26S proteasome non-ATPase regula	N/A	N/A	-22.774217	N/A	N/A	N/A
818	P55060	CSE1L	Exportin-2 OS=Homo sapiens GN=C	N/A	N/A	N/A	N/A	N/A	N/A
819	P55957	BID	BH3-interacting domain death agon	-21.37739	-21.597604	-21.289358	N/A	N/A	N/A
820	P56134	ATP5MF	ATP synthase subunit f, mitochondr	N/A	N/A	N/A	N/A	N/A	N/A
821	P56937	HSD17B7	3-keto-steroid reductase OS=Homo	-21.87545	N/A	N/A	N/A	N/A	N/A
822	P57088	TMEM33	Transmembrane protein 33 OS=Ho	-20.756593	N/A	N/A	N/A	N/A	N/A
823	P60468	SEC61B	Protein transport protein Sec61 sub	N/A	N/A	N/A	N/A	N/A	N/A
824	P60866	RPS20	40S ribosomal protein S20 OS=Hom	-18.819988	-21.002559	N/A	N/A	N/A	N/A
825	P60903	S100A10	Protein S100-A10 OS=Homo sapien	-20.883726	N/A	N/A	N/A	N/A	N/A
826	P61224	RAP1B	Ras-related protein Rap-1b OS=Ho	0	0	0	0	0	N/A
827	P61457	PCBD1	Pterin-4-alpha-carbinolamine dehy	-20.734419	N/A	N/A	N/A	N/A	N/A
828	P61513	RPL37A	60S ribosomal protein L37a OS=Ho	-21.508804	N/A	N/A	N/A	N/A	N/A
829	P61927	RPL37	60S ribosomal protein L37 OS=Hom	N/A	N/A	N/A	N/A	N/A	N/A
830	P62081	RPS7	40S ribosomal protein S7 OS=Homo	-18.779924	N/A	-18.075325	N/A	N/A	N/A
831	P62280	RPS11	40S ribosomal protein S11 OS=Hom	-20.37341	-21.435267	-20.157754	N/A	N/A	N/A
832	P62310	LSM3	U6 snRNA-associated Sm-like prote	N/A	-20.29093	-19.650578	N/A	N/A	N/A
833	P62316	SNRPD2	Small nuclear ribonucleoprotein Sm	N/A	N/A	-17.898697	N/A	N/A	N/A
834	P62333	PSMC6	26S protease regulatory subunit 10	N/A	-24.020635	N/A	N/A	N/A	N/A
835	P62714	PPP2CB	Serine/threonine-protein phosphat	N/A	-21.090428	-20.521574	N/A	N/A	N/A
836	P62736	ACTA2	Actin, aortic smooth muscle OS=Ho	N/A	N/A	N/A	N/A	N/A	N/A
837	P62847	RPS24	40S ribosomal protein S24 OS=Hom	-18.866824	N/A	N/A	N/A	N/A	N/A
838	P62854	RPS26	40S ribosomal protein S26 OS=Hom	N/A	N/A	N/A	N/A	N/A	N/A
839	P62891	RPL39	60S ribosomal protein L39 OS=Hom	N/A	N/A	-18.753056	N/A	N/A	N/A
840	P62913	RPL11	60S ribosomal protein L11 OS=Hom	-20.867445	N/A	N/A	N/A	N/A	N/A
841	P62993	GRB2	Growth factor receptor-bound prot	N/A	N/A	-21.832657	N/A	N/A	N/A
842	P63167	DYNLL1	Dynein light chain 1, cytoplasmic O	-19.522742	N/A	0	N/A	N/A	N/A
843	P63173	RPL38	60S ribosomal protein L38 OS=Hom	N/A	N/A	-19.46808	N/A	N/A	N/A
844	P63267	ACTG2	Actin, aortic smooth muscle OS=Ho	N/A	N/A	N/A	N/A	N/A	N/A
845	P68366	TUBA4A	Tubulin alpha-4A chain OS=Homo s	N/A	N/A	N/A	N/A	0	N/A
846	P69849	NOMO3	Nodal modulator 3 OS=Homo sapie	0	N/A	N/A	N/A	N/A	N/A
847	P80303	NUCB2	Nucleobindin-2 OS=Homo sapiens	N/A	N/A	-23.627178	N/A	N/A	N/A
848	P83111	LACTB	Serine beta-lactamase-like protein	-22.12074	N/A	N/A	N/A	N/A	N/A
849	Q00059	TFAM	Transcription factor A, mitochondri	-22.96415	N/A	N/A	N/A	N/A	N/A
850	Q00688	FKBP3	Peptidyl-prolyl cis-trans isomerase	-22.108306	N/A	N/A	N/A	N/A	N/A
851	Q01546	KRT76	Keratin, type II cytoskeletal 2 oral O	N/A	N/A	0	N/A	N/A	N/A
852	Q02218	OGDH	2-oxoglutarate dehydrogenase, mit	-25.693467	N/A	N/A	N/A	N/A	N/A
853	Q02224	CENPE	Centromere-associated protein E O	N/A	N/A	N/A	N/A	N/A	N/A
854	Q02388	COL7A1	Collagen alpha-1(VII) chain OS=Hom	N/A	N/A	N/A	0	N/A	N/A
855	Q02543	RPL18A	60S ribosomal protein L18a OS=Ho	-20.88539	-22.080318	-21.952139	N/A	N/A	N/A
856	Q02809	PLOD1	Procollagen-lysine,2-oxoglutarate 5	-21.87438	N/A	N/A	N/A	N/A	N/A
857	Q02978	SLC25A11	Mitochondrial 2-oxoglutarate/mala	-22.470944	N/A	N/A	N/A	N/A	N/A

858	Q03405	PLAUR	Urokinase plasminogen activator su	-21.795085	N/A	N/A	N/A	N/A	N/A
859	Q03518	TAP1	Antigen peptide transporter 1 OS=H	-22.39995	N/A	N/A	0	N/A	N/A
860	Q05639	EEF1A2	Elongation factor 1-alpha 1 OS=Ho	N/A	N/A	N/A	N/A	N/A	N/A
861	Q07666	KHDRBS1	KH domain-containing, RNA-bindin	N/A	N/A	N/A	N/A	N/A	N/A
862	Q10471	GALNT2	Polypeptide N-acetylgalactosaminy	N/A	N/A	N/A	N/A	N/A	N/A
863	Q10588	BST1	ADP-ribosyl cyclase/cyclic ADP-ribo	N/A	N/A	N/A	N/A	N/A	N/A
864	Q10713	PMPCA	Mitochondrial-processing peptidas	-21.607735	N/A	N/A	N/A	N/A	N/A
865	Q13162	PRDX4	Peroxisredoxin-4 OS=Homo sapiens	-22.002167	N/A	-22.214831	N/A	N/A	N/A
866	Q13217	DNAJC3	DnaJ homolog subfamily C member	-22.87751	N/A	N/A	N/A	N/A	N/A
867	Q13228	SELENBP1	Selenium-binding protein 1 OS=Ho	-18.012046	N/A	N/A	N/A	N/A	N/A
868	Q13232	NME3	Nucleoside diphosphate kinase 3 O	-21.917037	N/A	N/A	N/A	N/A	N/A
869	Q13405	MRPL49	39S ribosomal protein L49, mitocho	-21.427238	N/A	N/A	N/A	N/A	N/A
870	Q13423	NNT	NAD(P) transhydrogenase, mitocho	-22.437049	N/A	N/A	N/A	N/A	N/A
871	Q13442	PDAP1	28 kDa heat- and acid-stable phosp	N/A	-20.30857	N/A	N/A	N/A	N/A
872	Q13561	DCTN2	Dynactin subunit 2 OS=Homo sapie	N/A	N/A	-23.886871	N/A	N/A	N/A
873	Q13724	MOGS	Mannosyl-oligosaccharide glucosid	-23.160976	N/A	N/A	N/A	N/A	N/A
874	Q13740	ALCAM	CD166 antigen OS=Homo sapiens G	-24.473805	N/A	N/A	N/A	N/A	N/A
875	Q14008	CKAP5	Cytoskeleton-associated protein 5	-24.903323	N/A	N/A	N/A	N/A	N/A
876	Q14157	UBAP2L	Ubiquitin-associated protein 2-like	N/A	N/A	-23.63354	N/A	N/A	N/A
877	Q14197	MRPL58	Peptidyl-tRNA hydrolase ICT1, mito	-22.104985	N/A	N/A	N/A	N/A	N/A
878	Q14667	KIAA0100	Protein KIAA0100 OS=Homo sapien	N/A	N/A	0	N/A	N/A	N/A
879	Q14749	GNMT	Glycine N-methyltransferase OS=H	-18.058284	N/A	N/A	N/A	N/A	N/A
880	Q14964	RAB39A	Ras-related protein Rab-39A OS=Ho	-24.234607	N/A	N/A	N/A	N/A	N/A
881	Q14978	NOLC1	Nucleolar and coiled-body phospho	N/A	N/A	N/A	N/A	N/A	N/A
882	Q14C86	GAPVD1	GTPase-activating protein and VPS9	N/A	-25.330652	N/A	N/A	N/A	N/A
883	Q15005	SPCS2	Signal peptidase complex subunit 2	-19.711134	N/A	N/A	N/A	N/A	N/A
884	Q15006	EMC2	ER membrane protein complex sub	N/A	N/A	N/A	N/A	N/A	N/A
885	Q15027	ACAP1	Arf-GAP with coiled-coil, ANK repea	-24.522969	N/A	N/A	N/A	N/A	N/A
886	Q15041	ARL6IP1	ADP-ribosylation factor-like protein	N/A	N/A	N/A	N/A	N/A	N/A
887	Q15067	ACOX1	Peroxisomal acyl-coenzyme A oxida	-21.903037	N/A	N/A	N/A	N/A	N/A
888	Q15155	NOMO1	Nodal modulator 3 OS=Homo sapie	N/A	N/A	N/A	N/A	N/A	N/A
889	Q15233	NONO	Non-POU domain-containing octam	-22.843402	-23.147941	-19.819724	N/A	N/A	N/A
890	Q15286	RAB35	Ras-related protein Rab-35 OS=Ho	-23.979947	N/A	N/A	N/A	N/A	N/A
891	Q15323	KRT31	Keratin, type I cuticular Ha1 OS=Ho	N/A	N/A	N/A	N/A	N/A	N/A
892	Q15413	RYR3	Ryanodine receptor 3 OS=Homo sa	-30.845389	N/A	N/A	N/A	N/A	N/A
893	Q15493	RGN	Regucalcin OS=Homo sapiens GN=R	-20.677897	N/A	N/A	N/A	N/A	N/A
894	Q15637	SF1	Splicing factor 1 OS=Homo sapiens	N/A	-24.305975	-23.664486	N/A	N/A	N/A
895	Q15843	NEDD8	NEDD8 OS=Homo sapiens GN=NED	N/A	N/A	-19.781817	N/A	N/A	N/A
896	Q16181	SEPTIN7	Septin-7 OS=Homo sapiens GN=SEP	0	N/A	N/A	N/A	N/A	N/A
897	Q16630	CPSF6	Cleavage and polyadenylation spec	N/A	N/A	-22.923916	N/A	N/A	N/A
898	Q16762	TST	Thiosulfate sulfurtransferase OS=H	-22.343418	N/A	N/A	N/A	N/A	N/A
899	Q16822	PCK2	Phosphoenolpyruvate carboxykinas	-22.296825	N/A	N/A	N/A	N/A	N/A
900	Q16831	UPP1	Uridine phosphorylase 1 OS=Homo	N/A	-24.314156	N/A	N/A	N/A	N/A
901	Q16881	TXNRD1	Thioredoxin reductase 1, cytoplasm	N/A	-22.957079	-21.891439	N/A	N/A	N/A
902	Q1KMD3	HNRNPUL2	Heterogeneous nuclear ribonucleo	N/A	N/A	-22.830249	N/A	N/A	N/A
903	Q29940	HLA-B	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
904	Q32P51	HNRNPA1L2	Heterogeneous nuclear ribonucleo	N/A	N/A	N/A	N/A	N/A	N/A
905	Q3SY69	ALDH1L2	Cytosolic 10-formyltetrahydrofolat	-24.625679	N/A	N/A	N/A	N/A	N/A
906	Q3ZCM7	TUBB8	Tubulin beta-8 chain-like protein LO	N/A	N/A	N/A	N/A	N/A	N/A
907	Q4VC31	MIX23	Coiled-coil domain-containing prot	-20.259389	N/A	N/A	N/A	N/A	N/A
908	Q53H82	LACTB2	Endoribonuclease LACTB2 OS=Hom	-23.072865	N/A	N/A	N/A	N/A	N/A

909	Q59GN2	<i>RPL39P5</i>	60S ribosomal protein L39 OS=Hom	N/A	N/A	-18.753056	N/A	N/A	N/A
910	Q5HYI8	<i>RABL3</i>	Rab-like protein 3 OS=Homo sapien	-24.229136	N/A	N/A	N/A	N/A	N/A
911	Q5JNZ5	<i>RPS26P11</i>	40S ribosomal protein S26 OS=Hom	N/A	N/A	N/A	N/A	N/A	N/A
912	Q5JPE7	<i>NOMO2</i>	Nodal modulator 3 OS=Homo sapie	0	N/A	N/A	N/A	N/A	N/A
913	Q5JRA6	<i>MIA3</i>	Melanoma inhibitory activity protei	N/A	N/A	N/A	N/A	N/A	N/A
914	Q5JTH9	<i>RRP12</i>	RRP12-like protein OS=Homo sapie	N/A	N/A	N/A	N/A	N/A	N/A
915	Q5JTJ3	<i>COA6</i>	Cytochrome c oxidase assembly fac	-20.617073	-21.000054	-20.82702	N/A	N/A	N/A
916	Q5T1J5	<i>CHCHD2P9</i>	Putative coiled-coil-helix-coiled-coil	N/A	N/A	N/A	N/A	N/A	N/A
917	Q5TB30	<i>DEPDC1</i>	DEP domain-containing protein 1A	N/A	N/A	N/A	N/A	N/A	N/A
918	Q5VYS8	<i>TUT7</i>	Terminal uridylyltransferase 7 OS=H	N/A	N/A	N/A	0	N/A	N/A
919	Q66K74	<i>MAP1S</i>	Microtubule-associated protein 1S	N/A	-25.320084	N/A	N/A	N/A	N/A
920	Q6DD88	<i>ATL3</i>	Atlastin-3 OS=Homo sapiens GN=A	-23.671346	N/A	N/A	N/A	N/A	N/A
921	Q6NVY1	<i>HIBCH</i>	3-hydroxyisobutyryl-CoA hydrolase	-25.491164	N/A	N/A	N/A	N/A	N/A
922	Q6PIU2	<i>NCEH1</i>	Neutral cholesterol ester hydrolase	-23.050466	N/A	N/A	N/A	N/A	N/A
923	Q6S8J3	<i>POTEE</i>	POTE ankyrin domain family memb	0	N/A	N/A	N/A	N/A	N/A
924	Q7LGA3	<i>HS2ST1</i>	Heparan sulfate 2-O-sulfotransfera	-23.944286	N/A	N/A	N/A	N/A	N/A
925	Q7Z2K8	<i>GPRIN1</i>	G protein-regulated inducer of neu	N/A	0	N/A	N/A	N/A	N/A
926	Q7Z2W4	<i>ZC3HAV1</i>	Zinc finger CCCH-type antiviral prot	-25.855075	N/A	N/A	N/A	N/A	N/A
927	Q7Z3Y9	<i>KRT26</i>	Keratin, type I cytoskeletal 26 OS=H	N/A	-25.276665	N/A	N/A	N/A	N/A
928	Q7Z4P5	<i>GDF7</i>	Growth/differentiation factor 7 OS=	N/A	N/A	N/A	N/A	N/A	N/A
929	Q7Z7K0	<i>CMC1</i>	COX assembly mitochondrial protei	-20.730009	N/A	N/A	N/A	N/A	N/A
930	Q86SQ9	<i>DHDDS</i>	Dehydrodolichyl diphosphate synth	-21.114755	N/A	N/A	N/A	N/A	N/A
931	Q86SX6	<i>GLRX5</i>	Glutaredoxin-related protein 5, mit	-21.468202	N/A	N/A	N/A	N/A	N/A
932	Q86VB7	<i>CD163</i>	Scavenger receptor cysteine-rich ty	-24.826458	N/A	N/A	N/A	N/A	N/A
933	Q86Y46	<i>KRT73</i>	Keratin, type II cytoskeletal 74 OS=	N/A	0	N/A	N/A	0	N/A
934	Q86Y82	<i>STX12</i>	Syntaxin-12 OS=Homo sapiens GN=	-22.864431	N/A	N/A	N/A	N/A	N/A
935	Q8IXI1	<i>RHOT2</i>	Mitochondrial Rho GTPase 2 OS=Ho	-25.175391	N/A	N/A	N/A	N/A	N/A
936	Q8IY84	<i>NIM1K</i>	Serine/threonine-protein kinase NI	-25.521837	N/A	N/A	N/A	N/A	N/A
937	Q8IZ81	<i>ELMOD2</i>	ELMO domain-containing protein 2	-23.410215	N/A	N/A	N/A	N/A	N/A
938	Q8IZH2	<i>XRN1</i>	5'-3' exoribonuclease 1 OS=Homo s	N/A	N/A	N/A	N/A	N/A	N/A
939	Q8N128	<i>FAM177A1</i>	Protein FAM177A1 OS=Homo sapie	-20.929403	N/A	N/A	N/A	N/A	N/A
940	Q8N2K0	<i>ABHD12</i>	Monoacylglycerol lipase ABHD12 O	-22.8626	N/A	N/A	N/A	N/A	N/A
941	Q8N5K1	<i>CISD2</i>	CDGSH iron-sulfur domain-containi	-23.071267	N/A	N/A	N/A	N/A	N/A
942	Q8N6T3	<i>ARFGAP1</i>	ADP-ribosylation factor GTPase-act	-22.648884	N/A	N/A	N/A	N/A	N/A
943	Q8N766	<i>EMC1</i>	ER membrane protein complex sub	N/A	N/A	N/A	N/A	N/A	N/A
944	Q8NB25	<i>FAM184A</i>	Protein FAM184A OS=Homo sapien	0	N/A	-20.823653	N/A	N/A	N/A
945	Q8NF37	<i>LPCAT1</i>	Lysophosphatidylcholine acyltransf	-22.866009	N/A	N/A	N/A	N/A	N/A
946	Q8TB22	<i>SPATA20</i>	Spermatogenesis-associated protei	N/A	N/A	N/A	N/A	N/A	N/A
947	Q8TCJ2	<i>STT3B</i>	Dolichyl-diphosphooligosaccharide-	N/A	N/A	N/A	N/A	N/A	N/A
948	Q8TCT9	<i>HM13</i>	Minor histocompatibility antigen H	-22.411764	N/A	N/A	N/A	N/A	N/A
949	Q8TD55	<i>PLEKHO2</i>	Pleckstrin homology domain-contai	N/A	N/A	N/A	N/A	N/A	N/A
950	Q8WUH6	<i>TMEM263</i>	Transmembrane protein 263 OS=H	-17.340333	N/A	N/A	N/A	N/A	N/A
951	Q8WVM8	<i>SCFD1</i>	Sec1 family domain-containing prot	-26.102821	N/A	N/A	N/A	N/A	N/A
952	Q8WW12	<i>PCNP</i>	PEST proteolytic signal-containing n	N/A	-21.397544	N/A	N/A	N/A	N/A
953	Q8WVG9	<i>ADGRV1</i>	G-protein coupled receptor 98 OS=	N/A	-24.882402	N/A	N/A	N/A	N/A
954	Q92522	<i>H1-10</i>	Histone H1x OS=Homo sapiens GN=	N/A	N/A	N/A	N/A	N/A	N/A
955	Q92542	<i>NCSTN</i>	Nicastrin OS=Homo sapiens GN=NC	N/A	N/A	N/A	N/A	N/A	N/A
956	Q92575	<i>UBXN4</i>	UBX domain-containing protein 4 O	-23.851228	N/A	N/A	N/A	N/A	N/A
957	Q92598	<i>HSPH1</i>	Heat shock protein 105 kDa OS=Ho	N/A	-24.880981	-24.646244	N/A	N/A	N/A
958	Q92841	<i>DDX17</i>	Probable ATP-dependent RNA helic	-24.646588	-24.582785	-23.663046	N/A	N/A	N/A
959	Q92882	<i>OSTF1</i>	Osteoclast-stimulating factor 1 OS=	N/A	-23.609833	-22.810415	N/A	N/A	N/A

960	Q92896	GLG1	Golgi apparatus protein 1 OS=Hom	-24.638161	N/A	N/A	N/A	N/A	N/A
961	Q93050	ATP6V0A1	V-type proton ATPase 116 kDa sub	N/A	N/A	N/A	N/A	N/A	N/A
962	Q93088	BHMT	Betaine--homocysteine S-methyltra	-19.361314	N/A	N/A	N/A	N/A	N/A
963	Q95604	HLA-C	HLA class I histocompatibility antige	N/A	N/A	N/A	0	N/A	N/A
964	Q969V3	NCLN	Nicalin OS=Homo sapiens GN=NCLN	N/A	N/A	N/A	N/A	N/A	N/A
965	Q969X5	ERGIC1	Endoplasmic reticulum-Golgi interm	-24.132609	N/A	N/A	N/A	N/A	N/A
966	Q96A33	CCDC47	Coiled-coil domain-containing prot	-22.921635	N/A	N/A	N/A	N/A	N/A
967	Q96AD5	PNPLA2	Patatin-like phospholipase domain-	-21.891932	-24.174781	N/A	N/A	N/A	N/A
968	Q96AT9	RPE	Ribulose-phosphate 3-epimerase O	N/A	N/A	-22.907918	N/A	N/A	N/A
969	Q96B49	TOMM6	Mitochondrial import receptor sub	-22.020913	N/A	N/A	N/A	N/A	N/A
970	Q96BI1	SLC22A18	Solute carrier family 22 member 18	N/A	N/A	N/A	N/A	N/A	N/A
971	Q96BM9	ARL8A	ADP-ribosylation factor-like protein	-20.661916	N/A	N/A	N/A	N/A	N/A
972	Q96BR5	COA7	Cytochrome c oxidase assembly fac	-21.353066	N/A	-22.37014	N/A	N/A	N/A
973	Q96C01	FAM136A	Protein FAM136A OS=Homo sapien	-21.031501	N/A	N/A	N/A	N/A	N/A
974	Q96C19	EFHD2	EF-hand domain-containing protein	-22.484096	-20.389814	-18.904111	N/A	N/A	N/A
975	Q96CS4	ZNF689	Zinc finger protein 689 OS=Homo s	N/A	N/A	N/A	N/A	N/A	N/A
976	Q96DA0	ZG16B	Zymogen granule protein 16 homol	N/A	N/A	-21.102212	N/A	N/A	N/A
977	Q96DB9	FXVD5	FXVD domain-containing ion transp	N/A	N/A	N/A	N/A	N/A	N/A
978	Q96E22	NUS1	Dehydrodolichyl diphosphate synth	-22.197371	N/A	N/A	N/A	N/A	N/A
979	Q96FJ2	DYNLL2	Dynein light chain 1, cytoplasmic O	-19.522742	N/A	N/A	N/A	N/A	N/A
980	Q96FZ7	CHMP6	Charged multivesicular body protei	-22.460305	N/A	N/A	N/A	N/A	N/A
981	Q96G23	CERS2	Ceramide synthase 2 OS=Homo sap	-21.875732	N/A	N/A	N/A	N/A	N/A
982	Q96HE7	ERO1A	ERO1-like protein alpha OS=Homo	-23.391191	N/A	N/A	N/A	N/A	N/A
983	Q96HS1	PGAM5	Serine/threonine-protein phosphat	-22.494057	N/A	N/A	N/A	N/A	N/A
984	Q96HY6	DDRKG1	DDRKG domain-containing protein	-21.962199	N/A	N/A	N/A	N/A	N/A
985	Q96JB5	CDK5RAP3	CDK5 regulatory subunit-associated	-22.889098	N/A	N/A	N/A	N/A	N/A
986	Q96JJ7	TMX3	Protein disulfide-isomerase TMX3 O	-22.462372	N/A	N/A	N/A	N/A	N/A
987	Q99442	SEC62	Translocation protein SEC62 OS=Ho	-22.460132	N/A	N/A	N/A	N/A	N/A
988	Q99489	DDO	D-aspartate oxidase OS=Homo sapi	N/A	N/A	N/A	N/A	N/A	N/A
989	Q99714	HSD17B10	3-hydroxyacyl-CoA dehydrogenase	-20.935906	N/A	N/A	N/A	N/A	N/A
990	Q99805	TM9SF2	Transmembrane 9 superfamily me	N/A	N/A	N/A	N/A	N/A	N/A
991	Q9BQA1	WDR77	Methylosome protein 50 OS=Homo	N/A	N/A	-22.863814	N/A	N/A	N/A
992	Q9BQE5	APOL2	Apolipoprotein L2 OS=Homo sapien	-23.655994	N/A	N/A	N/A	N/A	N/A
993	Q9BRP8	PYM1	Partner of Y14 and mago OS=Homo	N/A	N/A	-21.840654	N/A	N/A	N/A
994	Q9BRQ8	AIFM2	Apoptosis-inducing factor 2 OS=Ho	-22.150857	N/A	N/A	N/A	N/A	N/A
995	Q9BTV4	TMEM43	Transmembrane protein 43 OS=Ho	-21.049764	N/A	N/A	N/A	N/A	N/A
996	Q9BUF5	TUBB6	Tubulin beta-6 chain OS=Homo sap	N/A	N/A	N/A	N/A	N/A	N/A
997	Q9BV40	VAMP8	Vesicle-associated membrane prot	-22.128181	N/A	N/A	N/A	N/A	N/A
998	Q9BVG4	PBDC1	Protein PBDC1 OS=Homo sapiens G	N/A	N/A	-21.934594	N/A	N/A	N/A
999	Q9BVK6	TMED9	Transmembrane emp24 domain-co	-22.93876	N/A	N/A	N/A	N/A	N/A
1000	Q9BW60	ELOVL1	Elongation of very long chain fatty	-21.582217	N/A	N/A	N/A	N/A	N/A
1001	Q9BWM7	SFXN3	Sideroflexin-3 OS=Homo sapiens G	-20.766677	N/A	N/A	N/A	N/A	N/A
1002	Q9BYX7	POTEKP	Putative beta-actin-like protein 3 O	N/A	N/A	N/A	N/A	N/A	N/A
1003	Q9BYZ2	LDHAL6B	L-lactate dehydrogenase A-like 6B	0	N/A	N/A	N/A	N/A	N/A
1004	Q9C005	DPY30	Protein dpy-30 homolog OS=Homo	N/A	-18.814558	-19.715029	N/A	N/A	N/A
1005	Q9C037	TRIM4	E3 ubiquitin-protein ligase TRIM4 O	-25.848243	N/A	N/A	N/A	N/A	N/A
1006	Q9H0C2	SLC25A31	ADP/ATP translocase 4 OS=Homo s	0	N/A	N/A	N/A	N/A	N/A
1007	Q9H0U3	MAGT1	Magnesium transporter protein 1 O	-21.68709	N/A	N/A	N/A	N/A	N/A
1008	Q9H3K6	BOLA2	BolA-like protein 2 OS=Homo sapie	N/A	N/A	-19.800014	N/A	N/A	N/A
1009	Q9H444	CHMP4B	Charged multivesicular body protei	N/A	-20.417618	-20.006748	N/A	N/A	N/A
1010	Q9H4G4	GLIPR2	Golgi-associated plant pathogenesi	-21.351343	N/A	N/A	N/A	N/A	N/A

1011	Q9H845	ACAD9	Acyl-CoA dehydrogenase family me	-23.525114	N/A	N/A	N/A	N/A	N/A
1012	Q9HBL7	PLGRKT	Plasminogen receptor (KT) OS=Hom	N/A	N/A	N/A	N/A	N/A	N/A
1013	Q9HC07	TMEM165	Transmembrane protein 165 OS=H	N/A	N/A	N/A	N/A	N/A	N/A
1014	Q9HC84	MUC5B	Mucin-5B OS=Homo sapiens GN=M	N/A	N/A	-26.099436	N/A	N/A	N/A
1015	Q9HCC0	MCCC2	Methylcrotonoyl-CoA carboxylase b	-24.068762	N/A	N/A	N/A	N/A	N/A
1016	Q9HDC9	APMAP	Adipocyte plasma membrane-assoc	-20.732663	N/A	N/A	N/A	N/A	N/A
1017	Q9NPJ3	ACOT13	Acyl-coenzyme A thioesterase 13 O	-21.804515	N/A	N/A	N/A	N/A	N/A
1018	Q9NQ50	MRPL40	39S ribosomal protein L40, mitocho	-23.121516	N/A	N/A	N/A	N/A	N/A
1019	Q9NQX3	GPHN	Gephyrin OS=Homo sapiens GN=GP	-24.806338	N/A	N/A	N/A	N/A	N/A
1020	Q9NSE4	IARS2	Isoleucine--tRNA ligase, mitochond	-22.46278	N/A	N/A	N/A	N/A	N/A
1021	Q9NUJ1	ABHD10	Mycophenolic acid acyl-glucuronide	-23.500988	N/A	N/A	N/A	N/A	N/A
1022	Q9NVI7	ATAD3A	ATPase family AAA domain-contain	-23.087365	N/A	N/A	N/A	N/A	N/A
1023	Q9NVJ2	ARL8B	ADP-ribosylation factor-like protein	-20.661916	N/A	N/A	0	N/A	N/A
1024	Q9NWB6	ARGLU1	Arginine and glutamate-rich protei	N/A	N/A	N/A	N/A	N/A	N/A
1025	Q9NXH8	TOR4A	Torsin-4A OS=Homo sapiens GN=T	N/A	N/A	N/A	N/A	N/A	N/A
1026	Q9NYV4	CDK12	Cyclin-dependent kinase 12 OS=Ho	N/A	N/A	N/A	N/A	N/A	N/A
1027	Q9NZ01	TECR	Very-long-chain enoyl-CoA reducta	-21.318797	N/A	N/A	N/A	N/A	N/A
1028	Q9NZJ7	MTCH1	Mitochondrial carrier homolog 1 O	N/A	N/A	N/A	N/A	N/A	N/A
1029	Q9NZR1	TMOD2	Tropomodulin-2 OS=Homo sapiens	N/A	N/A	0	N/A	N/A	N/A
1030	Q9P0P8	MTRES1	Uncharacterized protein C6orf203	-23.226651	N/A	N/A	N/A	N/A	N/A
1031	Q9P107	GMIP	GEM-interacting protein OS=Homo	-26.383171	N/A	N/A	N/A	N/A	N/A
1032	Q9UBM7	DHCR7	7-dehydrocholesterol reductase OS	-23.897566	N/A	N/A	N/A	N/A	N/A
1033	Q9UBV2	SEL1L	Protein sel-1 homolog 1 OS=Homo	-22.482312	N/A	N/A	N/A	N/A	N/A
1034	Q9UHG3	PCYOX1	Preylcysteine oxidase 1 OS=Homo	-23.412429	N/A	N/A	N/A	N/A	N/A
1035	Q9UHQ9	CYB5R1	NADH-cytochrome b5 reductase 1	N/A	N/A	N/A	N/A	N/A	N/A
1036	Q9UI08	EVL	Ena/VASP-like protein OS=Homo sa	-26.436917	N/A	N/A	N/A	N/A	N/A
1037	Q9UI12	ATP6V1H	V-type proton ATPase subunit H OS	N/A	N/A	N/A	N/A	N/A	N/A
1038	Q9UIJ7	AK3	GTP:AMP phosphotransferase AK3,	-22.837644	N/A	N/A	N/A	N/A	N/A
1039	Q9UKK9	NUDT5	ADP-sugar pyrophosphatase OS=Ho	N/A	-21.695695	N/A	N/A	N/A	N/A
1040	Q9UKM9	RALY	RNA-binding protein Raly OS=Hom	N/A	N/A	-23.768141	N/A	N/A	N/A
1041	Q9UKT9	IKZF3	Zinc finger protein Aiolos OS=Homo	N/A	N/A	N/A	N/A	N/A	N/A
1042	Q9UKV3	ACIN1	Apoptotic chromatin condensation	N/A	N/A	N/A	N/A	N/A	N/A
1043	Q9UL25	RAB21	Ras-related protein Rab-21 OS=Ho	-19.937115	-21.872207	N/A	N/A	N/A	N/A
1044	Q9ULV4	CORO1C	Coronin-1C OS=Homo sapiens GN=	-21.696319	N/A	-22.973302	N/A	N/A	N/A
1045	Q9UM00	TMCO1	Calcium load-activated calcium cha	-20.462875	N/A	N/A	N/A	N/A	N/A
1046	Q9UNL2	SSR3	Translocon-associated protein subu	N/A	N/A	N/A	N/A	N/A	N/A
1047	Q9UNP9	PPIE	Peptidyl-prolyl cis-trans isomerase	-21.957474	N/A	N/A	N/A	N/A	N/A
1048	Q9UQ80	PA2G4	Proliferation-associated protein 2G	N/A	-23.520154	N/A	N/A	N/A	N/A
1049	Q9Y230	RUVBL2	RuvB-like 2 OS=Homo sapiens GN=	N/A	N/A	-22.09063	N/A	N/A	N/A
1050	Q9Y2Q3	GSTK1	Glutathione S-transferase kappa 1	-21.201124	N/A	N/A	N/A	N/A	N/A
1051	Q9Y3A6	TMED5	Transmembrane emp24 domain-co	N/A	N/A	N/A	N/A	N/A	N/A
1052	Q9Y3B3	TMED7	Transmembrane emp24 domain-co	-21.381609	N/A	N/A	N/A	N/A	N/A
1053	Q9Y3Z3	SAMHD1	Deoxynucleoside triphosphate triph	-21.753562	N/A	N/A	N/A	N/A	N/A
1054	Q9Y4B5	MTCL1	Microtubule cross-linking factor 1 O	N/A	N/A	N/A	N/A	N/A	N/A
1055	Q9Y4P3	TBL2	Transducin beta-like protein 2 OS=	-23.110596	N/A	N/A	N/A	N/A	N/A
1056	Q9Y4W6	AFG3L2	AFG3-like protein 2 OS=Homo sapie	-22.963965	N/A	N/A	N/A	N/A	N/A
1057	Q9Y512	SAMM50	Sorting and assembly machinery co	N/A	N/A	N/A	N/A	N/A	N/A
1058	Q9Y520	PRRC2C	Protein PRRC2C OS=Homo sapiens	-30.674364	N/A	N/A	N/A	N/A	N/A
1059	Q9Y5L4	TIMM13	Mitochondrial import inner membr	-18.902033	N/A	N/A	N/A	N/A	N/A
1060	Q9Y5S9	RBM8A	RNA-binding protein 8A OS=Homo	N/A	N/A	-20.592903	N/A	N/A	N/A
1061	Q9Y600	CSAD	Cysteine sulfinic acid decarboxylase	-23.458666	N/A	N/A	N/A	N/A	N/A

1062	Q9Y639	<i>NPTN</i>	Neuroplastin OS=Homo sapiens GN	-23.832661	N/A	N/A	N/A	N/A	N/A
1063	Q9Y673	<i>ALG5</i>	Dolichyl-phosphate beta-glucosyltr	N/A	N/A	N/A	N/A	N/A	N/A
1064	P05455	<i>SSB</i>	Lupus La protein OS=Homo sapiens	N/A	N/A	-22.685066	N/A	N/A	0
1065	P06899	<i>H2BC11</i>	Histone H2B type 1-J OS=Homo sap	0	0	0	0	0	0
1066	P09429	<i>HMGB1</i>	Putative high mobility group protei	0	-22.18288	-22.915109	N/A	N/A	0
1067	P09972	<i>ALDOC</i>	Fructose-bisphosphate aldolase C O	N/A	N/A	0	N/A	N/A	0
1068	P11940	<i>PABPC1</i>	Polyadenylate-binding protein 1 OS	0	N/A	N/A	N/A	N/A	0
1069	P23527	<i>H2BC17</i>	Histone H2B type 1-J OS=Homo sap	0	0	0	0	0	0
1070	P27635	<i>RPL10</i>	60S ribosomal protein L10 OS=Hom	0	0	0	0	N/A	0
1071	P33778	<i>H2BC3</i>	Histone H2B type 1-J OS=Homo sap	0	0	0	0	0	0
1072	P48668	<i>KRT6C</i>	Keratin, type II cytoskeletal 6C OS=	N/A	N/A	N/A	N/A	N/A	0
1073	P62136	<i>PPP1CA</i>	Serine/threonine-protein phosphat	N/A	N/A	N/A	N/A	N/A	0
1074	P67775	<i>PPP2CA</i>	Serine/threonine-protein phosphat	N/A	-21.090428	-20.521574	N/A	N/A	0
1075	P68032	<i>ACTC1</i>	Actin, aortic smooth muscle OS=Ho	0	0	0	0	0	0
1076	P68133	<i>ACTA1</i>	Actin, alpha cardiac muscle 1 OS=H	0	N/A	0	0	0	0
1077	Q16778	<i>H2BC21</i>	Histone H2B type 1-J OS=Homo sap	0	0	0	0	0	0
1078	Q562R1	<i>ACTBL2</i>	Beta-actin-like protein 2 OS=Homo	N/A	N/A	N/A	N/A	N/A	0
1079	Q7RTS7	<i>KRT74</i>	Keratin, type II cytoskeletal 74 OS=	0	0	0	0	0	0
1080	Q8WZ42	<i>TTN</i>	Titin OS=Homo sapiens GN=TTN PE	N/A	N/A	N/A	0	N/A	0
1081	Q9H0E9	<i>BRD8</i>	Bromodomain-containing protein 8	N/A	N/A	N/A	N/A	N/A	0
1082	Q9H0U4	<i>RAB1B</i>	Ras-related protein Rab-1A OS=Ho	0	N/A	-23.08613	0	N/A	0
1083	Q9ULJ1	<i>ODF2L</i>	Outer dense fiber protein 2-like OS	N/A	N/A	N/A	N/A	N/A	0
1084	Q16629	<i>SRSF7</i>	Serine/arginine-rich splicing factor	-20.373247	-20.143061	-18.869506	N/A	N/A	-16.175258
1085	P14174	<i>MIF</i>	Macrophage migration inhibitory fa	-20.905105	-18.590209	-17.089191	N/A	N/A	-17.470117
1086	P63220	<i>RPS21</i>	40S ribosomal protein S21 OS=Hom	-22.072422	-17.702589	-17.134151	N/A	N/A	-17.623411
1087	P25398	<i>RPS12</i>	40S ribosomal protein S12 OS=Hom	-19.792615	-19.824648	-17.516242	N/A	N/A	-17.864474
1088	P62318	<i>SNRPD3</i>	Small nuclear ribonucleoprotein Sm	N/A	-16.019515	-17.860219	N/A	N/A	-17.885668
1089	P51858	<i>HDGF</i>	Hepatoma-derived growth factor O	-22.538471	-18.080614	-18.589362	N/A	N/A	-17.936773
1090	P84090	<i>ERH</i>	Enhancer of rudimentary homolog	N/A	-18.383367	-16.92911	N/A	N/A	-18.163966
1091	Q86UA1	<i>PRPF39</i>	Pre-mRNA-processing factor 39 OS	N/A	N/A	N/A	N/A	N/A	-18.187702
1092	Q04760	<i>GLO1</i>	Lactoylglutathione lyase OS=Homo	N/A	-18.654935	-18.166128	N/A	0	-18.41362
1093	Q9HCY8	<i>S100A14</i>	Protein S100-A14 OS=Homo sapien	N/A	N/A	N/A	N/A	N/A	-18.895226
1094	Q15185	<i>PTGES3</i>	Prostaglandin E synthase 3 OS=Hom	-21.438802	-18.76471	-18.130109	N/A	N/A	-18.906242
1095	O75368	<i>SH3BGR1</i>	SH3 domain-binding glutamic acid-	N/A	-18.431478	-17.966268	N/A	N/A	-19.108907
1096	P22528	<i>SPRR1B</i>	Cornifin-B OS=Homo sapiens GN=S	-20.199055	-19.229756	-19.804603	N/A	N/A	-19.109367
1097	P26447	<i>S100A4</i>	Protein S100-A4 OS=Homo sapiens	-20.244666	-20.277784	-17.820122	N/A	N/A	-19.166673
1098	P49458	<i>SRP9</i>	Signal recognition particle 9 kDa pr	N/A	N/A	N/A	N/A	N/A	-19.169
1099	P62995	<i>TRA2B</i>	Transformer-2 protein homolog be	-21.572014	-19.987345	-18.985983	N/A	N/A	-19.385894
1100	A8MWD9	<i>SNRPGP15</i>	Putative small nuclear ribonucleopr	N/A	-18.650212	-21.772884	N/A	N/A	-19.516424
1101	P62308	<i>SNRPG</i>	Putative small nuclear ribonucleopr	N/A	-18.650212	-21.772884	N/A	N/A	-19.516424
1102	P30086	<i>PEBP1</i>	Phosphatidylethanolamine-binding	-22.464655	-19.441017	-19.036188	N/A	N/A	-19.659907
1103	O14950	<i>MYL12B</i>	Myosin regulatory light chain 12B O	-20.805741	-20.480115	-19.721539	N/A	N/A	-19.663479
1104	P19105	<i>MYL12A</i>	Myosin regulatory light chain 12B O	-20.805741	-20.480115	-19.721539	N/A	N/A	-19.663479
1105	P62910	<i>RPL32</i>	60S ribosomal protein L32 OS=Hom	-19.114375	-22.967311	-18.173939	N/A	N/A	-19.802088
1106	P84098	<i>RPL19</i>	60S ribosomal protein L19 OS=Hom	-21.548747	-19.790933	-20.025179	N/A	N/A	-19.818499
1107	O60506	<i>SYNCRIP</i>	Heterogeneous nuclear ribonucleo	-23.424919	-23.934601	-22.804514	N/A	N/A	-19.957466
1108	P37802	<i>TAGLN2</i>	Transgelin-2 OS=Homo sapiens GN	-21.254905	-19.125167	-17.975905	N/A	N/A	-19.962884
1109	Q9Y3U8	<i>RPL36</i>	60S ribosomal protein L36 OS=Hom	-19.682209	-19.197283	-18.868554	N/A	N/A	-19.965814
1110	P52566	<i>ARHGD1B</i>	Rho GDP-dissociation inhibitor 2 OS	N/A	-20.343645	-21.207974	N/A	N/A	-20.10375
1111	Q15121	<i>PEA15</i>	Astrocytic phosphoprotein PEA-15	N/A	-20.113475	-19.888534	N/A	N/A	-20.164768
1112	P29401	<i>TKT</i>	Transketolase OS=Homo sapiens G	-21.204652	-21.375258	-18.810462	N/A	0	-20.201993

1113	Q13185	CBX3	Chromobox protein homolog 3 OS=	N/A	-20.743083	-19.633954	N/A	N/A	-20.228971
1114	P62906	RPL10A	60S ribosomal protein L10a OS=Ho	-21.664458	-20.222665	-20.207232	N/A	N/A	-20.243566
1115	P28072	PSMB6	Proteasome subunit beta type-6 OS	-22.664561	-21.296123	-19.410941	N/A	N/A	-20.251849
1116	P06703	S100A6	Protein S100-A6 OS=Homo sapiens	N/A	-19.793622	N/A	N/A	N/A	-20.279648
1117	P22531	SPRR2E	Small proline-rich protein 2E OS=H	-20.967083	-20.63818	-21.006257	N/A	0	-20.279936
1118	P22532	SPRR2D	Small proline-rich protein 2E OS=H	-20.967083	-20.63818	-21.006257	N/A	N/A	-20.279936
1119	P35325	SPRR2B	Small proline-rich protein 2E OS=H	-20.967083	-20.63818	-21.006257	N/A	N/A	-20.279936
1120	P35326	SPRR2A	Small proline-rich protein 2E OS=H	-20.967083	-20.63818	-21.006257	N/A	N/A	-20.279936
1121	Q9BYE4	SPRR2G	Small proline-rich protein 2E OS=H	-20.967083	-20.63818	-21.006257	N/A	N/A	-20.279936
1122	Q7RTV0	PHF5A	PHD finger-like domain-containing	N/A	N/A	-20.219828	N/A	N/A	-20.385473
1123	P43487	RANBP1	Ran-specific GTPase-activating prot	-21.878962	-19.421237	-19.453155	N/A	N/A	-20.439523
1124	P58546	MTPN	Myotrophin OS=Homo sapiens GN=	N/A	N/A	-19.405493	N/A	N/A	-20.512481
1125	P20962	PTMS	Parathymosin OS=Homo sapiens G	N/A	-20.029846	-20.0153	N/A	N/A	-20.554769
1126	P56537	EIF6	Eukaryotic translation initiation fac	N/A	-22.14172	-19.481736	N/A	N/A	-20.596781
1127	P31948	STIP1	Stress-induced-phosphoprotein 1 O	-23.482617	-21.970987	-20.344121	N/A	N/A	-20.622874
1128	Q14019	COTL1	Coactosin-like protein OS=Homo sa	N/A	N/A	-19.891912	N/A	N/A	-20.62703
1129	P83731	RPL24	60S ribosomal protein L24 OS=Hom	-20.367411	-19.823151	-19.801347	N/A	N/A	-20.682713
1130	P28838	LAP3	Cytosol aminopeptidase OS=Homo	-22.340141	-22.150625	-20.836792	N/A	N/A	-20.690941
1131	P45973	CBX5	Chromobox protein homolog 5 OS=	N/A	-21.636474	-20.513596	N/A	N/A	-20.716365
1132	P62942	FKBP1A	Peptidyl-prolyl cis-trans isomerase	N/A	N/A	-20.681847	N/A	N/A	-20.723082
1133	P08865	RPSA	40S ribosomal protein SA OS=Hom	-20.841794	-19.362908	-18.38281	N/A	N/A	-20.795596
1134	P62841	RPS15	40S ribosomal protein S15 OS=Hom	-20.993498	-20.885941	-20.443818	N/A	N/A	-20.804347
1135	P31943	HNRNPH1	Heterogeneous nuclear ribonucleo	-23.327434	-21.121433	-20.610797	N/A	N/A	-20.807752
1136	P59665	DEFA1	Neutrophil defensin 1 OS=Homo sa	N/A	N/A	-19.006482	N/A	N/A	-20.811786
1137	P59666	DEFA3	Neutrophil defensin 1 OS=Homo sa	N/A	N/A	-19.006482	N/A	N/A	-20.811786
1138	P78371	CCT2	T-complex protein 1 subunit beta O	-22.040701	-22.055827	-20.883996	N/A	N/A	-20.896096
1139	P62263	RPS14	40S ribosomal protein S14 OS=Hom	-19.243384	-21.669923	-20.445749	N/A	N/A	-20.907505
1140	P14866	HNRNPL	Heterogeneous nuclear ribonucleo	-24.137881	-21.686799	-21.3119	N/A	0	-20.98129
1141	P49773	HINT1	Histidine triad nucleotide-binding p	-21.496278	-21.373153	-20.678617	N/A	N/A	-20.994046
1142	Q9BRA2	TXNDC17	Thioredoxin domain-containing pro	N/A	-21.183321	-20.153288	N/A	N/A	-21.022296
1143	P51397	DAP	Death-associated protein 1 OS=Ho	N/A	-20.732255	-20.566541	N/A	N/A	-21.069558
1144	P14317	HCLS1	Hematopoietic lineage cell-specific	N/A	-20.430263	-19.529765	N/A	N/A	-21.081383
1145	P13489	RNH1	Ribonuclease inhibitor OS=Homo sa	-25.17867	-23.645033	-21.583925	N/A	N/A	-21.107488
1146	P39687	ANP32A	Acidic leucine-rich nuclear phospho	N/A	-21.077935	-18.508517	N/A	N/A	-21.128505
1147	P40429	RPL13A	60S ribosomal protein L13a OS=Ho	-21.0292	N/A	-22.138735	N/A	N/A	-21.131889
1148	Q92945	KHSRP	Far upstream element-binding prot	-23.601432	-22.725496	-21.107637	N/A	N/A	-21.211143
1149	P62633	CNBP	Cellular nucleic acid-binding protein	N/A	N/A	N/A	N/A	N/A	-21.224619
1150	P52565	ARHGDIA	Rho GDP-dissociation inhibitor 1 OS	N/A	-21.011999	-20.937413	N/A	N/A	-21.277037
1151	O00299	CLIC1	Chloride intracellular channel prote	-23.06615	-20.343057	-19.230691	N/A	N/A	-21.292743
1152	Q99729	HNRNPAB	Heterogeneous nuclear ribonucleo	-22.683135	-21.167652	-20.953918	N/A	N/A	-21.319249
1153	Q86U42	PABPN1	Polyadenylate-binding protein 2 OS	N/A	-22.404907	-21.536679	N/A	N/A	-21.391407
1154	P47914	RPL29	60S ribosomal protein L29 OS=Hom	N/A	-19.641062	-19.287839	N/A	N/A	-21.459452
1155	Q9BUJ2	HNRNPUL1	Heterogeneous nuclear ribonucleo	-23.667652	-21.428806	-19.863818	N/A	N/A	-21.493792
1156	P63244	RACK1	Receptor of activated protein C kin	-23.173841	-22.464667	-22.05385	N/A	N/A	-21.494462
1157	Q96EP5	DAZAP1	DAZ-associated protein 1 OS=Homo	N/A	-24.237695	-21.69578	N/A	N/A	-21.527769
1158	P37108	SRP14	Signal recognition particle 14 kDa p	N/A	N/A	N/A	N/A	N/A	-21.544109
1159	Q9UHV9	PFDN2	Prefoldin subunit 2 OS=Homo sapie	N/A	N/A	N/A	N/A	N/A	-21.632523
1160	O43707	ACTN4	Alpha-actinin-4 OS=Homo sapiens	-23.196691	-21.971817	-21.189237	N/A	N/A	-21.658112
1161	O75083	WDR1	WD repeat-containing protein 1 OS	N/A	-22.810956	-20.79829	N/A	N/A	-21.690525
1162	O60234	GMFG	Glia maturation factor gamma OS=	N/A	N/A	-21.37182	N/A	N/A	-21.737388
1163	Q15056	EIF4H	Eukaryotic translation initiation fac	N/A	-20.685712	-20.607562	N/A	N/A	-21.738465

1164	P83881	<i>RPL36A</i>	60S ribosomal protein L36a OS=Ho	-22.431771	N/A	-19.953711	N/A	N/A	-21.774562
1165	Q969Q0	<i>RPL36AL</i>	60S ribosomal protein L36a OS=Ho	N/A	N/A	-19.953711	N/A	N/A	-21.774562
1166	P61956	<i>SUMO2</i>	Small ubiquitin-related modifier 2	N/A	N/A	N/A	N/A	N/A	-21.836374
1167	Q9Y2W1	<i>THRAP3</i>	Thyroid hormone receptor-associat	-24.554753	-21.971646	-21.120542	N/A	N/A	-21.840434
1168	P61970	<i>NUTF2</i>	Nuclear transport factor 2 OS=Hom	N/A	N/A	N/A	N/A	N/A	-21.856274
1169	P49368	<i>CCT3</i>	T-complex protein 1 subunit gamm	-22.108223	-21.699186	-21.464509	N/A	N/A	-21.914709
1170	Q12905	<i>ILF2</i>	Interleukin enhancer-binding factor	N/A	N/A	-21.313424	N/A	N/A	-21.920399
1171	P01036	<i>CST4</i>	Cystatin-S OS=Homo sapiens GN=C	N/A	N/A	N/A	N/A	N/A	-21.953549
1172	P01037	<i>CST1</i>	Cystatin-S OS=Homo sapiens GN=C	N/A	N/A	N/A	N/A	N/A	-21.953549
1173	P49321	<i>NASP</i>	Nuclear autoantigenic sperm protei	N/A	-24.162996	-20.880479	N/A	N/A	-21.969519
1174	P25787	<i>PSMA2</i>	Proteasome subunit alpha type-2 O	N/A	-21.885947	-19.152898	N/A	N/A	-21.986296
1175	P0DN76	<i>U2AF1L5</i>	Splicing factor U2AF 35 kDa subunit	N/A	N/A	N/A	N/A	N/A	-22.049553
1176	Q01081	<i>U2AF1</i>	Splicing factor U2AF 35 kDa subunit	N/A	N/A	N/A	N/A	N/A	-22.049553
1177	Q8IYS4	<i>C16orf71</i>	Uncharacterized protein C16orf71	N/A	N/A	N/A	N/A	N/A	-22.070652
1178	P50502	<i>ST13</i>	Hsc70-interacting protein OS=Hom	-23.086609	-22.253092	-22.32931	N/A	N/A	-22.084003
1179	Q8IZP2	<i>ST13P4</i>	Hsc70-interacting protein OS=Hom	-23.086609	-22.253092	-22.32931	N/A	N/A	-22.084003
1180	Q13151	<i>HNRNPA0</i>	Heterogeneous nuclear ribonucleo	N/A	-22.47108	-21.937555	N/A	N/A	-22.114108
1181	Q86UX7	<i>FERMT3</i>	Fermitin family homolog 3 OS=Hom	-22.391037	-22.160934	-21.857287	N/A	N/A	-22.136082
1182	Q00610	<i>CLTC</i>	Clathrin heavy chain 1 OS=Homo sa	-22.503299	-23.604052	-23.001408	N/A	N/A	-22.140352
1183	O14737	<i>PDCD5</i>	Programmed cell death protein 5 O	N/A	-21.525517	-21.232817	N/A	N/A	-22.185119
1184	Q9UJU6	<i>DBNL</i>	Drebrin-like protein OS=Homo sapi	-24.159005	-22.636818	-22.038867	N/A	N/A	-22.204948
1185	P61160	<i>ACTR2</i>	Actin-related protein 2 OS=Homo s	-23.275641	-22.951896	-21.347022	N/A	N/A	-22.246273
1186	P37837	<i>TALDO1</i>	Transaldolase OS=Homo sapiens G	-21.165217	-21.078029	-19.937979	N/A	N/A	-22.268823
1187	Q16555	<i>DPYSL2</i>	Dihydropyrimidinase-related protei	N/A	-22.192423	-21.257084	N/A	N/A	-22.337627
1188	P46777	<i>RPL5</i>	60S ribosomal protein L5 OS=Homo	-21.961531	-22.104127	-21.097346	N/A	N/A	-22.410315
1189	Q13435	<i>SF3B2</i>	Splicing factor 3B subunit 2 OS=Ho	N/A	-23.433252	-21.611299	N/A	N/A	-22.444349
1190	P31153	<i>MAT2A</i>	S-adenosylmethionine synthase iso	N/A	-24.388062	-23.334662	N/A	N/A	-22.467769
1191	Q08211	<i>DHX9</i>	ATP-dependent RNA helicase A OS=	-24.351841	-24.287907	-22.826552	N/A	N/A	-22.508904
1192	P01040	<i>CSTA</i>	Cystatin-A OS=Homo sapiens GN=C	N/A	N/A	N/A	N/A	N/A	-22.561014
1193	P13693	<i>TPT1</i>	Translationally-controlled tumor pr	0	0	-22.219895	N/A	N/A	-22.615636
1194	Q96AE4	<i>FUBP1</i>	Far upstream element-binding prot	N/A	-22.418754	-21.509676	N/A	N/A	-22.63663
1195	P50991	<i>CCT4</i>	T-complex protein 1 subunit delta O	-24.635112	0	-22.32953	N/A	N/A	-22.720667
1196	P40227	<i>CCT6A</i>	T-complex protein 1 subunit zeta O	-25.225571	N/A	-23.083708	N/A	N/A	-22.729067
1197	P0CG05	<i>IGLC2</i>	Ig lambda-2 chain C regions OS=Ho	N/A	-23.09529	-22.156815	N/A	N/A	-22.7405
1198	P0CG06	<i>IGLC3</i>	Ig lambda-2 chain C regions OS=Ho	N/A	-23.09529	-22.156815	N/A	N/A	-22.7405
1199	P63241	<i>EIF5A</i>	Eukaryotic translation initiation fac	-24.026929	-22.790715	-22.398045	N/A	N/A	-22.753617
1200	Q9GZV4	<i>EIF5A2</i>	Eukaryotic translation initiation fac	-24.026929	-22.790715	-22.398045	N/A	N/A	-22.753617
1201	P55209	<i>NAP1L1</i>	Nucleosome assembly protein 1-lik	N/A	-20.743974	-21.381649	N/A	N/A	-22.782272
1202	Q06323	<i>PSME1</i>	Proteasome activator complex sub	-24.384819	-22.972546	-21.799379	N/A	N/A	-22.792576
1203	P07355	<i>ANXA2</i>	Annexin A2 OS=Homo sapiens GN=	-21.747538	-19.062872	-21.624225	0	0	-22.889613
1204	Q08188	<i>TGM3</i>	Protein-glutamine gamma-glutamyl	-23.035517	N/A	-22.705152	N/A	N/A	-22.919537
1205	P17987	<i>TCP1</i>	T-complex protein 1 subunit alpha	N/A	-22.927976	-21.858522	N/A	N/A	-22.949478
1206	Q9HB71	<i>CACYBP</i>	Calcyclin-binding protein OS=Homo	N/A	N/A	-22.539002	N/A	N/A	-22.977739
1207	Q14974	<i>KPNB1</i>	Importin subunit beta-1 OS=Homo	-24.044581	N/A	-21.730262	N/A	N/A	-23.010248
1208	P17980	<i>PSMC3</i>	26S protease regulatory subunit 6A	N/A	-23.197391	-22.483053	N/A	N/A	-23.014481
1209	Q9H0E2	<i>TOLLIP</i>	Toll-interacting protein OS=Homo s	N/A	N/A	-22.093066	N/A	N/A	-23.088039
1210	P48643	<i>CCT5</i>	T-complex protein 1 subunit epsilo	-23.124249	-23.272489	-22.925645	N/A	N/A	-23.113271
1211	P52597	<i>HNRNPF</i>	Heterogeneous nuclear ribonucleo	-24.229151	N/A	-21.58272	N/A	N/A	-23.116878
1212	P43686	<i>PSMC4</i>	26S protease regulatory subunit 6B	N/A	-24.691919	-22.421755	N/A	N/A	-23.123044
1213	P34932	<i>HSPA4</i>	Heat shock 70 kDa protein 4 OS=Ho	N/A	N/A	-24.000147	N/A	N/A	-23.151398
1214	Q9NYF8	<i>BCLAF1</i>	Bcl-2-associated transcription facto	-24.797507	-23.117818	-22.847144	N/A	N/A	-23.19377

1215	Q9UNF0	PACSLN2	Protein kinase C and casein kinase s	-23.584973	N/A	-22.278538	N/A	N/A	-23.211943
1216	P46782	RPS5	40S ribosomal protein S5 OS=Homo	-23.076357	-21.44402	-19.550116	N/A	N/A	-23.303185
1217	Q9UBS4	DNAJB11	DnaJ homolog subfamily B member	-21.835548	N/A	N/A	N/A	N/A	-23.30744
1218	Q9BTT0	ANP32E	Acidic leucine-rich nuclear phospho	N/A	N/A	N/A	N/A	N/A	-23.325096
1219	P09960	LTA4H	Leukotriene A-4 hydrolase OS=Hom	N/A	N/A	-22.243692	N/A	N/A	-23.333105
1220	P60900	PSMA6	Proteasome subunit alpha type-6 O	-21.544982	-21.74942	-19.348759	N/A	N/A	-23.354196
1221	P01857	IGHG1	Ig gamma-1 chain C region OS=Hom	N/A	N/A	N/A	N/A	N/A	-23.484543
1222	P01860	IGHG3	Ig gamma-1 chain C region OS=Hom	N/A	N/A	N/A	N/A	N/A	-23.484543
1223	P52209	PGD	6-phosphogluconate dehydrogenas	-22.148202	N/A	N/A	N/A	N/A	-23.489929
1224	Q01844	EWSR1	RNA-binding protein EWS OS=Hom	N/A	N/A	N/A	N/A	N/A	-23.503108
1225	P12956	XRCC6	X-ray repair cross-complementing p	-24.159155	-24.84427	-23.073341	N/A	N/A	-23.516843
1226	Q99832	CCT7	T-complex protein 1 subunit eta OS	N/A	-24.803493	-20.603316	N/A	N/A	-23.521243
1227	P49189	ALDH9A1	4-trimethylaminobutyraldehyde de	-22.606562	N/A	-22.628774	N/A	N/A	-23.660442
1228	P17844	DDX5	Probable ATP-dependent RNA helic	-24.525815	-24.11557	-23.780751	N/A	N/A	-23.692724
1229	P22314	UBA1	Ubiquitin-like modifier-activating e	N/A	N/A	-23.609435	N/A	N/A	-23.697738
1230	P09012	SNRPA	U1 small nuclear ribonucleoprotein	N/A	-22.930511	-23.669367	N/A	N/A	-23.7867
1231	P0CG39	POTEJ	POTE ankyrin domain family memb	N/A	N/A	-23.56392	N/A	N/A	-23.796243
1232	Q9UMS4	PRPF19	Pre-mRNA-processing factor 19 OS	N/A	N/A	-23.699746	N/A	N/A	-23.80829
1233	P17812	CTPS1	CTP synthase 1 OS=Homo sapiens G	N/A	-23.030708	-23.898144	N/A	N/A	-23.811301
1234	Q96KP4	CNDP2	Cytosolic non-specific dipeptidase O	N/A	N/A	N/A	N/A	N/A	-23.819979
1235	P23526	AHCY	Adenosylhomocysteinase OS=Hom	-18.560561	-21.775206	-21.838415	N/A	N/A	-23.844947
1236	P23588	EIF4B	Eukaryotic translation initiation fac	-24.848903	-21.597989	-20.997094	N/A	N/A	-23.872561
1237	P17931	LGALS3	Galectin-3 OS=Homo sapiens GN=L	-22.127644	-22.592514	-24.082897	N/A	N/A	-23.946633
1238	Q15366	PCBP2	Poly(rC)-binding protein 2 OS=Hom	-23.700609	-24.037763	-23.781646	N/A	N/A	-24.033345
1239	Q92688	ANP32B	Acidic leucine-rich nuclear phospho	N/A	N/A	N/A	N/A	N/A	-24.040835
1240	Q15424	SAFB	Scaffold attachment factor B1 OS=H/N/A	-24.243681	N/A	N/A	N/A	N/A	-24.054835
1241	O14818	PSMA7	Proteasome subunit alpha type-7 O	N/A	N/A	-21.055646	N/A	N/A	-24.298221
1242	P25786	PSMA1	Proteasome subunit alpha type-1 O	N/A	N/A	-20.992119	N/A	N/A	-24.307135
1243	Q724V5	HDGFL2	Hepatoma-derived growth factor-r	N/A	N/A	-24.365065	N/A	N/A	-24.313289
1244	Q8WXF1	PSPC1	Paraspeckle component 1 OS=Hom	N/A	N/A	N/A	N/A	N/A	-24.322157
1245	Q15393	SF3B3	Splicing factor 3B subunit 3 OS=Ho	N/A	N/A	-24.698521	N/A	N/A	-24.323759
1246	P60842	EIF4A1	Eukaryotic initiation factor 4A-I OS=	-21.940136	-24.04409	-23.918551	N/A	N/A	-24.363019
1247	Q14240	EIF4A2	Eukaryotic initiation factor 4A-I OS=	N/A	-24.04409	-23.918551	N/A	N/A	-24.363019
1248	Q13310	PABPC4	Polyadenylate-binding protein 4 OS	-24.063002	0	-23.416572	N/A	N/A	-24.405268
1249	O15068	MCF2L	Guanine nucleotide exchange facto	-25.458063	-23.79129	-24.777163	N/A	N/A	-24.497504
1250	Q5J TZ9	AARS2	Alanine--tRNA ligase, mitochondria	-24.366243	N/A	N/A	N/A	N/A	-24.547544
1251	P62826	RAN	GTP-binding nuclear protein Ran O	-20.117571	N/A	-20.003248	N/A	N/A	-24.627508
1252	Q13838	DDX39B	Spliceosome RNA helicase DDX39B	N/A	-25.359373	-23.556564	N/A	N/A	-24.976658
1253	Q13243	SRSF5	Serine/arginine-rich splicing factor	N/A	N/A	N/A	N/A	N/A	-24.997359
1254	Q13428	TCOF1	Treacle protein OS=Homo sapiens G	N/A	N/A	-24.928148	N/A	N/A	-25.308798
1255	P62195	PSMC5	26S protease regulatory subunit 8 O	N/A	N/A	N/A	N/A	N/A	-25.42757
1256	Q8IYB9	ZNF595	Zinc finger protein 595 OS=Homo s	N/A	N/A	N/A	N/A	N/A	-25.4324
1257	P01023	A2M	Alpha-2-macroglobulin OS=Homo s	N/A	N/A	N/A	N/A	N/A	-25.437757
1258	Q9NYU2	UGGT1	UDP-glucose:glycoprotein glucosylt	-24.429561	N/A	N/A	N/A	N/A	-25.447624
1259	P46940	IQGAP1	Ras GTPase-activating-like protein I	N/A	N/A	N/A	N/A	N/A	-25.557627
1260	Q15459	SF3A1	Splicing factor 3A subunit 1 OS=Ho	N/A	N/A	N/A	N/A	N/A	-25.656003
1261	Q92804	TAF15	TATA-binding protein-associated fa	-25.746178	-23.881848	-23.709648	N/A	N/A	-25.744564
1262	O76021	RSL1D1	Ribosomal L1 domain-containing pr	-26.633904	N/A	N/A	N/A	N/A	-25.795559
1263	P12270	TPR	Nucleoprotein TPR OS=Homo sapie	N/A	N/A	-24.781754	N/A	N/A	-26.704919
1264	Q15075	EEA1	Early endosome antigen 1 OS=Hom	N/A	N/A	N/A	N/A	N/A	-27.019939
1265	Q9UQ35	SRRM2	Serine/arginine repetitive matrix pr	-26.927024	-26.018487	-27.222022	N/A	N/A	-27.890253

Table 6.2. Gene Ontology (GO) Enrichment Analysis of Lipid Droplets Enriched Following Chloroquine Treatment

LDs were purified from agLDL-loaded THP-1 macrophages by density gradient centrifugation and subjected to mass spectrometry. GO names of pathways are shown. Table has been shortened to fit this format. Full table can be found in the supplemental data of the published manuscript.

GO_Name	p.adjusted	Protein ID	Output
negative regulation of chemokine production	8.77E-04	APOD_HUMAN	A LEG9_HUMAN Ga
alpha-amylase activity (releasing maltohexaose)	0.001470588	AMY1_HUMAN	A AMYP_HUMAN AI
amylase activity	0.001470588	AMYP_HUMAN	Alpha-amylase 1 ,
cellular developmental process	0.001692571	HMGB2_HUMAN	MMP14_HUMAN
purine nucleoside biosynthetic process	0.001694915	NDKA_HUMAN	N NDKB_HUMAN Nu
GTP biosynthetic process	0.001814882	NDKA_HUMAN	N NDKB_HUMAN Nu
developmental process	0.001956942	HMGB2_HUMAN	MMP14_HUMAN
negative regulation of cell junction assembly	0.002283105	APOD_HUMAN	A MMP14_HUMAN
condensed chromosome	0.002569667	HMGB2_HUMAN	H2AY_HUMAN Co
multicellular organismal process	0.002695298	HMGB2_HUMAN	K1C13_HUMAN K
cellular response to lipid	0.002988287	NDKA_HUMAN	N HMGB2_HUMAN
nucleoside biosynthetic process	0.003112532	NDKA_HUMAN	N NDKB_HUMAN Nu
positive regulation of epidermal cell differentiation	0.003322259	NDKB_HUMAN	N H2AY_HUMAN Co
serine-type peptidase activity	0.003356138	MMP9_HUMAN	MMP14_HUMAN
positive regulation of DNA binding	0.003363295	NDKA_HUMAN	N HMGB2_HUMAN
negative regulation of adherens junction organization	0.003389831	APOD_HUMAN	A MMP14_HUMAN
glycosyl compound biosynthetic process	0.003392501	NDKA_HUMAN	N NDKB_HUMAN Nu
inflammatory response	0.003546205	HMGB2_HUMAN	APOD_HUMAN A
PcG protein complex	0.00360055	H2AY_HUMAN	CoRBBP7_HUMAN H
serine hydrolase activity	0.003645949	MMP9_HUMAN	MMP14_HUMAN
collagen fibril organization	0.003751379	GT251_HUMAN	P SERPH_HUMAN S
cellular component movement	0.003989638	HMGB2_HUMAN	APOD_HUMAN A
positive regulation of multicellular organismal process	0.00407656	NDKA_HUMAN	N HMGB2_HUMAN
pyrimidine ribonucleotide metabolic process	0.004152098	NDKA_HUMAN	N NDKB_HUMAN Nu
cell proliferation	0.004330365	NDKA_HUMAN	N HMGB2_HUMAN
positive regulation of developmental process	0.004354121	NDKA_HUMAN	N HMGB2_HUMAN
negative regulation of MAPK cascade	0.004794882	PHB_HUMAN	Pro SPB3_HUMAN Ser
regulation of stress-activated protein kinase signaling cascade	0.004802503	LEG9_HUMAN	Ga SPB3_HUMAN Ser
positive regulation of cell differentiation	0.004991851	NDKA_HUMAN	N HMGB2_HUMAN
negative regulation of protein metabolic process	0.004994657	APOD_HUMAN	A MMP9_HUMAN M
pyrimidine ribonucleotide biosynthetic process	0.00519856	NDKA_HUMAN	N NDKB_HUMAN Nu
regulation of DNA binding	0.005315671	NDKA_HUMAN	N HMGB2_HUMAN
regulation of chemokine production	0.005347594	APOD_HUMAN	A LEG9_HUMAN Ga
response to oxygen-containing compound	0.00561518	NDKA_HUMAN	N HMGB2_HUMAN
pyrimidine nucleoside triphosphate metabolic process	0.005984372	NDKA_HUMAN	N NDKB_HUMAN Nu
pyrimidine ribonucleoside triphosphate metabolic process	0.006208676	NDKA_HUMAN	N NDKB_HUMAN Nu
pyrimidine nucleotide metabolic process	0.006368012	NDKA_HUMAN	N NDKB_HUMAN Nu
regulation of cell communication	0.006944778	HMGB2_HUMAN	HCDH_HUMAN H
response to lipid	0.006954607	NDKA_HUMAN	N HMGB2_HUMAN
negative regulation of protein serine/threonine kinase activity	0.007056452	SPB3_HUMAN	Se H2AY_HUMAN Co
trans-Golgi network transport vesicle	0.007056452	RAB8A_HUMAN	RTMEDA_HUMAN
pyrimidine nucleoside triphosphate biosynthetic process	0.007544507	NDKA_HUMAN	N NDKB_HUMAN Nu
positive regulation of epithelial cell differentiation	0.007574164	NDKB_HUMAN	N H2AY_HUMAN Co
pyrimidine ribonucleoside triphosphate biosynthetic process	0.00786309	NDKA_HUMAN	N NDKB_HUMAN Nu
regulation of signaling	0.007928378	HMGB2_HUMAN	HCDH_HUMAN H
cellular response to biotic stimulus	0.008317751	HMGB2_HUMAN	LYRIC_HUMAN Pr

regulation of cell proliferation	0.008455794	NDKA_HUMAN	N	HMGB2_HUMAN	R
negative regulation of cellular protein metabolic process	0.008557665	MMP9_HUMAN	PHB_HUMAN	Pro	
pyrimidine-containing compound biosynthetic process	0.008707162	NDKA_HUMAN	N	NDKB_HUMAN	Nu
cellular response to oxygen-containing compound	0.008728455	NDKA_HUMAN	N	HMGB2_HUMAN	
protein maturation	0.009031366	C2CD3_HUMAN	CPHB_HUMAN	Pro	
positive regulation of keratinocyte differentiation	0.009725906	NDKB_HUMAN	N	H2AY_HUMAN	Co
pyrimidine nucleotide biosynthetic process	0.00993571	NDKA_HUMAN	N	NDKB_HUMAN	Nu
Golgi vesicle fusion to target membrane	0.01012979	RAB8A_HUMAN	RRAB8B_HUMAN	R	
establishment of vesicle localization	0.010661561	C2CD3_HUMAN	CRAB8A_HUMAN	R	
vesicle targeting	0.010858305	C2CD3_HUMAN	CRAB8A_HUMAN	R	
peptidase regulator activity	0.010909514	MMP14_HUMAN	SPB3_HUMAN	Ser	
nucleoside diphosphate kinase activity	0.010989011	NDKA_HUMAN	N	NDKB_HUMAN	Nu
metalloendopeptidase activity	0.0111319	MMP9_HUMAN	MMP14_HUMAN		
regulation of locomotion	0.011252117	APOD_HUMAN	A	MMP9_HUMAN	M
negative regulation of signaling	0.011279794	HMGB2_HUMAN	HCDH_HUMAN	H	
pyrimidine-containing compound metabolic process	0.011474729	NDKA_HUMAN	N	NDKB_HUMAN	Nu
phosphotransferase activity, phosphate group as acceptor	0.011722509	NDKA_HUMAN	N	NDKB_HUMAN	Nu
negative regulation of cell proliferation	0.012169546	NDKA_HUMAN	N	APOD_HUMAN	A
negative regulation of nitrogen compound metabolic process	0.012249466	HMGB2_HUMAN	APOD_HUMAN	A	
negative regulation of cell-matrix adhesion	0.012820513	APOD_HUMAN	A	MMP14_HUMAN	
negative regulation of defense response	0.013098058	APOD_HUMAN	A	LEG9_HUMAN	Ga
serine-type endopeptidase activity	0.013594946	MMP9_HUMAN	MMP14_HUMAN		
nucleoplasm	0.01362792	HMGB2_HUMAN	HCDH_HUMAN	H	
nucleobase-containing small molecule interconversion	0.013753651	NDKA_HUMAN	N	NDKB_HUMAN	Nu
chemokine production	0.014042382	APOD_HUMAN	A	LEG9_HUMAN	Ga
GTP metabolic process	0.014400922	NDKA_HUMAN	N	NDKB_HUMAN	Nu
stress-activated protein kinase signaling cascade	0.014575671	LEG9_HUMAN	Ga	SPB3_HUMAN	Ser
negative regulation of cellular metabolic process	0.015150645	HMGB2_HUMAN	APOD_HUMAN	A	
negative regulation of protein phosphorylation	0.015975733	PHB_HUMAN	Pro	SPB3_HUMAN	Ser
cilium basal body	0.016678886	C2CD3_HUMAN	CRAB8A_HUMAN	R	
negative regulation of peptidase activity	0.016928508	MMP9_HUMAN	SPB3_HUMAN	Ser	
sister chromatid cohesion	0.016979793	H2AY_HUMAN	Co	PP1G_HUMAN	Ser
regulation of multicellular organismal process	0.017209433	HMGB2_HUMAN	MMP14_HUMAN		
glycosyl compound metabolic process	0.017281606	NDKA_HUMAN	N	NDKB_HUMAN	Nu
response to molecule of bacterial origin	0.017299512	HMGB2_HUMAN	LYRIC_HUMAN	Pr	
negative regulation of cell communication	0.017467461	HMGB2_HUMAN	HCDH_HUMAN	H	
intraflagellar transport	0.01814882	C2CD3_HUMAN	CRAB8A_HUMAN	R	
covalent chromatin modification	0.018435651	PHB_HUMAN	Pro	H2AY_HUMAN	Co
nucleobase-containing compound kinase activity	0.018759019	NDKA_HUMAN	N	NDKB_HUMAN	Nu
histone modification	0.018876645	PHB_HUMAN	Pro	H2AY_HUMAN	Co
purine nucleoside metabolic process	0.018956022	NDKA_HUMAN	N	NDKB_HUMAN	Nu
localization of cell	0.019130574	HMGB2_HUMAN	APOD_HUMAN	A	
positive regulation of lymphocyte differentiation	0.02	MMP14_HUMAN	LEG9_HUMAN	Gal	
Golgi-associated vesicle	0.020238565	RAB8A_HUMAN	RTMEDA_HUMAN		
vesicle docking involved in exocytosis	0.020722634	RAB8A_HUMAN	RRAB8B_HUMAN	R	
regulation of cysteine-type endopeptidase activity involved in apoptotic signal	0.021470343	MMP9_HUMAN	LEG9_HUMAN	Gal	
regulation of adherens junction organization	0.021608267	APOD_HUMAN	A	MMP14_HUMAN	
collagen metabolic process	0.022179138	MMP9_HUMAN	MMP14_HUMAN		
regulation of proteolysis	0.022264385	MMP9_HUMAN	C2CD3_HUMAN	C	
response to hormone stimulus	0.022786671	NDKA_HUMAN	N	HMGB2_HUMAN	
small nucleolar ribonucleoprotein complex assembly	0.0234375	NH2L1_HUMAN	NHP2-like protein 1		
plasma membrane part	0.023902954	NDKA_HUMAN	N	APOD_HUMAN	A
cysteine-type endopeptidase activity involved in apoptotic signaling pathway	0.02391521	MMP9_HUMAN	LEG9_HUMAN	Gal	
locomotion	0.024443205	HMGB2_HUMAN	APOD_HUMAN	A	

vesicle docking	0.024494465 RAB8A_HUMAN RRAB8B_HUMAN R
negative regulation of response to stimulus	0.024802971 HMGB2_HUMAN APOD_HUMAN A
positive regulation of epidermis development	0.025292529 NDKB_HUMAN N H2AY_HUMAN Co
negative regulation of cellular process	0.026100368 HMGB2_HUMAN HCDH_HUMAN H
cell growth	0.02650743 PHB_HUMAN Pro MMP14_HUMAN
small nucleolar ribonucleoprotein complex	0.026666667 NH2L1_HUMAN NHP2-like protein 1
bone morphogenesis	0.026710684 MMP14_HUMAN SERPH_HUMAN S
regulation of cellular component movement	0.027041665 APOD_HUMAN A MMP9_HUMAN M
macromolecule deacylation	0.027243262 PHB_HUMAN Pro RBBP7_HUMAN H
microtubule organizing center	0.027452521 NDKA_HUMAN N C2CD3_HUMAN C
negative regulation of JNK cascade	0.027777778 SPB3_HUMAN Se GSTP1_HUMAN G
regulation of stress-activated MAPK cascade	0.027808676 LEG9_HUMAN Ga SPB3_HUMAN Ser
protein deacylation	0.028073251 PHB_HUMAN Pro RBBP7_HUMAN H
peptidase inhibitor activity	0.028365566 SPB3_HUMAN Se SPB12_HUMAN S
organelle localization	0.028488233 C2CD3_HUMAN CLEG9_HUMAN Ga
monocyte chemotactic protein-1 production	0.028571429 APOD_HUMAN A LEG9_HUMAN Ga
plasma membrane region	0.028594627 NDKA_HUMAN N APOD_HUMAN A
negative regulation of phosphorylation	0.029012876 PHB_HUMAN Pro SPB3_HUMAN Ser
stress-activated MAPK cascade	0.029374729 LEG9_HUMAN Ga SPB3_HUMAN Ser
protein processing	0.029782379 C2CD3_HUMAN CPHB_HUMAN Pro
regulation of peptidase activity	0.030016064 MMP9_HUMAN MMP14_HUMAN
U4atac snRNP	0.03125 NH2L1_HUMAN NHP2-like protein 1
embryonic organ morphogenesis	0.031591252 C2CD3_HUMAN CMMP14_HUMAN
protein secretion	0.03168026 HCDH_HUMAN H LEG9_HUMAN Ga
metallopeptidase activity	0.032741619 MMP9_HUMAN MMP14_HUMAN
negative regulation of stress-activated protein kinase signaling cascade	0.033101045 SPB3_HUMAN Se GSTP1_HUMAN G
positive regulation of epithelial cell proliferation	0.033180961 NDKA_HUMAN N HMGB2_HUMAN
TRAF2-GSTP1 complex	0.033333333 GSTP1_HUMAN Glutathione S-transf
ciliary transition fiber	0.033333333 RAB8A_HUMAN Ras-related protein
snoRNA binding	0.03358209 NH2L1_HUMAN NHP2-like protein 1
establishment of organelle localization	0.033589545 C2CD3_HUMAN CLEG9_HUMAN Ga
regulation of protein metabolic process	0.033745157 MMP14_HUMAN H2AY_HUMAN Co
response to oxidative stress	0.033841556 APOD_HUMAN A MMP9_HUMAN M
protein targeting to peroxisome	0.034090909 RAB8B_HUMAN Ras-related protein
glutathione derivative biosynthetic process	0.034482759 GSTP1_HUMAN Glutathione S-transf
protein import into peroxisome membrane	0.034482759 RAB8B_HUMAN Ras-related protein
histone methyltransferase complex	0.034897851 H2AY_HUMAN CoRBBP7_HUMAN H
maintenance of mitotic sister chromatid cohesion	0.036363636 H2AY_HUMAN Core histone macro-
cell junction organization	0.036623379 APOD_HUMAN A LYRIC_HUMAN Pr
leukocyte migration	0.036781992 APOD_HUMAN A MMP9_HUMAN M
regulation of cell junction assembly	0.037172472 APOD_HUMAN A MMP14_HUMAN
positive regulation of humoral immune response	0.037974684 PHB_HUMAN Prohibitin ,
regulation of cell growth	0.03820053 PHB_HUMAN Pro MMP14_HUMAN
gamma-tubulin binding	0.038461538 NDKA_HUMAN Nucleoside diphosph
positive regulation of hemopoiesis	0.038830055 HMGB2_HUMAN MMP14_HUMAN
glutathione binding	0.03902439 GSTP1_HUMAN Glutathione S-transf
JUN phosphorylation	0.039312039 SPB3_HUMAN Se GSTP1_HUMAN G
positive regulation of binding	0.039760625 NDKA_HUMAN N HMGB2_HUMAN
regulation of CD4-positive, CD25-positive, alpha-beta regulatory T cell differen	0.04 LEG9_HUMAN Galectin-9 ,
nitric-oxide synthase biosynthetic process	0.040677966 GSTP1_HUMAN Glutathione S-transf
ERK1 and ERK2 cascade	0.04075497 PHB_HUMAN Pro LEG9_HUMAN Ga
DNA ligation involved in DNA repair	0.041666667 HMGB2_HUMAN High mobility grou
positive regulation of T cell activation via T cell receptor contact with antigen b	0.041666667 LEG9_HUMAN Galectin-9 ,
regulation of exocytosis	0.041821729 LEG9_HUMAN GaRAB8A_HUMAN R

peroxisomal transport	0.042056075 RAB8B_HUMAN Ras-related protein
cilium organization	0.04225635 C2CD3_HUMAN CRAB8A_HUMAN R
serine-type endopeptidase inhibitor activity	0.042490119 SPB3_HUMAN Se SPB12_HUMAN S
cellular response to interleukin-6	0.042553191 PHB_HUMAN Prohibitin ,
regulation of secretion by cell	0.043225438 HCDH_HUMAN H LEG9_HUMAN Ga
nucleoplasm part	0.043249364 LYRIC_HUMAN Pr H2AY_HUMAN Co
negative regulation of immune system process	0.043427583 NDKA_HUMAN N APOD_HUMAN A
positive regulation of CD4-positive, CD25-positive, alpha-beta regulatory T cell	0.043478261 LEG9_HUMAN Galectin-9 ,
gamma-secretase complex	0.043478261 TMEDA_HUMAN Transmembrane e
I-kappaB kinase/NF-kappaB cascade	0.04350224 LYRIC_HUMAN Pr LEG9_HUMAN Ga
SAP kinase activity	0.044117647 SPB3_HUMAN Se GSTP1_HUMAN G
regulation of JUN kinase activity	0.044117647 SPB3_HUMAN Se GSTP1_HUMAN G
regulation of activated T cell autonomous cell death	0.044368601 LEG9_HUMAN Galectin-9 ,
bile acid biosynthetic process	0.044444444 DHB4_HUMAN Peroxisomal multifun
establishment of protein localization to peroxisome	0.044642857 RAB8B_HUMAN Ras-related protein
regulation of nitrogen compound metabolic process	0.044695398 HMGB2_HUMAN MMP14_HUMAN
protein phosphatase type 1 complex	0.045045045 PP1G_HUMAN Serine/threonine-pro
interleukin-1 production	0.045117992 LEG9_HUMAN Ga GSTP1_HUMAN G
positive regulation of cell proliferation	0.045410328 NDKA_HUMAN N HMGB2_HUMAN
positive regulation of protein activation cascade	0.045454545 PHB_HUMAN Prohibitin ,
negative regulation of tumor necrosis factor superfamily cytokine production	0.045614035 LEG9_HUMAN Ga GSTP1_HUMAN G
nucleolar part	0.045786829 LYRIC_HUMAN Pr NH2L1_HUMAN N
regulation of localization	0.046497889 HCDH_HUMAN H APOD_HUMAN A
regulation of epidermal cell differentiation	0.046592659 NDKB_HUMAN N H2AY_HUMAN Co
negative regulation of signal transduction	0.046760148 HMGB2_HUMAN APOD_HUMAN A
embryonic morphogenesis	0.047523451 MMP9_HUMAN C2CD3_HUMAN C
positive regulation of G-protein coupled receptor protein signaling pathway	0.047619048 PHB_HUMAN Prohibitin ,
endocrine process	0.047619048 RAB8B_HUMAN Ras-related protein
purine ribonucleoside biosynthetic process	0.047619048 NDKA_HUMAN N NDKB_HUMAN Nu
negative regulation of tumor necrosis factor-mediated signaling pathway	0.047619048 GSTP1_HUMAN Glutathione S-transf
regulation of endocrine process	0.047619048 RAB8B_HUMAN Ras-related protein
protein localization to cilium	0.047684078 C2CD3_HUMAN CRAB8A_HUMAN R
peptide secretion	0.048009939 HCDH_HUMAN H LEG9_HUMAN Ga
regulation of leukocyte migration	0.048460843 APOD_HUMAN A MMP14_HUMAN
carbohydrate metabolic process	0.048476241 APOD_HUMAN A AMY1_HUMAN AI
protease binding	0.048476352 MMP14_HUMAN SPB3_HUMAN Ser
negative regulation of growth	0.048568967 PHB_HUMAN Pro RBBP7_HUMAN H
cilium part	0.048634804 C2CD3_HUMAN CRAB8A_HUMAN R
regulation of keratinocyte differentiation	0.048780488 NDKB_HUMAN N H2AY_HUMAN Co
regulation of nitric-oxide synthase biosynthetic process	0.049261084 GSTP1_HUMAN Glutathione S-transf
dinitrosyl-iron complex binding	0.049382716 GSTP1_HUMAN Glutathione S-transf
nitric oxide binding	0.049382716 GSTP1_HUMAN Glutathione S-transf
positive regulation of locomotion	0.049820279 MMP9_HUMAN MMP14_HUMAN
regulatory region nucleic acid binding	0.050849813 NDKA_HUMAN N HMGB2_HUMAN
nitric oxide storage	0.050955414 GSTP1_HUMAN Glutathione S-transf
regulation of hormone levels	0.051067062 HCDH_HUMAN H DHB4_HUMAN Pe
lipid oxidation	0.05117115 HCDH_HUMAN H APOD_HUMAN A
negative regulation of leukocyte degranulation	0.051282051 LEG9_HUMAN Galectin-9 ,
null	0.051756481 NDKA_HUMAN N MMP14_HUMAN
negative regulation of transferase activity	0.051845975 SPB3_HUMAN Se H2AY_HUMAN Co
positive regulation of activated T cell autonomous cell death	0.051948052 LEG9_HUMAN Galectin-9 ,
axoneme assembly	0.052210506 C2CD3_HUMAN CRAB8A_HUMAN R
null	0.052210506 C2CD3_HUMAN CRAB8A_HUMAN R
protein localization to peroxisome	0.052287582 RAB8B_HUMAN Ras-related protein

tolerance induction	0.052631579 LEG9_HUMAN Galectin-9 ,
mitotic sister chromatid cohesion	0.052631579 H2AY_HUMAN Core histone macro-
microbody part	0.053175487 DHB4_HUMAN PeRAB8B_HUMAN R
vesicle targeting, to, from or within Golgi	0.053470919 C2CD3_HUMAN CRAB8A_HUMAN R
transport vesicle	0.053522431 RAB8A_HUMAN RTMEDA_HUMAN
regulation of peptide transport	0.053828565 HCDH_HUMAN H APOD_HUMAN A
null	0.054009462 MMP14_HUMAN NDKB_HUMAN N
negative regulation of catalytic activity	0.054126397 MMP9_HUMAN SPB3_HUMAN Ser
methyltransferase complex	0.054134009 H2AY_HUMAN CoRBBP7_HUMAN H
null	0.054176072 GSTP1_HUMAN Glutathione S-transf
skeletal system morphogenesis	0.054190247 MMP14_HUMAN SERPH_HUMAN S
coated vesicle	0.054310044 RAB8A_HUMAN RTMEDA_HUMAN
deoxyribonuclease activity	0.054321754 NDKA_HUMAN N HMGB2_HUMAN
neural plate development	0.054545455 C2CD3_HUMAN C2 domain-containi
negative regulation of myeloid leukocyte mediated immunity	0.054545455 LEG9_HUMAN Galectin-9 ,
secretion	0.055375157 NDKA_HUMAN N HCDH_HUMAN H
3alpha,7alpha,12alpha-trihydroxy-5beta-cholest-24-enoyl-CoA hydratase activ	0.055555556 DHB4_HUMAN Peroxisomal multifun
determination of heart left/right asymmetry	0.055555556 C2CD3_HUMAN C2 domain-containi
male sex differentiation	0.055617633 HMGB2_HUMAN MMP14_HUMAN
negative regulation of cell-substrate adhesion	0.056451613 APOD_HUMAN A MMP14_HUMAN
lipoprotein oxidation	0.056451613 APOD_HUMAN Apolipoprotein D ,
null	0.056478405 MMP14_HUMAN Matrix metallopro
ribosomal large subunit biogenesis	0.056591627 RL14_HUMAN 60 NH2L1_HUMAN N
nucleoside metabolic process	0.0568595 NDKA_HUMAN N NDKB_HUMAN Nu
regulation of epithelial cell differentiation	0.056862811 NDKB_HUMAN N H2AY_HUMAN Co
nuclear chromosome part	0.056920176 HMGB2_HUMAN PHB_HUMAN Pro
intercellular canaliculus	0.057142857 LYRIC_HUMAN Protein LYRIC ,
regulation of macromolecule metabolic process	0.057754593 HMGB2_HUMAN MMP14_HUMAN
regulation of epidermis development	0.057843354 NDKB_HUMAN N H2AY_HUMAN Co
regulation of cell-matrix adhesion	0.058479532 APOD_HUMAN A MMP14_HUMAN
3-hydroxyacyl-CoA dehydrogenase activity	0.058479532 HCDH_HUMAN H DHB4_HUMAN Pe
null	0.058811881 RAB8A_HUMAN Ras-related protein
embryonic heart tube development	0.058823529 C2CD3_HUMAN C2 domain-containi
null	0.058823529 GT251_HUMAN Procollagen galacto
translation repressor activity	0.058823529 CIRBP_HUMAN Cold-inducible RNA-
regulation of interleukin-1 production	0.058888218 LEG9_HUMAN Ga GSTP1_HUMAN G
negative regulation of transport	0.058970915 HCDH_HUMAN H APOD_HUMAN A
regulation of lipoprotein metabolic process	0.059190031 APOD_HUMAN Apolipoprotein D ,
microbody membrane	0.059484481 DHB4_HUMAN PeRAB8B_HUMAN R
regulation of synapse assembly	0.05952381 AGRL3_HUMAN Adhesion G protein-
craniofacial suture morphogenesis	0.059701493 MMP14_HUMAN Matrix metallopro
negative regulation of cytokine production	0.059798515 APOD_HUMAN A LEG9_HUMAN Ga
quinone binding	0.06 SQRD_HUMAN Sulfide:quinone oxid
negative regulation of ion transmembrane transporter activity	0.06 MMP9_HUMAN Matrix metalloprot
regulation of lymphocyte differentiation	0.060066741 MMP14_HUMAN LEG9_HUMAN Gal
regulation of growth	0.060239495 PHB_HUMAN Pro MMP14_HUMAN
negative regulation of macromolecule metabolic process	0.060368425 HMGB2_HUMAN H2AY_HUMAN Co
endodermal cell differentiation	0.060513818 MMP9_HUMAN MMP14_HUMAN
positive regulation of synapse assembly	0.060606061 AGRL3_HUMAN Adhesion G protein-
collagen catabolic process	0.060822603 MMP9_HUMAN MMP14_HUMAN
regulation of transcription from RNA polymerase II promoter	0.061123422 HMGB2_HUMAN LYRIC_HUMAN Pr
toll-like receptor 2 signaling pathway	0.0625 LEG9_HUMAN Galectin-9 ,
regulation of keratinocyte migration	0.0625 MMP9_HUMAN Matrix metalloprot
negative regulation of phosphate metabolic process	0.062526634 PHB_HUMAN Pro SPB3_HUMAN Ser

negative regulation of cellular macromolecule biosynthetic process	0.062554047 HMGB2_HUMAN LYRIC_HUMAN Pr
embryonic organ development	0.062734575 C2CD3_HUMAN CMMP14_HUMAN
intermediate filament cytoskeleton	0.062944923 NDKA_HUMAN N MMP14_HUMAN
gas homeostasis	0.062980031 GSTP1_HUMAN Glutathione S-transf
null	0.063143445 RAB8A_HUMAN Ras-related protein
null	0.063218944 HMGB2_HUMAN MMP9_HUMAN M
null	0.063237269 HMGB2_HUMAN MMP14_HUMAN
paracrine signaling	0.063492063 SPB3_HUMAN Serpin B3 ,
cell projection organization	0.064034319 HMGB2_HUMAN NDKB_HUMAN N
intraflagellar transport particle	0.064331381 C2CD3_HUMAN CRAB8A_HUMAN R
negative regulation of transcription from RNA polymerase II promoter	0.064843041 LYRIC_HUMAN Pr PHB_HUMAN Pro
nuclear chromosome	0.065043829 HMGB2_HUMAN PHB_HUMAN Pro
growth	0.065140398 APOD_HUMAN A PHB_HUMAN Pro
JUN kinase binding	0.065217391 GSTP1_HUMAN Glutathione S-transf
negative regulation of molecular function	0.065691286 MMP9_HUMAN SPB3_HUMAN Ser
protein localization in plasma membrane	0.065800866 RAB8A_HUMAN RRAB8B_HUMAN R
regulation of defense response	0.066055294 HMGB2_HUMAN APOD_HUMAN A
maintenance of sister chromatid cohesion	0.066666667 H2AY_HUMAN Core histone macro-
null	0.066666667 C2CD3_HUMAN C2 domain-containi
oxidoreductase activity, acting on a sulfur group of donors, quinone or similar	0.066666667 SQRD_HUMAN Sulfide:quinone oxid
negative regulation of JUN kinase activity	0.066666667 SPB3_HUMAN Se GSTP1_HUMAN G
chondrocyte development	0.06741573 SERPH_HUMAN Serpin H1 ,
recycling endosome membrane	0.067567568 RAB8A_HUMAN RRAB8B_HUMAN R
stress granule assembly	0.067968906 CIRBP_HUMAN Cold-inducible RNA-
endopeptidase activity	0.068014805 MMP9_HUMAN MMP14_HUMAN
negative regulation of cell growth	0.0682473 PHB_HUMAN Pro RBBP7_HUMAN H
negative regulation of cell adhesion	0.068605673 APOD_HUMAN A MMP14_HUMAN
response to endogenous stimulus	0.068672825 NDKA_HUMAN N HMGB2_HUMAN
nucleolus	0.068781361 HMGB2_HUMAN LYRIC_HUMAN Pr
regulation of lipoprotein lipid oxidation	0.068965517 APOD_HUMAN Apolipoprotein D ,
DNA secondary structure binding	0.069565217 HMGB2_HUMAN High mobility grou
phosphotransferase activity, nitrogenous group as acceptor	0.070422535 NDKB_HUMAN Nucleoside diphosph
null	0.070567986 RAB8A_HUMAN Ras-related protein
carbohydrate binding	0.070666941 LEG9_HUMAN Ga LEG7_HUMAN Ga
homeostasis of number of cells	0.070773742 HMGB2_HUMAN LEG9_HUMAN Gal
null	0.07139872 APOD_HUMAN A H2AY_HUMAN Co
oligopeptide binding	0.071428571 GSTP1_HUMAN Glutathione S-transf
S-nitrosoglutathione binding	0.071428571 GSTP1_HUMAN Glutathione S-transf
alpha-beta T cell proliferation	0.071428571 LEG9_HUMAN Galectin-9 ,
entrainment of circadian clock	0.071428571 PP1G_HUMAN Serine/threonine-pro
collagen binding	0.071887329 MMP9_HUMAN SERPH_HUMAN S
DNA topological change	0.072289157 HMGB2_HUMAN High mobility grou
epithelial cell proliferation	0.072650814 NDKA_HUMAN N HMGB2_HUMAN
cation binding	0.073090988 NDKA_HUMAN N MMP9_HUMAN M
regulation of T cell activation via T cell receptor contact with antigen bound to	0.073148148 LEG9_HUMAN Galectin-9 ,
extrinsic apoptotic signaling pathway	0.073274966 HMGB2_HUMAN GSTP1_HUMAN G
cellular component organization	0.073469766 HMGB2_HUMAN K1C13_HUMAN K
hydrogen sulfide metabolic process	0.073708341 SQRD_HUMAN Sulfide:quinone oxid
NAD+ binding	0.074048242 HCDH_HUMAN Hydroxyacyl-coenzy
nucleosome assembly	0.074162679 HMGB2_HUMAN H2AY_HUMAN Co
beta-amyloid metabolic process	0.074600972 TMEDA_HUMAN Transmembrane e
negative regulation of intracellular transport	0.074771653 APOD_HUMAN A LEG9_HUMAN Gal
bile acid metabolic process	0.074975657 DHB4_HUMAN Peroxisomal multifun
regulation of platelet-derived growth factor receptor signaling pathway	0.075862069 APOD_HUMAN Apolipoprotein D ,

cell projection assembly
 mast cell granule
 regulation of I-kappaB kinase/NF-kappaB cascade
 positive regulation of leukocyte apoptotic process
 negative regulation of lipoprotein metabolic process
 negative regulation of transcription from RNA polymerase I promoter
 protein processing involved in protein targeting to mitochondrion
 spermatid nucleus differentiation
 chloride ion binding
 protein transport within lipid bilayer
 negative regulation of smooth muscle cell proliferation
 muscle cell proliferation
 negative regulation of transcription by competitive promoter binding
 regulation of epithelial to mesenchymal transition
 cellular response to acid
 null
 regulation of mast cell activation involved in immune response
 positive regulation of complement activation
 procollagen galactosyltransferase activity
 null
 null
 response to interleukin-6
 microtubule bundle formation

enzyme regulator activity
 positive regulation of proteolysis
 null
 negative regulation of protein modification process
 regulation of RNA biosynthetic process
 negative regulation of mast cell activation involved in immune response
 positive regulation of keratinocyte migration
 null
 cellular response to cell-matrix adhesion
 interleukin-12 secretion
 interferon-gamma secretion
 heat acclimation
 negative regulation of insulin secretion
 epithelial tube morphogenesis
 null
 regulation of natural killer cell mediated immunity
 negative regulation of leukocyte proliferation
 negative regulation of nitric-oxide synthase biosynthetic process
 cilium morphogenesis
 hormone transport
 regulation of smooth muscle cell proliferation
 nucleoside diphosphate metabolic process
 regulation of protein phosphorylation
 null
 cell tip
 protein serine/threonine phosphatase complex
 DNA binding
 cell projection part
 null
 null
 macrolide binding

0.075877137 NDKA_HUMAN N HMGB2_HUMAN
 0.075924076 LEG9_HUMAN Galectin-9 ,
 0.076168069 LYRIC_HUMAN Pr LEG9_HUMAN Ga
 0.076168929 LEG9_HUMAN Galectin-9 ,
 0.076923077 APOD_HUMAN Apolipoprotein D ,
 0.076923077 H2AY_HUMAN Core histone macro-
 0.076923077 QCR2_HUMAN Cytochrome b-c1 co
 0.076923077 HMGB2_HUMAN High mobility grou
 0.077318606 AMYP_HUMAN Alpha-amylase 1 ,
 0.077639602 RAB8A_HUMAN Ras-related protein
 0.077895894 APOD_HUMAN A GSTP1_HUMAN G
 0.077930572 APOD_HUMAN A MMP9_HUMAN M
 0.078125 PHB_HUMAN Prohibitin ,
 0.078947368 SPB3_HUMAN Serpin B3 ,
 0.078975238 NDKA_HUMAN N K1C13_HUMAN K
 0.079775102 C2CD3_HUMAN CRAB8A_HUMAN R
 0.079970381 LEG9_HUMAN Galectin-9 ,
 0.08 PHB_HUMAN Prohibitin ,
 0.080213904 GT251_HUMAN Procollagen galacto
 0.080258559 RAB8A_HUMAN Ras-related protein
 0.080338266 LEG7_HUMAN Ga AGRL3_HUMAN A
 0.080370292 PHB_HUMAN Prohibitin ,
 0.080606923 C2CD3_HUMAN CRAB8A_HUMAN R

0.080812591 MMP14_HUMAN SPB3_HUMAN Ser
 0.081015569 PHB_HUMAN Pro MMP14_HUMAN
 0.081185567 PP1G_HUMAN Serine/threonine-pro
 0.081427422 PHB_HUMAN Pro SPB3_HUMAN Ser
 0.081935648 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.083333333 LEG9_HUMAN Galectin-9 ,
 0.083333333 MMP9_HUMAN Matrix metalloprot
 0.083333333 GT251_HUMAN Procollagen galacto
 0.083333333 GSTP1_HUMAN Glutathione S-transf
 0.083333333 LEG9_HUMAN Galectin-9 ,
 0.083333333 LEG9_HUMAN Galectin-9 ,
 0.083333333 RBBP7_HUMAN Histone-binding pro
 0.083333333 HCDH_HUMAN Hydroxyacyl-coenzy
 0.084158752 C2CD3_HUMAN CMMP14_HUMAN
 0.084181314 TMEDA_HUMAN Transmembrane e
 0.084181314 LEG9_HUMAN Galectin-9 ,
 0.084288261 LEG9_HUMAN Ga GSTP1_HUMAN G
 0.084337349 GSTP1_HUMAN Glutathione S-transf
 0.084347885 C2CD3_HUMAN CRAB8A_HUMAN R
 0.08443018 HCDH_HUMAN H RAB8B_HUMAN R
 0.085211117 APOD_HUMAN A MMP9_HUMAN M
 0.085433855 NDKA_HUMAN N NDKB_HUMAN Nu
 0.085817133 MMP9_HUMAN PHB_HUMAN Pro
 0.085931486 RAB8A_HUMAN Ras-related protein
 0.086197859 RAB8B_HUMAN Ras-related protein
 0.086197859 PP1G_HUMAN Serine/threonine-pro
 0.086409823 NDKA_HUMAN N HMGB2_HUMAN
 0.086529668 NDKA_HUMAN N APOD_HUMAN A
 0.086725417 NDKA_HUMAN N HMGB2_HUMAN
 0.086956522 H2AY_HUMAN Core histone macro-
 0.087197741 FKBP2_HUMAN Peptidyl-prolyl cis-tr

negative regulation of metabolic process
 endopeptidase inhibitor activity
 mesoderm morphogenesis
 double-stranded methylated DNA binding
 DNA catabolic process
 toxin metabolic process
 biological adhesion
 regulation of binding
 positive regulation of cellular component movement
 cytoskeleton-dependent intracellular transport
 peptidyl-histidine phosphorylation
 lipid modification
 progesterone receptor signaling pathway
 negative regulation of leukocyte mediated cytotoxicity
 cellular heat acclimation
 interleukin-10 secretion
 positive regulation of erythrocyte differentiation
 cellular response to lipopolysaccharide
 regulation of nucleocytoplasmic transport
 membrane docking
 cell projection
 mast cell degranulation
 response to acid
 rRNA transcription
 null
 null

positive regulation of transforming growth factor beta production
 negative regulation of leukocyte mediated immunity
 cargo loading into vesicle
 viral entry into host cell
 secondary metabolic process
 locomotion involved in locomotory behavior
 sulfide oxidation
 response to organic cyclic compound
 androgen metabolic process
 cytokine production involved in inflammatory response
 protein histidine kinase activity
 interaction with host
 enzyme inhibitor activity
 centriole
 membrane fusion
 execution phase of apoptosis
 response to insulin stimulus
 negative regulation of proteolysis
 negative regulation of phosphorus metabolic process
 cell chemotaxis
 positive regulation of interleukin-8 secretion
 null
 regulation of interleukin-12 secretion
 interleukin-13 secretion
 tongue development
 proteinase activated receptor binding
 null
 negative regulation of focal adhesion assembly

0.087552338 HMGB2_HUMAN H2AY_HUMAN Co
 0.087696011 SPB3_HUMAN Se SPB12_HUMAN S
 0.087723043 MMP9_HUMAN MMP14_HUMAN
 0.088235294 H2AY_HUMAN Core histone macro-
 0.08861745 HMGB2_HUMAN High mobility grou
 0.088770053 SQRD_HUMAN Sulfide:quinone oxid
 0.088955291 APOD_HUMAN A MMP14_HUMAN
 0.08923063 NDKA_HUMAN N HMGB2_HUMAN
 0.089656539 MMP9_HUMAN MMP14_HUMAN
 0.089699467 C2CD3_HUMAN CRAB8A_HUMAN R
 0.08974359 NDKB_HUMAN Nucleoside diphosph
 0.090428345 HCDH_HUMAN H APOD_HUMAN A
 0.090909091 PHB_HUMAN Prohibitin ,
 0.090909091 LEG9_HUMAN Galectin-9 ,
 0.090909091 RBBP7_HUMAN Histone-binding pro
 0.090909091 LEG9_HUMAN Galectin-9 ,
 0.090909091 HMGB2_HUMAN High mobility grou
 0.091103582 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.091308165 APOD_HUMAN A PP1G_HUMAN Ser
 0.091914448 C2CD3_HUMAN CRAB8A_HUMAN R
 0.092200957 NDKA_HUMAN N APOD_HUMAN A
 0.092510307 LEG9_HUMAN Galectin-9 ,
 0.09361064 NDKA_HUMAN N K1C13_HUMAN K
 0.093701997 H2AY_HUMAN Core histone macro-
 0.094772134 H2AY_HUMAN Core histone macro-
 0.095238095 LEG9_HUMAN Galectin-9 ,

0.095238095 LEG9_HUMAN Galectin-9 ,
 0.095561775 LEG9_HUMAN Galectin-9 ,
 0.09591139 TMEDA_HUMAN Transmembrane e
 0.096194503 LEG9_HUMAN Ga SPB3_HUMAN Ser
 0.096214799 SQRD_HUMAN Sulfide:quinone oxid
 0.096385542 AGRL3_HUMAN Adhesion G protein-
 0.096493857 SQRD_HUMAN Sulfide:quinone oxid
 0.096699417 NDKA_HUMAN N HMGB2_HUMAN
 0.096774194 DHB4_HUMAN Peroxisomal multifu
 0.097560976 APOD_HUMAN Apolipoprotein D ,
 0.097560976 NDKB_HUMAN Nucleoside diphosph
 0.098164701 LEG9_HUMAN Ga SPB3_HUMAN Ser
 0.098273326 SPB3_HUMAN Se H2AY_HUMAN Co
 0.098445904 NDKA_HUMAN N C2CD3_HUMAN C
 0.098583666 MMP9_HUMAN LEG9_HUMAN Ga
 0.098902348 NDKA_HUMAN N HMGB2_HUMAN
 0.099099099 HCDH_HUMAN H RAB8A_HUMAN R
 0.099541651 MMP9_HUMAN SPB3_HUMAN Ser
 0.099570804 PHB_HUMAN Pro SPB3_HUMAN Ser
 0.099788969 HMGB2_HUMAN LEG9_HUMAN Ga
 0.1 LEG9_HUMAN Galectin-9 ,
 0.1 LEG9_HUMAN Galectin-9 ,
 0.1 LEG9_HUMAN Galectin-9 ,
 0.1 LEG9_HUMAN Galectin-9 ,
 0.1 K1C13_HUMAN Keratin, type I cytos
 0.1 PHB_HUMAN Prohibitin ,
 0.1 NDKA_HUMAN N NDKB_HUMAN Nu
 0.1 APOD_HUMAN A MMP14_HUMAN

regulation of monocyte chemotactic protein-1 production	0.1 APOD_HUMAN A LEG9_HUMAN Ga
zymogen granule membrane	0.1 TMEDA_HUMAN Transmembrane e
positive regulation of interleukin-13 production	0.1 LEG9_HUMAN Galectin-9 ,
positive regulation of interleukin-4 production	0.1 LEG9_HUMAN Galectin-9 ,
protein-DNA complex assembly	0.100496018 HMGB2_HUMAN H2AY_HUMAN Co
extracellular structure organization	0.101163762 MMP9_HUMAN GT251_HUMAN P
interleukin-13 production	0.102564103 LEG9_HUMAN Galectin-9 ,
interleukin-4 production	0.102564103 LEG9_HUMAN Galectin-9 ,
interleukin-5 production	0.102564103 LEG9_HUMAN Galectin-9 ,
myeloid leukocyte differentiation	0.102585558 NDKA_HUMAN N MMP9_HUMAN M
t-UTP complex	0.102890012 NH2L1_HUMAN NHP2-like protein 1
negative regulation of platelet-derived growth factor receptor signaling pathw	0.102941176 APOD_HUMAN Apolipoprotein D ,
transcription from RNA polymerase I promoter	0.103376262 H2AY_HUMAN Core histone macro-
mitochondrial protein processing	0.103530494 QCR2_HUMAN Cytochrome b-c1 co
microtubule organizing center part	0.103551906 NDKA_HUMAN N C2CD3_HUMAN C
negative regulation of endopeptidase activity	0.103665755 MMP9_HUMAN SPB3_HUMAN Ser
regulation of signal transduction	0.104147797 HMGB2_HUMAN APOD_HUMAN A
protein localization to cytoskeleton	0.104161074 C2CD3_HUMAN C2 domain-containi
regulation of cytokine production involved in inflammatory response	0.104166667 APOD_HUMAN Apolipoprotein D ,
null	0.104953816 HCDH_HUMAN H LEG9_HUMAN Gal
regulation of autophagy	0.1050339 LYRIC_HUMAN Pr RAB8A_HUMAN R
reproduction	0.105314879 HMGB2_HUMAN MMP9_HUMAN M
reproductive process	0.105314879 HMGB2_HUMAN MMP9_HUMAN M
nuclease activity	0.105522427 NDKA_HUMAN N HMGB2_HUMAN
negative regulation of response to cytokine stimulus	0.105534106 GSTP1_HUMAN Glutathione S-transf
regulation of protein transport	0.106134381 HCDH_HUMAN H APOD_HUMAN A
mast cell mediated immunity	0.106588824 LEG9_HUMAN Galectin-9 ,
mast cell activation	0.106588824 LEG9_HUMAN Galectin-9 ,
peptidyl-histidine modification	0.107070707 NDKB_HUMAN Nucleoside diphosph
pyrimidine ribonucleoside metabolic process	0.107142857 NDKA_HUMAN N NDKB_HUMAN Nu
regulation of histone phosphorylation	0.107142857 H2AY_HUMAN Core histone macro-
mast cell activation involved in immune response	0.107543323 LEG9_HUMAN Galectin-9 ,
chromatin assembly	0.108517033 HMGB2_HUMAN H2AY_HUMAN Co
hydrolase activity, acting on glycosyl bonds	0.108546078 AMY1_HUMAN A AMYP_HUMAN AI
leukocyte differentiation	0.1085659 NDKA_HUMAN N MMP9_HUMAN M
null	0.108763693 C2CD3_HUMAN C2 domain-containi
negative regulation of protein kinase activity	0.109094347 SPB3_HUMAN Se H2AY_HUMAN Co
regulation of MAPK cascade	0.109321581 PHB_HUMAN Pro LEG9_HUMAN Ga
response to external stimulus	0.109346405 NDKA_HUMAN N HMGB2_HUMAN
response to alkaloid	0.109536471 TMEDA_HUMAN AGRL3_HUMAN A
nucleosome organization	0.109838005 HMGB2_HUMAN H2AY_HUMAN Co
common myeloid progenitor cell proliferation	0.11 GSTP1_HUMAN Glutathione S-transf
protein phosphorylation	0.110153901 MMP9_HUMAN PHB_HUMAN Pro
sex differentiation	0.110671937 HMGB2_HUMAN MMP14_HUMAN
endopeptidase regulator activity	0.110829584 SPB3_HUMAN Se SPB12_HUMAN S
rDNA binding	0.111111111 H2AY_HUMAN Core histone macro-
embryonic heart tube morphogenesis	0.111111111 C2CD3_HUMAN C2 domain-containi
long-chain-enoyl-CoA hydratase activity	0.111111111 DHB4_HUMAN Peroxisomal multifun
regulation of interleukin-10 secretion	0.111111111 LEG9_HUMAN Galectin-9 ,
negative regulation of natural killer cell mediated immunity	0.111111111 LEG9_HUMAN Galectin-9 ,
galactosyltransferase activity	0.111111111 GT251_HUMAN Procollagen galacto
negative regulation of lipoprotein lipid oxidation	0.111111111 APOD_HUMAN Apolipoprotein D ,
regulation of alpha-beta T cell proliferation	0.111111111 LEG9_HUMAN Galectin-9 ,
dendritic cell apoptotic process	0.111111111 LEG9_HUMAN Galectin-9 ,

catalytic activity	0.111372287 HMGB2_HUMAN K1C13_HUMAN K
positive regulation of leukocyte migration	0.111624967 MMP14_HUMAN LEG9_HUMAN Gal
regulation of transcription from RNA polymerase I promoter	0.111709946 H2AY_HUMAN Core histone macro-
regulation of chromatin silencing	0.111709946 H2AY_HUMAN Core histone macro-
positive regulation of nuclear division	0.112045864 H2AY_HUMAN Core histone macro-
protein-DNA complex subunit organization	0.112310238 HMGB2_HUMAN H2AY_HUMAN Co
microtubule cytoskeleton organization	0.112397236 C2CD3_HUMAN CRAB8A_HUMAN R
circadian regulation of gene expression	0.112921731 PP1G_HUMAN Serine/threonine-pro
negative regulation of hydrolase activity	0.113555499 MMP9_HUMAN SPB3_HUMAN Ser
gonad development	0.11509666 HMGB2_HUMAN MMP14_HUMAN
regulation of cellular component organization	0.115213165 NDKA_HUMAN N APOD_HUMAN A
centriolar satellite	0.115384615 C2CD3_HUMAN C2 domain-containi
positive regulation of sister chromatid cohesion	0.115864528 H2AY_HUMAN Core histone macro-
alpha-linolenic acid metabolic process	0.116388374 DHB4_HUMAN Peroxisomal multifun
regulation of erythrocyte differentiation	0.116484232 HMGB2_HUMAN High mobility grou
positive regulation of nuclease activity	0.116683417 HMGB2_HUMAN High mobility grou
perinuclear region of cytoplasm	0.116734977 NDKA_HUMAN N HMGB2_HUMAN
null	0.117647059 H2AY_HUMAN Core histone macro-
non-sequence-specific DNA binding, bending	0.117647059 HMGB2_HUMAN High mobility grou
regulation of interleukin-13 production	0.117647059 LEG9_HUMAN Galectin-9 ,
regulation of interleukin-4 production	0.117647059 LEG9_HUMAN Galectin-9 ,
regulation of interleukin-5 production	0.117647059 LEG9_HUMAN Galectin-9 ,
negative regulation of macromolecule biosynthetic process	0.119762601 HMGB2_HUMAN LYRIC_HUMAN Pr
null	0.120471909 HMGB2_HUMAN LYRIC_HUMAN Pr
zymogen granule	0.120639535 TMEDA_HUMAN Transmembrane e
exocytic vesicle	0.121263948 RAB8A_HUMAN RRAB8B_HUMAN R
response to lipopolysaccharide	0.121405197 HMGB2_HUMAN LYRIC_HUMAN Pr
ossification	0.121794132 MMP9_HUMAN PHB_HUMAN Pro
neuron spine	0.122074139 RAB8A_HUMAN RPP1G_HUMAN Ser
negative regulation of kinase activity	0.122391702 SPB3_HUMAN Se H2AY_HUMAN Co
microbody	0.12283574 DHB4_HUMAN PeRAB8B_HUMAN R
positive regulation of cysteine-type endopeptidase activity involved in apoptot	0.122983871 LEG9_HUMAN Galectin-9 ,
regulation of cellular macromolecule biosynthetic process	0.123521407 HMGB2_HUMAN LYRIC_HUMAN Pr
positive regulation of epithelial to mesenchymal transition	0.123595506 SPB3_HUMAN Serpin B3 ,
null	0.12379992 NDKA_HUMAN N RAB8A_HUMAN R
autocrine signaling	0.12391193 SPB3_HUMAN Serpin B3 ,
pinosome	0.12391193 MMP14_HUMAN Matrix metallopro
extracellular matrix organization	0.124183007 MMP9_HUMAN GT251_HUMAN P
regulation of response to stimulus	0.12444888 HMGB2_HUMAN APOD_HUMAN A
chromosome, centromeric region	0.125 H2AY_HUMAN CoPP1G_HUMAN Ser
regulation of interleukin-13 secretion	0.125 LEG9_HUMAN Galectin-9 ,
very long-chain fatty-acyl-CoA metabolic process	0.125 DHB4_HUMAN Peroxisomal multifun
medium-chain fatty-acyl-CoA metabolic process	0.125 DHB4_HUMAN Peroxisomal multifun
negative regulation of protein import into nucleus	0.125 APOD_HUMAN Apolipoprotein D ,
negative regulation of androgen receptor signaling pathway	0.125 PHB_HUMAN Prohibitin ,
regulatory T cell differentiation	0.125 LEG9_HUMAN Galectin-9 ,
U4atac snRNA binding	0.125 NH2L1_HUMAN NHP2-like protein 1
U4/U6 snRNP	0.125 NH2L1_HUMAN NHP2-like protein 1
mitochondrial respiratory chain complex IV	0.125 QCR2_HUMAN Cytochrome b-c1 co
regulation of response to external stimulus	0.125006086 APOD_HUMAN A PHB_HUMAN Pro
regulation of inflammatory response	0.125823769 APOD_HUMAN A PHB_HUMAN Pro
positive regulation of immune effector process	0.125950185 PHB_HUMAN Pro LEG9_HUMAN Gal
sequence-specific DNA binding transcription factor activity	0.126567307 HMGB2_HUMAN LYRIC_HUMAN Pr
negative regulation of hormone secretion	0.126582278 HCDH_HUMAN Hydroxyacyl-coenzy

negative regulation of protein localization to nucleus
 monocarboxylic acid catabolic process
 response to axon injury
 DNA ligation
 preribosome
 negative regulation of protein transport
 secretion by cell
 regulation of extrinsic apoptotic signaling pathway
 estrogen metabolic process
 synaptic vesicle
 regulation of cell differentiation
 centrosome duplication
 centriole replication
 smooth muscle cell chemotaxis
 dosage compensation
 sister chromatid segregation
 cellular macromolecular complex assembly
 RNA biosynthetic process
 null
 lipid transporter activity
 development of primary sexual characteristics
 negative regulation of locomotion
 positive regulation of striated muscle cell differentiation
 negative regulation of lymphocyte mediated immunity
 regulation of cell-substrate junction assembly
 positive regulation of corticotropin secretion
 positive regulation of mitosis
 regulation of cellular process
 negative regulation of MAP kinase activity
 response to radiation
 negative regulation of cytokine-mediated signaling pathway
 regulation of hormone secretion
 regulation of nuclear division
 response to carbohydrate stimulus
 extracellular matrix disassembly

bone development
 receptor localization to synapse
 response to organic substance
 tube morphogenesis
 positive regulation of cellular process
 tumor necrosis factor superfamily cytokine production
 cell junction assembly
 modified amino acid binding
 regulation of cellular metabolic process
 negative regulation of inflammatory response
 protein localization to synapse
 null
 null
 negative regulation of lymphocyte migration
 regulation of dendritic cell apoptotic process
 keratinocyte migration
 galactose binding
 negative regulation of peptide hormone secretion
 ribonucleoside biosynthetic process

0.12688172 APOD_HUMAN Apolipoprotein D ,
 0.126966783 HCDH_HUMAN H DHB4_HUMAN Pe
 0.127272727 APOD_HUMAN Apolipoprotein D ,
 0.127868852 HMGB2_HUMAN High mobility grou
 0.128036515 NH2L1_HUMAN NHP2-like protein 1
 0.128404729 HCDH_HUMAN H APOD_HUMAN Ap
 0.128916965 HCDH_HUMAN H MMP9_HUMAN M
 0.128960278 HMGB2_HUMAN GSTP1_HUMAN G
 0.129032258 DHB4_HUMAN Peroxisomal multifun
 0.129153 RAB8A_HUMAN RRAB8B_HUMAN R
 0.12937329 NDKA_HUMAN N HMGB2_HUMAN
 0.12972973 C2CD3_HUMAN C2 domain-containi
 0.12972973 C2CD3_HUMAN C2 domain-containi
 0.130434783 GSTP1_HUMAN Glutathione S-transf
 0.131034483 H2AY_HUMAN Core histone macro-
 0.131243736 H2AY_HUMAN CoPP1G_HUMAN Ser
 0.131664849 HMGB2_HUMAN RL14_HUMAN 60
 0.132075381 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.132375776 GT251_HUMAN Procollagen galacto
 0.132445141 APOD_HUMAN Apolipoprotein D ,
 0.13293809 HMGB2_HUMAN MMP14_HUMAN
 0.133157218 APOD_HUMAN A GSTP1_HUMAN G
 0.133333333 MMP14_HUMAN Matrix metallopro
 0.133333333 LEG9_HUMAN Galectin-9 ,
 0.133333333 APOD_HUMAN A MMP14_HUMAN
 0.134146341 RAB8B_HUMAN Ras-related protein
 0.13430127 H2AY_HUMAN Core histone macro-
 0.135139625 HMGB2_HUMAN K1C13_HUMAN K
 0.135475051 SPB3_HUMAN Se GSTP1_HUMAN G
 0.135616142 K1C13_HUMAN K PP1G_HUMAN Se
 0.135757576 GSTP1_HUMAN Glutathione S-transf
 0.136811806 HCDH_HUMAN H RAB8B_HUMAN R
 0.137204301 H2AY_HUMAN Core histone macro-
 0.137248216 NDKA_HUMAN N GSTP1_HUMAN G
 0.138050921 MMP9_HUMAN MMP14_HUMAN
 0.139154334 MMP14_HUMAN SERPH_HUMAN S
 0.139372822 RAB8A_HUMAN Ras-related protein
 0.139907531 HMGB2_HUMAN HCDH_HUMAN H
 0.139938103 APOD_HUMAN A LYRIC_HUMAN Pr
 0.140054308 HMGB2_HUMAN MMP14_HUMAN
 0.140679141 LEG9_HUMAN Ga GSTP1_HUMAN G
 0.140801517 APOD_HUMAN A LYRIC_HUMAN Pr
 0.141136675 GSTP1_HUMAN Glutathione S-transf
 0.141346974 HMGB2_HUMAN MMP14_HUMAN
 0.141354794 APOD_HUMAN A GSTP1_HUMAN G
 0.141507281 RAB8A_HUMAN Ras-related protein
 0.14203194 GT251_HUMAN Procollagen galacto
 0.142142126 MMP9_HUMAN LEG9_HUMAN Ga
 0.142857143 APOD_HUMAN Apolipoprotein D ,
 0.142857143 LEG9_HUMAN Galectin-9 ,
 0.142857143 MMP9_HUMAN Matrix metalloprot
 0.142857143 LEG9_HUMAN Galectin-9 ,
 0.142857143 HCDH_HUMAN Hydroxyacyl-coenzy
 0.142857143 NDKA_HUMAN N NDKB_HUMAN Nu

regulation of B cell differentiation
 COPI-coated vesicle
 negative regulation of tumor necrosis factor production
 U4/U6 x U5 tri-snRNP complex
 integral to plasma membrane
 secretory granule membrane
 transferase activity, transferring phosphorus-containing groups
 kinetochore
 regulation of peptide hormone secretion
 natural killer cell mediated immunity
 reflex
 regulation of response to stress
 microtubule organizing center organization
 negative regulation of ion transmembrane transport
 immune system development
 endoderm development
 cellular component disassembly involved in apoptotic process
 regulation of smoothened signaling pathway
 replacement ossification
 ribosomal small subunit binding
 negative regulation of biosynthetic process
 regulation of ERK1 and ERK2 cascade
 null
 phosphorus metabolic process
 negative regulation of cytokine production involved in inflammatory response
 enzyme binding
 somatic diversification of immune receptors
 steroid dehydrogenase activity
 regulation of secretion
 chromatin organization
 skeletal system development
 chromosome segregation
 dendritic spine
 null
 endocrine hormone secretion
 positive regulation of B cell differentiation
 regulation of receptor binding
 negative regulation of cell killing

regulation of G0 to G1 transition
 null
 response to bacterium
 regulation of cell adhesion
 regulation of metabolic process
 positive regulation of hormone secretion
 regulation of intracellular transport
 neurogenesis
 transcriptional repressor complex
 regulation of cell motility
 null
 positive regulation of lymphocyte activation
 female sex differentiation
 cell-cell junction organization
 oxidoreductase activity, acting on CH-OH group of donors
 synapse assembly

0.142857143 MMP14_HUMAN Matrix metallopro
 0.142857143 TMEDA_HUMAN Transmembrane e
 0.142857143 LEG9_HUMAN Ga GSTP1_HUMAN G
 0.142857143 NH2L1_HUMAN NHP2-like protein 1
 0.14297209 PHB_HUMAN Pro MMP14_HUMAN
 0.143017265 RAB8A_HUMAN RTMEDA_HUMAN
 0.143036746 NDKA_HUMAN N NDKB_HUMAN N
 0.14325019 PP1G_HUMAN Serine/threonine-pro
 0.143280632 HCDH_HUMAN H RAB8B_HUMAN R
 0.143518519 LEG9_HUMAN Galectin-9 ,
 0.143801653 NDKA_HUMAN Nucleoside diphosph
 0.144229196 HMGB2_HUMAN APOD_HUMAN A
 0.144278018 C2CD3_HUMAN C2 domain-containi
 0.144352483 MMP9_HUMAN Matrix metalloprot
 0.14455155 NDKA_HUMAN N HMGB2_HUMAN
 0.144775114 MMP9_HUMAN MMP14_HUMAN
 0.145278124 HMGB2_HUMAN High mobility grou
 0.145488899 C2CD3_HUMAN C2 domain-containi
 0.148148148 MMP14_HUMAN Matrix metallopro
 0.148148148 NDKA_HUMAN Nucleoside diphosph
 0.14828694 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.148577785 PHB_HUMAN Pro LEG9_HUMAN Ga
 0.148678033 MMP14_HUMAN Matrix metallopro
 0.148860403 NDKA_HUMAN N MMP9_HUMAN M
 0.15 APOD_HUMAN Apolipoprotein D ,
 0.150337692 PHB_HUMAN Pro MMP14_HUMAN
 0.150356301 HMGB2_HUMAN High mobility grou
 0.151382893 DHB4_HUMAN Peroxisomal multifun
 0.151465375 HCDH_HUMAN H LEG9_HUMAN Ga
 0.151601928 HMGB2_HUMAN PHB_HUMAN Pro
 0.152081719 MMP9_HUMAN MMP14_HUMAN
 0.153124083 H2AY_HUMAN CoPP1G_HUMAN Ser
 0.153578656 RAB8A_HUMAN RPP1G_HUMAN Ser
 0.153627312 RAB8A_HUMAN Ras-related protein
 0.153846154 RAB8B_HUMAN Ras-related protein
 0.153846154 MMP14_HUMAN Matrix metallopro
 0.154354762 MMP9_HUMAN Matrix metalloprot
 0.154431955 LEG9_HUMAN Galectin-9 ,
 0.155102041 RBBP7_HUMAN Histone-binding pro
 0.155260026 LEG9_HUMAN Galectin-9 ,
 0.155667382 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.155735446 APOD_HUMAN A MMP14_HUMAN
 0.155773751 HMGB2_HUMAN MMP14_HUMAN
 0.156222778 RAB8B_HUMAN Ras-related protein
 0.156448348 APOD_HUMAN A LEG9_HUMAN Ga
 0.156494899 NDKA_HUMAN N HMGB2_HUMAN
 0.156617587 RBBP7_HUMAN Histone-binding pro
 0.157570503 APOD_HUMAN A MMP9_HUMAN M
 0.157645833 LEG9_HUMAN Ga RAB8B_HUMAN R
 0.157657658 MMP14_HUMAN LEG9_HUMAN Gal
 0.158221399 MMP14_HUMAN Matrix metallopro
 0.159197324 APOD_HUMAN A LYRIC_HUMAN Pr
 0.159499623 HCDH_HUMAN H DHB4_HUMAN Pe
 0.159926289 AGRL3_HUMAN Adhesion G protein-

regulation of developmental process
 positive regulation of receptor binding
 regulation of nuclease activity
 negative regulation of fibroblast proliferation
 protein deacetylase activity
 cell communication
 regulation of sister chromatid segregation
 respiratory chain complex IV
 COPII vesicle coating
 signal release
 long-chain fatty acid metabolic process
 amyloid precursor protein metabolic process
 cilium
 negative regulation of secretion
 response to other organism
 carboxylic acid catabolic process
 regulation of transport
 aromatic compound biosynthetic process
 multicellular organismal development
 regulation of phosphorylation
 collagen biosynthetic process
 regulation of mast cell degranulation
 negative regulation of mast cell activation
 regulation of glucocorticoid receptor signaling pathway
 negative regulation of T cell migration
 interleukin-1 secretion
 positive regulation of interleukin-1 secretion
 null
 positive regulation of interleukin-10 secretion
 positive regulation of interleukin-12 secretion
 positive regulation of lymphocyte apoptotic process
 regulation of smooth muscle cell chemotaxis
 lipoprotein modification
 photoperiodism
 regulation of chromatin silencing at rDNA
 negative regulation of natural killer cell mediated cytotoxicity
 negative regulation of histone methylation
 positive regulation of chromosome segregation
 nucleobase-containing small molecule biosynthetic process
 appendage morphogenesis
 null

negative regulation of transcription, DNA-dependent
 protein metabolic process
 positive regulation of interleukin-8 production
 heterocycle biosynthetic process
 ectoderm development
 mesoderm development
 null
 supercoiled DNA binding
 regulation of peptide secretion
 null
 response to cold
 chemotaxis
 cis-Golgi network

0.160421084 NDKA_HUMAN N HMGB2_HUMAN
 0.160966503 MMP9_HUMAN Matrix metalloprot
 0.161200357 HMGB2_HUMAN High mobility grou
 0.161290323 GSTP1_HUMAN Glutathione S-transf
 0.161737678 RBBP7_HUMAN Histone-binding pro
 0.161834067 HMGB2_HUMAN HCDH_HUMAN H
 0.161840609 H2AY_HUMAN Core histone macro-
 0.16259617 QCR2_HUMAN Cytochrome b-c1 co
 0.162613194 TMEDA_HUMAN Transmembrane e
 0.162849676 HCDH_HUMAN H RAB8B_HUMAN R
 0.163584637 GSTP1_HUMAN GDHB4_HUMAN Pe
 0.164133337 TMEDA_HUMAN Transmembrane e
 0.164438447 C2CD3_HUMAN CRAB8A_HUMAN R
 0.164529041 HCDH_HUMAN H LEG9_HUMAN Gal
 0.164686417 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.164811379 HCDH_HUMAN H DHB4_HUMAN Pe
 0.164817525 HCDH_HUMAN H APOD_HUMAN A
 0.164911351 NDKA_HUMAN N HMGB2_HUMAN
 0.165122214 HMGB2_HUMAN MMP14_HUMAN
 0.165652137 MMP9_HUMAN PHB_HUMAN Pro
 0.165701871 SERPH_HUMAN Serpin H1 ,
 0.166386555 LEG9_HUMAN Galectin-9 ,
 0.166666667 LEG9_HUMAN Galectin-9 ,
 0.166666667 PHB_HUMAN Prohibitin ,
 0.166666667 APOD_HUMAN Apolipoprotein D ,
 0.166666667 LEG9_HUMAN Galectin-9 ,
 0.166666667 LEG9_HUMAN Galectin-9 ,
 0.166666667 LEG9_HUMAN Galectin-9 ,
 0.166666667 LEG9_HUMAN Galectin-9 ,
 0.166666667 LEG9_HUMAN Galectin-9 ,
 0.166666667 GSTP1_HUMAN Glutathione S-transf
 0.166666667 APOD_HUMAN Apolipoprotein D ,
 0.166666667 PP1G_HUMAN Serine/threonine-pro
 0.166666667 H2AY_HUMAN Core histone macro-
 0.166666667 LEG9_HUMAN Galectin-9 ,
 0.166666667 H2AY_HUMAN Core histone macro-
 0.166837467 H2AY_HUMAN Core histone macro-
 0.167803738 NDKA_HUMAN N NDKB_HUMAN Nu
 0.168630532 C2CD3_HUMAN C2 domain-containi
 0.169112574 HCDH_HUMAN H APOD_HUMAN Ap

0.169474011 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.169944687 MMP14_HUMAN NDKB_HUMAN N
 0.169960474 LEG9_HUMAN Galectin-9 ,
 0.17026683 NDKA_HUMAN N HMGB2_HUMAN
 0.170304215 MMP9_HUMAN MMP14_HUMAN
 0.170304215 MMP9_HUMAN MMP14_HUMAN
 0.171122995 APOD_HUMAN Apolipoprotein D ,
 0.171122995 HMGB2_HUMAN High mobility grou
 0.17143707 HCDH_HUMAN H LEG9_HUMAN Ga
 0.17164714 NDKA_HUMAN N HCDH_HUMAN H
 0.171918506 CIRBP_HUMAN Cold-inducible RNA-
 0.172282816 HMGB2_HUMAN LEG9_HUMAN Ga
 0.173223148 TMEDA_HUMAN Transmembrane e

Golgi vesicle transport	0.173587277 C2CD3_HUMAN CRAB8A_HUMAN R
transcription from RNA polymerase II promoter	0.173600193 HMGB2_HUMAN LYRIC_HUMAN Pr
regulation of hydrolase activity	0.173617063 HMGB2_HUMAN MMP9_HUMAN M
null	0.17362613 HMGB2_HUMAN LYRIC_HUMAN Pr
SNARE binding	0.173840691 TMEDA_HUMAN Transmembrane e
opsonin binding	0.173840691 PHB_HUMAN Prohibitin ,
complement binding	0.173840691 PHB_HUMAN Prohibitin ,
protein localization to chromatin	0.175 H2AY_HUMAN CoRBBP7_HUMAN H
null	0.175084175 RBBP7_HUMAN Histone-binding pro
negative regulation of stress-activated MAPK cascade	0.175824176 SPB3_HUMAN Se GSTP1_HUMAN G
positive regulation of release of cytochrome c from mitochondria	0.176136364 MMP9_HUMAN Matrix metalloprot
platelet-derived growth factor receptor signaling pathway	0.176136364 APOD_HUMAN Apolipoprotein D ,
positive regulation of cellular metabolic process	0.176301443 HMGB2_HUMAN MMP9_HUMAN M
cellular response to epidermal growth factor stimulus	0.176352861 GSTP1_HUMAN Glutathione S-transf
lipoprotein lipid oxidation	0.176470588 APOD_HUMAN Apolipoprotein D ,
negative regulation of interleukin-1 production	0.176470588 GSTP1_HUMAN Glutathione S-transf
histone deacetylase complex	0.176660105 RBBP7_HUMAN Histone-binding pro
null	0.177059381 RAB8A_HUMAN RRAB8B_HUMAN R
translation regulator activity	0.17740382 CIRBP_HUMAN Cold-inducible RNA-
regulation of mast cell activation	0.177489177 LEG9_HUMAN Galectin-9 ,
null	0.177629585 HMGB2_HUMAN LYRIC_HUMAN Pr
positive regulation of cell activation	0.177694818 MMP14_HUMAN LEG9_HUMAN Gal
phosphorylation	0.17810401 NDKA_HUMAN N MMP9_HUMAN M
DNA binding, bending	0.178718993 HMGB2_HUMAN H2AY_HUMAN Co
transcription regulatory region DNA binding	0.178718993 NDKA_HUMAN N HMGB2_HUMAN
deacetylase activity	0.179305635 RBBP7_HUMAN Histone-binding pro
negative regulation of chemotaxis	0.179401993 GSTP1_HUMAN Glutathione S-transf
null	0.180071589 LEG9_HUMAN Ga GSTP1_HUMAN G
negative regulation of innate immune response	0.180316569 LEG9_HUMAN Galectin-9 ,
histone phosphorylation	0.180392157 H2AY_HUMAN Core histone macro-
secretion by tissue	0.180911317 NDKA_HUMAN Nucleoside diphosph
monocarboxylic acid metabolic process	0.180952381 HCDH_HUMAN H GSTP1_HUMAN G
microbody lumen	0.181304535 DHB4_HUMAN Peroxisomal multifun
COPI-coated vesicle budding	0.181818182 TMEDA_HUMAN Transmembrane e
negative regulation of mast cell degranulation	0.181818182 LEG9_HUMAN Galectin-9 ,
smooth muscle cell-matrix adhesion	0.181818182 APOD_HUMAN Apolipoprotein D ,
focal adhesion assembly	0.181818182 APOD_HUMAN A MMP14_HUMAN
Golgi transport vesicle coating	0.181818182 TMEDA_HUMAN Transmembrane e
cellular glucose homeostasis	0.181818182 NDKA_HUMAN Nucleoside diphosph
corticosteroid receptor signaling pathway	0.181818182 PHB_HUMAN Prohibitin ,
interferon-beta production	0.181818182 HMGB2_HUMAN High mobility grou
regulation of interferon-beta production	0.181818182 HMGB2_HUMAN High mobility grou
zinc ion binding	0.182550972 MMP9_HUMAN MMP14_HUMAN
nervous system development	0.182837927 NDKA_HUMAN N HMGB2_HUMAN
negative regulation of ion transport	0.183389454 MMP9_HUMAN Matrix metalloprot
sulfur compound metabolic process	0.183404772 SQRD_HUMAN Su GSTP1_HUMAN G
regulation of cellular protein metabolic process	0.184748951 MMP9_HUMAN PHB_HUMAN Pro
peroxisome organization	0.184854292 RAB8B_HUMAN Ras-related protein
T cell activation involved in immune response	0.185185185 LEG9_HUMAN Galectin-9 ,
lipopolysaccharide-mediated signaling pathway	0.185687706 LYRIC_HUMAN Protein LYRIC ,
apoptotic signaling pathway	0.186167034 NDKA_HUMAN N HMGB2_HUMAN
synapse part	0.186728496 RAB8A_HUMAN RPP1G_HUMAN Se
embryonic pattern specification	0.187096774 C2CD3_HUMAN C2 domain-containi
U2-type precatalytic spliceosome	0.1875 NH2L1_HUMAN NHP2-like protein 1

regulation of alpha-beta T cell activation	0.1875 LEG9_HUMAN Galectin-9 ,
null	0.1875 C2CD3_HUMAN C2 domain-containi
embryonic digit morphogenesis	0.1875 C2CD3_HUMAN C2 domain-containi
mitochondrial respiratory chain complex III	0.1875 QCR2_HUMAN Cytochrome b-c1 co
positive regulation of peptidase activity	0.188122208 MMP14_HUMAN LEG9_HUMAN Ga
regulation of synaptic plasticity	0.189059477 RAB8A_HUMAN Ras-related protein
centrosome cycle	0.189337282 C2CD3_HUMAN C2 domain-containi
smoothened signaling pathway	0.189586925 C2CD3_HUMAN C2 domain-containi
positive regulation of RNA metabolic process	0.190545842 HMGB2_HUMAN LYRIC_HUMAN Pr
regulation of molecular function	0.191118754 NDKA_HUMAN N HMGB2_HUMAN
null	0.192307692 NDKA_HUMAN Nucleoside diphosph
negative regulation of T cell activation	0.192307692 LEG9_HUMAN Galectin-9 ,
morphogenesis of a branching epithelium	0.192307692 MMP14_HUMAN Matrix metallopro
alpha-beta T cell activation	0.192307692 LEG9_HUMAN Galectin-9 ,
null	0.19298298 MMP9_HUMAN NDKB_HUMAN N
null	0.193568823 RBBP7_HUMAN Histone-binding pro
negative regulation of extrinsic apoptotic signaling pathway	0.193675889 HMGB2_HUMAN GSTP1_HUMAN G
formation of primary germ layer	0.194096375 MMP9_HUMAN MMP14_HUMAN
positive regulation of interferon-gamma production	0.194736842 LEG9_HUMAN Galectin-9 ,
positive regulation of interleukin-10 production	0.194736842 LEG9_HUMAN Galectin-9 ,
positive regulation of interleukin-12 production	0.194736842 LEG9_HUMAN Galectin-9 ,
positive regulation of leukocyte activation	0.194985547 MMP14_HUMAN LEG9_HUMAN Gal
unsaturated fatty acid metabolic process	0.195590327 GSTP1_HUMAN GDHB4_HUMAN Pe
regulation of transcription, DNA-dependent	0.196089753 HMGB2_HUMAN LYRIC_HUMAN Pr
regulation of transmembrane transporter activity	0.196721311 MMP9_HUMAN Matrix metalloprot
transforming growth factor beta production	0.197031039 LEG9_HUMAN Galectin-9 ,
interleukin-10 production	0.197031039 LEG9_HUMAN Galectin-9 ,
regulation of humoral immune response	0.197185851 PHB_HUMAN Prohibitin ,
secretory granule	0.197786875 MMP9_HUMAN NDKB_HUMAN N
positive regulation of nitrogen compound metabolic process	0.199001662 HMGB2_HUMAN MMP9_HUMAN M
null	0.199384826 MMP9_HUMAN Matrix metalloprot
regulation of interleukin-1 secretion	0.2 LEG9_HUMAN Galectin-9 ,
positive regulation of interleukin-13 secretion	0.2 LEG9_HUMAN Galectin-9 ,
regulation of striated muscle cell differentiation	0.2 MMP14_HUMAN Matrix metallopro
T cell differentiation involved in immune response	0.2 LEG9_HUMAN Galectin-9 ,
corticotropin secretion	0.2 RAB8B_HUMAN Ras-related protein
dendritic cell chemotaxis	0.2 LEG9_HUMAN Galectin-9 ,
regulation of histone H3-K4 methylation	0.2 H2AY_HUMAN Core histone macro-
negative regulation of cation channel activity	0.2 MMP9_HUMAN Matrix metalloprot
activated T cell autonomous cell death	0.2 LEG9_HUMAN Galectin-9 ,
null	0.2 MMP14_HUMAN Matrix metallopro
specification of symmetry	0.2 C2CD3_HUMAN C2 domain-containi
regulation of myotube differentiation	0.2 MMP14_HUMAN Matrix metallopro
neural plate pattern specification	0.2 C2CD3_HUMAN C2 domain-containi
regulation of histone H3-K27 methylation	0.2 H2AY_HUMAN Core histone macro-
ER to Golgi transport vesicle membrane	0.2 TMEDA_HUMAN Transmembrane e
positive regulation of alpha-beta T cell proliferation	0.2 LEG9_HUMAN Galectin-9 ,
small-subunit processome	0.2 NH2L1_HUMAN NHP2-like protein 1
negative regulation of acute inflammatory response	0.2 GSTP1_HUMAN Glutathione S-transf
null	0.200399462 H2AY_HUMAN CoPP1G_HUMAN Ser
cellular lipid catabolic process	0.200817782 HCDH_HUMAN H DHB4_HUMAN Pe
production of molecular mediator involved in inflammatory response	0.200941439 APOD_HUMAN Apolipoprotein D ,
regulation of myeloid leukocyte mediated immunity	0.201832358 LEG9_HUMAN Galectin-9 ,
midbody	0.202088502 RAB8A_HUMAN RPP1G_HUMAN Ser

GTP binding	0.202654475 NDKA_HUMAN N RAB8A_HUMAN R
negative regulation of protein catabolic process	0.202704981 PHB_HUMAN Pro SPB12_HUMAN Se
null	0.202908933 APOD_HUMAN A RAB8A_HUMAN R
neuron migration	0.203122026 AGRL3_HUMAN Adhesion G protein-
nucleotide phosphorylation	0.203629746 NDKA_HUMAN N NDKB_HUMAN Nu
digestion	0.203659397 AMYP_HUMAN Alpha-amylase 1 ,
transferase activity, transferring alkyl or aryl (other than methyl) groups	0.203933006 GSTP1_HUMAN Glutathione S-transf
protein localization to chromosome	0.204190877 H2AY_HUMAN CoRBBP7_HUMAN H
response to xenobiotic stimulus	0.204403082 GSTP1_HUMAN GAGRL3_HUMAN A
intracellular signal transduction	0.204898786 MMP9_HUMAN LYRIC_HUMAN Pr
response to L-ascorbic acid	0.206349206 GSTP1_HUMAN Glutathione S-transf
regulation of ion transmembrane transporter activity	0.20754717 MMP9_HUMAN Matrix metalloprot
chondrocyte proliferation	0.208888889 MMP14_HUMAN Matrix metallopro
regulation of leukocyte degranulation	0.20973173 LEG9_HUMAN Galectin-9 ,
cellular response to cadmium ion	0.210526316 MMP9_HUMAN Matrix metalloprot
inflammatory response to antigenic stimulus	0.211422754 HMGB2_HUMAN High mobility grou
maturation of LSU-rRNA	0.211469534 NH2L1_HUMAN NHP2-like protein 1
positive regulation of macromolecule biosynthetic process	0.21208116 HMGB2_HUMAN LYRIC_HUMAN Pr
negative regulation of catabolic process	0.212552807 PHB_HUMAN Pro SPB12_HUMAN S
regulation of mitosis	0.213505245 H2AY_HUMAN Core histone macro-
null	0.213523435 K1C13_HUMAN Keratin, type I cytos
positive regulation of I-kappaB kinase/NF-kappaB cascade	0.214026445 LYRIC_HUMAN Pr LEG9_HUMAN Gal
pyrimidine nucleoside metabolic process	0.214285714 NDKA_HUMAN N NDKB_HUMAN Nu
cytoplasmic stress granule	0.214285714 CIRBP_HUMAN Cold-inducible RNA-
myotube differentiation	0.214285714 MMP14_HUMAN Matrix metallopro
body fluid secretion	0.214819002 NDKA_HUMAN Nucleoside diphosph
signal transduction	0.214985822 HMGB2_HUMAN MMP14_HUMAN
ovarian follicle development	0.215686275 MMP14_HUMAN Matrix metallopro
cell surface receptor signaling pathway	0.215915204 HMGB2_HUMAN APOD_HUMAN A
vesicle localization	0.216064654 C2CD3_HUMAN CRAB8A_HUMAN R
positive regulation of molecular function	0.216918267 NDKA_HUMAN N HMGB2_HUMAN
GTPase activity	0.217199017 RAB8A_HUMAN RRAB8B_HUMAN R
peptidyl-lysine methylation	0.217391304 H2AY_HUMAN Core histone macro-
null	0.217391304 LEG9_HUMAN Galectin-9 ,
cellular response to peptide hormone stimulus	0.218039494 RAB8A_HUMAN RGSTP1_HUMAN G
adherens junction assembly	0.218394649 APOD_HUMAN A MMP14_HUMAN
nucleic acid phosphodiester bond hydrolysis	0.218470457 NDKA_HUMAN N HMGB2_HUMAN
null	0.218625455 RAB8A_HUMAN RPP1G_HUMAN Ser
positive regulation of hydrolase activity	0.218923174 HMGB2_HUMAN MMP14_HUMAN
negative regulation of biological process	0.21936639 HMGB2_HUMAN HCDH_HUMAN H
cation channel activity	0.219512195 MMP9_HUMAN Matrix metalloprot
neuron projection regeneration	0.220711905 APOD_HUMAN Apolipoprotein D ,
regulation of protein serine/threonine kinase activity	0.221468432 SPB3_HUMAN Se H2AY_HUMAN Co
cell-substrate adherens junction assembly	0.222222222 APOD_HUMAN A MMP14_HUMAN
dense fibrillar component	0.222222222 NH2L1_HUMAN NHP2-like protein 1
positive regulation of megakaryocyte differentiation	0.222222222 HMGB2_HUMAN High mobility grou
box C/D snoRNP complex	0.222222222 NH2L1_HUMAN NHP2-like protein 1
response to estrogen stimulus	0.222613475 MMP14_HUMAN Matrix metallopro
response to activity	0.222613475 HCDH_HUMAN Hydroxyacyl-coenzy
vesicle-mediated transport	0.223521307 NDKA_HUMAN N MMP9_HUMAN M
cell death	0.224369696 NDKA_HUMAN N HMGB2_HUMAN
RNA polymerase II transcription factor binding	0.22459893 LYRIC_HUMAN Protein LYRIC ,
NF-kappaB binding	0.22459893 LYRIC_HUMAN Protein LYRIC ,
regulation of transforming growth factor beta production	0.22459893 LEG9_HUMAN Galectin-9 ,

regulation of interleukin-10 production	0.22459893 LEG9_HUMAN Galectin-9 ,
guanyl nucleotide binding	0.225979172 NDKA_HUMAN N RAB8A_HUMAN R
cell cycle process	0.226033109 C2CD3_HUMAN CRAB8A_HUMAN R
null	0.226864093 APOD_HUMAN A RAB8A_HUMAN R
null	0.227089003 TMEDA_HUMAN Transmembrane e
intermediate filament	0.228395728 NDKA_HUMAN N K1C13_HUMAN K
response to temperature stimulus	0.229254571 RBBP7_HUMAN HCIRBP_HUMAN Co
null	0.229445333 HMGB2_HUMAN LYRIC_HUMAN Pr
null	0.229661362 MMP9_HUMAN PHB_HUMAN Pro
negative regulation of myeloid cell differentiation	0.229808945 NDKA_HUMAN Nucleoside diphosph
Golgi lumen	0.22981844 MMP14_HUMAN Matrix metallopro
protein localization to organelle	0.230189577 APOD_HUMAN A C2CD3_HUMAN C
RNA stabilization	0.230769231 CIRBP_HUMAN Cold-inducible RNA-
negative regulation of lymphocyte proliferation	0.230769231 LEG9_HUMAN Galectin-9 ,
null	0.230769231 MMP14_HUMAN Matrix metallopro
negative regulation of myeloid leukocyte differentiation	0.230769231 NDKA_HUMAN Nucleoside diphosph
response to chemical stimulus	0.2317772 HMGB2_HUMAN HCDH_HUMAN H
positive regulation of autophagy	0.232142857 LYRIC_HUMAN Protein LYRIC ,
mitochondrial membrane	0.232596041 NDKA_HUMAN N PHB_HUMAN Pro
myeloid cell differentiation	0.233895455 NDKA_HUMAN N HMGB2_HUMAN
respiratory chain complex III	0.233968849 QCR2_HUMAN Cytochrome b-c1 co
positive regulation of ERK1 and ERK2 cascade	0.234432234 PHB_HUMAN Pro LEG9_HUMAN Gal
peptide transport	0.234897861 HCDH_HUMAN H APOD_HUMAN A
regulation of chromosome segregation	0.235237182 H2AY_HUMAN Core histone macro-
SSU rRNA binding	0.235294118 CIRBP_HUMAN Cold-inducible RNA-
negative regulation of histone phosphorylation	0.235294118 H2AY_HUMAN Core histone macro-
null	0.2357624 LEG9_HUMAN Galectin-9 ,
mRNA stabilization	0.236842105 CIRBP_HUMAN Cold-inducible RNA-
Barr body	0.237011289 H2AY_HUMAN Core histone macro-
null	0.237011289 H2AY_HUMAN Core histone macro-
SWI/SNF-type complex	0.237011289 RBBP7_HUMAN Histone-binding pro
null	0.237014829 NDKA_HUMAN N HMGB2_HUMAN
synapse	0.237045215 RAB8A_HUMAN RPP1G_HUMAN Se
response to drug	0.237202384 NDKA_HUMAN N HMGB2_HUMAN
cell division site	0.237493219 PP1G_HUMAN Serine/threonine-pro
cell division site part	0.237493219 PP1G_HUMAN Serine/threonine-pro
chromosome	0.23759358 HMGB2_HUMAN PHB_HUMAN Pro
recycling endosome	0.237793055 RAB8A_HUMAN RRAB8B_HUMAN R
leukocyte homeostasis	0.237818895 LEG9_HUMAN Galectin-9 ,
negative regulation of G0 to G1 transition	0.237903226 RBBP7_HUMAN Histone-binding pro
embryonic skeletal system development	0.237903226 MMP14_HUMAN Matrix metallopro
cell-matrix adhesion	0.238095238 APOD_HUMAN A MMP14_HUMAN
negative regulation of apoptotic signaling pathway	0.238101288 HMGB2_HUMAN MMP9_HUMAN M
chondrocyte differentiation	0.238349309 SERPH_HUMAN Serpin H1 ,
cytochrome complex	0.239317701 QCR2_HUMAN Cytochrome b-c1 co
positive regulation of mitotic cell cycle	0.239631336 H2AY_HUMAN Core histone macro-
regulation of epithelial cell proliferation	0.239690585 NDKA_HUMAN N HMGB2_HUMAN
null	0.239718414 HMGB2_HUMAN LYRIC_HUMAN Pr
sex chromosome	0.239777577 H2AY_HUMAN Core histone macro-
response to amine stimulus	0.240225035 NDKA_HUMAN Nucleoside diphosph
intrinsic to plasma membrane	0.2408746 PHB_HUMAN Pro MMP14_HUMAN
adherens junction organization	0.241176471 APOD_HUMAN A MMP14_HUMAN
negative regulation of leukocyte migration	0.241443108 APOD_HUMAN Apolipoprotein D ,
intracellular organelle lumen	0.24187682 HMGB2_HUMAN HCDH_HUMAN H

snRNA binding	0.241924471 NH2L1_HUMAN NHP2-like protein 1
Golgi apparatus part	0.242709583 MMP14_HUMAN RAB8A_HUMAN R
Rab protein signal transduction	0.242857143 RAB8A_HUMAN RRAB8B_HUMAN R
myosin binding	0.243417367 RAB8A_HUMAN Ras-related protein
regulation of protein modification process	0.243424045 MMP9_HUMAN PHB_HUMAN Pro
negative regulation of transporter activity	0.243614464 MMP9_HUMAN Matrix metalloprot
mitochondrial part	0.243955178 NDKA_HUMAN N HCDH_HUMAN H
cation transport	0.244105409 MMP9_HUMAN PHB_HUMAN Pro
viral infectious cycle	0.244220023 LEG9_HUMAN GaSPB3_HUMAN Ser
null	0.244869853 MMP9_HUMAN Matrix metalloprot
alcohol binding	0.244878701 APOD_HUMAN Apolipoprotein D ,
guanyl ribonucleotide binding	0.245029261 NDKA_HUMAN N RAB8A_HUMAN R
stem cell division	0.24516129 HMGB2_HUMAN High mobility grou
Golgi-associated vesicle membrane	0.245558185 TMEDA_HUMAN Transmembrane e
G0 to G1 transition	0.245633305 RBBP7_HUMAN Histone-binding pro
negative regulation of cellular component movement	0.245688007 APOD_HUMAN A GSTP1_HUMAN G
regulation of histone modification	0.245817474 H2AY_HUMAN Core histone macro-
chromosome organization	0.246449149 HMGB2_HUMAN PHB_HUMAN Pro
hemopoietic or lymphoid organ development	0.246684623 NDKA_HUMAN N HMGB2_HUMAN
mitochondrial envelope	0.247011868 NDKA_HUMAN N PHB_HUMAN Pro
regulation of establishment of protein localization	0.248206062 HCDH_HUMAN H APOD_HUMAN A
response to organic nitrogen	0.248549975 NDKA_HUMAN N HCDH_HUMAN H
toll-like receptor 4 signaling pathway	0.25 LEG9_HUMAN Galectin-9 ,
alpha-beta T cell activation involved in immune response	0.25 LEG9_HUMAN Galectin-9 ,
ciliary transition zone	0.25 RAB8A_HUMAN Ras-related protein
interleukin-6 secretion	0.25 LEG9_HUMAN Galectin-9 ,
heterophilic cell-cell adhesion	0.25 LEG7_HUMAN Galectin-7 ,
lactation	0.25 NDKA_HUMAN Nucleoside diphosph
positive regulation of T cell proliferation	0.25 LEG9_HUMAN Galectin-9 ,
regulation of natural killer cell mediated cytotoxicity	0.25 LEG9_HUMAN Galectin-9 ,
negative regulation of alpha-beta T cell activation	0.25 LEG9_HUMAN Galectin-9 ,
oligodendrocyte development	0.25 GSTP1_HUMAN Glutathione S-transf
U4 snRNA binding	0.25 NH2L1_HUMAN NHP2-like protein 1
mature dendritic cell differentiation	0.25 LEG9_HUMAN Galectin-9 ,
null	0.25 RAB8A_HUMAN Ras-related protein
negative regulation of glucocorticoid receptor signaling pathway	0.25 PHB_HUMAN Prohibitin ,
complement component C3a binding	0.25 PHB_HUMAN Prohibitin ,
negative regulation of histone H3-K4 methylation	0.25 H2AY_HUMAN Core histone macro-
UDP-galactosyltransferase activity	0.25 GT251_HUMAN Procollagen galacto
positive regulation of T cell apoptotic process	0.25 LEG9_HUMAN Galectin-9 ,
positive regulation of myotube differentiation	0.25 MMP14_HUMAN Matrix metallopro
17-beta-hydroxysteroid dehydrogenase (NAD+) activity	0.25 DHB4_HUMAN Peroxisomal multifun
negative regulation of histone H3-K27 methylation	0.25 H2AY_HUMAN Core histone macro-
regulation of regulatory T cell differentiation	0.25 LEG9_HUMAN Galectin-9 ,
negative regulation of alpha-beta T cell proliferation	0.25 LEG9_HUMAN Galectin-9 ,
response to cadmium ion	0.25 MMP9_HUMAN Matrix metalloprot
null	0.25 NH2L1_HUMAN NHP2-like protein 1
chromosomal part	0.250039847 HMGB2_HUMAN PHB_HUMAN Pro
clathrin-coated vesicle	0.250877193 RAB8A_HUMAN RTMEDA_HUMAN
negative regulation of I-kappaB kinase/NF-kappaB cascade	0.251515152 GSTP1_HUMAN Glutathione S-transf
endoplasmic reticulum-Golgi intermediate compartment	0.251684966 TMEDA_HUMAN SERPH_HUMAN S
nucleobase-containing compound biosynthetic process	0.251824326 NDKA_HUMAN N HMGB2_HUMAN
regulation of catalytic activity	0.252891258 HMGB2_HUMAN MMP9_HUMAN M
outer membrane	0.252992683 NDKA_HUMAN N PP1G_HUMAN Ser
positive regulation of macromolecule metabolic process	0.253048944 HMGB2_HUMAN MMP9_HUMAN M

negative regulation of response to external stimulus

0.253250978 APOD_HUMAN A GSTP1_HUMAN G

negative regulation of lymphocyte activation

positive regulation of metabolic process

regulation of synapse structure and activity

acid-thiol ligase activity

regulation of circadian rhythm

fatty acid beta-oxidation using acyl-CoA oxidase

establishment of protein localization to chromatin

RAGE receptor binding

regulation of primary metabolic process

negative regulation of cell activation

mammary gland development

cellular component assembly

negative regulation of cysteine-type endopeptidase activity involved in apopto

negative regulation of nucleocytoplasmic transport

positive regulation of viral reproduction

fatty acid metabolic process

organelle outer membrane

chromatin silencing at rDNA

precatalytic spliceosome

cellular polysaccharide metabolic process

signal transduction by phosphorylation

fatty acid catabolic process

regulation of immune system process

cellular nitrogen compound biosynthetic process

nuclear chromosome, telomeric region

organelle membrane

regulation of phosphate metabolic process

gene expression

trans-Golgi network membrane

negative regulation of histone modification

regulation of programmed cell death

null

cellular process involved in reproduction in multicellular organism

organelle fusion

protein serine/threonine kinase inhibitor activity

maturation of SSU-rRNA

positive regulation of interleukin-1 production

cellular response to insulin stimulus

negative regulation of cellular biosynthetic process

negative regulation of leukocyte activation

regulation of tumor necrosis factor-mediated signaling pathway

activated T cell proliferation

regulation of interleukin-8 secretion

negative regulation of T cell proliferation

energy reserve metabolic process

envelope

null

ribosomal small subunit biogenesis

cellular component biogenesis

regulation of muscle cell differentiation

hippocampus development

appendage development

cis-trans isomerase activity

0.255255255 LEG9_HUMAN Galectin-9 ,

0.255849066 HMGB2_HUMAN MMP9_HUMAN M

0.255949809 AGRL3_HUMAN Adhesion G protein-

0.256097561 HCDH_HUMAN H DHB4_HUMAN Pe

0.25706421 PP1G_HUMAN Serine/threonine-pro

0.257142857 DHB4_HUMAN Peroxisomal multifun

0.257142857 H2AY_HUMAN Core histone macro-

0.25886819 HMGB2_HUMAN High mobility grou

0.259529073 HMGB2_HUMAN MMP14_HUMAN

0.26054844 LEG9_HUMAN Galectin-9 ,

0.260869565 NDKA_HUMAN Nucleoside diphosph

0.260878397 HMGB2_HUMAN MMP14_HUMAN

0.261083744 MMP9_HUMAN Matrix metalloprot

0.261675062 APOD_HUMAN Apolipoprotein D ,

0.261972003 LEG9_HUMAN Galectin-9 ,

0.262398572 HCDH_HUMAN H GSTP1_HUMAN G

0.262398861 NDKA_HUMAN N PP1G_HUMAN Ser

0.263157895 H2AY_HUMAN Core histone macro-

0.263157895 NH2L1_HUMAN NHP2-like protein 1

0.263599174 PP1G_HUMAN Serine/threonine-pro

0.264002263 PHB_HUMAN Pro LEG9_HUMAN Ga

0.264270613 HCDH_HUMAN H DHB4_HUMAN Pe

0.264609899 NDKA_HUMAN N HMGB2_HUMAN

0.265891393 HMGB2_HUMAN NDKB_HUMAN N

0.266073571 H2AY_HUMAN CoPP1G_HUMAN Ser

0.266968381 NDKA_HUMAN N LYRIC_HUMAN Pr

0.267632011 MMP9_HUMAN PHB_HUMAN Pro

0.268007321 HMGB2_HUMAN MMP14_HUMAN

0.268907563 RAB8A_HUMAN Ras-related protein

0.268907563 H2AY_HUMAN Core histone macro-

0.270155256 NDKA_HUMAN N HMGB2_HUMAN

0.270556 NDKA_HUMAN Nucleoside diphosph

0.270827836 HMGB2_HUMAN High mobility grou

0.270917258 MMP9_HUMAN LEG9_HUMAN Ga

0.271190211 H2AY_HUMAN Core histone macro-

0.271285034 NH2L1_HUMAN NHP2-like protein 1

0.271428571 LEG9_HUMAN Galectin-9 ,

0.272030651 RAB8A_HUMAN RGSTP1_HUMAN G

0.272379293 HMGB2_HUMAN LYRIC_HUMAN Pr

0.272513849 LEG9_HUMAN Galectin-9 ,

0.272727273 GSTP1_HUMAN Glutathione S-transf

0.272727273 LEG9_HUMAN Galectin-9 ,

0.272727273 LEG9_HUMAN Galectin-9 ,

0.272727273 LEG9_HUMAN Galectin-9 ,

0.274390244 PP1G_HUMAN Serine/threonine-pro

0.274600774 NDKA_HUMAN N LYRIC_HUMAN Pr

0.274725275 LEG9_HUMAN Galectin-9 ,

0.274800861 NH2L1_HUMAN NHP2-like protein 1

0.274905711 HMGB2_HUMAN MMP14_HUMAN

0.275349793 MMP14_HUMAN Matrix metallopro

0.27611071 NDKA_HUMAN Nucleoside diphosph

0.27611071 C2CD3_HUMAN C2 domain-containi

0.276292335 FKBP2_HUMAN Peptidyl-prolyl cis-tr

neural tube development
endosome
negative regulation of transmembrane transport
cellular hormone metabolic process

histone deacetylation
regulation of JNK cascade
organic acid catabolic process
cellular response to carbohydrate stimulus
regulation of vesicle-mediated transport
negative regulation of nucleobase-containing compound metabolic process
ion channel activity
ribose phosphate biosynthetic process
null
U4 snRNP
U6 snRNP
null
regulation of cell death
regulation of phosphorus metabolic process
interferon-gamma production
interleukin-12 production
cellular macromolecule metabolic process
positive regulation of transcription, DNA-dependent
DNA catabolic process, endonucleolytic
RNA polymerase II core promoter sequence-specific DNA binding
oligosaccharide binding
positive regulation of peptide hormone secretion
protein N-terminus binding
Rab GTPase binding
null
null
monosaccharide binding
GDP binding
phagocytic vesicle membrane
chromatin binding
null
positive regulation of cellular component organization
peptidase activator activity
vesicle coating
positive regulation of cellular biosynthetic process
null
positive regulation of secretion
passive transmembrane transporter activity
cellular response to molecule of bacterial origin
limbic system development
oligodendrocyte differentiation
cell differentiation
regulation of cell killing
lysosome localization
kinase activity
regulation of lymphocyte mediated immunity
null
taxi
negative regulation of gene expression, epigenetic
negative regulation of smooth muscle cell migration

0.277121459 C2CD3_HUMAN C2 domain-containi
0.27750823 PHB_HUMAN Pro MMP14_HUMAN
0.277867934 MMP9_HUMAN Matrix metalloprot
0.278750558 DHB4_HUMAN Peroxisomal multifun

0.278787879 PHB_HUMAN Pro RBBP7_HUMAN H
0.278787879 SPB3_HUMAN Se GSTP1_HUMAN G
0.278934831 HCDH_HUMAN H DHB4_HUMAN Pe
0.278977404 NDKA_HUMAN Nucleoside diphosph
0.279251469 LEG9_HUMAN GaRAB8A_HUMAN R
0.279288033 HMGB2_HUMAN LYRIC_HUMAN Pr
0.28 MMP9_HUMAN Matrix metalloprot
0.281183932 NDKA_HUMAN N NDKB_HUMAN N
0.28125 NH2L1_HUMAN NHP2-like protein 1
0.28125 NH2L1_HUMAN NHP2-like protein 1
0.28125 NH2L1_HUMAN NHP2-like protein 1
0.28206837 H2AY_HUMAN CoPP1G_HUMAN Ser
0.282352241 NDKA_HUMAN N HMGB2_HUMAN
0.282638574 MMP9_HUMAN PHB_HUMAN Pro
0.283838494 LEG9_HUMAN Galectin-9 ,
0.283838494 LEG9_HUMAN Galectin-9 ,
0.283920969 HMGB2_HUMAN MMP14_HUMAN
0.283984039 HMGB2_HUMAN PHB_HUMAN Pro
0.284210526 HMGB2_HUMAN High mobility grou
0.284210526 H2AY_HUMAN Core histone macro-
0.284210526 LEG9_HUMAN Galectin-9 ,
0.284210526 RAB8B_HUMAN Ras-related protein
0.284658738 PP1G_HUMAN Serine/threonine-pro
0.285714286 RAB8A_HUMAN Ras-related protein
0.285714286 LEG9_HUMAN Galectin-9 ,
0.285909713 MMP9_HUMAN PHB_HUMAN Pro
0.286737731 LEG9_HUMAN Galectin-9 ,
0.28736842 RAB8A_HUMAN RRAB8B_HUMAN R
0.287394958 RAB8A_HUMAN RRAB8B_HUMAN R
0.2876793 HMGB2_HUMAN H2AY_HUMAN Co
0.288451013 RAB8A_HUMAN Ras-related protein
0.288577815 NDKA_HUMAN N MMP9_HUMAN M
0.289411227 MMP14_HUMAN Matrix metallopro
0.289655574 TMEDA_HUMAN Transmembrane e
0.289801689 HMGB2_HUMAN LYRIC_HUMAN Pr
0.289962028 SPB3_HUMAN Serpin B3 ,
0.290710929 LEG9_HUMAN GaRAB8B_HUMAN R
0.290909091 MMP9_HUMAN Matrix metalloprot
0.291021672 HMGB2_HUMAN LYRIC_HUMAN Pr
0.29128827 NDKA_HUMAN Nucleoside diphosph
0.291424229 GSTP1_HUMAN Glutathione S-transf
0.291502323 HMGB2_HUMAN MMP14_HUMAN
0.291882055 LEG9_HUMAN Galectin-9 ,
0.292013568 LEG9_HUMAN Galectin-9 ,
0.292471241 NDKA_HUMAN N NDKB_HUMAN N
0.294117647 LEG9_HUMAN Galectin-9 ,
0.294145969 RAB8A_HUMAN RRAB8B_HUMAN R
0.294374257 HMGB2_HUMAN LEG9_HUMAN Ga
0.294633654 H2AY_HUMAN CoRBBP7_HUMAN H
0.294871795 GSTP1_HUMAN Glutathione S-transf

null
 lymphocyte differentiation
 Golgi vesicle budding
 positive regulation of viral entry into host cell
 proteolysis
 cellular response to hormone stimulus
 spermatid differentiation

regulation of mitotic sister chromatid segregation
 positive regulation of alpha-beta T cell activation
 mRNA 3'-UTR binding
 negative regulation of intracellular protein transport
 regulation of leukocyte proliferation
 regulation of cell activation
 lipoprotein metabolic process
 programmed cell death
 tissue regeneration
 protein complex localization
 cellular response to fatty acid
 signaling
 localization within membrane
 regulation of cell-substrate adhesion
 negative regulation of exocytosis
 response to retinoic acid
 microtubule cytoskeleton
 regulation of chemotaxis
 binding
 damaged DNA binding
 mitochondrial calcium ion transport
 regulation of stem cell proliferation
 morphogenesis of a branching structure
 cell-substrate junction assembly
 response to cocaine
 null
 null
 kinase regulator activity
 negative regulation of ERK1 and ERK2 cascade
 cellular lipid metabolic process
 cellular response to retinoic acid
 xenobiotic metabolic process
 cytokine production
 hydrolase activity
 cytoplasm
 positive regulation of biosynthetic process
 protein localization
 null
 regulation of lymphocyte activation
 regulation of superoxide metabolic process
 pattern specification process
 protein import
 steroid binding
 occluding junction
 null
 regulation of protein activation cascade
 endothelial cell differentiation

0.296203162 C2CD3_HUMAN CRAB8A_HUMAN R
 0.296653432 MMP14_HUMAN LEG9_HUMAN Gal
 0.296702468 TMEDA_HUMAN Transmembrane e
 0.298245614 LEG9_HUMAN Galectin-9 ,
 0.298337525 MMP9_HUMAN C2CD3_HUMAN C
 0.29865156 PHB_HUMAN Pro RAB8A_HUMAN R
 0.299095396 HMGB2_HUMAN High mobility grou

0.3 H2AY_HUMAN Core histone macro-
 0.3 LEG9_HUMAN Galectin-9 ,
 0.3 CIRBP_HUMAN Cold-inducible RNA-
 0.300764481 APOD_HUMAN Apolipoprotein D ,
 0.301137316 LEG9_HUMAN GaGSTP1_HUMAN G
 0.301909854 MMP14_HUMAN LEG9_HUMAN Gal
 0.30240505 APOD_HUMAN Apolipoprotein D ,
 0.302619182 NDKA_HUMAN N HMGB2_HUMAN
 0.302878706 APOD_HUMAN Apolipoprotein D ,
 0.304052989 C2CD3_HUMAN CRAB8A_HUMAN R
 0.30434518 NDKA_HUMAN Nucleoside diphosph
 0.305723035 HMGB2_HUMAN HCDH_HUMAN H
 0.305757255 RAB8A_HUMAN Ras-related protein
 0.305882353 APOD_HUMAN A MMP14_HUMAN
 0.305917657 LEG9_HUMAN Galectin-9 ,
 0.306280793 K1C13_HUMAN Keratin, type I cytos
 0.306323999 NDKA_HUMAN N C2CD3_HUMAN C
 0.306621378 LEG9_HUMAN GaGSTP1_HUMAN G
 0.306968603 HMGB2_HUMAN K1C13_HUMAN K
 0.30732842 HMGB2_HUMAN High mobility grou
 0.307692308 PHB_HUMAN Prohibitin ,
 0.307780827 HMGB2_HUMAN High mobility grou
 0.308132925 MMP14_HUMAN Matrix metallopro
 0.308300395 APOD_HUMAN A MMP14_HUMAN
 0.309067123 AGRL3_HUMAN Adhesion G protein-
 0.310380376 MMP9_HUMAN NDKB_HUMAN N
 0.310608287 QCR2_HUMAN Cytochrome b-c1 co
 0.310667047 H2AY_HUMAN CoGSTP1_HUMAN G
 0.310689311 PHB_HUMAN Pro GSTP1_HUMAN G
 0.310879443 HCDH_HUMAN H APOD_HUMAN A
 0.311293969 K1C13_HUMAN Keratin, type I cytos
 0.311869155 GSTP1_HUMAN Glutathione S-transf
 0.312518525 HMGB2_HUMAN APOD_HUMAN A
 0.314264887 HMGB2_HUMAN AMY1_HUMAN AI
 0.314725625 HMGB2_HUMAN K1C13_HUMAN K
 0.314906486 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.314924613 HCDH_HUMAN H APOD_HUMAN A
 0.315089607 NDKA_HUMAN N HMGB2_HUMAN
 0.315315315 MMP14_HUMAN LEG9_HUMAN Gal
 0.315789474 GSTP1_HUMAN Glutathione S-transf
 0.316622861 C2CD3_HUMAN C2 domain-containi
 0.31748335 APOD_HUMAN A RAB8B_HUMAN R
 0.317636695 APOD_HUMAN Apolipoprotein D ,
 0.317647059 LYRIC_HUMAN Protein LYRIC ,
 0.318181818 LEG9_HUMAN Galectin-9 ,
 0.318447335 PHB_HUMAN Prohibitin ,
 0.319153794 SETLP_HUMAN Protein SETSIP ,

cellular component organization or biogenesis
 carbohydrate homeostasis
 cytosolic ribosome
 response to steroid hormone stimulus
 regulation of interferon-gamma production
 regulation of interleukin-12 production
 neuronal cell body
 organelle lumen
 transport vesicle membrane
 polysaccharide metabolic process

regulation of cytokine production
 null
 MAPK cascade
 cartilage development
 Golgi organization
 null
 DNA conformation change
 negative regulation of immune response
 regulation of cell migration
 organelle inner membrane
 ATPase binding
 null
 mitotic sister chromatid segregation
 embryonic appendage morphogenesis
 negative regulation of interferon-gamma production
 positive regulation of nucleobase-containing compound metabolic process
 apoptotic process
 DNA metabolic process
 regulation of leukocyte mediated cytotoxicity
 glutathione peroxidase activity
 somatic cell DNA recombination
 regulation of maintenance of mitotic sister chromatid cohesion
 sex chromatin
 regulation of corticotropin secretion
 regulation of cation channel activity
 CD4-positive, alpha-beta T cell proliferation
 chondrocyte differentiation involved in endochondral bone morphogenesis
 negative regulation of smooth muscle cell chemotaxis
 UTP biosynthetic process
 tongue morphogenesis
 polysaccharide digestion
 positive regulation of regulatory T cell differentiation
 UTP metabolic process
 regulation of histone methylation
 positive regulation of interferon-beta production
 regulation of myeloid cell differentiation
 response to biotic stimulus
 regulation of leukocyte mediated immunity
 nucleus organization
 G-protein coupled receptor signaling pathway
 negative regulation of intracellular steroid hormone receptor signaling pathwa
 autophagy
 regulation of synapse organization
 peptidyl-proline modification

0.319751808 HMGB2_HUMAN K1C13_HUMAN K
 0.321044547 NDKA_HUMAN Nucleoside diphosph
 0.321070234 APOD_HUMAN A RL14_HUMAN 60
 0.321267646 HMGB2_HUMAN PHB_HUMAN Pro
 0.321524064 LEG9_HUMAN Galectin-9 ,
 0.321524064 LEG9_HUMAN Galectin-9 ,
 0.321609583 APOD_HUMAN A RAB8A_HUMAN R
 0.322232712 HMGB2_HUMAN HCDH_HUMAN H
 0.324152165 TMEDA_HUMAN Transmembrane e
 0.32436938 PP1G_HUMAN Serine/threonine-pro

0.324395929 HMGB2_HUMAN APOD_HUMAN A
 0.324705882 H2AY_HUMAN Core histone macro-
 0.324728996 PHB_HUMAN Pro LEG9_HUMAN Ga
 0.325596145 SERPH_HUMAN Serpin H1 ,
 0.326095744 TMEDA_HUMAN Transmembrane e
 0.327087831 H2AY_HUMAN CoRBBP7_HUMAN H
 0.327134094 HMGB2_HUMAN H2AY_HUMAN Co
 0.327343051 LEG9_HUMAN Galectin-9 ,
 0.328317399 APOD_HUMAN A MMP9_HUMAN M
 0.328521395 PHB_HUMAN Pro QCR2_HUMAN Cy
 0.329808109 NH2L1_HUMAN NHP2-like protein 1
 0.329843449 PHB_HUMAN Prohibitin ,
 0.330143541 H2AY_HUMAN Core histone macro-
 0.330882353 C2CD3_HUMAN C2 domain-containi
 0.330882353 LEG9_HUMAN Galectin-9 ,
 0.331238054 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.332261571 NDKA_HUMAN N HMGB2_HUMAN
 0.332756629 NDKA_HUMAN N HMGB2_HUMAN
 0.333333333 LEG9_HUMAN Galectin-9 ,
 0.333333333 GSTP1_HUMAN Glutathione S-transf
 0.333333333 HMGB2_HUMAN High mobility grou
 0.333333333 H2AY_HUMAN Core histone macro-
 0.333333333 H2AY_HUMAN Core histone macro-
 0.333333333 RAB8B_HUMAN Ras-related protein
 0.333333333 MMP9_HUMAN Matrix metalloprot
 0.333333333 LEG9_HUMAN Galectin-9 ,
 0.333333333 SERPH_HUMAN Serpin H1 ,
 0.333333333 GSTP1_HUMAN Glutathione S-transf
 0.333333333 NDKA_HUMAN N NDKB_HUMAN Nu
 0.333333333 K1C13_HUMAN Keratin, type I cytos
 0.333333333 AMYP_HUMAN Alpha-amylase 1 ,
 0.333333333 LEG9_HUMAN Galectin-9 ,
 0.333333333 NDKA_HUMAN N NDKB_HUMAN Nu
 0.333333333 H2AY_HUMAN Core histone macro-
 0.333333333 HMGB2_HUMAN High mobility grou
 0.33488551 NDKA_HUMAN N HMGB2_HUMAN
 0.335935716 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.336531737 LEG9_HUMAN Galectin-9 ,
 0.336938532 HMGB2_HUMAN High mobility grou
 0.337082051 PHB_HUMAN Pro AGRL3_HUMAN A
 0.337835231 PHB_HUMAN Prohibitin ,
 0.339094001 LYRIC_HUMAN Pr RAB8A_HUMAN R
 0.339956082 AGRL3_HUMAN Adhesion G protein-
 0.340728035 FKBP2_HUMAN Peptidyl-prolyl cis-tr

vesicle fusion
 endoplasmic reticulum-Golgi intermediate compartment membrane
 NAD binding
 cell cycle
 response to epidermal growth factor stimulus
 positive regulation of gene expression
 exocytosis
 peptidyl-prolyl cis-trans isomerase activity
 response to anesthetic
 centromere complex assembly
 complement activation
 fatty-acyl-CoA metabolic process
 null

null
 cysteine-type endopeptidase inhibitor activity
 positive regulation of cell motility
 positive regulation of protein binding
 glutathione metabolic process
 regulation of multicellular organismal development
 axon regeneration
 localization
 chromatin assembly or disassembly
 positive regulation of cytokine secretion
 glial cell development
 organelle envelope
 endothelium development
 positive regulation of peptide secretion
 null
 connective tissue development
 male gonad development
 development of primary male sexual characteristics
 germ cell development
 system development
 chaperone-mediated protein transport
 negative regulation of RNA metabolic process
 regulation of immune effector process
 mitochondrion
 cytoplasmic vesicle part
 response to abiotic stimulus
 regulation of viral reproduction
 protein targeting to mitochondrion
 endocytic vesicle membrane
 tight junction assembly
 chemoattractant activity
 null
 positive regulation of muscle cell differentiation
 cellular response to xenobiotic stimulus
 single-stranded DNA binding
 positive regulation of innate immune response
 null
 regulation of cellular localization
 microtubule-based process
 null
 cellular response to corticosteroid stimulus

0.340734784 MMP9_HUMAN LEG9_HUMAN Ga
 0.341237438 TMEDA_HUMAN Transmembrane e
 0.341825277 HCDH_HUMAN Hydroxyacyl-coenzy
 0.342034005 C2CD3_HUMAN CRAB8A_HUMAN R
 0.342244592 GSTP1_HUMAN Glutathione S-transf
 0.343958414 HMGB2_HUMAN PHB_HUMAN Pro
 0.344328372 MMP9_HUMAN LEG9_HUMAN Ga
 0.34479942 FKBP2_HUMAN Peptidyl-prolyl cis-tr
 0.34489208 AGRL3_HUMAN Adhesion G protein-
 0.347137821 RBBP7_HUMAN Histone-binding pro
 0.347552891 PHB_HUMAN Prohibitin ,
 0.347826087 DHB4_HUMAN Peroxisomal multifun
 0.347962546 MMP9_HUMAN LEG9_HUMAN Gal

0.348121268 RAB8A_HUMAN RGSTP1_HUMAN G
 0.348846454 SPB3_HUMAN Serpin B3 ,
 0.3503707 MMP9_HUMAN MMP14_HUMAN
 0.350400868 MMP9_HUMAN Matrix metalloprot
 0.350507124 GSTP1_HUMAN Glutathione S-transf
 0.350951055 NDKA_HUMAN N HMGB2_HUMAN
 0.352380952 APOD_HUMAN Apolipoprotein D ,
 0.352403184 HMGB2_HUMAN HCDH_HUMAN H
 0.352642613 HMGB2_HUMAN H2AY_HUMAN Co
 0.353846154 LEG9_HUMAN Galectin-9 ,
 0.35386302 GSTP1_HUMAN Glutathione S-transf
 0.353965671 NDKA_HUMAN N LYRIC_HUMAN Pr
 0.354566376 SETLP_HUMAN Protein SETSIP ,
 0.355742297 LEG9_HUMAN GaRAB8B_HUMAN R
 0.355768089 RAB8A_HUMAN Ras-related protein
 0.355851639 SERPH_HUMAN Serpin H1 ,
 0.357142857 HMGB2_HUMAN MMP14_HUMAN
 0.357142857 HMGB2_HUMAN MMP14_HUMAN
 0.357538767 HMGB2_HUMAN High mobility grou
 0.358732054 HMGB2_HUMAN MMP14_HUMAN
 0.360112519 TIM8A_HUMAN Mitochondrial impo
 0.361525913 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.361584989 PHB_HUMAN Pro LEG9_HUMAN Gal
 0.361588047 NDKA_HUMAN N HCDH_HUMAN H
 0.361652123 MMP9_HUMAN NDKB_HUMAN N
 0.361668063 MMP14_HUMAN K1C13_HUMAN K
 0.363303278 LEG9_HUMAN Galectin-9 ,
 0.363318315 QCR2_HUMAN Cytochrome b-c1 co
 0.363497599 RAB8A_HUMAN RRAB8B_HUMAN R
 0.363636364 LYRIC_HUMAN Protein LYRIC ,
 0.363636364 HMGB2_HUMAN High mobility grou
 0.363636364 LEG9_HUMAN Galectin-9 ,
 0.364033613 MMP14_HUMAN Matrix metallopro
 0.364439721 GSTP1_HUMAN Glutathione S-transf
 0.364731419 NDKA_HUMAN N HMGB2_HUMAN
 0.365820178 HMGB2_HUMAN LEG9_HUMAN Gal
 0.366278961 RAB8A_HUMAN Ras-related protein
 0.366459777 APOD_HUMAN A LEG9_HUMAN Ga
 0.366530803 C2CD3_HUMAN CRAB8A_HUMAN R
 0.367423369 MMP14_HUMAN LEG9_HUMAN Gal
 0.368421053 GSTP1_HUMAN Glutathione S-transf

U2-type spliceosomal complex
 positive regulation of cellular protein metabolic process
 macromolecule modification
 cell activation involved in immune response
 transferase activity, transferring glycosyl groups
 hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds
 regulation of intracellular steroid hormone receptor signaling pathway
 vacuole
 null
 head development
 lipid catabolic process
 nucleoside triphosphate metabolic process
 regulation of response to cytokine stimulus
 null
 protein serine/threonine phosphatase activity
 regulation of androgen receptor signaling pathway

 regulation of activated T cell proliferation
 U1 snRNP
 DNA packaging
 null
 stem cell proliferation
 glial cell migration
 regulation of Notch signaling pathway
 regulation of endopeptidase activity
 membrane part
 null
 regulation of leukocyte activation
 branching morphogenesis of a tube
 lung development
 respiratory tube development
 defense response to Gram-negative bacterium
 defense response to Gram-positive bacterium
 regulation of protein import into nucleus
 null
 core promoter sequence-specific DNA binding
 cytoplasmic part
 intracellular organelle part
 regeneration
 positive regulation of mononuclear cell proliferation
 superoxide metabolic process
 cell leading edge
 steroid dehydrogenase activity, acting on the CH-OH group of donors, NAD or
 regulation of T cell differentiation
 regulation of smooth muscle cell migration
 molecular transducer activity
 regulation of acute inflammatory response
 positive regulation of gene expression, epigenetic
 response to vitamin
 mitochondrial electron transport, ubiquinol to cytochrome c
 regulation of interleukin-8 production
 lymphocyte activation involved in immune response
 receptor binding
 regulation of release of cytochrome c from mitochondria
 negative regulation of cell motility

0.368421053 NH2L1_HUMAN NHP2-like protein 1
 0.368825381 MMP9_HUMAN PHB_HUMAN Pro
 0.369464429 MMP9_HUMAN PHB_HUMAN Pro
 0.371075588 MMP9_HUMAN LEG9_HUMAN Ga
 0.371127803 GT251_HUMAN Procollagen galacto
 0.371822922 RBBP7_HUMAN Histone-binding pro
 0.371920681 PHB_HUMAN Prohibitin ,
 0.372695974 MMP14_HUMAN NDKB_HUMAN N
 0.372924272 CIRBP_HUMAN Cold-inducible RNA-
 0.372928909 NDKA_HUMAN N APOD_HUMAN A
 0.373033071 HCDH_HUMAN H DHB4_HUMAN Pe
 0.373186344 NDKA_HUMAN N NDKB_HUMAN N
 0.373556162 GSTP1_HUMAN Glutathione S-transf
 0.374574999 H2AY_HUMAN Core histone macro-
 0.375 PP1G_HUMAN Serine/threonine-pro
 0.375 PHB_HUMAN Prohibitin ,

 0.375 LEG9_HUMAN Galectin-9 ,
 0.375 NH2L1_HUMAN NHP2-like protein 1
 0.375788024 HMGB2_HUMAN H2AY_HUMAN Co
 0.376063528 APOD_HUMAN A RAB8A_HUMAN R
 0.377330809 HMGB2_HUMAN High mobility grou
 0.377966429 MMP14_HUMAN Matrix metallopro
 0.378202064 MMP14_HUMAN Matrix metallopro
 0.378258914 MMP9_HUMAN LEG9_HUMAN Ga
 0.378914478 MMP14_HUMAN NDKB_HUMAN N
 0.379294434 RBBP7_HUMAN Histone-binding pro
 0.379443571 MMP14_HUMAN LEG9_HUMAN Gal
 0.380422439 MMP14_HUMAN Matrix metallopro
 0.380608198 MMP14_HUMAN Matrix metallopro
 0.380654609 MMP14_HUMAN Matrix metallopro
 0.380952381 HMGB2_HUMAN High mobility grou
 0.380952381 HMGB2_HUMAN High mobility grou
 0.381223329 APOD_HUMAN Apolipoprotein D ,
 0.381224894 CIRBP_HUMAN Cold-inducible RNA-
 0.382173382 H2AY_HUMAN Core histone macro-
 0.384060731 HMGB2_HUMAN K1C13_HUMAN K
 0.384060731 HMGB2_HUMAN K1C13_HUMAN K
 0.384520372 APOD_HUMAN A GSTP1_HUMAN G
 0.384615385 LEG9_HUMAN Galectin-9 ,
 0.384615385 GSTP1_HUMAN Glutathione S-transf
 0.385185829 NDKA_HUMAN N NDKB_HUMAN Nu
 0.385964912 DHB4_HUMAN Peroxisomal multifun
 0.385964912 LEG9_HUMAN Galectin-9 ,
 0.386022514 GSTP1_HUMAN Glutathione S-transf
 0.387337436 HMGB2_HUMAN PHB_HUMAN Pro
 0.387969925 PHB_HUMAN Pro GSTP1_HUMAN G
 0.388341684 H2AY_HUMAN Core histone macro-
 0.388888889 GSTP1_HUMAN Glutathione S-transf
 0.388888889 QCR2_HUMAN Cytochrome b-c1 co
 0.389525507 LEG9_HUMAN Galectin-9 ,
 0.390090631 LEG9_HUMAN Galectin-9 ,
 0.390121741 HMGB2_HUMAN MMP9_HUMAN M
 0.390881926 MMP9_HUMAN Matrix metalloprot
 0.391459573 APOD_HUMAN A GSTP1_HUMAN G

immune system process	0.393674883 NDKA_HUMAN N HMGB2_HUMAN
cellular response to organic nitrogen	0.393985939 RAB8A_HUMAN RGSTP1_HUMAN G
large ribosomal subunit	0.394230769 RL14_HUMAN 60 NH2L1_HUMAN N
negative regulation of programmed cell death	0.394352534 HMGB2_HUMAN MMP9_HUMAN M
null	0.394529915 LEG9_HUMAN Galectin-9 ,
null	0.394884063 LEG9_HUMAN Galectin-9 ,
negative regulation of mononuclear cell proliferation	0.395604396 LEG9_HUMAN Galectin-9 ,
centromeric heterochromatin	0.395604396 H2AY_HUMAN Core histone macro-
peroxisomal part	0.395604396 DHB4_HUMAN PeRAB8B_HUMAN R
positive regulation of T cell differentiation	0.395604396 LEG9_HUMAN Galectin-9 ,
coated vesicle membrane	0.39576518 TMEDA_HUMAN Transmembrane e
dendrite	0.398539404 APOD_HUMAN A RAB8A_HUMAN R
regulation of G-protein coupled receptor protein signaling pathway	0.399032019 PHB_HUMAN Prohibitin ,
regulation of protein localization	0.399502205 HCDH_HUMAN H APOD_HUMAN A
condensed chromosome kinetochore	0.4 PP1G_HUMAN Serine/threonine-pro
regulation of smooth muscle cell-matrix adhesion	0.4 APOD_HUMAN Apolipoprotein D ,
T cell activation via T cell receptor contact with antigen bound to MHC molecu	0.4 LEG9_HUMAN Galectin-9 ,
regulation of focal adhesion assembly	0.4 APOD_HUMAN A MMP14_HUMAN
UDP-glycosyltransferase activity	0.4 GT251_HUMAN Procollagen galacto
axis specification	0.4 C2CD3_HUMAN C2 domain-containi
CD4-positive, alpha-beta T cell differentiation	0.4 LEG9_HUMAN Galectin-9 ,
cell division	0.400251941 HMGB2_HUMAN PP1G_HUMAN Ser
negative regulation of multicellular organismal process	0.400980782 NDKA_HUMAN N APOD_HUMAN A
vesicle membrane	0.402236729 RAB8A_HUMAN RTMEDA_HUMAN
nucleoside triphosphate biosynthetic process	0.402622378 NDKA_HUMAN N NDKB_HUMAN N
regulation of gene silencing	0.40381962 H2AY_HUMAN Core histone macro-
positive regulation of sequence-specific DNA binding transcription factor activi	0.40472871 LYRIC_HUMAN Pr LEG9_HUMAN Ga
protein kinase activity	0.404745802 NDKB_HUMAN N SPB3_HUMAN Ser
cleavage furrow	0.40529856 PP1G_HUMAN Serine/threonine-pro
muscle cell migration	0.406212817 GSTP1_HUMAN Glutathione S-transf
regulation of symbiosis, encompassing mutualism through parasitism	0.406719717 LEG9_HUMAN Galectin-9 ,
peptide hormone secretion	0.407224959 HCDH_HUMAN H RAB8B_HUMAN R
calcium ion transmembrane transport	0.40726817 PHB_HUMAN Prohibitin ,
T cell proliferation	0.407407407 LEG9_HUMAN Galectin-9 ,
regulation of leukocyte apoptotic process	0.407729585 LEG9_HUMAN Galectin-9 ,
positive regulation of apoptotic signaling pathway	0.408016383 MMP9_HUMAN LEG9_HUMAN Gal
synapse organization	0.40817642 AGRL3_HUMAN Adhesion G protein-
purine-containing compound biosynthetic process	0.408871133 NDKA_HUMAN N NDKB_HUMAN N
respiratory electron transport chain	0.409090909 QCR2_HUMAN Cytochrome b-c1 co
metabolic process	0.409769675 HMGB2_HUMAN H2AY_HUMAN Co
apical junction complex	0.410084034 LYRIC_HUMAN Protein LYRIC ,
protein C-terminus binding	0.410284431 PHB_HUMAN Prohibitin ,
organ morphogenesis	0.410595874 C2CD3_HUMAN CMMP14_HUMAN
null	0.410969936 H2AY_HUMAN CoQCR2_HUMAN Cy
positive regulation of lymphocyte proliferation	0.411255411 LEG9_HUMAN Galectin-9 ,
regulation of viral entry into host cell	0.411255411 LEG9_HUMAN Galectin-9 ,
null	0.411422762 MMP9_HUMAN NDKB_HUMAN N
tube development	0.411711067 APOD_HUMAN A LYRIC_HUMAN Pr
oxidation-reduction process	0.412589176 HCDH_HUMAN H APOD_HUMAN A
cell-cell junction	0.412778515 LYRIC_HUMAN Pr AGRL3_HUMAN A
regulation of cytokine-mediated signaling pathway	0.413530202 GSTP1_HUMAN Glutathione S-transf
reproductive structure development	0.413565827 HMGB2_HUMAN MMP14_HUMAN
negative regulation of lipid metabolic process	0.416079287 APOD_HUMAN Apolipoprotein D ,
positive regulation of mitochondrion organization	0.418747986 MMP9_HUMAN Matrix metalloprot

negative regulation of Notch signaling pathway
 establishment of protein localization to chromosome
 null
 protein activation cascade
 positive regulation of T cell activation
 negative regulation of chromosome organization
 entry into other organism involved in symbiotic interaction
 angiogenesis
 vesicle organization
 cytosolic large ribosomal subunit
 cellular response to heat
 sulfur compound biosynthetic process
 null
 linoleic acid metabolic process
 regulation of apoptotic signaling pathway
 cellular response to extracellular stimulus
 regulation of dendritic cell chemotaxis
 positive regulation of interleukin-6 secretion
 null
 metalloaminopeptidase activity
 leukocyte mediated cytotoxicity
 null

 oxidoreductase activity, acting on peroxide as acceptor
 acute inflammatory response
 protein import into nucleus
 phagocytic vesicle
 regulation of protein secretion
 regulation of B cell activation
 positive regulation of type I interferon production
 double-stranded RNA binding
 cell-cell signaling
 regulation of gene expression, epigenetic
 positive regulation of reactive oxygen species metabolic process
 cell motility
 null
 lipid metabolic process
 Notch signaling pathway
 interleukin-6 production
 null
 positive regulation of chemokine production
 response to heat
 blood vessel development
 COPII-coated vesicle budding
 double-stranded DNA binding
 negative regulation of cell migration
 response to mechanical stimulus
 negative regulation of immune effector process
 chaperone-mediated protein folding
 alpha-beta T cell differentiation
 regulation of T cell proliferation
 positive regulation of myeloid cell differentiation
 apical part of cell
 release of cytochrome c from mitochondria
 positive regulation of cell migration

0.419248214 MMP14_HUMAN Matrix metallopro
 0.419280689 H2AY_HUMAN Core histone macro-
 0.419975951 APOD_HUMAN A RAB8A_HUMAN R
 0.420676135 PHB_HUMAN Prohibitin ,
 0.420677362 LEG9_HUMAN Galectin-9 ,
 0.420734147 H2AY_HUMAN Core histone macro-
 0.421182266 LEG9_HUMAN GaSPB3_HUMAN Ser
 0.421336064 APOD_HUMAN A LYRIC_HUMAN Pr
 0.421475999 NDKA_HUMAN N MMP9_HUMAN M
 0.421784046 RL14_HUMAN 60 NH2L1_HUMAN N
 0.422146172 RBBP7_HUMAN Histone-binding pro
 0.42244277 GSTP1_HUMAN Glutathione S-transf
 0.423076923 MMP14_HUMAN Matrix metallopro
 0.423076923 GSTP1_HUMAN Glutathione S-transf
 0.425879047 HMGB2_HUMAN MMP9_HUMAN M
 0.428199203 GSTP1_HUMAN Glutathione S-transf
 0.428571429 LEG9_HUMAN Galectin-9 ,
 0.428571429 LEG9_HUMAN Galectin-9 ,
 0.428571429 GSTP1_HUMAN Glutathione S-transf
 0.428571429 MMP14_HUMAN Matrix metallopro
 0.428571429 LEG9_HUMAN Galectin-9 ,
 0.428571429 LEG9_HUMAN Galectin-9 ,

 0.428867785 GSTP1_HUMAN Glutathione S-transf
 0.4289534 PHB_HUMAN Pro GSTP1_HUMAN G
 0.429010282 APOD_HUMAN Apolipoprotein D ,
 0.429175476 RAB8A_HUMAN RRAB8B_HUMAN R
 0.429535865 HCDH_HUMAN H LEG9_HUMAN Gal
 0.43 MMP14_HUMAN Matrix metallopro
 0.43 HMGB2_HUMAN High mobility grou
 0.430354223 LYRIC_HUMAN Protein LYRIC ,
 0.430439979 HCDH_HUMAN H RAB8A_HUMAN R
 0.431268183 H2AY_HUMAN CoRBBP7_HUMAN H
 0.431304271 GSTP1_HUMAN Glutathione S-transf
 0.432055341 HMGB2_HUMAN APOD_HUMAN A
 0.434243795 RAB8A_HUMAN RGSTP1_HUMAN G
 0.434637467 HCDH_HUMAN H APOD_HUMAN A
 0.435526178 MMP14_HUMAN Matrix metallopro
 0.43616491 LEG9_HUMAN Galectin-9 ,
 0.436684968 NDKA_HUMAN N APOD_HUMAN A
 0.436758893 LEG9_HUMAN Galectin-9 ,
 0.437334293 RBBP7_HUMAN Histone-binding pro
 0.437427541 APOD_HUMAN A LYRIC_HUMAN Pr
 0.4375 TMEDA_HUMAN Transmembrane e
 0.437929864 NDKA_HUMAN N HMGB2_HUMAN
 0.439972928 APOD_HUMAN A GSTP1_HUMAN G
 0.440340249 MMP14_HUMAN Matrix metallopro
 0.443031885 LEG9_HUMAN Galectin-9 ,
 0.444444444 FKBP2_HUMAN Peptidyl-prolyl cis-tr
 0.444444444 LEG9_HUMAN Galectin-9 ,
 0.444444444 LEG9_HUMAN Galectin-9 ,
 0.444820409 HMGB2_HUMAN High mobility grou
 0.445803669 LYRIC_HUMAN Protein LYRIC ,
 0.446027738 MMP9_HUMAN Matrix metalloprot
 0.446267832 MMP9_HUMAN MMP14_HUMAN

positive regulation of interleukin-6 production
 null
 MAP kinase activity
 rRNA binding
 protein dephosphorylation
 histone deacetylase binding
 membrane-enclosed lumen
 interleukin-8 secretion
 respiratory system development
 cofactor binding
 positive regulation of biological process
 nucleoside binding
 positive regulation of immune system process
 response to fatty acid
 embryo development
 organelle assembly
 transcription regulatory region sequence-specific DNA binding
 G-protein coupled receptor binding
 receptor agonist activity
 response to organophosphorus
 cellular protein metabolic process
 positive regulation of cell-cell adhesion
 female gonad development
 development of primary female sexual characteristics
 null

response to testosterone stimulus
 hormone metabolic process
 epidermal cell differentiation
 B cell differentiation
 U2 snRNP
 null
 regulation of cellular response to stress
 dendritic cell differentiation
 organ regeneration
 cytoplasmic vesicle membrane
 chromatin remodeling
 positive regulation of cell death
 DNA replication
 response to interferon-gamma
 primary metabolic process
 kidney development
 response to stimulus
 null
 regulation of complement activation
 cell activation
 endomembrane system
 null
 mitochondrial outer membrane
 leukocyte proliferation
 null
 transcription factor binding
 organophosphate biosynthetic process
 telencephalon development
 pallium development

0.447368421 LEG9_HUMAN Galectin-9 ,
 0.45 RAB8A_HUMAN RH2AY_HUMAN Co
 0.450006851 SPB3_HUMAN Se GSTP1_HUMAN G
 0.450698715 CIRBP_HUMAN Cold-inducible RNA-
 0.451968434 PP1G_HUMAN Serine/threonine-pro
 0.454408391 PHB_HUMAN Prohibitin ,
 0.454467293 HMGB2_HUMAN HCDH_HUMAN H
 0.454545455 LEG9_HUMAN Galectin-9 ,
 0.454736018 MMP14_HUMAN Matrix metallopro
 0.455235567 HCDH_HUMAN H SQRD_HUMAN Su
 0.455381452 HMGB2_HUMAN MMP14_HUMAN
 0.456296534 NDKA_HUMAN N RAB8A_HUMAN R
 0.457258264 HMGB2_HUMAN PHB_HUMAN Pro
 0.459307089 NDKA_HUMAN Nucleoside diphosph
 0.460315945 MMP9_HUMAN C2CD3_HUMAN C
 0.460489054 C2CD3_HUMAN CRAB8A_HUMAN R
 0.460519343 NDKA_HUMAN N HMGB2_HUMAN
 0.461118449 PHB_HUMAN Prohibitin ,
 0.461118449 HMGB2_HUMAN High mobility grou
 0.461164458 NDKA_HUMAN Nucleoside diphosph
 0.463184006 MMP14_HUMAN NDKB_HUMAN N
 0.463742944 LEG9_HUMAN Galectin-9 ,
 0.464285714 MMP14_HUMAN Matrix metallopro
 0.464285714 MMP14_HUMAN Matrix metallopro
 0.466453694 RAB8A_HUMAN RTMEDA_HUMAN T

0.466856765 NDKA_HUMAN Nucleoside diphosph
 0.466958948 DHB4_HUMAN Peroxisomal multifun
 0.467830169 NDKB_HUMAN N K1C13_HUMAN K
 0.467836257 MMP14_HUMAN Matrix metallopro
 0.46875 NH2L1_HUMAN NHP2-like protein 1
 0.469457443 LEG9_HUMAN Galectin-9 ,
 0.469863605 LEG9_HUMAN Ga SPB3_HUMAN Ser
 0.47012987 LEG9_HUMAN Galectin-9 ,
 0.470248821 GSTP1_HUMAN Glutathione S-transf
 0.470627107 RAB8A_HUMAN RTMEDA_HUMAN
 0.471797943 HMGB2_HUMAN RBBP7_HUMAN H
 0.471992152 MMP9_HUMAN PHB_HUMAN Pro
 0.472093021 RBBP7_HUMAN Histone-binding pro
 0.473212786 LEG9_HUMAN Galectin-9 ,
 0.473783063 HMGB2_HUMAN H2AY_HUMAN Co
 0.473841257 TMEDA_HUMAN Transmembrane e
 0.474387214 HMGB2_HUMAN K1C13_HUMAN K
 0.475633535 HMGB2_HUMAN MMP14_HUMAN
 0.475806452 PHB_HUMAN Prohibitin ,
 0.475860102 MMP9_HUMAN MMP14_HUMAN
 0.476491308 MMP14_HUMAN NDKB_HUMAN N
 0.476697863 MMP9_HUMAN Matrix metalloprot
 0.477104114 NDKA_HUMAN N PP1G_HUMAN Ser
 0.477250658 LEG9_HUMAN Ga GSTP1_HUMAN G
 0.477855648 LYRIC_HUMAN Pr RAB8A_HUMAN R
 0.478377583 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.478481486 NDKA_HUMAN N NDKB_HUMAN N
 0.478503546 NDKA_HUMAN Nucleoside diphosph
 0.478503546 NDKA_HUMAN Nucleoside diphosph

forebrain development	0.478503546 NDKA_HUMAN Nucleoside diphosph
regulation of mitochondrion organization	0.478510405 MMP9_HUMAN Matrix metalloprot
oxidoreductase activity, acting on a sulfur group of donors	0.479893704 SQRD_HUMAN Sulfide:quinone oxid
DNA recombination	0.481225628 HMGB2_HUMAN High mobility grou
ion transmembrane transport	0.482087683 MMP9_HUMAN PHB_HUMAN Pro
positive regulation of cysteine-type endopeptidase activity involved in apoptot	0.482180294 LEG9_HUMAN Galectin-9 ,
T cell differentiation	0.482758621 LEG9_HUMAN Galectin-9 ,
myeloid cell homeostasis	0.484389733 HMGB2_HUMAN High mobility grou
drug binding	0.485097581 NDKA_HUMAN N HMGB2_HUMAN
regulation of transporter activity	0.485966489 MMP9_HUMAN Matrix metalloprot
positive regulation of cell projection organization	0.486649768 NDKA_HUMAN N RAB8B_HUMAN R
nucleotide biosynthetic process	0.486770982 NDKA_HUMAN N NDKB_HUMAN N
phosphoprotein phosphatase activity	0.486836773 PP1G_HUMAN Serine/threonine-pro
leukocyte apoptotic process	0.486894544 LEG9_HUMAN Galectin-9 ,
regulation of interleukin-6 production	0.4878601 LEG9_HUMAN Galectin-9 ,
ncRNA processing	0.488188976 RL14_HUMAN 60 NH2L1_HUMAN N
positive chemotaxis	0.488461538 HMGB2_HUMAN High mobility grou
regulation of protein binding	0.490219417 MMP9_HUMAN Matrix metalloprot
p38MAPK cascade	0.490909091 LEG9_HUMAN Galectin-9 ,
positive regulation of smooth muscle cell proliferation	0.491180739 MMP9_HUMAN Matrix metalloprot
null	0.49122807 C2CD3_HUMAN CRAB8A_HUMAN R
ion binding	0.491565593 HCDH_HUMAN H AMY1_HUMAN AI
anatomical structure formation involved in morphogenesis	0.491929459 APOD_HUMAN A MMP9_HUMAN M
regulation of ion transmembrane transport	0.492339122 MMP9_HUMAN Matrix metalloprot
establishment of protein localization	0.492536717 HCDH_HUMAN H APOD_HUMAN A
macromolecule metabolic process	0.492772575 HMGB2_HUMAN H2AY_HUMAN Co
cell body	0.493795828 APOD_HUMAN A RAB8A_HUMAN R
null	0.494209977 NDKA_HUMAN N NDKB_HUMAN N
DNA biosynthetic process	0.495138141 PHB_HUMAN Pro RBBP7_HUMAN H
regulation of lipid metabolic process	0.495147909 APOD_HUMAN A H2AY_HUMAN Co
null	0.495482928 NDKA_HUMAN N NDKB_HUMAN N
null	0.496717509 MMP14_HUMAN NDKB_HUMAN N
behavior	0.496997543 AGRL3_HUMAN Adhesion G protein-
rhythmic process	0.496997543 PP1G_HUMAN Serine/threonine-pro
postsynaptic density	0.497636072 RAB8A_HUMAN Ras-related protein
tissue remodeling	0.498176754 MMP14_HUMAN Matrix metallopro
extracellular region part	0.499119399 HMGB2_HUMAN AMY1_HUMAN AI
regulation of cellular component biogenesis	0.499337403 APOD_HUMAN A MMP14_HUMAN
hormone-mediated signaling pathway	0.499704501 PHB_HUMAN Prohibitin ,
null	0.5 MMP9_HUMAN GSTP1_HUMAN G
null	0.5 MMP9_HUMAN GSTP1_HUMAN G
positive regulation of superoxide anion generation	0.5 GSTP1_HUMAN Glutathione S-transf
CenH3-containing nucleosome assembly at centromere	0.5 RBBP7_HUMAN Histone-binding pro
regulation of T cell migration	0.5 APOD_HUMAN Apolipoprotein D ,
positive regulation of dendritic cell chemotaxis	0.5 LEG9_HUMAN Galectin-9 ,
regulation of lymphocyte apoptotic process	0.5 LEG9_HUMAN Galectin-9 ,
regulation of CD4-positive, CD25-positive, alpha-beta regulatory T cell differen	0.5 LEG9_HUMAN Galectin-9 ,
positive regulation of CD4-positive, CD25-positive, alpha-beta regulatory T cell	0.5 LEG9_HUMAN Galectin-9 ,
NuRD complex	0.5 RBBP7_HUMAN Histone-binding pro
nitric oxide homeostasis	0.5 GSTP1_HUMAN Glutathione S-transf
null	0.5 H2AY_HUMAN Core histone macro-
regulation of maintenance of sister chromatid cohesion	0.5 H2AY_HUMAN Core histone macro-
positive regulation of maintenance of sister chromatid cohesion	0.5 H2AY_HUMAN Core histone macro-
positive regulation of maintenance of mitotic sister chromatid cohesion	0.5 H2AY_HUMAN Core histone macro-

positive regulation of interleukin-1 beta secretion	0.5 LEG9_HUMAN Galectin-9 ,
positive regulation of CD4-positive, alpha-beta T cell activation	0.5 LEG9_HUMAN Galectin-9 ,
regulation of CD4-positive, alpha-beta T cell activation	0.5 LEG9_HUMAN Galectin-9 ,
negative regulation of CD4-positive, alpha-beta T cell proliferation	0.5 LEG9_HUMAN Galectin-9 ,
positive regulation of CD4-positive, alpha-beta T cell proliferation	0.5 LEG9_HUMAN Galectin-9 ,
regulation of CD4-positive, alpha-beta T cell proliferation	0.5 LEG9_HUMAN Galectin-9 ,
positive regulation of dendritic cell apoptotic process	0.5 LEG9_HUMAN Galectin-9 ,
complement component C3b binding	0.5 PHB_HUMAN Prohibitin ,
alpha-beta T cell differentiation involved in immune response	0.5 LEG9_HUMAN Galectin-9 ,
CD4-positive, CD25-positive, alpha-beta regulatory T cell differentiation involve ESC/E(Z) complex	0.5 LEG9_HUMAN Galectin-9 ,
CD4-positive, CD25-positive, alpha-beta regulatory T cell differentiation	0.5 H2AY_HUMAN CoRBBP7_HUMAN H
null	0.5 LEG9_HUMAN Galectin-9 ,
neural plate axis specification	0.5 C2CD3_HUMAN C2 domain-containi
PTW/PP1 phosphatase complex	0.5 C2CD3_HUMAN C2 domain-containi
astrocyte cell migration	0.5 PP1G_HUMAN Serine/threonine-pro
milk ejection	0.5 MMP14_HUMAN Matrix metallopro
null	0.5 NDKA_HUMAN Nucleoside diphosph
negative regulation of activated T cell proliferation	0.5 MMP14_HUMAN SERPH_HUMAN S
pyrimidine nucleoside biosynthetic process	0.5 LEG9_HUMAN Galectin-9 ,
peripheral nervous system axon regeneration	0.5 NDKA_HUMAN N NDKB_HUMAN Nu
interleukin-1 beta production	0.5 APOD_HUMAN Apolipoprotein D ,
regulation of interleukin-1 beta production	0.5 LEG9_HUMAN Ga GSTP1_HUMAN G
ATP synthesis coupled electron transport	0.5 LEG9_HUMAN Ga GSTP1_HUMAN G
negative regulation of cell death	0.5 QCR2_HUMAN Cytochrome b-c1 co
interleukin-8 production	0.501626538 HMGB2_HUMAN MMP9_HUMAN M
null	0.50249845 LEG9_HUMAN Galectin-9 ,
cellular response to interferon-gamma	0.502961864 NDKA_HUMAN N HMGB2_HUMAN
null	0.50320162 LEG9_HUMAN Galectin-9 ,
vasculature development	0.503263915 DHB4_HUMAN Peroxisomal multifun
negative regulation of cysteine-type endopeptidase activity involved in apopto	0.503913166 APOD_HUMAN A LYRIC_HUMAN Pr
	0.504628063 MMP9_HUMAN Matrix metalloprot
cellular protein modification process	0.504970248 MMP9_HUMAN PHB_HUMAN Pro
small molecule biosynthetic process	0.505939331 NDKA_HUMAN N NDKB_HUMAN N
nuclear heterochromatin	0.506162465 H2AY_HUMAN Core histone macro-
positive regulation of leukocyte proliferation	0.507783375 LEG9_HUMAN Galectin-9 ,
nucleosome binding	0.507939782 H2AY_HUMAN Core histone macro-
magnesium ion binding	0.508498117 NDKA_HUMAN Nucleoside diphosph
null	0.511091626 GSTP1_HUMAN Glutathione S-transf
positive regulation of phosphorus metabolic process	0.511882559 MMP9_HUMAN PHB_HUMAN Pro
fibroblast proliferation	0.512515567 GSTP1_HUMAN Glutathione S-transf
response to ammonium ion	0.51278713 AGRL3_HUMAN Adhesion G protein-
null	0.513173316 HMGB2_HUMAN LYRIC_HUMAN Pr
integrin-mediated signaling pathway	0.513584844 NDKB_HUMAN Nucleoside diphosph
ruffle	0.514311148 NDKA_HUMAN N NDKB_HUMAN Nu
macromolecule methylation	0.516056674 H2AY_HUMAN Core histone macro-
null	0.517083933 QCR2_HUMAN Cytochrome b-c1 co
regulation of cysteine-type endopeptidase activity involved in apoptotic proces	0.517238384 MMP9_HUMAN LEG9_HUMAN Gal
ribosome biogenesis	0.517576337 RL14_HUMAN 60 NH2L1_HUMAN N
positive regulation of protein metabolic process	0.517836978 MMP9_HUMAN PHB_HUMAN Pro
multicellular organism reproduction	0.518356089 HMGB2_HUMAN LEG9_HUMAN Gal
cysteine-type endopeptidase activity involved in apoptotic process	0.519232981 MMP9_HUMAN LEG9_HUMAN Gal
cytoplasmic vesicle	0.52066676 MMP9_HUMAN PHB_HUMAN Pro
tubulin binding	0.520728291 NDKA_HUMAN Nucleoside diphosph
regulation of protein localization to nucleus	0.520748101 APOD_HUMAN Apolipoprotein D ,

null
 ERBB signaling pathway
 heart morphogenesis
 null
 positive regulation of protein kinase B signaling cascade
 cellular response to oxidative stress
 thioester metabolic process
 positive regulation of transcription from RNA polymerase II promoter
 ER to Golgi vesicle-mediated transport
 regulation of biological process
 cardiovascular system development
 regulation of angiogenesis
 nucleic acid binding
 null
 positive regulation of protein secretion
 retrograde vesicle-mediated transport, Golgi to ER
 negative regulation of cellular component organization
 regulation of fibroblast proliferation
 response to light stimulus
 nuclear chromatin
 DNA replication-independent nucleosome organization
 regulation of catabolic process
 Golgi apparatus
 mesenchyme development
 embryo implantation
 developmental process involved in reproduction
 positive regulation of defense response
 Golgi membrane
 tumor necrosis factor-mediated signaling pathway
 positive regulation of protein processing
 negative regulation of apoptotic process
 heterochromatin
 positive regulation of multi-organism process
 kinase inhibitor activity

protein localization to chromosome, centromeric region
 renal system development
 protein kinase regulator activity
 null
 positive regulation of phosphate metabolic process
 positive regulation of B cell activation
 positive regulation of leukocyte chemotaxis
 cell migration
 peptidase activity
 innate immune response-activating signal transduction
 aminopeptidase activity
 androgen receptor signaling pathway
 negative regulation of cell-cell adhesion
 response to cAMP
 fatty acid oxidation
 small molecule catabolic process
 null
 B cell activation
 brain development
 positive regulation of cell growth

0.520772947 LEG9_HUMAN Galectin-9 ,
 0.520833333 MMP9_HUMAN Matrix metalloprot
 0.521528786 C2CD3_HUMAN C2 domain-containi
 0.52173913 LEG9_HUMAN Galectin-9 ,
 0.522222222 LYRIC_HUMAN Protein LYRIC ,
 0.52222416 MMP9_HUMAN GSTP1_HUMAN G
 0.522675855 DHB4_HUMAN Peroxisomal multifun
 0.524679608 HMGB2_HUMAN NDKB_HUMAN N
 0.524701805 TMEDA_HUMAN Transmembrane e
 0.526312535 HMGB2_HUMAN K1C13_HUMAN K
 0.526590387 APOD_HUMAN A LYRIC_HUMAN Pr
 0.526920448 LYRIC_HUMAN Protein LYRIC ,
 0.527292442 HMGB2_HUMAN NDKB_HUMAN N
 0.529642735 PHB_HUMAN Prohibitin ,
 0.530321719 LEG9_HUMAN Galectin-9 ,
 0.53220763 TMEDA_HUMAN Transmembrane e
 0.532773734 APOD_HUMAN A MMP14_HUMAN
 0.533281185 GSTP1_HUMAN Glutathione S-transf
 0.534161491 PP1G_HUMAN Se CIRBP_HUMAN Co
 0.534186529 HMGB2_HUMAN PHB_HUMAN Pro
 0.534759358 RBBP7_HUMAN Histone-binding pro
 0.53504207 LYRIC_HUMAN Pr PHB_HUMAN Pro
 0.535897938 MMP14_HUMAN RAB8A_HUMAN R
 0.536017813 SPB3_HUMAN Serpin B3 ,
 0.536052808 MMP9_HUMAN Matrix metalloprot
 0.53642443 HMGB2_HUMAN MMP14_HUMAN
 0.536684901 HMGB2_HUMAN LEG9_HUMAN Gal
 0.537101393 RAB8A_HUMAN RTMEDA_HUMAN T
 0.537634409 GSTP1_HUMAN Glutathione S-transf
 0.537810035 PHB_HUMAN Prohibitin ,
 0.538428663 HMGB2_HUMAN MMP9_HUMAN M
 0.53913116 H2AY_HUMAN Core histone macro-
 0.541081785 LEG9_HUMAN Galectin-9 ,
 0.541219836 H2AY_HUMAN Core histone macro-

0.541666667 RBBP7_HUMAN Histone-binding pro
 0.543059191 TMEDA_HUMAN Transmembrane e
 0.543073074 H2AY_HUMAN Core histone macro-
 0.543680514 MMP9_HUMAN Matrix metalloprot
 0.543814483 MMP9_HUMAN PHB_HUMAN Pro
 0.543859649 MMP14_HUMAN Matrix metallopro
 0.543859649 LEG9_HUMAN Galectin-9 ,
 0.544937142 HMGB2_HUMAN APOD_HUMAN A
 0.5453956 MMP9_HUMAN MMP14_HUMAN
 0.545454545 LEG9_HUMAN Galectin-9 ,
 0.545454545 MMP14_HUMAN Matrix metallopro
 0.545454545 PHB_HUMAN Prohibitin ,
 0.545678604 LEG9_HUMAN Galectin-9 ,
 0.547779282 NDKA_HUMAN Nucleoside diphosph
 0.547835285 HCDH_HUMAN H DHB4_HUMAN Pe
 0.548501211 HCDH_HUMAN H DHB4_HUMAN Pe
 0.549458183 MMP9_HUMAN Matrix metalloprot
 0.54954955 MMP14_HUMAN Matrix metallopro
 0.55121586 NDKA_HUMAN N APOD_HUMAN A
 0.551829183 MMP14_HUMAN Matrix metallopro

positive regulation of protein modification process
 sensory organ development
 lymphocyte apoptotic process
 positive regulation of epidermal growth factor receptor signaling pathway
 cell part morphogenesis
 null
 respiratory chain
 null
 catabolic process
 small GTPase binding
 carbohydrate catabolic process
 ribonucleotide binding
 ephrin receptor signaling pathway
 cellular carbohydrate metabolic process
 nucleus
 microtubule-based movement
 null
 cellular response to inorganic substance
 organelle
 regulation of sequence-specific DNA binding transcription factor activity
 protein kinase B signaling cascade
 positive regulation of cell adhesion
 positive regulation of MAPK cascade
 lymphocyte mediated immunity
 null
 transition metal ion binding
 pattern recognition receptor signaling pathway
 mesenchymal cell differentiation
 endosome membrane
 organelle envelope lumen
 nitrogen compound transport
 transcription, DNA-dependent
 positive regulation of signaling
 hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear
 locomotory behavior
 phosphatase activity
 peroxidase activity

nucleosomal DNA binding
 sequence-specific DNA binding
 cellular response to stimulus
 nuclear membrane
 anatomical structure development
 plasma membrane
 protein serine/threonine kinase activity
 cellular modified amino acid metabolic process
 intracellular steroid hormone receptor signaling pathway
 positive regulation of cell communication
 divalent metal ion transport
 amide binding
 protein alkylation
 null
 biological regulation
 null
 protein modification process

0.553607382 MMP9_HUMAN PHB_HUMAN Pro
 0.553842002 K1C13_HUMAN Keratin, type I cytos
 0.555555556 LEG9_HUMAN Galectin-9 ,
 0.555555556 MMP9_HUMAN Matrix metalloprot
 0.555555556 RAB8A_HUMAN Ras-related protein
 0.557850899 MMP9_HUMAN LYRIC_HUMAN Pr
 0.55828924 QCR2_HUMAN Cytochrome b-c1 co
 0.558441558 LEG9_HUMAN Galectin-9 ,
 0.558906823 HMGB2_HUMAN HCDH_HUMAN H
 0.56 RAB8A_HUMAN Ras-related protein
 0.560643511 AMYP_HUMAN Alpha-amylase 1 ,
 0.562158233 NDKA_HUMAN N NDKB_HUMAN N
 0.5625 MMP9_HUMAN Matrix metalloprot
 0.563938003 PP1G_HUMAN Serine/threonine-pro
 0.564026732 HMGB2_HUMAN HCDH_HUMAN H
 0.564581827 C2CD3_HUMAN CRAB8A_HUMAN R
 0.564595323 CIRBP_HUMAN Cold-inducible RNA-
 0.564837208 MMP9_HUMAN Matrix metalloprot
 0.564912555 HMGB2_HUMAN K1C13_HUMAN K
 0.56545898 LYRIC_HUMAN Pr LEG9_HUMAN Ga
 0.565525356 LYRIC_HUMAN Protein LYRIC ,
 0.565559475 LEG9_HUMAN Galectin-9 ,
 0.565560265 PHB_HUMAN Pro LEG9_HUMAN Gal
 0.566076624 LEG9_HUMAN Galectin-9 ,
 0.566158077 NDKA_HUMAN N HMGB2_HUMAN
 0.56617141 MMP9_HUMAN MMP14_HUMAN
 0.566666667 LEG9_HUMAN Galectin-9 ,
 0.566958634 SPB3_HUMAN Serpin B3 ,
 0.567408118 RAB8A_HUMAN RRAB8B_HUMAN R
 0.568580586 TIM8A_HUMAN Mitochondrial impo
 0.569595276 HCDH_HUMAN H APOD_HUMAN A
 0.570300984 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.570852581 MMP9_HUMAN LYRIC_HUMAN Pr
 0.571428571 RBBP7_HUMAN Histone-binding pro
 0.571428571 AGRL3_HUMAN Adhesion G protein-
 0.571428571 PP1G_HUMAN Serine/threonine-pro
 0.571428571 GSTP1_HUMAN Glutathione S-transf

0.571428571 H2AY_HUMAN Core histone macro-
 0.571714886 NDKA_HUMAN N HMGB2_HUMAN
 0.573229074 HMGB2_HUMAN MMP14_HUMAN
 0.573740132 LYRIC_HUMAN Protein LYRIC ,
 0.574157039 HMGB2_HUMAN MMP14_HUMAN
 0.575259512 MMP14_HUMAN NDKB_HUMAN N
 0.577663671 SPB3_HUMAN Se H2AY_HUMAN Co
 0.577898058 GSTP1_HUMAN Glutathione S-transf
 0.578947368 PHB_HUMAN Prohibitin ,
 0.5794691 MMP9_HUMAN LYRIC_HUMAN Pr
 0.580645161 PHB_HUMAN Prohibitin ,
 0.581378211 GSTP1_HUMAN Glutathione S-transf
 0.581378987 H2AY_HUMAN Core histone macro-
 0.581954837 HCDH_HUMAN H RAB8A_HUMAN R
 0.582069638 HMGB2_HUMAN K1C13_HUMAN K
 0.582529472 DHB4_HUMAN Peroxisomal multifun
 0.583090885 MMP9_HUMAN PHB_HUMAN Pro

response to metal ion
 purine nucleotide biosynthetic process
 leukocyte activation
 negative regulation of peptide secretion
 transmembrane receptor protein tyrosine kinase signaling pathway
 lymphocyte proliferation
 positive regulation of immune response
 establishment of protein localization in mitochondrion
 molecular_function
 phosphatase binding
 defense response
 leukocyte degranulation
 cellular response to stress
 null
 isomerase activity
 mitochondrial ATP synthesis coupled electron transport
 peptidyl-lysine modification
 peptidyl-tyrosine modification
 acyl-CoA metabolic process
 exopeptidase activity
 phosphotransferase activity, alcohol group as acceptor
 regulation of transferase activity
 regulation of reactive oxygen species metabolic process
 intracellular transport
 positive regulation of phosphorylation
 positive regulation of response to external stimulus
 chromatin remodeling at centromere
 null
 DNA replication-independent nucleosome assembly
 vesicle targeting, rough ER to cis-Golgi
 circulatory system development
 central nervous system development
 CD4-positive, alpha-beta T cell activation
 histone deacetylase activity
 regulation of T cell apoptotic process
 cellular response to glucose stimulus
 positive regulation of monocyte chemotactic protein-1 production
 protein deacetylation
 regulation of sister chromatid cohesion
 superoxide anion generation

cartilage development involved in endochondral bone morphogenesis
 regulation of alpha-beta T cell differentiation
 regulation of neuronal synaptic plasticity
 cellular macromolecule localization
 histone methylation
 regulation of insulin secretion
 myelin sheath
 purine-containing compound metabolic process
 null
 endoplasmic reticulum lumen
 steroid biosynthetic process
 hormone secretion
 negative regulation of translation
 response to purine-containing compound

0.583333333 MMP9_HUMAN Matrix metalloprot
 0.583845478 NDKA_HUMAN N NDKB_HUMAN N
 0.583857202 MMP9_HUMAN MMP14_HUMAN
 0.584260289 HCDH_HUMAN Hydroxyacyl-coenzy
 0.584717608 APOD_HUMAN A MMP9_HUMAN M
 0.585585586 LEG9_HUMAN Galectin-9 ,
 0.586930068 HMGB2_HUMAN PHB_HUMAN Pro
 0.587213962 QCR2_HUMAN Cytochrome b-c1 co
 0.587551562 HMGB2_HUMAN K1C13_HUMAN K
 0.587808943 PP1G_HUMAN Serine/threonine-pro
 0.588179619 HMGB2_HUMAN APOD_HUMAN A
 0.588188093 MMP9_HUMAN LEG9_HUMAN Ga
 0.590156727 HMGB2_HUMAN APOD_HUMAN A
 0.590336134 RAB8A_HUMAN Ras-related protein
 0.593012499 DHB4_HUMAN PeFKBP2_HUMAN P
 0.593196452 QCR2_HUMAN Cytochrome b-c1 co
 0.593672575 H2AY_HUMAN Core histone macro-
 0.593672575 H2AY_HUMAN Core histone macro-
 0.593998148 DHB4_HUMAN Peroxisomal multifun
 0.594375518 MMP14_HUMAN Matrix metallopro
 0.594684264 NDKB_HUMAN N SPB3_HUMAN Ser
 0.594815535 SPB3_HUMAN Se H2AY_HUMAN Co
 0.594839445 GSTP1_HUMAN Glutathione S-transf
 0.594993521 APOD_HUMAN A C2CD3_HUMAN C
 0.595384861 MMP9_HUMAN PHB_HUMAN Pro
 0.5954922 LEG9_HUMAN Galectin-9 ,
 0.595588235 RBBP7_HUMAN Histone-binding pro
 0.596977573 H2AY_HUMAN Core histone macro-
 0.597965994 RBBP7_HUMAN Histone-binding pro
 0.597965994 TMEDA_HUMAN Transmembrane e
 0.599749009 APOD_HUMAN A LYRIC_HUMAN Pr
 0.599749009 NDKA_HUMAN N APOD_HUMAN A
 0.6 LEG9_HUMAN Galectin-9 ,
 0.6 RBBP7_HUMAN Histone-binding pro
 0.6 LEG9_HUMAN Galectin-9 ,
 0.6 NDKA_HUMAN Nucleoside diphosph
 0.6 LEG9_HUMAN Galectin-9 ,
 0.6 PHB_HUMAN Pro RBBP7_HUMAN H
 0.6 H2AY_HUMAN Core histone macro-
 0.6 GSTP1_HUMAN Glutathione S-transf

0.6 SERPH_HUMAN Serpin H1 ,
 0.6 LEG9_HUMAN Galectin-9 ,
 0.6 RAB8A_HUMAN Ras-related protein
 0.600336176 APOD_HUMAN A C2CD3_HUMAN C
 0.600877193 H2AY_HUMAN Core histone macro-
 0.600877193 HCDH_HUMAN Hydroxyacyl-coenzy
 0.601200739 NDKA_HUMAN N PHB_HUMAN Pro
 0.601915434 NDKA_HUMAN N NDKB_HUMAN N
 0.60284066 MMP9_HUMAN PHB_HUMAN Pro
 0.604275577 GT251_HUMAN P SERPH_HUMAN S
 0.604292874 DHB4_HUMAN Peroxisomal multifun
 0.604395604 HCDH_HUMAN H RAB8B_HUMAN R
 0.60538368 CIRBP_HUMAN Cold-inducible RNA-
 0.605822937 NDKA_HUMAN Nucleoside diphosph

response to estradiol stimulus	0.606068582 GSTP1_HUMAN Glutathione S-transf
glial cell differentiation	0.606326031 GSTP1_HUMAN Glutathione S-transf
cell periphery	0.606488893 MMP14_HUMAN NDKB_HUMAN N
mitochondrion organization	0.607078737 MMP9_HUMAN PHB_HUMAN Pro
cellular response to metal ion	0.607142857 MMP9_HUMAN Matrix metalloprot
null	0.611944745 NDKA_HUMAN N MMP9_HUMAN M
response to amino acid stimulus	0.612740922 GSTP1_HUMAN Glutathione S-transf
transmembrane signaling receptor activity	0.613320999 PHB_HUMAN Pro AGRL3_HUMAN A
fatty acid beta-oxidation	0.614035088 HCDH_HUMAN H DHB4_HUMAN Pe
protein tyrosine kinase activity	0.614293817 H2AY_HUMAN Core histone macro-
tertiary granule	0.615390161 MMP9_HUMAN SPB12_HUMAN Se
null	0.615862247 GSTP1_HUMAN Glutathione S-transf
female pregnancy	0.616642959 MMP9_HUMAN LEG9_HUMAN Gal
myeloid leukocyte activation	0.616676767 MMP9_HUMAN LEG9_HUMAN Ga
biological_process	0.616849955 HMGB2_HUMAN K1C13_HUMAN K
regulation of myeloid leukocyte differentiation	0.616996047 NDKA_HUMAN Nucleoside diphosph
urogenital system development	0.61767873 TMEDA_HUMAN Transmembrane e
centrosome	0.617826618 NDKA_HUMAN N C2CD3_HUMAN C
purine nucleoside triphosphate biosynthetic process	0.618067467 NDKA_HUMAN N NDKB_HUMAN N
regulation of apoptotic process	0.618538711 NDKA_HUMAN N HMGB2_HUMAN
lymphocyte activation	0.618721717 MMP14_HUMAN LEG9_HUMAN Gal
osteoblast differentiation	0.619333063 PHB_HUMAN Pro DHB4_HUMAN Pe
regulation of lymphocyte proliferation	0.619565217 LEG9_HUMAN Galectin-9 ,
purine nucleotide metabolic process	0.621337127 NDKA_HUMAN N NDKB_HUMAN N
regulation of receptor activity	0.62194436 HMGB2_HUMAN High mobility grou
divalent inorganic cation transport	0.622027972 PHB_HUMAN Prohibitin ,
methylation	0.622676626 H2AY_HUMAN Core histone macro-
apical plasma membrane	0.623546752 LYRIC_HUMAN Protein LYRIC ,
intracellular receptor mediated signaling pathway	0.624995518 PHB_HUMAN Prohibitin ,
positive regulation of growth	0.625170385 MMP14_HUMAN Matrix metallopro
response to interleukin-1	0.625368189 LEG9_HUMAN Galectin-9 ,
ribosome	0.625978787 APOD_HUMAN A RL14_HUMAN 60
negative regulation of cellular amide metabolic process	0.626287492 CIRBP_HUMAN Cold-inducible RNA-
null	0.626307147 APOD_HUMAN Apolipoprotein D ,
intracellular non-membrane-bounded organelle	0.62667278 HMGB2_HUMAN MMP14_HUMAN
ncRNA metabolic process	0.628368286 RL14_HUMAN 60 H2AY_HUMAN Co
null	0.629300019 NDKA_HUMAN Nucleoside diphosph
endoplasmic reticulum part	0.630104533 LYRIC_HUMAN Pr GT251_HUMAN P
kinase binding	0.632928655 H2AY_HUMAN CoPP1G_HUMAN Se
negative regulation of protein secretion	0.634185786 HCDH_HUMAN Hydroxyacyl-coenzy
positive regulation of translation	0.634630872 CIRBP_HUMAN Cold-inducible RNA-
positive regulation of cellular catabolic process	0.635260823 LYRIC_HUMAN Protein LYRIC ,
mitochondrial intermembrane space	0.636178796 TIM8A_HUMAN Mitochondrial impo
apical junction assembly	0.636363636 LYRIC_HUMAN Protein LYRIC ,
myosin V binding	0.636363636 RAB8A_HUMAN Ras-related protein
extracellular matrix	0.636675875 MMP14_HUMAN Matrix metallopro
null	0.637349525 RAB8A_HUMAN Ras-related protein
regulation of lymphocyte migration	0.637362637 APOD_HUMAN Apolipoprotein D ,
carbon-oxygen lyase activity	0.638888889 DHB4_HUMAN Peroxisomal multifun
post-translational protein modification	0.639607744 RAB8A_HUMAN RRBPP7_HUMAN H
transcription coactivator activity	0.639792111 LYRIC_HUMAN Protein LYRIC ,
nuclear import	0.640070922 APOD_HUMAN Apolipoprotein D ,
ruffle membrane	0.64021164 NDKA_HUMAN Nucleoside diphosph
apoptotic mitochondrial changes	0.64070177 MMP9_HUMAN Matrix metalloprot

dephosphorylation	0.64070177 PP1G_HUMAN Serine/threonine-pro
positive regulation of protein transport	0.642486685 LEG9_HUMAN Galectin-9 ,
positive regulation of dendritic cell differentiation	0.642857143 LEG9_HUMAN Galectin-9 ,
circadian rhythm	0.642857143 PP1G_HUMAN Serine/threonine-pro
null	0.642985994 LYRIC_HUMAN Pr PHB_HUMAN Pro
regulation of cytokine secretion	0.643221781 LEG9_HUMAN Galectin-9 ,
extracellular region	0.643852027 HMGB2_HUMAN AMY1_HUMAN AI
endocytic vesicle	0.64399112 RAB8A_HUMAN RRAB8B_HUMAN R
ribonucleotide biosynthetic process	0.644108131 NDKA_HUMAN N NDKB_HUMAN N
null	0.644982095 GSTP1_HUMAN Glutathione S-transf
cell killing	0.644982095 LEG9_HUMAN Galectin-9 ,
cellular response to chemical stimulus	0.645361273 NDKA_HUMAN N HMGB2_HUMAN
antioxidant activity	0.645472549 GSTP1_HUMAN Glutathione S-transf
ribonucleotide metabolic process	0.646871529 NDKA_HUMAN N NDKB_HUMAN N
regulation of nervous system development	0.646977395 NDKA_HUMAN N HMGB2_HUMAN
regulation of synaptic transmission	0.647058824 RAB8A_HUMAN Ras-related protein
gliogenesis	0.648586527 MMP14_HUMAN GSTP1_HUMAN G
chemical homeostasis	0.649565782 NDKA_HUMAN N GSTP1_HUMAN G
hydrolase activity, acting on ester bonds	0.649752883 NDKA_HUMAN N HMGB2_HUMAN
myeloid leukocyte mediated immunity	0.650002291 MMP9_HUMAN LEG9_HUMAN Ga
positive regulation of chromosome organization	0.650326102 H2AY_HUMAN Core histone macro-
nuclear transport	0.651619779 APOD_HUMAN A PP1G_HUMAN Ser
lipid transport	0.651886275 APOD_HUMAN Apolipoprotein D ,
maternal process involved in female pregnancy	0.652014652 LEG9_HUMAN Galectin-9 ,
nucleobase-containing small molecule metabolic process	0.65281927 NDKA_HUMAN N NDKB_HUMAN N
purine ribonucleoside triphosphate biosynthetic process	0.653725206 NDKA_HUMAN N NDKB_HUMAN N
cellular amide metabolic process	0.653823212 RL14_HUMAN 60 TMEDA_HUMAN
organ development	0.655375777 HMGB2_HUMAN MMP14_HUMAN
regulation of protein kinase B signaling cascade	0.655952246 LYRIC_HUMAN Protein LYRIC ,
neuron differentiation	0.6569384 NDKA_HUMAN N APOD_HUMAN A
response to nutrient	0.657196599 GSTP1_HUMAN Glutathione S-transf
receptor regulator activity	0.657805247 HMGB2_HUMAN High mobility grou
tissue morphogenesis	0.658237823 MMP9_HUMAN C2CD3_HUMAN C
lymphocyte migration	0.658974359 APOD_HUMAN Apolipoprotein D ,
response to reactive oxygen species	0.659183297 APOD_HUMAN A MMP9_HUMAN M
organelle organization	0.659219565 HMGB2_HUMAN NDKB_HUMAN N
ribonucleoside triphosphate biosynthetic process	0.659271965 NDKA_HUMAN N NDKB_HUMAN N
cell-substrate adhesion	0.659347923 APOD_HUMAN A MMP14_HUMAN
regulation of chromosome organization	0.66016287 H2AY_HUMAN Core histone macro-
regulation of dendritic cell differentiation	0.660606061 LEG9_HUMAN Galectin-9 ,
RNA metabolic process	0.660774057 HMGB2_HUMAN LYRIC_HUMAN Pr
mitochondrial respiratory chain	0.66535421 QCR2_HUMAN Cytochrome b-c1 co
null	0.666599772 NDKA_HUMAN N RAB8A_HUMAN R
V(D)J recombination	0.666666667 HMGB2_HUMAN High mobility grou
null	0.666666667 NDKA_HUMAN N NDKB_HUMAN Nu
beta-amyloid formation	0.666666667 TMEDA_HUMAN Transmembrane e
interleukin-1 beta secretion	0.666666667 LEG9_HUMAN Galectin-9 ,
regulation of interleukin-1 beta secretion	0.666666667 LEG9_HUMAN Galectin-9 ,
negative regulation of CD4-positive, alpha-beta T cell activation	0.666666667 LEG9_HUMAN Galectin-9 ,
endochondral ossification	0.666666667 MMP14_HUMAN Matrix metallopro
cellular response to glucocorticoid stimulus	0.666666667 GSTP1_HUMAN Glutathione S-transf
spermatid development	0.666666667 HMGB2_HUMAN High mobility grou
null	0.666666667 RBBP7_HUMAN Histone-binding pro
natural killer cell mediated cytotoxicity	0.666666667 LEG9_HUMAN Galectin-9 ,

regulation of CD4-positive, alpha-beta T cell differentiation
 positive regulation of CD4-positive, alpha-beta T cell differentiation
 embryonic limb morphogenesis
 regulation of long-term neuronal synaptic plasticity
 negative regulation of interleukin-1 beta production
 leading edge membrane
 positive regulation of endopeptidase activity
 response to ethanol
 formation of translation initiation ternary complex
 purine ribonucleotide biosynthetic process
 peptidyl-amino acid modification
 protein localization in mitochondrion
 pigment granule
 cellular response to steroid hormone stimulus
 regulation of innate immune response
 energy derivation by oxidation of organic compounds
 regulation of T cell activation
 null
 neuron projection development
 mitochondrial inner membrane
 nucleic acid metabolic process
 positive regulation of cellular amide metabolic process
 sequence-specific DNA binding RNA polymerase II transcription factor activity
 plasma membrane fusion
 trans-Golgi network
 endosomal part
 response to tumor necrosis factor
 cellular response to external stimulus
 immune effector process
 establishment of localization in cell
 positive regulation of protein phosphorylation
 negative regulation of cell cycle process
 null
 cellular protein complex disassembly
 positive regulation of catalytic activity
 amide transport
 humoral immune response
 microtubule-based transport
 megakaryocyte differentiation
 positive regulation of signal transduction
 regulation of intracellular protein transport
 regulation of protein catabolic process
 gene silencing
 gastrulation
 nuclear lumen
 positive regulation of nervous system development
 regulation of transmembrane transport
 cellular response to tumor necrosis factor
 hydrolase activity, hydrolyzing O-glycosyl compounds

embryo development ending in birth or egg hatching
 histone exchange
 response to external biotic stimulus
 regulation of epithelial cell migration
 organic substance metabolic process

0.666666667 LEG9_HUMAN Galectin-9 ,
 0.666666667 LEG9_HUMAN Galectin-9 ,
 0.666666667 C2CD3_HUMAN C2 domain-containi
 0.666666667 RAB8A_HUMAN Ras-related protein
 0.666666667 GSTP1_HUMAN Glutathione S-transf
 0.667133074 NDKA_HUMAN Nucleoside diphosph
 0.667484372 LEG9_HUMAN Ga SPB3_HUMAN Ser
 0.667502031 GSTP1_HUMAN Glutathione S-transf
 0.667650919 RL14_HUMAN 60 NH2L1_HUMAN N
 0.669871795 NDKA_HUMAN N NDKB_HUMAN N
 0.670334315 NDKB_HUMAN N H2AY_HUMAN Co
 0.670963311 QCR2_HUMAN Cytochrome b-c1 co
 0.671033338 MMP14_HUMAN TMEDA_HUMAN T
 0.673220982 PHB_HUMAN Pro GSTP1_HUMAN G
 0.674862194 HMGB2_HUMAN LEG9_HUMAN Gal
 0.675375495 QCR2_HUMAN CyPP1G_HUMAN Ser
 0.676136364 LEG9_HUMAN Galectin-9 ,
 0.676245446 NH2L1_HUMAN NHP2-like protein 1
 0.676562594 NDKA_HUMAN N APOD_HUMAN A
 0.676689271 PHB_HUMAN Pro QCR2_HUMAN Cy
 0.677315523 HMGB2_HUMAN NDKB_HUMAN N
 0.677501692 CIRBP_HUMAN Cold-inducible RNA-
 0.677523805 PHB_HUMAN Prohibitin ,
 0.679835287 MMP9_HUMAN LEG9_HUMAN Ga
 0.680672269 RAB8A_HUMAN Ras-related protein
 0.681459791 RAB8A_HUMAN RRAB8B_HUMAN R
 0.681468214 GSTP1_HUMAN Glutathione S-transf
 0.682615528 GSTP1_HUMAN Glutathione S-transf
 0.682978168 MMP9_HUMAN PHB_HUMAN Pro
 0.684971898 APOD_HUMAN A C2CD3_HUMAN C
 0.686117035 MMP9_HUMAN PHB_HUMAN Pro
 0.68771513 H2AY_HUMAN CoRBBP7_HUMAN H
 0.687927311 H2AY_HUMAN Core histone macro-
 0.688950287 RL14_HUMAN 60 NH2L1_HUMAN N
 0.689773854 HMGB2_HUMAN MMP14_HUMAN
 0.689869766 HCDH_HUMAN H APOD_HUMAN A
 0.690220001 PHB_HUMAN Prohibitin ,
 0.691965812 C2CD3_HUMAN CRAB8A_HUMAN R
 0.692012968 HMGB2_HUMAN High mobility grou
 0.693142971 MMP9_HUMAN LYRIC_HUMAN Pr
 0.693168347 APOD_HUMAN Apolipoprotein D ,
 0.695264694 PHB_HUMAN Pro SPB12_HUMAN Se
 0.695383292 H2AY_HUMAN Core histone macro-
 0.696428571 MMP9_HUMAN MMP14_HUMAN
 0.696840994 HMGB2_HUMAN HCDH_HUMAN H
 0.698395669 NDKA_HUMAN N AGRL3_HUMAN A
 0.699000075 MMP9_HUMAN Matrix metalloprot
 0.699329497 GSTP1_HUMAN Glutathione S-transf
 0.701754386 AMY1_HUMAN A AMYP_HUMAN AI

 0.701970443 C2CD3_HUMAN CMMP14_HUMAN
 0.702062643 RBBP7_HUMAN Histone-binding pro
 0.70282403 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.703282386 MMP9_HUMAN Matrix metalloprot
 0.703874343 HMGB2_HUMAN H2AY_HUMAN Co

structural constituent of ribosome	0.704371964 RL14_HUMAN 60S ribosomal protein
keratin filament	0.704433498 K1C13_HUMAN Keratin, type I cytos
positive regulation of chemotaxis	0.705270167 LEG9_HUMAN Galectin-9 ,
metalloexopeptidase activity	0.705357143 MMP14_HUMAN Matrix metallopro
response to stress	0.705626492 HMGB2_HUMAN APOD_HUMAN A
aerobic respiration	0.705882353 QCR2_HUMAN Cytochrome b-c1 co
null	0.706919743 NDKA_HUMAN N APOD_HUMAN A
keratinocyte differentiation	0.707986432 NDKB_HUMAN N K1C13_HUMAN K
steroid metabolic process	0.708199311 DHB4_HUMAN Peroxisomal multifun
macrophage differentiation	0.708333333 MMP9_HUMAN Matrix metalloprot
DNA geometric change	0.708613565 HMGB2_HUMAN High mobility grou
reactive oxygen species metabolic process	0.708929462 GSTP1_HUMAN Glutathione S-transf
transcription cofactor activity	0.709223266 LYRIC_HUMAN Protein LYRIC ,
epidermis development	0.709318099 NDKB_HUMAN N K1C13_HUMAN K
null	0.711272106 SPB12_HUMAN Serpin B12 ,
negative regulation of cellular catabolic process	0.711820553 CIRBP_HUMAN Cold-inducible RNA-
signal transduction by p53 class mediator	0.712365278 RBBP7_HUMAN Histone-binding pro
protein heterodimerization activity	0.712820513 H2AY_HUMAN Core histone macro-
regulation of p38MAPK cascade	0.714285714 LEG9_HUMAN Galectin-9 ,
embryonic skeletal system morphogenesis	0.714285714 MMP14_HUMAN Matrix metallopro
regulation of superoxide anion generation	0.714285714 GSTP1_HUMAN Glutathione S-transf
protein peptidyl-prolyl isomerization	0.714285714 FKBP2_HUMAN Peptidyl-prolyl cis-tr
purine ribonucleoside metabolic process	0.714285714 NDKA_HUMAN N NDKB_HUMAN Nu
endothelial cell proliferation	0.715384615 HMGB2_HUMAN MMP14_HUMAN
enzyme activator activity	0.715813927 MMP14_HUMAN Matrix metallopro
multicellular organismal reproductive process	0.717437731 HMGB2_HUMAN LEG9_HUMAN Gal
regulation of cellular biosynthetic process	0.717623627 HMGB2_HUMAN LYRIC_HUMAN Pr
lipid particle	0.718537758 SETLP_HUMAN Protein SETSIP ,
myeloid cell activation involved in immune response	0.71969372 MMP9_HUMAN LEG9_HUMAN Ga
cell development	0.720169614 NDKA_HUMAN N HMGB2_HUMAN
G-protein coupled receptor activity	0.720797721 PHB_HUMAN Pro AGRL3_HUMAN A
cellular response to organic substance	0.721549409 NDKA_HUMAN N HMGB2_HUMAN
peroxisomal matrix	0.722222222 DHB4_HUMAN Peroxisomal multifun
null	0.722485372 MMP9_HUMAN LEG9_HUMAN Ga
skin development	0.722913869 NDKB_HUMAN N K1C13_HUMAN K
leukocyte activation involved in immune response	0.723012277 MMP9_HUMAN LEG9_HUMAN Ga
cellular chemical homeostasis	0.723336006 NDKA_HUMAN Nucleoside diphosph
response to alcohol	0.723867483 GSTP1_HUMAN Glutathione S-transf
ribonucleoprotein granule	0.725543279 CIRBP_HUMAN Cold-inducible RNA-
non-membrane-bounded organelle	0.725666278 HMGB2_HUMAN MMP14_HUMAN
null	0.72604568 RAB8A_HUMAN Ras-related protein
ER to Golgi transport vesicle	0.726315789 TMEDA_HUMAN Transmembrane e
positive regulation of epithelial cell migration	0.726764583 MMP9_HUMAN Matrix metalloprot
histone displacement	0.727229602 RBBP7_HUMAN Histone-binding pro
enhancer binding	0.727229602 HMGB2_HUMAN High mobility grou
small GTPase mediated signal transduction	0.727465576 RAB8A_HUMAN RRAB8B_HUMAN R
azurophil granule lumen	0.728294178 SPB3_HUMAN Serpin B3 ,
monosaccharide metabolic process	0.728490168 APOD_HUMAN Apolipoprotein D ,
fatty acid ligase activity	0.728571429 HCDH_HUMAN H DHB4_HUMAN Pe
positive regulation of catabolic process	0.728571698 LYRIC_HUMAN Protein LYRIC ,
null	0.7293203 RBBP7_HUMAN Histone-binding pro
lamellipodium	0.729658609 NDKB_HUMAN Nucleoside diphosph
regulation of nucleobase-containing compound metabolic process	0.730348053 HMGB2_HUMAN LYRIC_HUMAN Pr
null	0.730423021 GT251_HUMAN Procollagen galacto

regulation of cellular amide metabolic process	0.730987876 TMEDA_HUMAN CIRBP_HUMAN Co
null	0.731803768 CIRBP_HUMAN Cold-inducible RNA-
leukocyte mediated immunity	0.732158036 MMP9_HUMAN LEG9_HUMAN Ga
purine ribonucleoside triphosphate binding	0.733074872 NDKA_HUMAN N NDKB_HUMAN N
ATP synthesis coupled proton transport	0.733333333 QCR2_HUMAN Cytochrome b-c1 co
immune response	0.733739451 HMGB2_HUMAN MMP9_HUMAN M
RNA splicing	0.735641227 NH2L1_HUMAN NHP2-like protein 1
cellular metabolic process	0.737926368 HMGB2_HUMAN H2AY_HUMAN Co
protein oligomerization	0.737948566 TMEDA_HUMAN Transmembrane e
cellular response to organic cyclic compound	0.739523132 PHB_HUMAN Pro GSTP1_HUMAN G
regulation of RNA metabolic process	0.740465123 HMGB2_HUMAN LYRIC_HUMAN Pr
positive regulation of endothelial cell proliferation	0.745454545 HMGB2_HUMAN High mobility grou
cellular macromolecule biosynthetic process	0.745790911 HMGB2_HUMAN LYRIC_HUMAN Pr
neuron projection	0.746416697 APOD_HUMAN A RAB8A_HUMAN R
phosphoric ester hydrolase activity	0.746938776 PP1G_HUMAN Serine/threonine-pro
cellular response to drug	0.74884655 NDKA_HUMAN Nucleoside diphosph
ligase activity	0.749360686 HCDH_HUMAN H DHB4_HUMAN Pe
null	0.75 H2AY_HUMAN Core histone macro-
X chromosome	0.75 H2AY_HUMAN Core histone macro-
response to glucocorticoid stimulus	0.75 GSTP1_HUMAN Glutathione S-transf
limb morphogenesis	0.75 C2CD3_HUMAN C2 domain-containi
cellular response to hexose stimulus	0.75 NDKA_HUMAN Nucleoside diphosph
tight junction	0.75 LYRIC_HUMAN Protein LYRIC ,
ribonucleoside metabolic process	0.75 NDKA_HUMAN N NDKB_HUMAN Nu
T cell homeostasis	0.75 LEG9_HUMAN Galectin-9 ,
positive regulation of alpha-beta T cell differentiation	0.75 LEG9_HUMAN Galectin-9 ,
smooth muscle cell migration	0.75 GSTP1_HUMAN Glutathione S-transf
organic substance transport	0.75066072 HCDH_HUMAN H APOD_HUMAN A
type I interferon production	0.751066856 HMGB2_HUMAN High mobility grou
regulation of cell projection organization	0.752955076 NDKA_HUMAN N RAB8B_HUMAN R
cell junction	0.755078467 LYRIC_HUMAN Pr MMP14_HUMAN
intrinsic to membrane	0.755536814 LYRIC_HUMAN Pr PHB_HUMAN Pro
lipid localization	0.756053393 APOD_HUMAN Apolipoprotein D ,
regulation of MAP kinase activity	0.756756757 SPB3_HUMAN Se GSTP1_HUMAN G
chromatin silencing	0.756967027 H2AY_HUMAN Core histone macro-
purine nucleotide binding	0.757008072 NDKA_HUMAN N NDKB_HUMAN N
chromosome, telomeric region	0.758333333 H2AY_HUMAN CoPP1G_HUMAN Ser
small nuclear ribonucleoprotein complex	0.75862911 NH2L1_HUMAN NHP2-like protein 1
null	0.758661644 SERPH_HUMAN Serpin H1 ,
regulation of kinase activity	0.75879913 SPB3_HUMAN Se H2AY_HUMAN Co
protein-DNA complex	0.759372045 H2AY_HUMAN Core histone macro-
response to peptide hormone stimulus	0.760066773 HCDH_HUMAN H RAB8A_HUMAN R
morphogenesis of an epithelium	0.760908796 C2CD3_HUMAN CMMP14_HUMAN
positive regulation of NF-kappaB transcription factor activity	0.761085415 LYRIC_HUMAN Pr LEG9_HUMAN Ga
cytosolic part	0.761935716 APOD_HUMAN A RL14_HUMAN 60
tissue development	0.763094656 APOD_HUMAN A MMP9_HUMAN M
protein domain specific binding	0.76361693 HMGB2_HUMAN PP1G_HUMAN Ser
positive regulation of neuron differentiation	0.764285714 NDKA_HUMAN Nucleoside diphosph
gamete generation	0.764705882 HMGB2_HUMAN High mobility grou
carboxylic acid biosynthetic process	0.76611008 DHB4_HUMAN Peroxisomal multifun
transmembrane transport	0.768386532 MMP9_HUMAN LYRIC_HUMAN Pr
viral receptor activity	0.769230769 SPB3_HUMAN Serpin B3 ,
regulation of megakaryocyte differentiation	0.769230769 HMGB2_HUMAN High mobility grou
protein phosphatase binding	0.769230769 PP1G_HUMAN Serine/threonine-pro
male gamete generation	0.769230769 HMGB2_HUMAN High mobility grou

endoplasmic reticulum membrane
 ribose phosphate metabolic process
 peptidyl-tyrosine phosphorylation
 regulation of cellular catabolic process
 regulation of immune response
 cell morphogenesis involved in neuron differentiation
 null
 translational termination
 mRNA binding
 ribonucleoprotein complex binding
 regulation of multi-organism process
 heart development
 neuron development
 nuclear part
 cellular response to toxin
 sexual reproduction
 cytokine secretion
 positive regulation of angiogenesis
 null
 sterol binding
 epithelial to mesenchymal transition
 peroxisomal membrane
 regulation of epidermal growth factor receptor signaling pathway
 primary lysosome
 regulation of macromolecule biosynthetic process
 insulin secretion
 vesicle lumen
 regulation of protein stability
 negative regulation of cysteine-type endopeptidase activity
 erythrocyte differentiation
 inorganic solute uptake transmembrane transporter activity
 cytoplasmic membrane-bounded vesicle lumen
 intracellular membrane-bounded organelle
 hydro-lyase activity
 null
 organic acid biosynthetic process
 RNA polymerase II regulatory region DNA binding
 adenylyl ribonucleotide binding
 protein homodimerization activity
 positive regulation of transport
 mRNA processing
 energy coupled proton transport, down electrochemical gradient
 intracellular protein transport
 negative regulation of cell cycle
 ribosomal subunit
 positive regulation of cell cycle process
 macromolecule biosynthetic process
 null
 regulation of RNA stability
 null
 membrane-bounded organelle
 small molecule metabolic process
 unfolded protein binding
 multi-organism process
 fertilization

0.769504383 LYRIC_HUMAN Pr TMEDA_HUMAN
 0.769919156 NDKA_HUMAN N NDKB_HUMAN N
 0.76993149 H2AY_HUMAN Core histone macro-
 0.770692242 LYRIC_HUMAN Pr RAB8A_HUMAN R
 0.770928788 HMGB2_HUMAN PHB_HUMAN Pro
 0.771907757 RAB8A_HUMAN Ras-related protein
 0.771929825 C2CD3_HUMAN CRAB8A_HUMAN R
 0.771982116 RL14_HUMAN 60 NH2L1_HUMAN N
 0.772110622 CIRBP_HUMAN Cold-inducible RNA-
 0.772488612 NDKA_HUMAN Nucleoside diphosph
 0.772949675 LEG9_HUMAN Galectin-9 ,
 0.77295661 C2CD3_HUMAN C2 domain-containi
 0.773659581 NDKA_HUMAN N APOD_HUMAN A
 0.773797851 HMGB2_HUMAN HCDH_HUMAN H
 0.7741588 GSTP1_HUMAN Glutathione S-transf
 0.774694784 HMGB2_HUMAN NH2L1_HUMAN N
 0.776375456 LEG9_HUMAN Galectin-9 ,
 0.776683087 LYRIC_HUMAN Protein LYRIC ,
 0.777197547 HMGB2_HUMAN High mobility grou
 0.777777778 APOD_HUMAN Apolipoprotein D ,
 0.777777778 SPB3_HUMAN Serpin B3 ,
 0.777777778 DHB4_HUMAN PeRAB8B_HUMAN R
 0.777777778 MMP9_HUMAN Matrix metalloprot
 0.778221778 SPB3_HUMAN Serpin B3 ,
 0.778485319 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.779145299 HCDH_HUMAN Hydroxyacyl-coenzy
 0.77982804 NDKB_HUMAN N SPB3_HUMAN Ser
 0.780591144 PHB_HUMAN Prohibitin ,
 0.781460173 MMP9_HUMAN Matrix metalloprot
 0.781609195 HMGB2_HUMAN High mobility grou
 0.781818182 MMP9_HUMAN Matrix metalloprot
 0.782282333 NDKB_HUMAN N SPB3_HUMAN Ser
 0.782542744 HMGB2_HUMAN K1C13_HUMAN K
 0.782608696 DHB4_HUMAN Peroxisomal multifun
 0.7836428 APOD_HUMAN A H2AY_HUMAN Co
 0.783666249 DHB4_HUMAN Peroxisomal multifun
 0.784155598 NDKA_HUMAN N H2AY_HUMAN Co
 0.78422294 NDKA_HUMAN N NDKB_HUMAN Nu
 0.785227689 DHB4_HUMAN Peroxisomal multifun
 0.785389633 LEG9_HUMAN GaRAB8B_HUMAN R
 0.785434564 NH2L1_HUMAN NHP2-like protein 1
 0.785714286 QCR2_HUMAN Cytochrome b-c1 co
 0.786014342 APOD_HUMAN A RL14_HUMAN 60
 0.786860514 H2AY_HUMAN CoRBBP7_HUMAN H
 0.787228896 RL14_HUMAN 60 NH2L1_HUMAN N
 0.788078489 H2AY_HUMAN Core histone macro-
 0.789585596 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.789683736 DHB4_HUMAN Peroxisomal multifun
 0.78990269 CIRBP_HUMAN Cold-inducible RNA-
 0.789910812 AMY1_HUMAN A AMYP_HUMAN AI
 0.789939629 HMGB2_HUMAN K1C13_HUMAN K
 0.7900683 NDKA_HUMAN N HCDH_HUMAN H
 0.790474408 SERPH_HUMAN Serpin H1 ,
 0.791665122 HMGB2_HUMAN MMP9_HUMAN M
 0.791934887 NH2L1_HUMAN NHP2-like protein 1

rRNA metabolic process
 zymogen activation

null
 null
 muscle cell differentiation
 chromatin DNA binding
 negative regulation of intrinsic apoptotic signaling pathway
 metal ion binding
 membrane organization
 regulation of system process
 positive regulation of neuron projection development
 null
 nuclear outer membrane-endoplasmic reticulum membrane network
 establishment of protein localization to organelle
 null
 lymphocyte homeostasis
 histone H3-K4 methylation
 glutathione transferase activity
 T cell apoptotic process
 histone H3-K27 methylation
 epidermal growth factor receptor signaling pathway
 hexose metabolic process
 null
 regulation of biosynthetic process
 regulation of cell-cell adhesion
 NIK/NF-kappaB cascade
 drug metabolic process
 protein localization to nucleus
 cytoskeleton organization
 DNA repair
 tissue migration
 positive regulation of cysteine-type endopeptidase activity
 electron transport chain
 epithelial cell migration
 negative regulation of cell differentiation
 regulation of type I interferon production
 positive regulation of response to stimulus
 negative regulation of organelle organization
 organelle part
 blood vessel morphogenesis
 regulation of biological quality
 response to corticosteroid stimulus
 protein targeting
 regulation of leukocyte chemotaxis
 macromolecule localization
 positive regulation of cytokine production
 enhancer sequence-specific DNA binding
 positive regulation of cellular component biogenesis
 nuclear envelope
 organelle subcompartment
 null
 endoplasmic reticulum
 cofactor metabolic process
 positive regulation of programmed cell death

0.792381675 RL14_HUMAN 60 H2AY_HUMAN Co
 0.793395253 MMP14_HUMAN Matrix metallopro

0.793811452 PHB_HUMAN Pro QCR2_HUMAN Cy
 0.794106818 TMEDA_HUMAN Transmembrane e
 0.795932194 MMP14_HUMAN Matrix metallopro
 0.796252977 H2AY_HUMAN Core histone macro-
 0.796836287 MMP9_HUMAN Matrix metalloprot
 0.796985012 NDKA_HUMAN N MMP9_HUMAN M
 0.797465872 NDKA_HUMAN N MMP9_HUMAN M
 0.797688243 RAB8B_HUMAN Ras-related protein
 0.79839094 NDKA_HUMAN Nucleoside diphosph
 0.798521387 QCR2_HUMAN Cytochrome b-c1 co
 0.798943017 LYRIC_HUMAN Pr TMEDA_HUMAN
 0.799634063 APOD_HUMAN A RL14_HUMAN 60
 0.79998226 APOD_HUMAN A RL14_HUMAN 60
 0.8 LEG9_HUMAN Galectin-9 ,
 0.8 H2AY_HUMAN Core histone macro-
 0.8 GSTP1_HUMAN Glutathione S-transf
 0.8 LEG9_HUMAN Galectin-9 ,
 0.8 H2AY_HUMAN Core histone macro-
 0.8 MMP9_HUMAN Matrix metalloprot
 0.8 APOD_HUMAN Apolipoprotein D ,
 0.800865966 LYRIC_HUMAN Protein LYRIC ,
 0.802487809 HMGB2_HUMAN LYRIC_HUMAN Pr
 0.803813063 LEG9_HUMAN Galectin-9 ,
 0.803983344 LEG9_HUMAN Galectin-9 ,
 0.804025688 QCR2_HUMAN CySQRD_HUMAN Su
 0.804102717 APOD_HUMAN Apolipoprotein D ,
 0.80518489 C2CD3_HUMAN CK1C13_HUMAN K
 0.805337964 HMGB2_HUMAN High mobility grou
 0.805919785 MMP9_HUMAN Matrix metalloprot
 0.806215122 LEG9_HUMAN Galectin-9 ,
 0.806266444 QCR2_HUMAN Cytochrome b-c1 co
 0.807692308 MMP9_HUMAN Matrix metalloprot
 0.807779987 NDKA_HUMAN Nucleoside diphosph
 0.809060721 HMGB2_HUMAN High mobility grou
 0.809828383 HMGB2_HUMAN MMP9_HUMAN M
 0.810374406 H2AY_HUMAN Core histone macro-
 0.810761598 HMGB2_HUMAN K1C13_HUMAN K
 0.811284969 APOD_HUMAN A LYRIC_HUMAN Pr
 0.811329945 NDKA_HUMAN N HMGB2_HUMAN
 0.811857708 GSTP1_HUMAN Glutathione S-transf
 0.812263126 RL14_HUMAN 60 QCR2_HUMAN Cy
 0.813397129 LEG9_HUMAN Galectin-9 ,
 0.81363329 HCDH_HUMAN H APOD_HUMAN A
 0.814003617 HMGB2_HUMAN LEG9_HUMAN Gal
 0.814285714 HMGB2_HUMAN High mobility grou
 0.815453736 AGRL3_HUMAN Adhesion G protein-
 0.81633343 LYRIC_HUMAN Protein LYRIC ,
 0.816418651 LYRIC_HUMAN Pr RAB8A_HUMAN R
 0.816532258 H2AY_HUMAN Core histone macro-
 0.816847151 APOD_HUMAN A LYRIC_HUMAN Pr
 0.816967156 GSTP1_HUMAN GDHB4_HUMAN Pe
 0.817079845 MMP9_HUMAN LEG9_HUMAN Gal

regulation of protein processing
ectoderm formation
spliceosomal complex
viral reproduction
condensed chromosome, centromeric region

0.817442155 PHB_HUMAN Prohibitin ,
0.818181818 MMP9_HUMAN MMP14_HUMAN
0.818189048 NH2L1_HUMAN NHP2-like protein 1
0.820467486 RL14_HUMAN 60 LEG9_HUMAN Ga
0.821428571 PP1G_HUMAN Serine/threonine-pro

adenyl nucleotide binding
protein stabilization
striated muscle cell differentiation
peptide binding
protein kinase inhibitor activity
protein transport
mitochondrial transport
null
chromatin
organelle membrane fusion
cell projection membrane
response to hypoxia
organelle fission
ligase activity, forming carbon-sulfur bonds
positive regulation of cell cycle
regulation of ion transport
GTPase binding
endomembrane system organization
small molecule binding
erythrocyte homeostasis
null
regulation of neuron projection development
establishment of protein localization in membrane
monocarboxylic acid biosynthetic process
inorganic cation transmembrane transporter activity
positive regulation of organelle organization
cellular response to cytokine stimulus
plasma membrane organization
null
intracellular organelle
membrane assembly
organophosphate metabolic process
response to virus
antigen processing and presentation
enzyme linked receptor protein signaling pathway
endonuclease activity
JNK cascade
Ras protein signal transduction
mitochondrial membrane part
viral transcription
purine ribonucleotide binding
null
peptide metabolic process
lipid binding
gland development
metal ion transport
cell surface
null
glucan metabolic process

0.822673779 NDKA_HUMAN N NDKB_HUMAN Nu
0.823529412 PHB_HUMAN Prohibitin ,
0.823529412 MMP14_HUMAN Matrix metallopro
0.823529412 GSTP1_HUMAN Glutathione S-transf
0.823529412 H2AY_HUMAN Core histone macro-
0.823540594 HCDH_HUMAN H APOD_HUMAN A
0.824388565 QCR2_HUMAN Cytochrome b-c1 co
0.825 K1C13_HUMAN Keratin, type I cytos
0.825394104 HMGB2_HUMAN PHB_HUMAN Pro
0.826228876 MMP9_HUMAN LEG9_HUMAN Ga
0.828072677 NDKA_HUMAN Nucleoside diphosph
0.828100215 MMP14_HUMAN Matrix metallopro
0.828171154 RAB8A_HUMAN RH2AY_HUMAN Co
0.828282828 HCDH_HUMAN H DHB4_HUMAN Pe
0.828372822 H2AY_HUMAN Core histone macro-
0.828596615 MMP9_HUMAN Matrix metalloprot
0.830186469 RAB8A_HUMAN Ras-related protein
0.830493352 K1C13_HUMAN K TMEDA_HUMAN T
0.83227939 NDKA_HUMAN N HCDH_HUMAN H
0.833333333 HMGB2_HUMAN High mobility grou
0.834260549 QCR2_HUMAN Cytochrome b-c1 co
0.834660633 NDKA_HUMAN Nucleoside diphosph
0.835888488 RL14_HUMAN 60 RAB8A_HUMAN R
0.836538462 DHB4_HUMAN Peroxisomal multifun
0.837209302 MMP9_HUMAN Matrix metalloprot
0.83750707 MMP9_HUMAN H2AY_HUMAN Co
0.83923354 MMP9_HUMAN PHB_HUMAN Pro
0.839568209 K1C13_HUMAN Keratin, type I cytos
0.840243553 LYRIC_HUMAN Protein LYRIC ,
0.840634395 HMGB2_HUMAN K1C13_HUMAN K
0.842023718 K1C13_HUMAN Keratin, type I cytos
0.842691494 NDKA_HUMAN N NDKB_HUMAN N
0.844332618 LEG9_HUMAN Galectin-9 ,
0.844921698 RAB8B_HUMAN Ras-related protein
0.847857205 APOD_HUMAN A MMP9_HUMAN M
0.848484848 HMGB2_HUMAN High mobility grou
0.848484848 SPB3_HUMAN Se GSTP1_HUMAN G
0.850202429 RAB8A_HUMAN RRAB8B_HUMAN R
0.850438712 QCR2_HUMAN Cytochrome b-c1 co
0.850889368 RL14_HUMAN 60S ribosomal protein
0.851015186 NDKA_HUMAN N NDKB_HUMAN N
0.851452191 GT251_HUMAN P SERPH_HUMAN S
0.852157962 RL14_HUMAN 60 TMEDA_HUMAN
0.853827436 APOD_HUMAN Apolipoprotein D ,
0.854235885 NDKA_HUMAN Nucleoside diphosph
0.856971154 PHB_HUMAN Prohibitin ,
0.857020964 PHB_HUMAN Prohibitin ,
0.857142857 HMGB2_HUMAN High mobility grou
0.857142857 PP1G_HUMAN Serine/threonine-pro

nucleoside diphosphate phosphorylation
viral genome expression
transporter activity
lipid biosynthetic process
cadherin binding
regulation of cell cycle
null
early endosome

transferase activity
cell-substrate adherens junction
regulation of mRNA catabolic process
cellular component disassembly
activation of immune response
cellular_component
leukocyte cell-cell adhesion
cellular response to monosaccharide stimulus
negative regulation of developmental process
coenzyme metabolic process
null
nuclear-transcribed mRNA catabolic process
null
adherens junction
cysteine-type endopeptidase activity
null
cellular response to reactive oxygen species
response to DNA damage stimulus
core promoter binding
epithelium development
axonogenesis
regulation of body fluid levels
wound healing
response to topologically incorrect protein
cellular biosynthetic process
extracellular organelle
cytoskeletal part
protein localization to chromosome, telomeric region
substrate-specific channel activity
U5 snRNP
membrane biogenesis
anatomical structure morphogenesis
null
vesicle
negative regulation of gene expression
G2/M transition of mitotic cell cycle
cellular response to growth factor stimulus
protein kinase binding
null
cell projection morphogenesis
heterocycle metabolic process
ion transport
generation of precursor metabolites and energy
symbiosis, encompassing mutualism through parasitism
oxidative phosphorylation
null

0.857142857 NDKA_HUMAN N NDKB_HUMAN Nu
0.857189817 RL14_HUMAN 60S ribosomal protein
0.858452728 APOD_HUMAN A MMP9_HUMAN M
0.85864583 DHB4_HUMAN Peroxisomal multifun
0.858974359 RL14_HUMAN 60S ribosomal protein
0.859445801 H2AY_HUMAN CoRBBP7_HUMAN H
0.859842555 RAB8A_HUMAN RH2AY_HUMAN Co
0.861917353 PHB_HUMAN Prohibitin ,

0.862233766 NDKA_HUMAN N GT251_HUMAN P
0.862584018 MMP14_HUMAN NDKB_HUMAN N
0.862639516 CIRBP_HUMAN Cold-inducible RNA-
0.863651737 HMGB2_HUMAN MMP9_HUMAN M
0.864300259 PHB_HUMAN Pro LEG9_HUMAN Gal
0.865647518 HMGB2_HUMAN K1C13_HUMAN K
0.86627907 LEG9_HUMAN Galectin-9 ,
0.866666667 NDKA_HUMAN Nucleoside diphosph
0.866695497 NDKA_HUMAN Nucleoside diphosph
0.867307692 DHB4_HUMAN Peroxisomal multifun
0.867355035 NDKA_HUMAN N HCDH_HUMAN H
0.867452135 RL14_HUMAN 60S ribosomal protein
0.867913501 RAB8A_HUMAN RH2AY_HUMAN Co
0.868594199 MMP14_HUMAN NDKB_HUMAN N
0.869233422 MMP9_HUMAN LEG9_HUMAN Gal
0.869620158 LEG9_HUMAN Galectin-9 ,
0.86965287 MMP9_HUMAN Matrix metalloprot
0.870792157 HMGB2_HUMAN High mobility grou
0.870946179 H2AY_HUMAN Core histone macro-
0.871742518 C2CD3_HUMAN CMMP14_HUMAN
0.871794872 RAB8A_HUMAN Ras-related protein
0.872036325 NDKA_HUMAN Nucleoside diphosph
0.872727273 APOD_HUMAN Apolipoprotein D ,
0.873334148 SERPH_HUMAN Serpin H1 ,
0.873336468 HMGB2_HUMAN NDKB_HUMAN N
0.87379422 AMY1_HUMAN A AMYP_HUMAN AI
0.874897622 NDKA_HUMAN N C2CD3_HUMAN C
0.875 H2AY_HUMAN Core histone macro-
0.875 MMP9_HUMAN Matrix metalloprot
0.875 NH2L1_HUMAN NHP2-like protein 1
0.875000429 K1C13_HUMAN Keratin, type I cytos
0.875842938 APOD_HUMAN A MMP9_HUMAN M
0.876417608 QCR2_HUMAN CyTMEDA_HUMAN T
0.876426935 AMY1_HUMAN A MMP14_HUMAN
0.876907188 NDKA_HUMAN N HMGB2_HUMAN
0.877192982 RAB8A_HUMAN Ras-related protein
0.877624346 GSTP1_HUMAN Glutathione S-transf
0.877659574 H2AY_HUMAN CoPP1G_HUMAN Se
0.877744992 HMGB2_HUMAN HCDH_HUMAN H
0.879224983 RAB8A_HUMAN Ras-related protein
0.880502536 HMGB2_HUMAN NDKB_HUMAN N
0.880964118 MMP9_HUMAN PHB_HUMAN Pro
0.882064383 QCR2_HUMAN CyPP1G_HUMAN Ser
0.882477389 RL14_HUMAN 60 LEG9_HUMAN Ga
0.882851568 QCR2_HUMAN Cytochrome b-c1 co
0.882978723 H2AY_HUMAN Core histone macro-

oxidoreductase activity
 organic cyclic compound binding
 homeostatic process
 null
 cation transmembrane transporter activity
 null
 membrane
 null
 muscle structure development
 lyase activity
 protein localization in membrane

0.884123876 HCDH_HUMAN H SQRD_HUMAN Su
 0.884246089 HMGB2_HUMAN HCDH_HUMAN H
 0.884314516 NDKA_HUMAN N HMGB2_HUMAN
 0.885286764 NDKA_HUMAN N NDKB_HUMAN N
 0.885486834 MMP9_HUMAN Matrix metalloprot
 0.885644769 NDKA_HUMAN N K1C13_HUMAN K
 0.88662688 MMP14_HUMAN NDKB_HUMAN N
 0.88666956 NDKA_HUMAN N RL14_HUMAN 60
 0.888137004 MMP14_HUMAN Matrix metallopro
 0.888887929 DHB4_HUMAN Peroxisomal multifun
 0.889364693 RL14_HUMAN 60 RAB8A_HUMAN R

ATP-dependent chromatin remodeling
 regulation of mononuclear cell proliferation
 cellular nitrogen compound catabolic process
 cellular aromatic compound metabolic process
 protein binding
 vacuolar lumen
 heterocycle catabolic process
 positive regulation of apoptotic process
 aging
 null
 induction of apoptosis via death domain receptors
 purine nucleoside triphosphate metabolic process
 chordate embryonic development
 aromatic compound catabolic process
 null
 ribonucleoprotein complex subunit organization
 cornification
 null
 cellular localization
 epithelial cell differentiation
 cytoskeleton
 nucleobase-containing compound catabolic process
 nucleoside phosphate metabolic process
 regulation of mitotic cell cycle
 protein targeting to membrane
 regulation of cell development
 transferase activity, transferring hexosyl groups
 null
 phosphate-containing compound metabolic process
 in utero embryonic development
 steroid hormone mediated signaling pathway
 membrane budding
 peptide biosynthetic process
 regulation of cysteine-type endopeptidase activity
 developmental growth
 translational elongation
 response to antibiotic
 regulation of intrinsic apoptotic signaling pathway
 organic acid metabolic process
 cell morphogenesis
 transferase activity, transferring acyl groups
 cell-cell junction assembly
 intrinsic apoptotic signaling pathway

0.889705882 RBBP7_HUMAN Histone-binding pro
 0.89010989 LEG9_HUMAN Galectin-9 ,
 0.891082138 HMGB2_HUMAN RL14_HUMAN 60
 0.891358186 HMGB2_HUMAN NDKB_HUMAN N
 0.891765969 HMGB2_HUMAN K1C13_HUMAN K
 0.89179216 SPB3_HUMAN Serpin B3 ,
 0.892091138 HMGB2_HUMAN RL14_HUMAN 60
 0.89219453 MMP9_HUMAN LEG9_HUMAN Gal
 0.89243613 APOD_HUMAN Apolipoprotein D ,
 0.892857143 HMGB2_HUMAN High mobility grou
 0.892857143 HMGB2_HUMAN High mobility grou
 0.892929293 NDKA_HUMAN N NDKB_HUMAN N
 0.894736842 C2CD3_HUMAN CMMP14_HUMAN
 0.894818255 HMGB2_HUMAN RL14_HUMAN 60
 0.895081616 LYRIC_HUMAN Pr TMEDA_HUMAN
 0.896418904 RL14_HUMAN 60 NH2L1_HUMAN N
 0.896777604 K1C13_HUMAN Keratin, type I cytos
 0.897189299 GT251_HUMAN Procollagen galacto
 0.897255646 APOD_HUMAN A C2CD3_HUMAN C
 0.897480812 NDKB_HUMAN N K1C13_HUMAN K
 0.897666193 NDKA_HUMAN N C2CD3_HUMAN C
 0.897964921 HMGB2_HUMAN RL14_HUMAN 60
 0.898039213 NDKA_HUMAN N NDKB_HUMAN N
 0.898435277 H2AY_HUMAN Core histone macro-
 0.899420864 RL14_HUMAN 60 RAB8B_HUMAN R
 0.899823078 NDKA_HUMAN N HMGB2_HUMAN
 0.9 GT251_HUMAN Procollagen galacto
 0.900649351 RAB8A_HUMAN Ras-related protein
 0.901379589 NDKA_HUMAN N MMP9_HUMAN M
 0.901960784 C2CD3_HUMAN C2 domain-containi
 0.901960784 PHB_HUMAN Prohibitin ,
 0.902364932 NDKA_HUMAN N TMEDA_HUMAN T
 0.903830768 RL14_HUMAN 60 NH2L1_HUMAN N
 0.904612243 MMP9_HUMAN LEG9_HUMAN Gal
 0.905061722 APOD_HUMAN Apolipoprotein D ,
 0.905605695 RL14_HUMAN 60 NH2L1_HUMAN N
 0.905788735 GSTP1_HUMAN Glutathione S-transf
 0.905955191 MMP9_HUMAN Matrix metalloprot
 0.906308451 HCDH_HUMAN H GSTP1_HUMAN G
 0.907407407 RAB8A_HUMAN Ras-related protein
 0.907712066 K1C13_HUMAN Keratin, type I cytos
 0.908695652 LYRIC_HUMAN Protein LYRIC ,
 0.909004308 MMP9_HUMAN Matrix metalloprot

response to UV
 secretory granule lumen
 null
 cell adhesion
 purine ribonucleotide metabolic process
 SRP-dependent cotranslational protein targeting to membrane
 axon
 translational initiation
 bleb assembly
 cell morphogenesis involved in differentiation
 fibrillar center
 regulation of translation
 T cell activation
 activation of innate immune response

nuclear-transcribed mRNA catabolic process, nonsense-mediated decay
 purine nucleoside monophosphate biosynthetic process
 ribonucleoside monophosphate biosynthetic process
 purine ribonucleoside monophosphate biosynthetic process
 hemopoietic progenitor cell differentiation
 anion binding
 regulation of cell cycle process
 null
 null
 response to growth factor stimulus
 cellular protein localization
 lytic vacuole
 cytoskeletal protein binding
 null
 amide biosynthetic process
 cell-substrate junction
 peptidase activity, acting on L-amino acid peptides
 regulation of protein kinase activity
 neuron projection morphogenesis
 calcium ion binding
 null
 response to extracellular stimulus
 positive regulation of neurogenesis
 intracellular
 intracellular part
 nitrogen compound metabolic process
 identical protein binding
 protein catabolic process
 nuclear mRNA splicing, via spliceosome
 innate immune response
 mitotic cell cycle
 glucose metabolic process
 keratinization
 defense response to bacterium
 response to cytokine stimulus
 toll-like receptor signaling pathway
 immune response-regulating signaling pathway
 RNA catabolic process
 nucleosome
 protein dimerization activity

0.909090909 CIRBP_HUMAN Cold-inducible RNA-
 0.910028002 NDKB_HUMAN N SPB3_HUMAN Ser
 0.910264037 LEG9_HUMAN GaLEG7_HUMAN Ga
 0.910447761 APOD_HUMAN A MMP14_HUMAN
 0.91048951 NDKA_HUMAN N NDKB_HUMAN N
 0.911055967 RL14_HUMAN 60S ribosomal protein
 0.911132403 AGRL3_HUMAN Adhesion G protein-
 0.911391584 RL14_HUMAN 60 NH2L1_HUMAN N
 0.915643724 NDKA_HUMAN N HMGB2_HUMAN
 0.916412478 RAB8A_HUMAN Ras-related protein
 0.916666667 LYRIC_HUMAN Protein LYRIC ,
 0.917054532 CIRBP_HUMAN Cold-inducible RNA-
 0.917417417 LEG9_HUMAN Galectin-9 ,
 0.917503966 LEG9_HUMAN Galectin-9 ,

0.919354839 RL14_HUMAN 60S ribosomal protein
 0.92 QCR2_HUMAN Cytochrome b-c1 co
 0.92 QCR2_HUMAN Cytochrome b-c1 co
 0.92 QCR2_HUMAN Cytochrome b-c1 co
 0.920029894 SPB12_HUMAN Serpin B12 ,
 0.920451585 NDKA_HUMAN N HCDH_HUMAN H
 0.921357551 H2AY_HUMAN CoRBBP7_HUMAN H
 0.921621679 HMGB2_HUMAN HCDH_HUMAN H
 0.921770737 HMGB2_HUMAN RL14_HUMAN 60
 0.922072514 GSTP1_HUMAN Glutathione S-transf
 0.923426208 APOD_HUMAN A C2CD3_HUMAN C
 0.923610363 LEG9_HUMAN Ga SPB3_HUMAN Ser
 0.92475554 NDKA_HUMAN N RAB8A_HUMAN R
 0.924855491 HMGB2_HUMAN NDKB_HUMAN N
 0.92507579 RL14_HUMAN 60 NH2L1_HUMAN N
 0.925223173 MMP14_HUMAN NDKB_HUMAN N
 0.926315789 MMP9_HUMAN MMP14_HUMAN
 0.926489346 SPB3_HUMAN Se H2AY_HUMAN Co
 0.926834793 RAB8A_HUMAN Ras-related protein
 0.928239821 AMYP_HUMAN A AGRL3_HUMAN A
 0.928571429 H2AY_HUMAN Core histone macro-
 0.930620683 GSTP1_HUMAN Glutathione S-transf
 0.930856629 NDKA_HUMAN Nucleoside diphosph
 0.932104559 HMGB2_HUMAN K1C13_HUMAN K
 0.932104559 HMGB2_HUMAN K1C13_HUMAN K
 0.932388333 HMGB2_HUMAN H2AY_HUMAN Co
 0.93300368 NDKA_HUMAN N MMP9_HUMAN M
 0.933911278 PHB_HUMAN Pro SPB12_HUMAN Se
 0.93442623 NH2L1_HUMAN NHP2-like protein 1
 0.935493394 HMGB2_HUMAN LEG9_HUMAN Gal
 0.936492961 RAB8A_HUMAN RH2AY_HUMAN Co
 0.9375 APOD_HUMAN Apolipoprotein D ,
 0.938913258 K1C13_HUMAN Keratin, type I cytos
 0.940726035 HMGB2_HUMAN High mobility grou
 0.941111127 MMP9_HUMAN PHB_HUMAN Pro
 0.941176471 LEG9_HUMAN Galectin-9 ,
 0.941649805 LEG9_HUMAN Galectin-9 ,
 0.9417929 RL14_HUMAN 60 CIRBP_HUMAN Co
 0.942127147 H2AY_HUMAN Core histone macro-
 0.942305666 H2AY_HUMAN CoDHB4_HUMAN Pe

biosynthetic process	0.942496794 HMGB2_HUMAN NDKB_HUMAN N
null	0.942962963 MMP14_HUMAN Matrix metallopro
regulation of neurogenesis	0.942975341 NDKA_HUMAN N HMGB2_HUMAN
null	0.943919147 NDKA_HUMAN N NDKB_HUMAN N
ion transmembrane transporter activity	0.943992773 MMP9_HUMAN Matrix metalloprot
null	0.944041604 NDKA_HUMAN Nucleoside diphosph
calcium ion transport	0.944444444 PHB_HUMAN Prohibitin ,
purine ribonucleoside triphosphate metabolic process	0.944444444 NDKA_HUMAN N NDKB_HUMAN N
nucleotide binding	0.944586585 NDKA_HUMAN N HCDH_HUMAN H
response to wounding	0.944797438 APOD_HUMAN Apolipoprotein D ,
transmembrane transporter activity	0.945014594 MMP9_HUMAN Matrix metalloprot
ribonucleoside triphosphate metabolic process	0.945454545 NDKA_HUMAN N NDKB_HUMAN N
generation of neurons	0.945502288 NDKA_HUMAN N HMGB2_HUMAN
cellular component morphogenesis	0.945744258 RAB8A_HUMAN Ras-related protein
purine nucleoside binding	0.947368421 NDKA_HUMAN N RAB8A_HUMAN R
ribonucleoside binding	0.947368421 NDKA_HUMAN N RAB8A_HUMAN R
null	0.947474747 QCR2_HUMAN Cytochrome b-c1 co
protein folding	0.948064098 FKBP2_HUMAN Peptidyl-prolyl cis-tr
cellular nitrogen compound metabolic process	0.948395122 HMGB2_HUMAN NDKB_HUMAN N
regulation of endothelial cell proliferation	0.948717949 HMGB2_HUMAN High mobility grou
ribonucleoprotein complex assembly	0.948758336 RL14_HUMAN 60 NH2L1_HUMAN N
ATP binding	0.948769361 NDKA_HUMAN N NDKB_HUMAN Nu
transferase activity, transferring amino-acyl groups	0.950808766 K1C13_HUMAN Keratin, type I cytos
translation	0.950842667 RL14_HUMAN 60 NH2L1_HUMAN N
cellular catabolic process	0.951347234 HMGB2_HUMAN HCDH_HUMAN H
cellular homeostasis	0.951480867 NDKA_HUMAN Nucleoside diphosph
extracellular space	0.951724138 HMGB2_HUMAN AMY1_HUMAN AI
response to inorganic substance	0.952287779 MMP9_HUMAN Matrix metalloprot
mitochondrial matrix	0.95230428 HCDH_HUMAN Hydroxyacyl-coenzy
response to oxygen levels	0.952315266 MMP14_HUMAN Matrix metallopro
nucleobase-containing compound metabolic process	0.952337811 HMGB2_HUMAN NDKB_HUMAN N
null	0.952380952 RBBP7_HUMAN Histone-binding pro
positive regulation of tumor necrosis factor production	0.952380952 LEG9_HUMAN Galectin-9 ,
null	0.953409264 HMGB2_HUMAN NDKB_HUMAN N
positive regulation of cell development	0.954564566 NDKA_HUMAN Nucleoside diphosph
integral to membrane	0.955357143 LYRIC_HUMAN Pr PHB_HUMAN Pro
response to toxin	0.955493739 GSTP1_HUMAN Glutathione S-transf
regulation of gene expression	0.956005068 HMGB2_HUMAN NDKB_HUMAN N
cytokine-mediated signaling pathway	0.956888842 MMP9_HUMAN GSTP1_HUMAN G
azurophil granule	0.957829868 SPB3_HUMAN Serpin B3 ,
null	0.958032988 NDKA_HUMAN N K1C13_HUMAN K
rRNA processing	0.958348557 RL14_HUMAN 60 NH2L1_HUMAN N
coenzyme binding	0.958367347 HCDH_HUMAN Hydroxyacyl-coenzy
null	0.959008614 CIRBP_HUMAN Cold-inducible RNA-
nucleocytoplasmic transport	0.959183673 APOD_HUMAN A PP1G_HUMAN Ser
nucleotide metabolic process	0.960526316 NDKA_HUMAN N NDKB_HUMAN N
null	0.96093372 H2AY_HUMAN Core histone macro-
RNA polymerase II regulatory region sequence-specific DNA binding	0.962337662 NDKA_HUMAN N H2AY_HUMAN Co
null	0.962962963 RAB8A_HUMAN Ras-related protein
nucleoside monophosphate metabolic process	0.963015647 QCR2_HUMAN Cytochrome b-c1 co
ATP metabolic process	0.963874057 QCR2_HUMAN Cytochrome b-c1 co
endodeoxyribonuclease activity	0.964285714 HMGB2_HUMAN High mobility grou
macromolecule catabolic process	0.964415454 HMGB2_HUMAN RL14_HUMAN 60
vacuolar part	0.965600121 SPB3_HUMAN Serpin B3 ,

macromolecular complex disassembly
nuclear division
RNA splicing, via transesterification reactions
response to nutrient levels
null
regulation of neuron differentiation
cytosol
regulated secretory pathway
carboxylic acid metabolic process
response to unfolded protein
null
null
regulation of mRNA stability
nuclear speck
null
cellular respiration
regulation of organelle organization
monovalent inorganic cation transport
membrane invagination
null

macromolecular complex subunit organization
protein targeting to ER
establishment of localization
anchoring junction
response to hexose stimulus
nucleoside monophosphate biosynthetic process
immune response-activating signal transduction
cell adhesion molecule binding
mRNA catabolic process
transport
RNA splicing, via transesterification reactions with bulged adenosine as nucle
establishment of protein localization in endoplasmic reticulum
macromolecular complex
leukocyte chemotaxis
null
ATP biosynthetic process
ameboidal cell migration
neutrophil degranulation
posttranscriptional regulation of gene expression
cellular response to endogenous stimulus
RNA binding
null
ribonucleoprotein complex biogenesis
cellular macromolecule catabolic process
endocytosis
cysteine-type peptidase activity
cellular process
structural molecule activity
granulocyte activation
mRNA metabolic process
protein localization in endoplasmic reticulum
interspecies interaction between organisms
system process
nuclear body

0.96561652 RL14_HUMAN 60 NH2L1_HUMAN N
0.966666667 RAB8A_HUMAN RH2AY_HUMAN Co
0.966666667 NH2L1_HUMAN NHP2-like protein 1
0.966666667 GSTP1_HUMAN Glutathione S-transf
0.96875 SERPH_HUMAN Serpin H1 ,
0.968923418 NDKA_HUMAN Nucleoside diphosph
0.969529557 MMP14_HUMAN NDKB_HUMAN N
0.969561484 MMP9_HUMAN LEG9_HUMAN Ga
0.97029703 HCDH_HUMAN H GSTP1_HUMAN G
0.970588235 SERPH_HUMAN Serpin H1 ,
0.971156018 NDKA_HUMAN Nucleoside diphosph
0.972222222 MMP14_HUMAN Matrix metallopro
0.973684211 CIRBP_HUMAN Cold-inducible RNA-
0.97369334 PP1G_HUMAN Serine/threonine-pro
0.974114461 HMGB2_HUMAN NDKB_HUMAN N
0.974390244 QCR2_HUMAN Cytochrome b-c1 co
0.974729214 MMP9_HUMAN H2AY_HUMAN Co
0.975087413 QCR2_HUMAN Cytochrome b-c1 co
0.975327712 NDKA_HUMAN Nucleoside diphosph
0.97598553 PHB_HUMAN Pro SPB12_HUMAN Se

0.975988502 HMGB2_HUMAN GT251_HUMAN P
0.977105421 RL14_HUMAN 60S ribosomal protein
0.977282189 HCDH_HUMAN H NDKB_HUMAN N
0.977302463 MMP14_HUMAN NDKB_HUMAN N
0.977777778 NDKA_HUMAN Nucleoside diphosph
0.977815934 QCR2_HUMAN Cytochrome b-c1 co
0.978959673 LEG9_HUMAN Galectin-9 ,
0.98098727 RL14_HUMAN 60S ribosomal protein
0.981519208 RL14_HUMAN 60 CIRBP_HUMAN Co
0.981658383 HCDH_HUMAN H NDKB_HUMAN N
0.982758621 NH2L1_HUMAN NHP2-like protein 1
0.982872638 RL14_HUMAN 60S ribosomal protein
0.98300075 HMGB2_HUMAN H2AY_HUMAN Co
0.983238952 LEG9_HUMAN Galectin-9 ,
0.983662285 RAB8A_HUMAN RH2AY_HUMAN Co
0.984440122 QCR2_HUMAN Cytochrome b-c1 co
0.985350667 MMP9_HUMAN Matrix metalloprot
0.985971793 MMP9_HUMAN NDKB_HUMAN N
0.988251185 CIRBP_HUMAN Cold-inducible RNA-
0.988572163 PHB_HUMAN Pro RAB8A_HUMAN R
0.988598416 NDKA_HUMAN N HMGB2_HUMAN
0.989369977 PHB_HUMAN Pro QCR2_HUMAN Cy
0.989481279 RL14_HUMAN 60 NH2L1_HUMAN N
0.989739483 HMGB2_HUMAN RL14_HUMAN 60
0.990497038 NDKA_HUMAN Nucleoside diphosph
0.991106747 MMP9_HUMAN LEG9_HUMAN Ga
0.991895727 HMGB2_HUMAN K1C13_HUMAN K
0.992269177 RL14_HUMAN 60 K1C13_HUMAN K
0.992679084 MMP9_HUMAN NDKB_HUMAN N
0.993796215 RL14_HUMAN 60 NH2L1_HUMAN N
0.993881879 RL14_HUMAN 60S ribosomal protein
0.994427684 RL14_HUMAN 60 LEG9_HUMAN Ga
0.994914721 RAB8B_HUMAN Ras-related protein
0.995985274 LYRIC_HUMAN Pr PP1G_HUMAN Se

extracellular vesicular exosome	0.996589369 AMY1_HUMAN A AMYP_HUMAN AI
cell	0.997026487 HMGB2_HUMAN K1C13_HUMAN K
cell part	0.997026487 HMGB2_HUMAN K1C13_HUMAN K
cotranslational protein targeting to membrane	0.997202797 RL14_HUMAN 60S ribosomal protein
neutrophil activation involved in immune response	0.99745158 MMP9_HUMAN NDKB_HUMAN N
neutrophil mediated immunity	0.997497915 MMP9_HUMAN NDKB_HUMAN N
RNA processing	0.998604365 RL14_HUMAN 60 NH2L1_HUMAN N
hydrolase activity, acting on acid anhydrides	0.999064235 RAB8A_HUMAN RRAB8B_HUMAN R
regulation of anatomical structure morphogenesis	0.999520033 LYRIC_HUMAN Protein LYRIC ,
macromolecular complex assembly	0.99989725 HMGB2_HUMAN RL14_HUMAN 60
null	1 MMP9_HUMAN Matrix metalloprot
null	1 AMY1_HUMAN A TIM8A_HUMAN M
pyrophosphatase activity	1 RAB8A_HUMAN RRAB8B_HUMAN R
mononuclear cell proliferation	1 LEG9_HUMAN Galectin-9 ,
oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as	1 HCDH_HUMAN H DHB4_HUMAN Pe
null	1 H2AY_HUMAN Core histone macro-
four-way junction DNA binding	1 HMGB2_HUMAN High mobility grou
hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydr	1 RAB8A_HUMAN RRAB8B_HUMAN R
box C/D snoRNP assembly	1 NH2L1_HUMAN NHP2-like protein 1
null	1 GSTP1_HUMAN Glutathione S-transf
embryonic axis specification	1 C2CD3_HUMAN C2 domain-containi
null	1 RAB8A_HUMAN Ras-related protein
Ras GTPase binding	1 RAB8A_HUMAN Ras-related protein
null	1 RAB8A_HUMAN Ras-related protein
nucleoside-triphosphatase activity	1 RAB8A_HUMAN RRAB8B_HUMAN R
null	1 NDKA_HUMAN N K1C13_HUMAN K
null	1 NDKA_HUMAN N K1C13_HUMAN K
null	1 RAB8A_HUMAN Ras-related protein
negative regulation of smooth muscle cell-matrix adhesion	1 APOD_HUMAN Apolipoprotein D ,
null	1 H2AY_HUMAN Core histone macro-
response to monosaccharide stimulus	1 NDKA_HUMAN N GSTP1_HUMAN G
regulation of lipoprotein oxidation	1 APOD_HUMAN Apolipoprotein D ,
negative regulation of lipoprotein oxidation	1 APOD_HUMAN Apolipoprotein D ,
endoderm formation	1 MMP9_HUMAN MMP14_HUMAN
mesoderm formation	1 MMP9_HUMAN MMP14_HUMAN
U3 snoRNA binding	1 NH2L1_HUMAN NHP2-like protein 1
box C/D snoRNA binding	1 NH2L1_HUMAN NHP2-like protein 1
heart looping	1 C2CD3_HUMAN C2 domain-containi
histone lysine methylation	1 H2AY_HUMAN Core histone macro-
CD4-positive, alpha-beta T cell differentiation involved in immune response	1 LEG9_HUMAN Galectin-9 ,
natural killer cell tolerance induction	1 LEG9_HUMAN Galectin-9 ,
somatic diversification of immune receptors via germline recombination within	1 HMGB2_HUMAN High mobility grou
entry into cell of other organism involved in symbiotic interaction	1 LEG9_HUMAN GaSPB3_HUMAN Ser
intraflagellar transport involved in cilium morphogenesis	1 C2CD3_HUMAN CRAB8A_HUMAN R
chondrocyte development involved in endochondral bone morphogenesis	1 SERPH_HUMAN Serpin H1 ,
syntaxin binding	1 TMEDA_HUMAN Transmembrane e
protein-glutamine gamma-glutamyltransferase activity	1 K1C13_HUMAN Keratin, type I cytos
alpha-amylase activity	1 AMY1_HUMAN A AMYP_HUMAN AI
sulfide oxidation, using sulfide:quinone oxidoreductase	1 SQRD_HUMAN Sulfide:quinone oxid
sulfide:quinone oxidoreductase activity	1 SQRD_HUMAN Sulfide:quinone oxid
JUN kinase activity	1 SPB3_HUMAN Se GSTP1_HUMAN G
signaling receptor activity	1 HMGB2_HUMAN PHB_HUMAN Pro
FK506 binding	1 FKBP2_HUMAN Peptidyl-prolyl cis-tr
lysosome	1 LEG9_HUMAN GaSPB3_HUMAN Ser

peroxisome
 focal adhesion
 glycogen metabolic process
 protein localization to centrosome
 cellular glucan metabolic process
 negative regulation of monocyte chemotactic protein-1 production
 CTP biosynthetic process
 apoptotic DNA fragmentation
 protein methylation
 T cell migration
 protein localization to microtubule cytoskeleton
 synaptic transmission
 spermatogenesis
 single fertilization
 determination of left/right symmetry
 epithelium migration
 purine nucleoside monophosphate metabolic process
 ribonucleoside monophosphate metabolic process
 purine ribonucleoside monophosphate metabolic process
 neutrophil activation
 melanosome
 response to glucose stimulus
 glucose homeostasis
 determination of bilateral symmetry
 transcription of nuclear large rRNA transcript from RNA polymerase I promote
 glucocorticoid receptor signaling pathway

amyloid precursor protein catabolic process
 entrainment of circadian clock by photoperiod
 oxoacid metabolic process
 limb development
 endochondral bone morphogenesis
 macropinosome
 entry into host
 membrane raft
 CTP metabolic process
 pyrimidine ribonucleoside biosynthetic process
 hemopoiesis
 entry into host cell
 apoptotic nuclear change
 disaccharide binding
 channel activity
 COPI coating of Golgi vesicle
 cholesterol binding
 null
 null
 smooth muscle cell proliferation
 asymmetric synapse
 peroxisomal membrane transport
 embryonic cranial skeleton morphogenesis
 purine ribonucleoside binding
 tumor necrosis factor production
 regulation of tumor necrosis factor production
 positive regulation of interleukin-1 beta production

1 DHB4_HUMAN PeRAB8B_HUMAN R
 1 MMP14_HUMAN NDKB_HUMAN N
 1 PP1G_HUMAN Serine/threonine-pro
 1 C2CD3_HUMAN C2 domain-containi
 1 PP1G_HUMAN Serine/threonine-pro
 1 APOD_HUMAN A GSTP1_HUMAN G
 1 NDKA_HUMAN N NDKB_HUMAN Nu
 1 HMGB2_HUMAN High mobility grou
 1 H2AY_HUMAN Core histone macro-
 1 APOD_HUMAN Apolipoprotein D ,
 1 C2CD3_HUMAN C2 domain-containi
 1 RAB8A_HUMAN Ras-related protein
 1 HMGB2_HUMAN High mobility grou
 1 NH2L1_HUMAN NHP2-like protein 1
 1 C2CD3_HUMAN C2 domain-containi
 1 MMP9_HUMAN Matrix metalloprot
 1 QCR2_HUMAN Cytochrome b-c1 co
 1 QCR2_HUMAN Cytochrome b-c1 co
 1 QCR2_HUMAN Cytochrome b-c1 co
 1 MMP9_HUMAN NDKB_HUMAN N
 1 MMP14_HUMAN TMEDA_HUMAN T
 1 NDKA_HUMAN Nucleoside diphosph
 1 NDKA_HUMAN Nucleoside diphosph
 1 C2CD3_HUMAN C2 domain-containi
 1 H2AY_HUMAN Core histone macro-
 1 PHB_HUMAN Prohibitin ,

1 TMEDA_HUMAN Transmembrane e
 1 PP1G_HUMAN Serine/threonine-pro
 1 HCDH_HUMAN H GSTP1_HUMAN G
 1 C2CD3_HUMAN C2 domain-containi
 1 MMP14_HUMAN SERPH_HUMAN S
 1 MMP14_HUMAN Matrix metallopro
 1 LEG9_HUMAN Ga SPB3_HUMAN Ser
 1 SERPH_HUMAN Serpin H1 ,
 1 NDKA_HUMAN N NDKB_HUMAN Nu
 1 NDKA_HUMAN N NDKB_HUMAN Nu
 1 NDKA_HUMAN N HMGB2_HUMAN
 1 LEG9_HUMAN Ga SPB3_HUMAN Ser
 1 HMGB2_HUMAN High mobility grou
 1 LEG9_HUMAN Galectin-9 ,
 1 MMP9_HUMAN Matrix metalloprot
 1 TMEDA_HUMAN Transmembrane e
 1 APOD_HUMAN Apolipoprotein D ,
 1 NH2L1_HUMAN NHP2-like protein 1
 1 HMGB2_HUMAN LYRIC_HUMAN Pr
 1 APOD_HUMAN A MMP9_HUMAN M
 1 RAB8A_HUMAN Ras-related protein
 1 RAB8B_HUMAN Ras-related protein
 1 MMP14_HUMAN Matrix metallopro
 1 NDKA_HUMAN N RAB8A_HUMAN R
 1 LEG9_HUMAN Ga GSTP1_HUMAN G
 1 LEG9_HUMAN Ga GSTP1_HUMAN G
 1 LEG9_HUMAN Galectin-9 ,

Table 6.3. Efflux of 3H-Cholesterol from agLDL-Loaded Mouse Peritoneal Macrophages Transfected with siRNA Against Indicated Target Genes

Gene	Ex1	Ex2	Ex3	Ex4	average	P-value two tail	
<i>Coro1a</i>	0.697949	1.040459	0.978811	1.04888628	0.9415262	0.50595598	
<i>Lamtor5</i>	0.736664	1.271825	0.74043	0.99277871	0.93542458	0.62965176	
<i>Park7</i>	1.005485	1.082703	0.743695	0.97688695	0.95219226	0.53683353	
<i>Vdac2</i>	0.718873	0.687289	1.094211	1.00690352	0.87681903	0.27296735	
<i>Ywhaz</i>	1.060335	1.002585	0.807238	1.09645942	0.99165447	0.90118756	
<i>Ywhag</i>	0.627237	1.175298	0.496325	0.911124	0.80249591	0.2399898	
<i>Ywhae</i>	1.107197	1.066771	0.674748	0.99703916	0.96143878	0.70822251	
<i>Ywhaq</i>	0.757372	0.95623	0.531212	1.13096755	0.84394539	0.27252135	
<i>Dap</i>	0.615941	0.960355	1.049151	0.93583217	0.89031979	0.29053706	
<i>Hmgb1</i>	0.694515	0.795589	0.839422	0.91851072	0.81200907	0.00691427	*
<i>Hsp90aa1</i>	0.875558	0.698689	0.787312	0.83734634	0.79972642	0.00193814	**
<i>Hspa8</i>	0.902042	0.647711	0.782423	1.19290913	0.88127119	0.34609284	
<i>Hspb1</i>	0.992445	0.878453	0.697474	0.90500116	0.86834343	0.07769773	#
<i>Hspa5</i>	0.830077	0.882969	0.511476	0.91532799	0.78496234	0.05975732	#
<i>Hsph1</i>	0.527234	0.864957	0.574916	0.91811416	0.72130498	0.03096062	*
<i>Scram1</i>	1.274119	0.896269	0.525312	1.19964386	0.97383587	0.88298832	
<i>Dsc1</i>	0.900524	0.868917	1.093373	0.7843011	0.91177847	0.22549496	
<i>Psap</i>	0.774075	0.739708		0.71118999	0.74165793	1.2942E-05	***
<i>Slc25a5</i>	0.80668	0.965233	1.561461	1.77211875	1.27637333	0.2780519	
<i>Slc25a26</i>	0.764342	0.902942	1.450793	1.48653654	1.15115332	0.44659196	
<i>Myl6</i>	0.820676	1.020892	1.535244	1.58651815	1.24083235	0.25089633	
<i>Npc2</i>	0.92527	0.975438	0.615151	0.87970173	0.84889021	0.10898056	#
<i>Scarb2</i>	0.850764	0.814676	0.738487	0.81060221	0.80363219	0.00016026	***
<i>Ces1</i>	1.004642	0.868361	0.563633	0.81465005	0.81282152	0.08860772	#
<i>Ldah</i>	0.840365	1.028554	0.755534	0.99998107	0.90610842	0.19910464	
<i>Pnpla1</i>	0.809758	1.038898	1.015549	0.72450151	0.89717665	0.23135832	
<i>Faf2</i>	0.921729	0.962247	1.330397	0.79593835	1.00257783	0.98282296	
<i>Rab1a</i>	0.711877	1.081402	1.351064	0.97806846	1.03060284	0.82458661	
<i>Kif11</i>	0.791256	0.782425	1.362511	1.03319213	0.99234614	0.95706724	
<i>Rab2a</i>	0.943896	0.739902	0.607931	0.80933673	0.7752664	0.01838644	*
<i>Rab5c</i>	0.691558	1.005194	0.746437	0.973401	0.85414728	0.11473944	#
<i>Rab6a</i>	0.794585	0.861196	0.620151	0.80197293	0.76947622	0.00439164	**
<i>Rab6b</i>	1.044892	1.177369	0.885932	0.95318741	1.01534513	0.81587955	
<i>Rab7</i>	0.837786	0.791248	1.573535	0.65175627	0.96358144	0.86621153	
<i>Rab10</i>	0.892077	0.906825		0.85747896	0.88546038	0.00023439	***
<i>Rab11a</i>	1.036885	0.663146	1.455671	0.63751864	0.94830526	0.79694095	
<i>Rab11b</i>	1.105707	0.912877	1.521284	0.85732992	1.09929961	0.53359346	
<i>Rab14</i>	1.352804	0.810415	0.790409	0.55680911	0.87760928	0.4950518	
<i>Rab18</i>	1.350514	0.915289	0.766029	0.62119033	0.91325555	0.60198672	
<i>Rab21</i>	1.667611	0.863457	0.644055	0.76106538	0.98404708	0.94746283	
<i>Rab31</i>	0.81751	1.172257	1.162955	1.02136248	1.04352097	0.61829657	

<i>Rab33b</i>	0.625049	0.971298	1.625488	0.55485286	0.94417196	0.82708329
<i>Scram2</i>	1.04194	1.038303	1.547073	0.82473986	1.11301423	0.48890669

<i>Rab13</i>	0.774149	0.865371		0.81126702	0.81692892	0.00042374	***
<i>Rab711</i>	0.919354	0.941115	1.352298	0.81626912	1.00725896	0.953022	
<i>Fus</i>	0.99448	0.875812	0.700503	0.67639355	0.81179714	0.04674186	*
<i>Aup1</i>	1.200498	0.883497	0.749598	0.78362563	0.9043046	0.38756484	
<i>Vcp</i>	1.28307	0.758788	0.545513	0.91852713	0.87647473	0.45746681	
<i>Tpd52l2</i>	0.741877	1.034802	1.377998	0.9436747	1.02458799	0.85916036	
<i>Apod</i>	0.623657	1.099435	1.481591	0.77577536	0.99511447	0.9803297	
<i>Ppp1cc</i>	0.686077	0.835832	1.346202	0.6940784	0.89054742	0.50849693	
<i>Rab8b</i>	0.978401	0.799471	1.34789	0.68860061	0.9535907	0.75874054	
<i>Nme1</i>	1.113843	0.76213	1.37325	1.15190664	1.10028238	0.45775963	
<i>Nme2</i>	1.233484	0.77656	0.526699	0.62500803	0.79043787	0.22870032	
<i>Hmgb2</i>	0.948582	0.851019	0.789657	0.73364946	0.83072688	0.01035062	*
<i>C2cd3</i>	1.225297	1.025736	0.714355	0.8252787	0.94766656	0.6589371	
<i>Rbbp7</i>	0.930335	0.921419		0.86552302	0.90575898	0.00260163	**
<i>Mmp14</i>	0.830953	1.04606	1.644686	0.76523636	1.07173374	0.73235113	
<i>Serp1nb12</i>	0.913154	0.802406	1.673023	0.68802342	1.01915163	0.93428101	
<i>Colgalt1</i>	1.018039	0.900509	1.580188	1.03004931	1.13219645	0.41835691	
<i>Mmp9</i>	0.915739	1.093283	1.173393	0.90838181	1.02269916	0.74246383	
<i>Phb</i>	1.028399	1.010837	0.711751	0.65152046	0.85062707	0.17982309	
<i>Tmed10</i>		0.759008	0.746757	0.66888353	0.72488286	8.1796E-05	***
<i>Rpl14</i>	1.207979	0.755012	0.509721	0.86908684	0.83544973	0.29989534	
<i>Hsd17b4</i>	0.703763	0.987663	1.617495	1.04967659	1.08964942	0.656011	
<i>Rab8a</i>	0.725704	0.924254	1.824351	0.67660753	1.03772925	0.89249776	
<i>Mtdh</i>	1.075715	0.874833	1.5629	0.83391329	1.08684017	0.62219568	
<i>Sqor</i>	1.041715	0.934931	1.458266	0.95875773	1.09841745	0.45102189	
<i>Fkbp2</i>	1.357942	1.018857	1.000311	0.74629713	1.03085178	0.81402212	
<i>Kif11</i>	0.968687	0.95637	1.029845	0.89542992	0.96258285	0.22329676	
<i>Serpinh1</i>	1.097804	0.972725	0.742926	0.65847828	0.8679836	0.24057688	
<i>Lgals9</i>	1.16967	0.994499	0.58843	0.7409922	0.87339786	0.36594717	
<i>Adgrl3</i>	0.597061	1.015309	1.027743	1.1024655	0.93564476	0.59440677	
<i>Lgals7</i>	0.680389	0.842935	1.452675	0.73932878	0.92883181	0.70284516	
<i>Snu13</i>	0.592911	0.944189	1.035082	0.76320301	0.83384622	0.14158561	
<i>Cirbp</i>	0.846174	0.843608	0.927526	0.66454782	0.82046411	0.01780663	*
<i>Serpinb3</i>	0.977386	0.857803	0.781436	1.25414216	0.96769182	0.76580748	
<i>Macroh2a1</i>		0.767518	0.803831	0.76784253	0.77973061	3.7291E-06	***
<i>Atg5</i>		0.837226	0.748562	0.76741683	0.78440167	0.0002125	***
<i>Plin2</i>	1.371786	0.917129	0.718899	1.15384224	1.04041413	0.78520195	
<i>Ube2g2</i>	0.344131	0.81701		0.84947188	0.6702043	0.0605819	#
<i>Sqstm1</i>	0.533189	0.77834	1.169991	0.96711782	0.86215951	0.34898383	
<i>Tfeb</i>	0.821578	0.842709	0.746447	0.86623072	0.8192412	0.00043237	***
<i>Nbr1</i>	0.794986	0.758993	0.619965	0.75839547	0.73308511	0.00045664	***
<i>Calcoco2</i>	0.916168	0.756236	0.748253	1.10306063	0.8809293	0.20392119	
<i>Tax1bp1</i>	1.076812		0.990941	0.99775414	1.02183565	0.38711375	
<i>Optn</i>	0.909131	0.932532	0.866218	0.83474999	0.88565793	0.00194168	**

<i>Bnip3l</i>	1.084915	1.023144	0.698215	1.17959411	0.99646724	0.97412634	
<i>Scram3</i>	0.68394	1.065428	0.927615	1.25843156	0.98385372	0.89805439	
<i>Lipa</i>	0.599589	0.884047	1.172914	0.99332396	0.91246847	0.49354437	
<i>Map1lc3a</i>	0.611016	0.635428	0.615159		0.6205342	2.4068E-08	***
<i>Mcoln1</i>	0.805269	0.836656	0.790709	0.92668288	0.83982907	0.00191608	***
<i>Prkaa1</i>	0.920483	0.875658	0.631458	1.18628054	0.90346972	0.42831693	
<i>Ppfia1</i>	1.021014	0.881421	0.455394	0.85932872	0.80428932	0.15887543	
<i>Ppm1d</i>	1.017828	0.925444	0.639394	0.97859452	0.89031523	0.24812059	
<i>Plin3</i>	1.140761	0.708148	0.569791	0.97407526	0.84819374	0.28261058	

6.2 Supplemental Material Manuscript #2

6.2.1 Expanded Materials & Methods

Plasma Cholesterol Measurements. Blood was collected in EDTA coated capillary tubes following an overnight fast by saphenous vein bleed or at sacrifice by cardiac puncture. Whole blood was centrifuged at 900 xg for 10 minutes at 4°C and plasma was collected and frozen at -20°C until analysis. Plasma cholesterol was measured following the manufacturers recommendations with the Infinity Cholesterol Liquid Stable Reagent (Thermo Fisher).

Oil Red O Staining. Aortic sinuses were prepared and sectioned as previously described⁷². Neutral lipid content was performed by oil red O staining at 60°C for 12 minutes. Lesion quantification was blinded with respect to the sex of the mice from which the sections were obtained. However, because of the large differences between the sizes of the lesions and time of sacrifice between the early and late cohorts, blinding was not possible for these analyses, although numbers were used for the mice and did not contain identifiers for their respective cohort.

Aortic Digest and Flow Cytometry Staining. Blood was collected by cardiac puncture and mice were perfused with HBSS. Aortic arches (including the descending thoracic aorta and the bracioencephalic, left common carotid and left subclavian arteries) were isolated and cleaned of all surrounding tissue. Aortic arches were digested in an enzyme mixture containing 0.4 units/mL Liberase TM (Roche), 40 units/mL hyaluronidase (Sigma) and 20 units/mL DNase (Sigma) in staining buffer (1 mM EDTA, 5% BSA in HBSS). Aortic arches minced in the enzyme mixture were transferred into individual GentleMACS C-

tubes (Milteny Biotec) and mechanically digested using the program m_spleen_02_01 on a GentleMACS dissociator (Milteny Biotec) followed by a 15-minute incubation at 37°C on a rotator. Arches were then further digested using the program m_spleen_03.01 on the GentleMACS dissociator. The cell suspension was then passed through a 70µm cell strainer to get rid of debris and centrifuged at 500xg for staining.

Cell culture. Bone-marrow derived macrophages were harvested and cultured in DMEM supplemented with 10% FBS, 20% L929 conditioned and 1% P/S as previously described⁷³. Primary smooth muscle cells were isolated from the thoracic aorta of C57BL/6N or GFP-LC3 mice. The aorta was subjected to enzymatic digestion for 20-30 minutes at 37°C (2U/mL of Liberase TM [Roche], 2U/mL of Elastase [Worthington Biochemical] in HBSS). Cells were plated on 0.1% gelatin coated plates for the first passage, and cultured in DMEM supplemented with 20% FBS, 1% Antibiotic-Antimycotic (Gibco), 1x L-glutamine, 10ng/mL of recombinant mouse leukemia inhibitory factor (LIF; Peprotech) and 0.1mM 2-mercaptoethanol (Gibco). E14TG2a (E14) mESCs were cultivated in DMEM (Gibco) supplemented with 15% fetal bovine serum (FBS), 200mM L-glutamine (Gibco), 100mM sodium pyruvate (Gibco), 1x MEM non-essential amino acids (NEAA; Gibco), 1x penicillin-streptomycin (P/S; Gibco), 46µM 2-mercaptoethanol (Sigma), 1000U/mL mouse LIF (Chemicon).

Methyl-beta cyclodextrin: cholesterol complexes. The methyl-beta cyclodextrin (mβ-CD) cholesterol was prepared as previously described⁷⁴ by adding methyl-beta cyclodextrin (Sigma) and cholesterol (Sigma) with a mβ-CD/cholesterol molar ratio of 8:1 in DMEM. This solution was sonicated using the Ultrasonic Bath 2.8L (Fisher Scientific)

for 3 min at 37°C, and was incubated on a rotator overnight at 37°C. The complex was filter sterilized prior to use.

Macrophage Conditioned Media. Macrophages were washed in HBSS twice. DMEM media containing 10% FBS, 1% P/S and 50µg/mL of agLDL was added onto the cells and incubated for 24 hours. Conditioned media was harvested and filtered using a 40µm cell strainer. Conditioned media was kept at 4°C until ready to be used.

Lipid loading of cells. Macrophages were lipid loaded for 24 to 30 hours with 50µg/mL of aggregated low-density lipoprotein (agLDL) in growth media followed by an overnight equilibration in 2mg/mL of fatty acid free BSA in DMEM, unless otherwise specified. Vascular smooth muscle cells were lipid loaded with 80µg/mL of cholesterol from mβ-CD-cholesterol complexes prepared in house as described above or purchased from Sigma, unless otherwise specified, for 72 hours in DMEM media supplemented with 10% FBS and 1% P/S. For comparative loading of BMDMs and VSMCs with mβ-CD-cholesterol, cells were treated for 48 hours with 35µg/mL of mβ-CD-cholesterol complexes to induce cytosolic LD accumulation in both cell types. For loading of VSMCs with agLDL, VSMCs were treated for 24 hours with 50µg/mL of agLDL diluted in conditioned media collected from agLDL-loaded BMDM foam cells.

Flow cytometry of primary cultured cells. Primary macrophages and VSMCs were loaded with either agLDL or mβ-CD-cholesterol complexes as described above. Macrophages were lifted using 5mM EDTA/HBSS for 20 minutes at 4°C and centrifuged at 200xg for 5 minutes. VSMCs were detached using 0.5% trypsin/EDTA for 1 minute. Cells were resuspended in warm media with or without 200nM LysoTracker for 30 minutes

at 37°C on a rotator. Cells were centrifuged at 500xg for 5 minutes and resuspended in staining buffer (1mM EDTA, 2% FBS in HBSS) before acquisition using a BD FACS Aria IIIu (Becton Dickinson). All flow cytometry analysis was performed using FlowJo (Becton Dickinson). Positive staining was confirmed by use of unstained or non-transgenic (ie. no GFP) controls.

Fluorescence microscopy. All antibody concentrations can be found in the Major Resources Table. Macrophages and VSMCs were plated onto #1.5 coverslips or 18-well μ -slide (Ibidi) and treated as indicated. Cells were washed in PBS and fixed for 10 minutes at 37°C with 4% PFA and quenched with five washes with PBS. Cells were blocked in 5% BSA in PBS or blocked and permeabilized in 5% BSA with 0.1% Triton X-100. Primary antibody incubation was done at 4°C overnight in either 1% BSA or 1% BSA with 0.05% saponin. Coverslips were washed three times with 1% BSA supplemented or without saponin and incubated with the appropriate secondary fluorescent antibody for 1 hour. Lipids were stained using 10 μ g/mL of BODIPY for 20 minutes at room temperature. Actin was stained using 1U per coverslip of phalloidin conjugated to Alexa Fluor 647 for 20 minutes at room temperature. Nuclei were stained using DAPI (Invitrogen) for 5 minutes at room temperature. Cells were imaged using the Zeiss LSM 880 with or without the Airyscan module at 20x (NA 0.8). For immunofluorescence of mESC derived macrophages and VSMCs, cells were plated onto 24 well plates coated with 0.1% gelatin and treated as described and the cells were imaged using the Cytation 5 Cell Imaging Multi-Mode Reader (BioTek) with the 20x objective (Olympus, 0.45). Aortic roots were fixed for 10 minutes in 4% PFA and washed three times in PBS. The tissues were blocked and permeabilized in 10% horse serum with 0.1% Triton X-100 for 30 minutes at room

temperature. Primary antibodies were added in 1% horse serum and were incubated on the aortic roots overnight at 4°C. Slides were washed three times in PBS and incubated with the appropriate secondary antibodies for 1 hour at room temperature. Simultaneously, lipids were stained using Monodansylpentane 1:500 (MDH) (Abcepta). Nuclei were stained using DRAQ5 (1:1000, Biolegend) for 20 minutes at room temperature. Slides were mounted using #1.5 coverslip and imaged using the Zeiss LSM 880 at 20x (NA 0.8). Positive staining was confirmed by staining of only secondary antibody or unstained controls. Representative images were selected as those best reflecting the average for each of the treatment groups as determined in consensus by multiple authors.

Western blot. For autophagy flux analyses, cells were treated with 30μM chloroquine or 10nM of bafilomycin for 2 hours prior harvest. Cells were lysed in 2X Laemmli Sample Buffer (Bio-Rad, 1610737) containing β-mercaptoethanol and boiled at 95°C for 5 minutes. For ABCA1, cells were harvested in RIPA buffer (Thermo Scientific) and incubated at 37°C for 20 minutes. Samples were run on 8-16% Criterion TGX Stain Free Pre-cast Gels (BioRad) or a 12% TGX Stain Free gels and were UV activated for total protein quantification using the ChemiDoc XRS+ System (Bio-Rad), according to manufacturer's protocol. To assess autophagy-related components, the proteins were transferred onto 0.22μm PVDF Membranes (Bio-Rad) using the Trans-Blot Turbo Transfer System (Bio-Rad). For ABCA1 quantification, the proteins were transferred onto 0.45μm nitrocellulose membrane (Bio-Rad) at 125V for 4 hours. Immunoblotting for proteins as indicated was performed with primary antibodies overnight at 4°C followed by horseradish peroxidase conjugated secondary antibodies for 1 hour at room temperature.

All antibody information can be found in the Major Resources Table. Proteins were developed using either Clarity (Bio-Rad) or Clarity Max (Bio-Rad) ECL Substrates and imaged on the ChemiDoc XRS+ system (Bio-Rad).

Atherosclerotic plaque immunofluorescence quantification. Microscopy images of atherosclerotic plaques were quantified by a blinded individual using Image J. Positive thresholds were first established manually for all markers and thereafter kept consistent throughout the analysis. Each lesion was outlined while excluding regions of acellularity, using the nuclear stain (DRAQ5) as a reference. This therefore excluded acellular areas that may retain MDH or other markers, while not representing the autophagic capacities of leukocyte/non-leukocyte foam cells (extracellular matrix, lipid deposits, necrotic core). From this outlined region, CD45+ (leukocytes) and CD45- (non-leukocytes) areas were determined. The expression of LC3, p62, pATG16L1, ABCA1 and ABCG1 was assessed in CD45+ and CD45- regions, yielding an area for the marker of interest within the region of interest. These results were normalized to the ratio of nucleus staining from the specific region, as a mean to account for variations in cellularity and ensure that the observed differences are not due to differences in the number of cells between regions (see schematic in **Online Figure II**).

Atherosclerotic Plaque Bioinformatic Analysis. Single cell RNA sequencing analysis was performed on previously published data of the atherosclerotic plaque²⁷, accessed from the Gene Expression Omnibus (GEO) database under the following accession number GSE131780. Single cell analysis was performed using Seurat v3.1.5. Cells with mitochondrial reads greater than 6% and less than 100 genes were excluded from the analyses. Dimensionality reduction was performed using the tSNE algorithm with the top

15 principle components. TSNE data was clustered using the top 15 principle components and the Louvain algorithm to find the nearest neighbour clusters based with a resolution of 0.2. Clusters were identified using genes as per *Wirka et al.*²⁷ Genes of interest were interrogated in combined wildtype smooth muscle (tdTomato⁺) and non-smooth muscle (tdTomato⁻) samples over a time course of atherogenic diet feeding, with comparative analyses between identified macrophage and modulated smooth muscle cell clusters.

Differentiation of vascular smooth muscle cells from mouse embryonic stem cells.

Embryoid bodies (EB) were generated by cultivating mESCs via hanging drops on bacterial petri dishes in vascular smooth muscle cell differentiation media comprised of DMEM containing 20% FBS, 1x P/S, 1x NEAA, 1x L-glutamine, 46µM 2-mercaptoethanol for 2 days. Hanging drops containing the EB were collected and grown for an additional 5 days in a bacteriological petri dish. EBs were collected and transferred into assay dishes and differentiated for up to 14 days in vascular smooth muscle cells differentiation media supplemented with 10nM retinoic acid (ACROS Organics), 0.5mM dibutyryl-cAMP (Sigma).

Differentiation of macrophages from mouse embryonic stem cells. Embryoid bodies (EB) were generated by cultivating mESCs in bacteriological petri dishes in macrophage differentiation media comprised of DMEM containing 15% FBS, 20% L929-conditioned media, 1x P/S, 1x NEAA, 0.1mM 2-mercaptoethanol, 1ng/mL mouse recombinant IL-3 (R&D) for 7 days. Floating EBs were plated on gelatin-coated plates in macrophage differentiation media, as previously described⁷⁵. EBs were incubated for 7 days after which the macrophages progenitors in suspensions were harvested and filtered through

a 40µm cell strainer and plated on gelatin-coated plates in the macrophage differentiation media for assays.

RNA extraction and qPCR. RNA was isolated following manufactures' recommendation using the Direct-zol RNA Miniprep (Zymo). Three hundred and fifty nanograms of RNA was reverse transcribed using iScript™ Reverse Transcription Supermix for RT-qPCR (Bio-Rad). Gene expression was assessed by qPCR using the SsoAdvanced Universal SYBR® Green Supermix (Bio-Rad) on the CFX Connect Real-Time PCR Detection System (Bio Rad) using the primers sequences as indicated below. Expression of the genes of interest were normalized to the geometric mean of the housekeeping genes Srp14 and Rps4x. ABCG1 (Mm00437390_m1) gene expression was quantified using TaqMan probes (Applied Biosystems) according to the manufacturers protocol using TaqMan™ Fast Advanced Master Mix and normalized to the expression of the housekeeping gene HRPT (Mm00446968_m1).

mAtg5-For	AAAGATGTGCTTCGAGATGTGT
mAtg5-Rev	CACTTTGTCAGTTACCAACGTCA
mTfeb-For	GCAGCCACCTGAACGTGTA
mTfeb-Rev	TGTTAGCTCTCGCTTCTGAGT
mAbca1-For	GTGGGCTCCTCCCTGTTTTT
mAbca1-Rev	CTCTGAGAAACACTGTCCTCCTTT
mSrp14-For	GAGAGCGAGCAGTTCCTGAC
mSrp14-Rev	TGTTAGCTCTCGCTTCTGAGT
mRps4x-For	TGCTGGGTTTATGGATGTCA
mRps4x-Rev	CCTCCTCCGGTGTAATACGA

mActa2-For	GCTTCGCTGGTGATGATGCTC
mActa2-Rev	AGTTGGTGATGATGCCGTGTTC
mTpm1-For	AGCTCGACAAAGAGAACGCC
mTpm1-Rev	ATCTTCCAGCTGCTTGCTCC
mLgals3-For	AGGAGAGGGAATGATGTTGCC
mLgals3-Rev	GGTTTGCCACTCTCAAAGGG
mCD68-For	TGTCTGATCTTGCTAGGACCG
mCdD68-Rev	GAGAGTAACGGCCTTTTTGTGA
mCnn1-for	TCTGCACATTTTAACCGAGGTC
mCnn1-rev	GCCAGCTTGTTCTTTACTTCAGC

Cholesterol efflux. mESC-derived and primary macrophages were loaded with aggregated or acetylated LDL (50µg/mL, generated as previously⁷³) and 0.5µCi/mL ³H-cholesterol (Perkin-Elmer, NET139001MC) in DMEM media containing 10% FBS for 30 hours after which the media was replaced with 2mg/mL fatty acid-free BSA (Sigma) DMEM and cells were equilibrated overnight. For mESC derived VSMC, ³H-cholesterol was added to growth media containing mβ-CD-cholesterol complexes (80µg/mL) during cholesterol loading for 72 hours. Cholesterol efflux to human recombinant apoA-I (50µg/mL) or HDL (50µg/mL, generated as previously⁷³) was carried out for 24 hours in 2mg/mL BSA DMEM media, in the presence or absence of 30µM of chloroquine (Sigma) or 100µM of metformin (Tocris), following which the supernatants were removed and cells were lysed in 0.5M NaOH. Aliquots of supernatants and cell lysates were transferred onto 96-well Lumaplates (Perkin Elmer), dried overnight and radioactivity from the samples was quantified using the Hydex Sense plate reader (Gamble). Cholesterol efflux was

calculated as a percentage of ^3H -cholesterol in the supernatant/ $(^3\text{H}$ -cholesterol in the supernatant + ^3H -cholesterol in cells) $\times 100\%$.

Gene silencing. For gene knockdown using siRNA, primary VSMCs were plated at 80% confluency and allowed to adhere overnight. VSMCs were then transfected for 24 hours with 10nM ON-TARGET pool of Scramble siRNA or Atg5 siRNA (Dharmacon) using Lipofectamine RNAiMax Transfection Reagent (Invitrogen) in OPTI-MEM (Gibco). 24 hours post-transfection, an equal volume of DMEM media containing 20% FBS and 2% P/S was added, and cells were harvested for RNA isolation 48 hours after transfection.

6.2.2 Online Figures

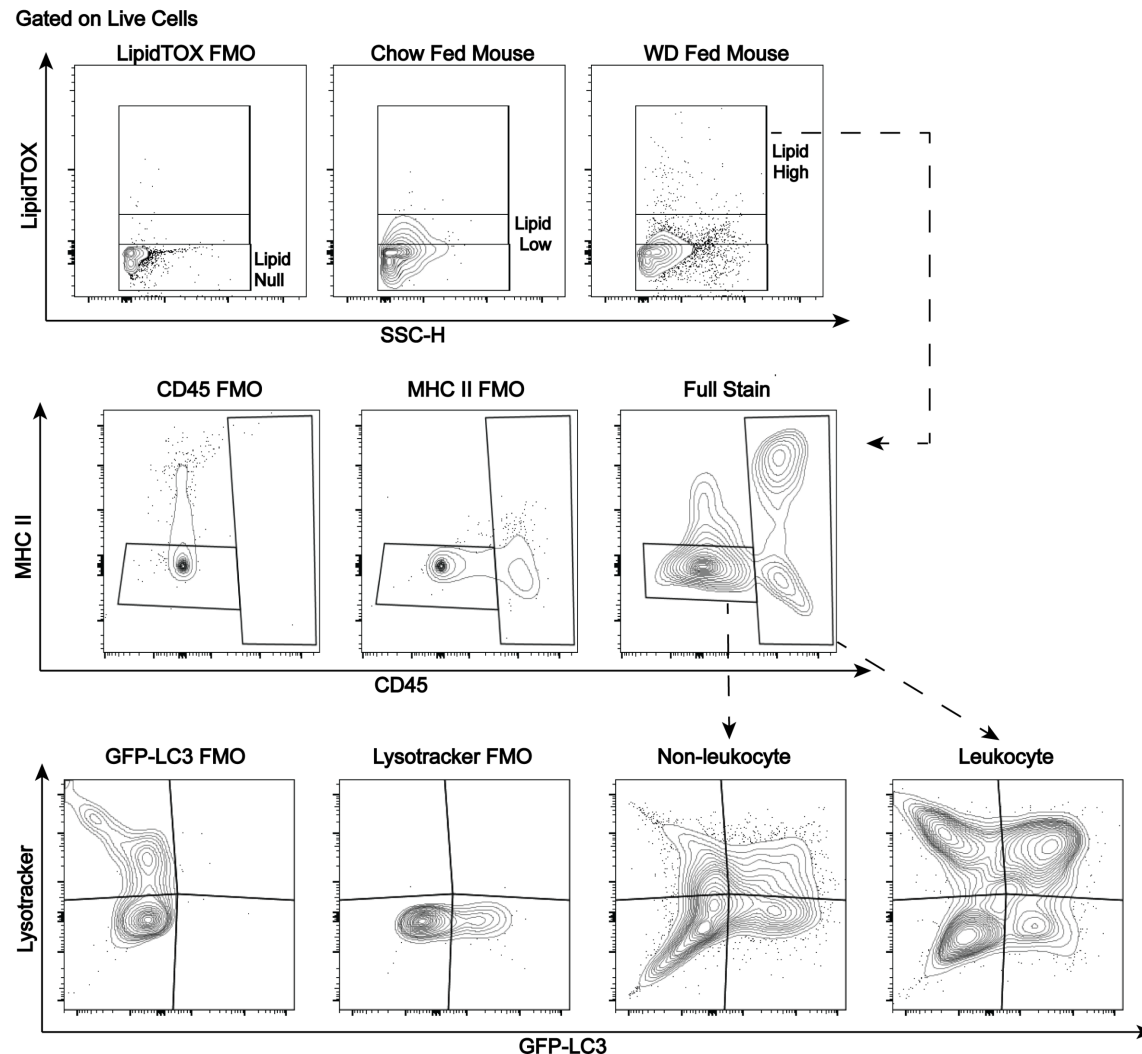


Figure 6-6: Gating Scheme for Leukocyte and Nonleukocyte Derived Foam Cells

Identification of foam cell subtypes by flow cytometry was performed as previously described¹⁶. Viable cells were stained with LipidTOX for identification of lipid content (*top Panel*). LipidTOX fluorescence minus one (FMO) was used to gate the negative population. Aortic digests from chow-fed mice were used to identify cells with a low lipid content, and aortic digests from Western diet fed mice for cells with a high lipid content. Leukocyte and nonleukocyte foam cells were identified from the lipid high population using MHC-II and CD45 (*mid panel*). Autophagic flux was then determined within each of these foam cells subtypes using GFP-LC3 and LysoTracker staining (*bottom panel*).

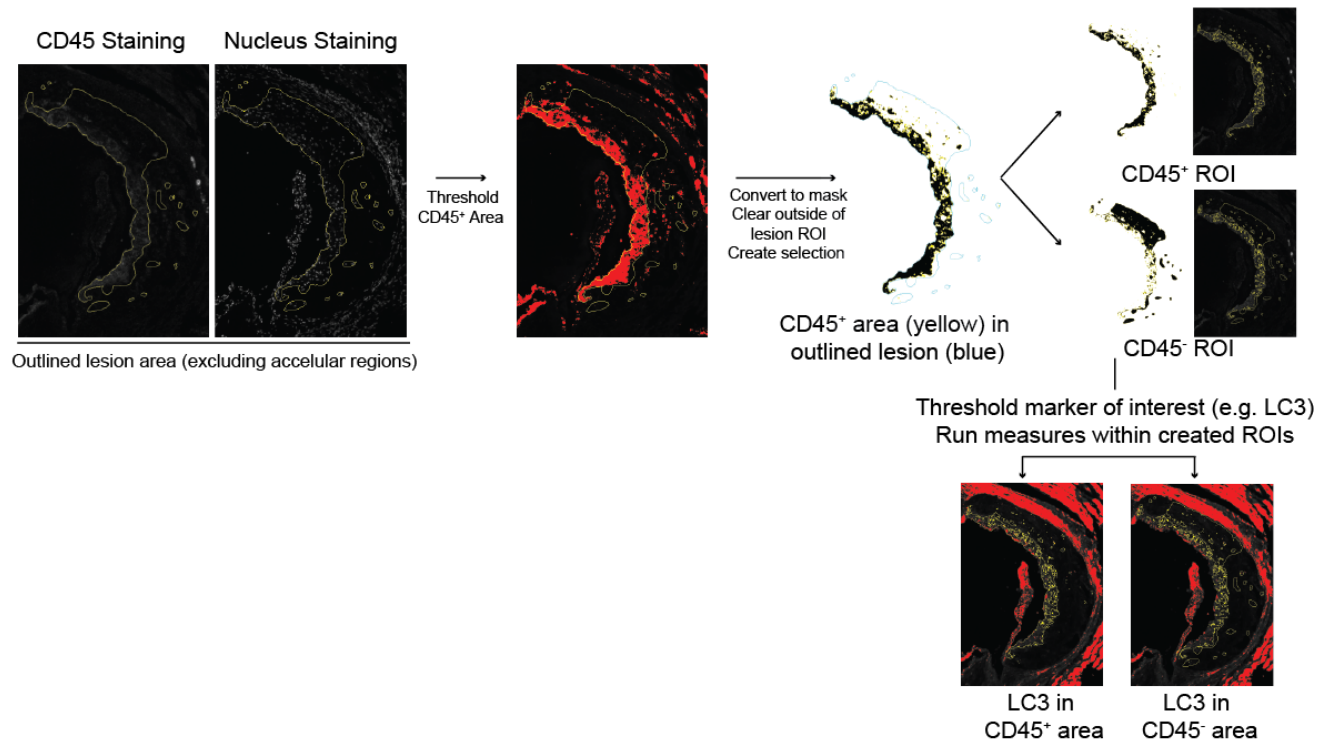


Figure 6-7. Pipeline for Quantification of Autophagy Markers in Leukocyte and Nonleukocyte Foam Cells by Immunofluorescence Staining

Cellular regions of the plaque were manually drawn (*top left*) and a threshold and mask was applied for CD45 staining to distinguish the leukocyte and nonleukocyte foam cells (*top right*). Within each of these foam cell subtypes, a threshold was applied for the autophagic or cholesterol efflux transporter marker of interest (*bottom right*).

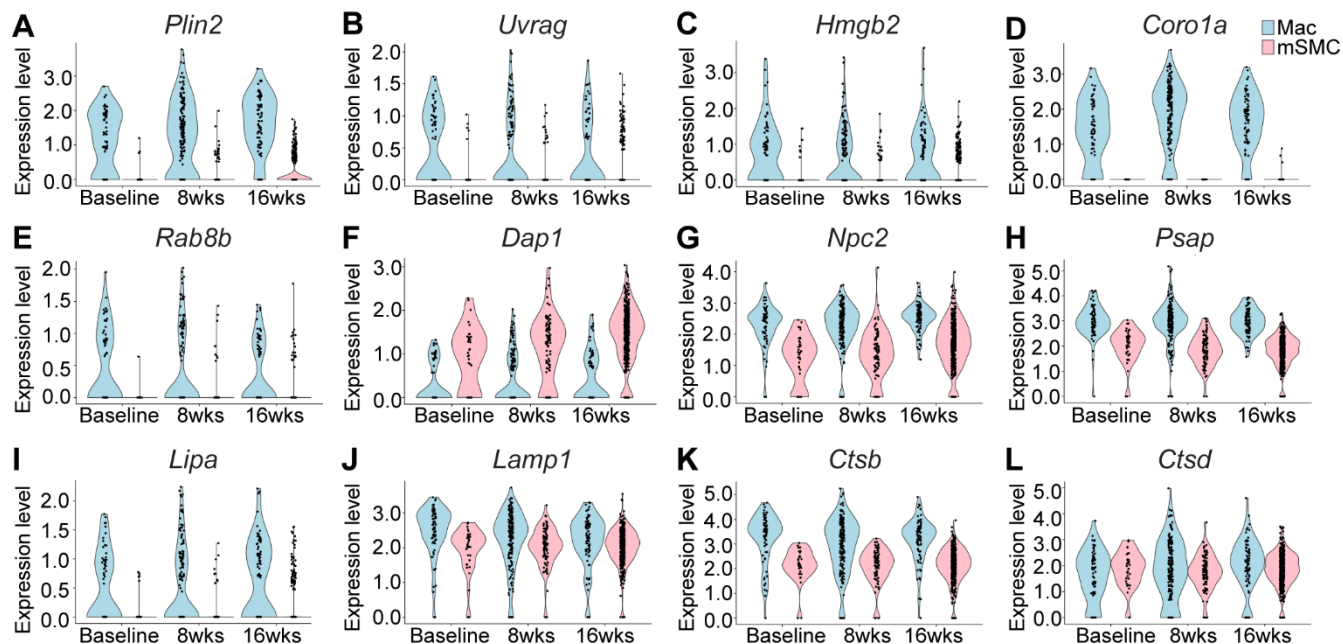


Figure 6-8. Transcriptomic analysis of autophagy markers in the atherosclerotic plaque

A-L, Expression of autophagy and lysosomal genes in macrophages and modulated smooth muscle cell (mSMC) clusters from single cell RNA sequencing of the mouse aortic root of *Apoe*^{-/-} mice at baseline and after 8 and 16 weeks of atherogenic diet feeding. Data was acquired from Wirka et al.²⁷

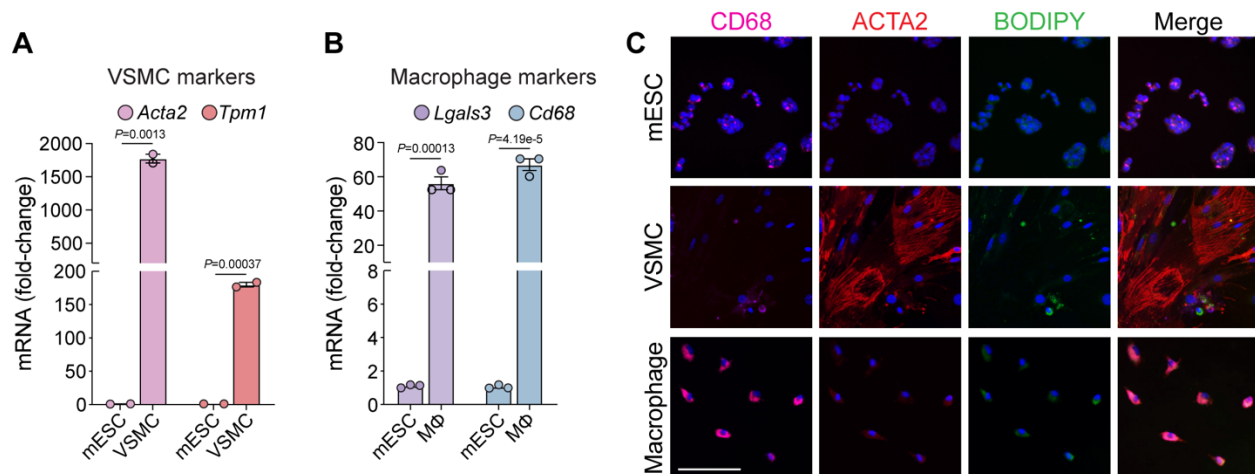


Figure 6-9. Embryonic-Derived VSMC and Macrophage Express Associated Lineage Markers.

Online Figure IV. Embryonic-Derived VSMC and Macrophage Express Associated Lineage Markers. **A-B**, Gene expression was determined for (A) VSMCs (n=2 technical replicates) and (B) macrophages (Mφ, n=3 technical replicates) derived from embryonic stem cells using qPCR analysis. An unpaired t-test was used to determine statistical significance. **C**, ESC-derived VSMC and macrophages were stained for macrophage, VSMC and lipid markers. Images are representative of 2 independent experiments. Scale bar=100μm.

6.2.3 Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse	Charles River	C57Bl/6N	M/F	https://www.criver.com/products-services/find-model/c57bl6-mouse?region=3611

Genetically Modified Animals

Parents	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	Mouse	Riken	C57BL/6JJcl	GFP-LC3B	https://knowledge.brc.riken.jp/resource/animal/card?__lang__=en&brc_no=RBRC00806
Parent Female	Mouse	Riken	C57BL/6JJcl	GFP-LC3B	https://knowledge.brc.riken.jp/resource/animal/card?__lang__=en&brc_no=RBRC00806

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
Purified Rat Anti-Mouse CD16/CD32	BD Bioscience	553141	1:50		https://www.bdbiosciences.com/ca/applications/research/b-cell-research/surface-markers/mouse/purified-rat-anti-mouse-cd16cd32-mouse-bd-fc-block-24g2/p/553141

CD45 APC/Fire 750	Biolegend	147714	1:200		https://www.biolegend.com/en-us/products/apc-fire-750-anti-mouse-cd45-antibody-16472
I-A/I-E BV785	Biolegend	107645	1:200		https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-mouse-i-a-i-e-antibody-12087
CD45 BV605	Biolegend	103139	1:50		https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd45-antibody-8721
CD68	Bio-Rad	MCA1957	1:500		https://www.bio-rad-antibodies.com/mo-noclonal/mouse-cd68-antibody-fa-11-mca1957.html?f=purified
ACTA2	Sigma	A2547	1:400		https://www.sigmaaldrich.com/catalog/product/sigma/a2547?lang=en&region=CA&gclid=Cj0KCQiA7YyCBhD_ARIsALkj54pYw5bWqgeNls5RQjzYZZE_VYtpD_d7je8d4-IUZaiid48en7r7r5LEaApE_EALw_wcB
PLIN2	Fitzgerald	20R-AP002	1:200		https://www.fitzgerald-fii.com/adipophilin-antibody-20r-ap002.html
LC3	MBL International	PM036	1:750		https://www.mblintl.com/products/pm036/
P62	Abnova	H00008878-M01	1:500; 1:2000		https://www.novusbio.com/products/p62-sgstm1-antibody-

					2c11_h00008878-m01
p-Atg16L1	abcam	ab195242	1:200; 1:1000		https://www.abcam.com/atg16l1-phospho-s278-antibody-epr19016-ab195242.html
Atg16l1	Abcam	ab187671	1:1000		https://www.abcam.com/atg16l1-antibody-epr15638-n-terminal-ab187671.html
LC3	MBL International	PD014	1:1000		https://www.mblintl.com/products/pd014/
P62	Abcam	ab109012	1:200		https://www.abcam.com/sqstm1--p62-antibody-epr4844-autophagosome-marker-ab109012.html
ABCA1	Novus	NB400-105	1:100; 1:500		https://www.novusbio.com/products/abca1-antibody_nb400-105
ABCG1	Novus	NB400-132	1:100; 1:500		https://www.novusbio.com/products/abcg1-antibody_nb400-132
Peroxidase-AffiniPure Goat Anti-Rabbit IgG	Jackson Immuno Research Laboratories	111-035-144	1:5000;1:10000		https://www.jacksonimmuno.com/catalog/products/111-035-144
Peroxidase-AffiniPure Goat Anti-Mouse IgG (H+L)	Jackson Immuno Research Laboratories	115-035-146	1:10000		https://www.jacksonimmuno.com/catalog/products/115-035-146
Goat anti-Rabbit IgG (H+L) Highly Cross-	Invitrogen	A32732	1:500-1:1000		https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-

Adsorbed Secondary Antibody, Alexa Fluor Plus 555					Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32732
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 647	Invitrogen	A32795	1:1000		https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32795
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	Invitrogen	A-31571	1:1000		https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31571
Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Cyanine5	Invitrogen	A10525	1:1000		https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A10525
Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	Invitrogen	A-21434	1:1000		https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21434
Goat anti-Guinea Pig IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	Invitrogen	A-21435	1:1000		https://www.thermofisher.com/antibody/product/Goat-anti-Guinea-Pig-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21435

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
ES-E14TG2a	uOttawa Stem Cell Core	Unknown	
Vascular Smooth Muscle Cells	Mouse	M and F	
Bone-Marrow Derived Macrophages	Mouse	M an F	

6.3 Supplementary Materials Manuscript #3

6.3.1 Supplementary Data

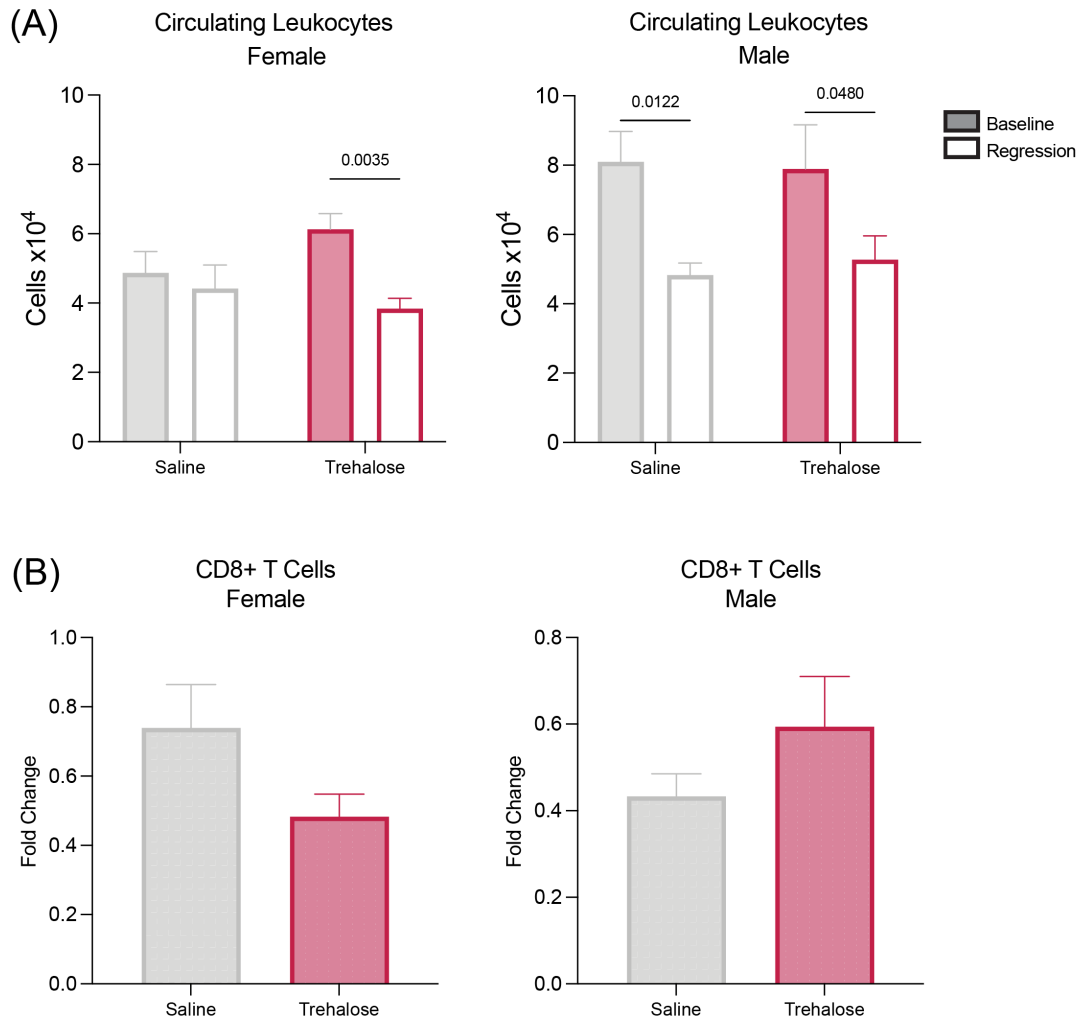


Figure 6-10: Circulating Leukocytes

(A) Circulating leukocytes numbers taken at baseline (16 weeks of diet) and 5 weeks of chow diet feeding. (B) Fold change of the total circulating CD8+ T cells following five weeks of chow diet and treatment over the baseline total cells. Statistical significance was assessed with an unpaired t-test when comparing two groups or two-way ANOVA when comparing more than two groups.

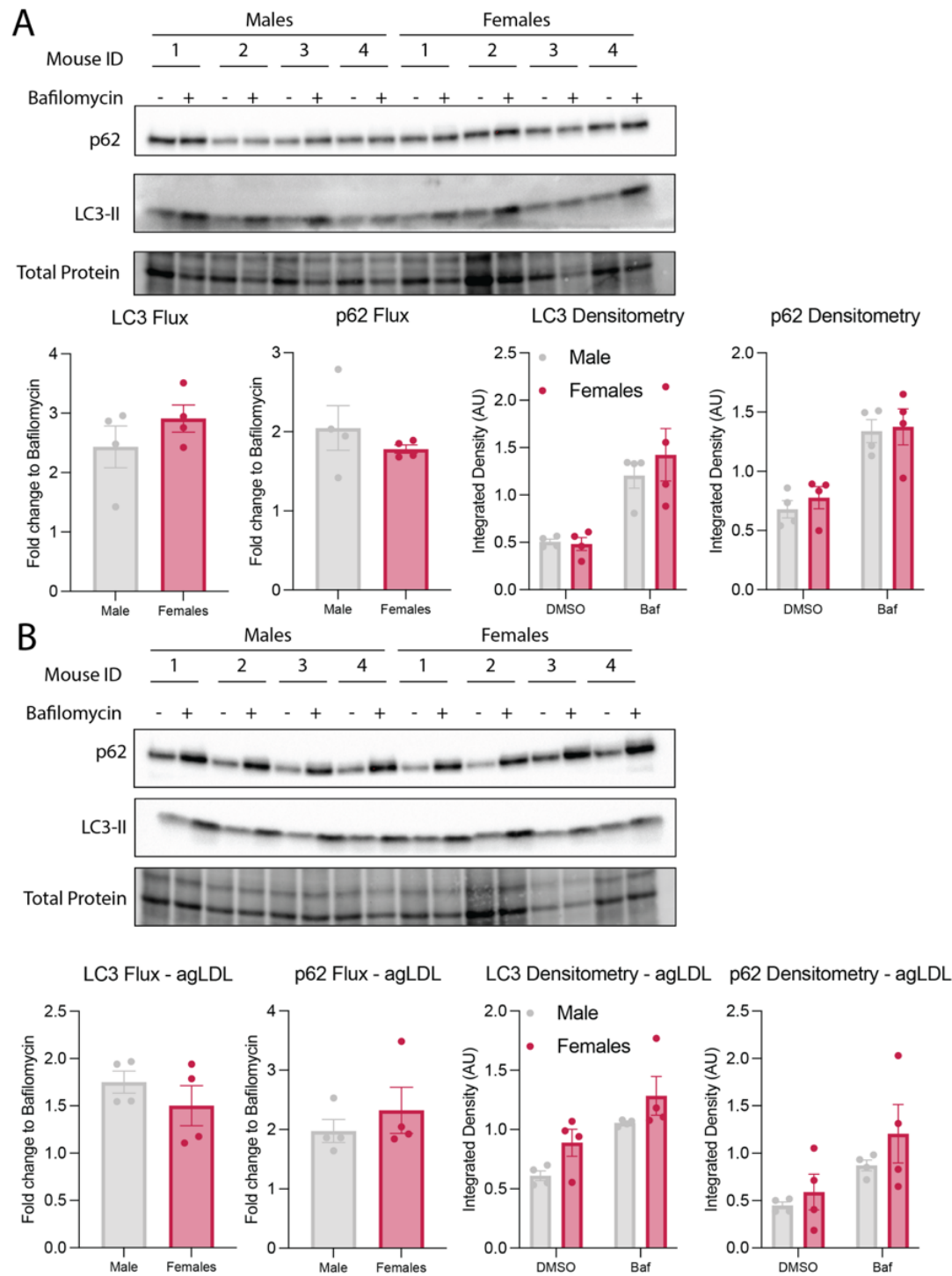


Figure 6-11: Autophagy Flux is Equivalent in Mouse Macrophages Isolated From Males as Compared to Female Mice. (A) Immunoblotting of peritoneal macrophages isolated from male or female mice and cultured for 2h in the presence or absence of Bafilomycin (n=4). (B) Immunoblotting of male or female mouse peritoneal macrophages lipid-loaded with 50 μ g/mL of aggregated LDLs for 24h before treatment with or without Bafilomycin (n=4).

6.3.2 Supplementary Table

Table 6.4: List of Antibodies

Target Antigen	Catalog #	Supplier
Ly6C-BV610	563011	BD Bioscience
CD8A-BV510	563068	BD Bioscience
CD11b-BV421	562605	BD Bioscience
CD4-PerCP-Cy5.5	561115	BD Bioscience
CD45-APC-Cy7	561037	BD Bioscience
CD3e-APC	553066	BD Bioscience
Purified Rat Anti-Mouse CD16/CD32	553141	BD Bioscience
CD45-APC-Fire 750	147714	Biolegend
I-A/I-E-BF785	107645	Biolegend
LC3	PM036	MBL International
CD45-AF647	103124	Biolegend
CD68-AF750	NBP2-33337AF750	Novus
Il-1β	ab254360	Abcam
CD38	ab61400	Abcam
Goat anti-rabbit Alexa Fluor 555+	A32732	Invitrogen
Donkey anti-rabbit Alexa Fluor 555+	A32794	Invitrogen
Donkey anti-rat Alexa Fluor 488+	A48269	Invitrogen
Alexa Fluor® 647 Rat IgG2a, κ Isotype Ctrl Antibody	400526	Biolegend
Rat IgG2a Isotype Control (54447) [Alexa Fluor® 750]	IC006S	Novus

7 Copyrights

Manuscript #1

Type of use: Academic

Article title: Identification of novel lipid droplet factors that regulate lipophagy and cholesterol efflux in macrophage foam cells

Article DOI: 10.1080/15548627.2021.1886839

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Autophagy Is Differentially Regulated in Leukocyte and Nonleukocyte Foam Cells During Atherosclerosis

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BACK

CLOSE WINDOW

Education

- 2018-present Ph.D. in Biochemistry**
University of Ottawa, Ottawa, Canada
Supervisor: Dr. Mireille Ouimet
Transferred from Master's Degree in April 2020
- 2013-2018 B.Sc. with Honours in Translational and Molecular Medicine**
University of Ottawa, Ottawa, Canada
cGPA: 9.5 out of 10.0

Funding

- 2021-2024 Vanier Canada Graduate Scholarship - CIHR**
University of Ottawa, Ottawa, Canada
\$150 000 CAD over 3 years
- 2021-2024 Frederick Banting and Charles Best Canada Graduate Scholarships - Doctoral Award**
University of Ottawa Heart Institute, Ottawa, Canada
\$105 000 CAD over 3 years
*Declined due to Vanier Scholarship acceptance
- 2019-2024 Excellence Scholarship**
Faculty of Medicine, University of Ottawa, Ottawa, Canada
\$10 000 CAD per year
- 2020 CIHR Canada Graduate Scholarship - Master's**
University of Ottawa, Ottawa, Canada
\$17 500 CAD
- 2019-2020 Ontario Graduate Scholarship**
University of Ottawa, Ottawa, Canada
\$15 000 CAD
- 2018 Admission Scholarship**
Faculty of Medicine, University of Ottawa, Ottawa, Canada
\$7 500 CAD
- 2018 Undergraduate Student Research Award Program - NSERC**
University of Ottawa, Ottawa, Canada
\$7 000 CAD
- 2017 Work in Biomedical Research Program - Translational and Molecular Medicine**
Faculty of Medicine, University of Ottawa, Ottawa, Canada
\$6 500 CAD

Honours and Awards

- 2023 Ottawa Region Cardiovascular Trainee of the Year**
University of Ottawa Heart Institute, Ottawa, Canada
\$1 000 CAD

2023 1st place, Biochemistry PhD Student Category Poster Presentation Award
BMI Seminar Day, Faculty of Medicine, University of Ottawa, Ottawa, Canada
 \$250 CAD

2023 Travel Award
University of Ottawa Heart Institute, Ottawa, Canada
 \$1 000 CAD

2022 Finalist, Emerging Scientist Award for Women
Vascular Discovery 2022, Seattle, USA
 \$500 USD

2022 Syed Sattar PhD Student Award
BMI Awards, Faculty of Medicine, University of Ottawa, Ottawa, Canada
 \$500 CAD

2022 2nd place, Biochemistry PhD Student Category Presentation Award
BMI Seminar Day, Faculty of Medicine, University of Ottawa, Ottawa, Canada
 \$100 CAD

2022 Travel Award
University of Ottawa Heart Institute, Ottawa, Canada
 \$1 000 CAD

2022 Young Investigator Travel Subsidy Award
Canadian Society of Atherosclerosis, Thrombosis and Vascular Biology, Montréal, Canada
 \$450 CAD

2021 5th place, Biochemistry PhD Student Category Presentation Award
BMI Seminar Day, Faculty of Medicine, University of Ottawa, Ottawa, Canada
 \$100 CAD

2019 2nd place, BMI Image Contest
Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Canada
 \$100 CAD

2019 Travel Award
University of Ottawa Heart Institute, Ottawa, Canada
 \$1 000 CAD

2018 Best Science Picture Award
Research Celebration, University of Ottawa Heart Institute, Ottawa, Canada

2018 Travel Award - CIHR Institute of Circulatory and Respiratory Health
Canadian Institute of Health Research, Ottawa, Canada
 \$1 000 CAD

2017 Excellence Award in Translational and Molecular Medicine
Awards in Education, Faculty of Medicine, University of Ottawa, Ottawa, Canada
 \$250 CAD

Publications

First Author

- 1 **Robichaud, S***, Rasheed, A*, Pietrangelo, A., Doyoung Kim, A., Boucher, D. M., Emerton, C., Vijithakumar, V., Gharibeh, L., Fairman, G., Mak, E., Nguyen, M. A., Geoffrion, M., Wirka, R., Rayner, K. J., & Ouimet, M. (2022). Autophagy Is Differentially Regulated in Leukocyte and Nonleukocyte Foam Cells During Atherosclerosis. *Circ Res*, 130(6), 831-847. <https://doi.org/10.1161/CIRCRESAHA.121.320047>
*Authors contributed equally
 - Selected as a Featured Article
 - Editorial by Francis, G. & Razani, B. *CircRes* (2022) <https://doi.org/10.1161/CIRCRESAHA.122.320857>
 - Selected for the Circulation Research panel discussion at AHA Scientific Session 2022
 - Featured in the Discover CircRes Podcast
 - Recipient of the Robert Roberts Award for Research Excellence at the University of Ottawa Heart Institute
- 2 **Robichaud, S.**, Fairman, G., Vijithakumar, V., Mak, E., Cook, D. P., Pelletier, A. R., Huard, S., Vanderhyden, B. C., Figeys, D., Lavalley-Adam, M., Baetz, K., & Ouimet, M. (2021). Identification of novel lipid droplet factors that regulate lipophagy and cholesterol efflux in macrophage foam cells. *Autophagy*, 17(11), 3671-3689. <https://doi.org/10.1080/15548627.2021.1886839>
- 3 Fairman, G*, **Robichaud, S***, & Ouimet, M. (2020). Metabolic Regulators of Vascular Inflammation. *Arterioscler Thromb Vasc Biol*, 40(2), e22-e30. <https://doi.org/10.1161/ATVBAHA.119.312582>
*Authors contributed equally
- 4 **Robichaud, S.**, & Ouimet, M. (2019). Quantifying Cellular Cholesterol Efflux. *Methods Mol Biol*, 1951, 111-133. https://doi.org/10.1007/978-1-4939-9130-3_9
- 5 **Robichaud, S.**, Rochon, V., Emerton, C., Ouimet, M. Trehalose Promotes Atherosclerosis Regression in Female Mice (Submitted)

Contributing author

- 1 Nguyen, M. A., Hoang, H. D., Rasheed, A., Duchez, A. C., Wyatt, H., Cottee, M. L., Graber, T. E., Susser, L., **Robichaud, S.**, Berber, I., Geoffrion, M., Ouimet, M., Kazan, H., Maegdefessel, L., Mulvihill, E. E., Alain, T., & Rayner, K. J. (2022). miR-223 Exerts Translational Control of Proatherogenic Genes in Macrophages. *Circ Res*, 131(1), 42-58. <https://doi.org/10.1161/CIRCRESAHA.121.319120>
- 2 Yildirim, Z., Baboo, S., Hamid, S. M., Dogan, A. E., Tufanli, O., **Robichaud, S.**, Emerton, C., Diedrich, J. K., Vatandaslar, H., Nikolos, F., Gu, Y., Iwawaki, T., Tarling, E., Ouimet, M., Nelson, D. L., Yates, J. R., 3rd, Walter, P., & Erbay, E. (2022). Intercepting IRE1 kinase-FMRP signaling prevents atherosclerosis progression. *EMBO Mol Med*, 14(4), e15344. <https://doi.org/10.15252/emmm.202115344>
- 3 Jung, R. G., Duchez, A., Simard, T., Dhaliwal, S., Gillmore, T., Di Santo, P., Labinaz, A., Ramirez, D., Rasheed, A., **Robichaud, S.**, Ouimet, M., Short, S., Clifford, C., Xiao, F., Lordkipanidzé, M., Burger, D., Gadde, G., Rayner, K. J., & Hibbert, B. (2022). Plasminogen activator inhibitor-1 platelet-derived extracellular vesicles predicts major adverse cardiac events and promotes the pro-inflammatory smooth muscle cell phenotype *JACC Basic Transl Sci*, 7(10), 985-997. <https://doi.org/10.1016/j.jacbts.2022.05.002>
- 4 LeBlond, N. D., Nunes, J. R. C., Smith, T. K. T., O'Dwyer, C., **Robichaud, S.**, Gadde, S., Cote, M., Kemp, B. E., Ouimet, M., & Fullerton, M. D. (2020). Foam Cell Induction Activates AMPK But Uncouples Its Regulation of Autophagy and Lysosomal Homeostasis. *Int J Mol Sci*, 21(23). <https://doi.org/10.3390/ijms21239033>
- 5 Rasheed, A., **Robichaud, S.**, Nguyen, M. A., Geoffrion, M., Wyatt, H., Cottee, M. L., Dennison, T., Pietrangelo, A., Lee, R., Lagace, T. A., Ouimet, M., & Rayner, K. J. (2020). Loss of MLKL (Mixed Lineage Kinase Domain-Like Protein) Decreases Necrotic Core but Increases Macrophage Lipid Accumulation

in Atherosclerosis. *Arterioscler Thromb Vasc Biol*, 40(5), 1155-1167.
<https://doi.org/10.1161/ATVBAHA.119.313640>

- 6 Boucher, D. M., **Robichaud, S.**, Suliman, I., Rasheed, A., Susser, L., Emerton, C., Geoffrion, M., De Jong, E., Bowdish, D., Rayner, K. J., & Ouimet, M. Age-dependent Immune Cell Autophagy Dysfunction Impairs Atherosclerosis Regression. (In revision)
- 8 Rasheed, A., **Robichaud, S.**, Dennison, T., Wyatt, H., Nguyen, M. A., Geoffrion, M., Marouf, Y., Baxi, A., Lee, R., Kazan, H., Solingen, C., Ouimet, M., & Rayner, K. J. Hyperlipidemia-induced Hematopoiesis is Repressed by MLKL in Endothelial Cells of the Splenic Niche. (Submitted)
- 7 Smyth, D., Al-Khalaf, M., Zhang, L., **Robichaud, S.**, Seymour, R., Geoffrion, M., Jung, R. G., Parlow, S., Du, F., McNeil, B., Du, Q., Beauregard, C., Zhao, X., Beug, S., LaCasse, E., Rayner, K. J., Mak, T., Hibbert, B., Korneluk, R., & Liu, P. cIAP2 is a Critical Driver of Inflammation and Heart Failure Following Myocardial Infarction. (In revision)
- 9 Vijithakumar, V., Kastongo, A., Abd Elrahman, K., Prabhune, R., Gharibeh, L., Emerton, C., Robillard, I., **Robichaud, S.**, Asif, S., Gibbings, D., Moore, K., Ruiz, M., Ferguson, S., Lagace, D., & Ouimet, M. ORP6 regulates astrocytic cholesterol metabolism and brain A β deposition (Submitted)

Conference Presentations

National and International Conferences

Podium Presentations

- 1 **Robichaud, S.**, Rasheed, A., Pietrangelo, A., Kim, A., Boucher, D., Emerton, C., Vijithakumar, V., Gharibeh, L., Fairman, G., Mak, E., Nguyen, M., Geoffrion, M., Wirka, R., Rayner, K.J., Ouimet, M. *Autophagy is Differentially Regulated in Leukocyte and Nonleukocyte Foam Cells during Atherosclerosis*. Vascular Discovery 2022. Seattle, WA, USA. May 12th-14th, 2022
- 2 **Robichaud, S.**, Fairman, G., Vijithakumar, V., Mak, E., Cook, D., Pelletier, A., Huard, S., Vanderhyden, B., Figeys, D., Lavallée-Adam, M., Baetz, K., Ouimet, M. *Identification of Novel Lipid Droplet Cargo Receptors That Regulate Autophagy and Cholesterol Efflux in Macrophage Foam Cell*. European Atherosclerosis Society Congress. Online. May 30th - June 2nd, 2021.
- 3 **Robichaud, S.**, Fairman, G., Vijithakumar, V., Mak, E., Cook, D., Pelletier, A., Huard, S., Vanderhyden, B., Figeys, D., Lavallée-Adam, M., Baetz, K., Ouimet, M. *Identification of Novel Lipid Droplet Cargo Receptors That Regulate Autophagy and Cholesterol Efflux in Macrophage Foam Cell*. Vascular Discovery 2020. Chicago, IL, USA. May 5th -7th, 2020. *Could not present due to COVID-19
- 4 **Robichaud, S.**, Mak, E., Pelletier, A., Cook, D., Vanderhyden, B., Figeys, D., Lavallée-Adam, M., Ouimet, M. *Eating Up the Plaque: Defining the Mechanisms of Foam Cell Lipid Droplet Catabolism*. Canadian Vascular and Lipid Summit. Banff, AB. October 3rd-6th, 2019.

Poster Presentations

- 1 **Robichaud, S.**, Rochon, V., Emerton, C., Ouimet, M. *Autophagy Activation Promotes Atherosclerosis Regression in Female Mice*. Gordon Research Conferences on Atherosclerosis, Barcelona, Spain, June 18th - 23rd 2023.
- 2 **Robichaud, S.**, Rochon, V., Emerton, C., Ouimet, M. *Autophagy Activation Promotes Atherosclerosis Regression in Female Mice*. Gordon Research Seminars on Atherosclerosis, Barcelona, Spain, June 17th 2023.
- 3 **Robichaud, S.**, Rochon, V., Emerton, C., Ouimet, M. *Autophagy Activation Promotes Atherosclerosis Regression in Female Mice*. Vascular Discovery 2023, Boston, MA, USA, May 10th -13th 2023.
- 4 **Robichaud, S.**, Rochon, V., Emerton, C., Ouimet, M. *Autophagy Activation Promotes Atherosclerosis Regression in Female Mice*. Canadian Vascular and Lipid Summit. Whistler, BC. October 13th-16th 2022.

- 5 **Robichaud, S.**, Rasheed, A., Pietrangelo, A., Kim, A., Boucher, D., Emerton, C., Vijithakumar, V., Gharibeh, L., Fairman, G., Mak, E., Nguyen, M., Geoffrion, M., Wirka, R., Rayner, K.J., Ouimet, M. *Autophagy is Differentially Regulated in Leukocyte and Nonleukocyte Foam Cells during Atherosclerosis*. Vascular Discovery 2022. Seattle, WA, USA. May 12th-14th, 2022
- 6 **Robichaud, S.**, Rasheed, A., Pietrangelo, A., Kim, A., Boucher, D., Emerton, C., Vijithakumar, V., Gharibeh, L., Fairman, G., Mak, E., Nguyen, M., Geoffrion, M., Wirka, R., Rayner, K.J., Ouimet, M. *Autophagy is Differentially Regulated in Leukocyte and Nonleukocyte Foam Cells during Atherosclerosis*. European Atherosclerosis Society Congress. Hybrid, Milan, Italy. May 22th -25th 2022.
- 7 **Robichaud, S.**, Rasheed, A., Pietrangelo, A., Kim, A., Emerton, C., Vijithakumar, V., Gharibeh, L., Mak, E., Nguyen, M., Geoffrion, M., Wirka, R., Rayner, K.J., Ouimet, M. *Autophagy is Impaired in Arterial Foam Cell Populations during Atherosclerosis*. Canadian Student Health Research Forum. Online. June 14th-18th 2021
- 8 **Robichaud, S.**, Fairman, G., Vijithakumar, V., Mak, E., Cook, D., Pelletier, A., Huard, S., Vanderhyden, B., Figeys, D., Lavallée-Adam, M., Baetz, K., Ouimet, M. *Identification of Novel Lipid Droplet Cargo Receptors That Regulate Autophagy and Cholesterol Efflux in Macrophage Foam Cell*. Canadian Vascular and Lipid Summit. Online. October 15th -16th, 2020.
- 9 **Robichaud, S.**, Fairman, G., Vijithakumar, V., Mak, E., Cook, D., Pelletier, A., Huard, S., Vanderhyden, B., Figeys, D., Lavallée-Adam, M., Baetz, K., Ouimet, M. *Identification of Novel Lipid Droplet Cargo Receptors That Regulate Autophagy and Cholesterol Efflux in Macrophage Foam Cell*. Canadian Student Health Research Forum. Online. August 24th -28th, 2020.
- 10 **Robichaud, S.**, Mak, E., Ouimet, M. *Lipophagy regulates macrophage foam cell metabolism and inflammation*. Gordon Research Conference on Atherosclerosis. Newry, ME. June 16th-21st, 2019.
- 11 **Robichaud, S.**, Mak, E., Ouimet, M. *Lipophagy regulates macrophage foam cell metabolism and inflammation*. Gordon Research Seminar on Atherosclerosis. Newry, ME. June 16th, 2019.
- 12 **Robichaud, S.**, Mak, E., Pelletier, A., Cook, D., Vanderhyden, B., Figeys, D., Lavallée-Adam, M., Ouimet, M. *Eating up the plaque: Investigating the Metabolism of Foam Cell Lipid Droplets*. International Ottawa Heart Conference. Ottawa, ON. April 13th, 2019.
- 13 **Robichaud, S.**, Mak, E., Pelletier, A., Figeys, D., Lavallée-Adam, M., Ouimet, M. *Eating up the plaque: Investigating the Metabolism of Foam Cell Lipid Droplets*. International Ottawa Heart Conference. Ottawa, ON. April 26th-27th, 2018.

Local Conferences

Podium Presentations

- 1 **Robichaud, S.**, Rochon, V., Emerton, C., Ouimet, M. *Autophagy Activation Promotes Atherosclerosis Regression in Female Mice*. Regenerative Medicine Day, University of Ottawa Heart Institute, March 23rd 2023.
- 2 **Robichaud, S.**, Rasheed, A., Pietrangelo, A., Kim, A., Boucher, D., Emerton, C., Vijithakumar, V., Gharibeh, L., Fairman, G., Mak, E., Nguyen, M., Geoffrion, M., Wirka, R., Rayner, K.J., Ouimet, M. *Autophagy is Differentially Regulated in Leukocyte and Nonleukocyte Foam Cells during Atherosclerosis*. uOttawa BMI Symposium. Montebello, QC,. May 30th-31st 2022
- 3 **Robichaud, S.**, Rasheed, A., Pietrangelo, A., Kim, A., Emerton, C., Vijithakumar, V., Gharibeh, L., Mak, E., Nguyen, M., Geoffrion, M., Wirka, R., Rayner, K.J., Ouimet, M. *Autophagy is Impaired in Arterial Foam Cell Populations during Atherosclerosis*. Ottawa Region Cardiovascular Day. Online. May 10th 2021.

Poster Presentations

- 1 **Robichaud, S.**, Rochon, V., Rasheed, A., Emerton, C., Ouimet, M. *Autophagy Activation Promotes Atherosclerosis Regression in Female Mice*. Ottawa Cardiovascular Research Day, University of Ottawa Heart Institute, Ottawa, ON, May 15th -16th 2023.
- 2 **Robichaud, S.**, Mak, E., Ouimet, M. *Lipophagy regulates macrophage foam cell metabolism and inflammation*. Faculty of Medicine Research Day. Ottawa, ON. September 25th, 2019.
- 3 **Robichaud, S.**, Mak, E., Pelletier, A., Cook, D., Vanderhyden, B., Figeys, D., Lavallée-Adam, M., Ouimet, M. *Eating up the plaque: Investigating the Metabolism of Foam Cell Lipid Droplets*. Ottawa Cardiovascular Research Day. University of Ottawa Heart Institute, Ottawa, ON. May 13th, 2019.
- 4 **Robichaud, S.**, Mak, E., Pelletier, A., Figeys, D., Lavallée-Adam, M., Ouimet, M. *Eating up the plaque: Investigating the Metabolism of Foam Cell Lipid Droplets*. uOttawa BMI Symposium, Montebello, QC. May 17th 2018.
- 5 **Robichaud, S.**, Mak, E., Pelletier, A., Figeys, D., Lavallée-Adam, M., Ouimet, M. *Eating up the plaque: Investigating the Metabolism of Foam Cell Lipid Droplets*. Ottawa Cardiovascular Research Day. University of Ottawa Heart Institute, Ottawa, ON. April 30th, 2018.

Departmental and Institutional Seminars

- 1 **Robichaud, S.**, Rasheed, A., Pietrangelo, A., Kim, A., Emerton, C., Vijithakumar, V., Gharibeh, L., Mak, E., Nguyen, M., Geoffrion, M., Wirka, R., Rayner, K.J., Ouimet, M. *Autophagy is Differentially Regulated in Leukocyte and Nonleukocyte Foam Cells during Atherosclerosis*. University of Ottawa Heart Institute Work-in-Progress Rounds September 18th 2021.
- 2 **Robichaud, S.**, Fairman, G., Vijithakumar, V., Mak, E., Cook, D., Pelletier, A., Huard, S., Vanderhyden, B., Figeys, D., Lavallée-Adam, M., Baetz, K., Ouimet, M. *Identification of Novel Lipid Droplet Cargo Receptors That Regulate Autophagy and Cholesterol Efflux in Macrophage Foam Cell*. Biochemistry, Microbiology and Immunology Work-in-Progress Rounds. November 23rd 2020.
- 3 **Robichaud, S.**, Fairman, G., Vijithakumar, V., Mak, E., Cook, D., Pelletier, A., Huard, S., Vanderhyden, B., Figeys, D., Lavallée-Adam, M., Baetz, K., Ouimet, M. *Identification of Novel Lipid Droplet Cargo Receptors That Regulate Autophagy and Cholesterol Efflux in Macrophage Foam Cell*. University of Ottawa Heart Institute Work-in-Progress Rounds. September 16th 2020.
- 4 **Robichaud, S.**, Mak, E., Pelletier, A., Cook, D., Vanderhyden, B., Figeys, D., Lavallée-Adam, M., Ouimet, M. *Eating up the plaque: Investigating the Metabolism of Foam Cell Lipid Droplets*. Biochemistry, Microbiology and Immunology Work-in-Progress Rounds. November 18th 2019.
- 5 **Robichaud, S.**, Mak, E., Pelletier, A., Cook, D., Vanderhyden, B., Figeys, D., Lavallée-Adam, M., Ouimet, M. *Eating up the plaque: Investigating the Metabolism of Foam Cell Lipid Droplets*. University of Ottawa Heart Institute Work-in-Progress Rounds. October 30th 2019.

Invited Presentations

- 2023** **Foam Cell Autophagy to Promote Lipid Metabolism in Atherosclerosis Regression**
Medical College of Georgia at Augusta University, Augusta, GA, USA

Leadership Positions

2018-2022	University of Ottawa Heart Institute Trainee Committee
2021-2022	<i>Chair</i>
2020-2021	<i>Co-Chair</i>
2018-2020	<i>Social Coordinator</i>
2021-2022	MetabTrainees
	<i>Organizer</i>
2019-2020	Graduate Student Experience Committee
	<i>Member</i>
2018-2019	Biochemistry, Microbiology and Immunology Graduate Student Association
	<i>University of Ottawa Heart Institute Representative</i>
2016-2018	Translational and Molecular Medicine Student Association
	<i>Vice President of Internal Affairs</i>

Community Outreach and Knowledge Translation

2023	Gordon Research Seminar on Atherosclerosis
	<i>Discussion Leader</i>
2023	Vascular Discovery 2023
	<i>Social Media Ambassador</i>
2019-2020; 2023	Canadian Medical Hall of Fame Discovery Day
	<i>Volunteer</i>
2022	Circulation Research: 2022 Highlights and Initiative Session, Scientific Session 2022
	<i>Invited Panelist</i>
2022	Discover CircRes Podcast, Episode 34
	<i>Featured Author</i>
2019-2021	University of Ottawa Heart Institute Research Services
	<i>Social Media Translator</i>
2018-2020	Let's Talk Science
	<i>Volunteer</i>
2019	Soapbox Science Ottawa
	<i>Volunteer</i>
2016-2018	Translational and Molecular Medicine
	<i>Program Ambassador</i>

Event Organization

2018-2022	Ottawa Cardiovascular Research Day Organizing Committee
	<i>Program Committee Member</i>
2021	MetabTrainees Symposium
	<i>MetabTrainee Organizer</i>
2018-2019	Faculty of Medicine Bio-Med Discovery Day
	<i>Program Committee Member</i>

Teaching Experience

Mar-Apr 2021 Instructor
TMM3009: 3rd Year Rotation Class - 6 students
Faculty of Medicine, University of Ottawa

Peer Review Experience

2023 Inflammation (1 article reviewed independently)
2022-2023 Scientific Reports (2 article reviewed independently)
2020-2022 Circulation Research (2 articles with PI)
2019-2022 Arteriosclerosis, Thrombosis and Vascular Biology (2 articles with PI)
2021 Journal of Physiology and Biochemistry (1 article with PI)
2021 Autophagy (1 article with PI)

Trainee Supervision

2022-2023	Master Student	Valérie Rochon
2021-2022	Honours Thesis	Valérie Rochon
2020-2021	Honours Thesis	Cameron Bosveld
2018-2019	Honours Thesis	Anne Kim
2018	Rotation Student	Shaza Asif

Previous Research Experience

2017-2018 Honours Student
University of Ottawa, Ottawa, Canada
Supervisor: Dr. Mireille Ouimet
Lipid Droplet Metabolism in Foam Cells During Atherosclerosis

2017 Translational and Molecular Medicine Rotation Student
University of Ottawa Heart Institute, Ottawa, Canada
Supervisor: Dr. Katey J. Rayner
Cell Death of Macrophages Following Exposure to Atherogenic Ligands

2017 Translational and Molecular Medicine Rotation Student
University of Ottawa, Ottawa, Canada
Supervisor: Dr. Martin Pelchat
The Effect of Hepatitis Delta Virus Infection on HEK293 cells