

Supplementary Information

Analysis of O-GlcNAc-modified proteins in murine CD8+ Effector and Memory T cells

Aime Lopez Aguilar, Yu Gao, Xiaomeng Hou, Gregoire Lauvau, John R. Yates, Peng Wu

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S1. Complete list and analysis of identified high confidence O-GlcNAcylated proteins

Ref = Identification Reference, Phos = Known phosphorylation, Eff = Effector, Mem = Memory, Det = Detected.

Protein	Gene	Group	Ref	Location	Phos	Family	Pathways
Plastin-2	Lcp1	eff	-	Cytoplasm	Y	other	-
40S ribosomal protein S23	Rps23	eff	6	Cytoplasm	Y	translation regulator	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
Cell division cycle and apoptosis regulator protein 1	Ccar1	mem	1,6	Nucleus	Y	transcription regulator	-
Cathepsin B	Ctsb	mem	6	Cytoplasm	Y	peptidase	autophagy; Inflammasome pathway; Phagosome Maturation
Hexokinase-1	Hk1	mem	-	Cytoplasm	-	kinase	GDP-glucose Biosynthesis; Glucose and Glucose-1-phosphate Degradation; Trehalose Degradation II (Trehalase); UDP-N-acetyl-D-galactosamine Biosynthesis II
E3 ubiquitin-protein ligase HUWE1	Huwe1	mem	2,5,6	Nucleus	Y	transcription regulator	-
DNA mismatch repair protein Msh6	Msh6	mem	-	Nucleus	Y	enzyme	Colorectal Cancer Metastasis Signaling; Hereditary Breast Cancer Signaling; Mismatch Repair in Eukaryotes; Ovarian Cancer Signaling; Role of BRCA1 in DNA Damage Response
Nuclear pore complex protein Nup214	Nup214	mem	1,6	Nucleus	Y	transporter	-
Nucleoporin p58/p45	Nup58	mem	1,4,5		-		-
Phosphatidylinositol-binding clathrin assembly protein	Picalm	mem	1,5,6	Cytoplasm	Y	other	Clathrin-mediated Endocytosis Signaling
Pleckstrin homology domain-containing, family A member 5	Plekha5	mem	5	Cytoplasm	-	other	-
Protein phosphatase 1 regulatory subunit 12A	Ppp1r12a	mem	1,5,6	Cytoplasm	Y	phosphatase	3-phosphoinositide Biosynthesis; 3-phosphoinositide Degradation; Actin Cytoskeleton Signaling; Actin Nucleation by ARP-WASP Complex; Breast Cancer Regulation by Stathmin1; Cardiac β -adrenergic Signaling; CCR3 Signaling in Eosinophils; Cdc42 Signaling; CDK5 Signaling; Cellular Effects of Sildenafil (Viagra); Chemokine Signaling; D-myo-inositol (1,4,5,6)-Tetrakisphosphate Biosynthesis; D-myo-inositol (3,4,5,6)-tetrakisphosphate Biosynthesis; D-myo-inositol-5-phosphate Metabolism; Dopamine-DARPP32 Feedback in cAMP Signaling; Dopamine Receptor Signaling; ERK/MAPK Signaling; HIPPO signaling; ILK Signaling; Insulin Receptor Signaling; Integrin Signaling; Phospholipase C Signaling; Production of Nitric Oxide and Reactive Oxygen Species in Macrophages; Protein Kinase A Signaling; Regulation of Actin-based Motility by Rho; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases; Superpathway of Inositol Phosphate Compounds; Synaptic Long Term Potentiation; Thrombin Signaling

RNA-binding protein 27	Rbm27	mem	1,5,6	Nucleus	Y	other	-
Deoxynucleoside triphosphate triphosphohydrolase SAMHD1	Samhd1	mem	6	Nucleus	Y	enzyme	-
SAP30-binding protein	Sap30bp	mem	1,5,6	Nucleus	Y	transcription regulator	-
Splicing factor 1	Sf1	mem	1,6	Nucleus	Y	other	-
Transcription factor Sp1	Sp1	mem	6	Nucleus	Y	transcription regulator	Aryl Hydrocarbon Receptor Signaling; ErbB2-ErbB3 Signaling; Estrogen-Dependent Breast Cancer Signaling; Gap Junction Signaling; HMGB1 Signaling; Huntington's Disease Signaling; IL-10 Signaling; Prolactin Signaling; Sumoylation Pathway; Telomerase Signaling; VDR/RXR Activation
Spectrin beta chain, non-erythrocytic 1	Sptbn1	mem	1,3,6	Plasma Membrane	-	other	Sertoli Cell-Sertoli Cell Junction Signaling
Transcription initiation factor TFIID subunit 6	Taf6	mem	1,6	Nucleus	Y	transcription regulator	Assembly of RNA Polymerase II Complex; Estrogen Receptor Signaling; Glucocorticoid Receptor Signaling
Thy-1 membrane glycoprotein	Thy1	mem	-	Plasma Membrane	-	other	Granulocyte Adhesion and Diapedesis; Leukocyte Extravasation Signaling
Ubiquitin-associated protein 2	Ubap2	mem	5,6	Cytoplasm	Y	other	-
Ubiquilin-2	Ubqln2	mem	6	Nucleus	Y	other	-
L-lactate dehydrogenase A chain	Ldha	det	-	Cytoplasm	Y	enzyme	HIF1 α Signaling; Hypoxia Signaling in the Cardiovascular System; Pyruvate Fermentation to Lactate
60S ribosomal protein L7	Rpl7	det	6	Nucleus	Y	transcription regulator	EIF2 Signaling
Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial	Sdha	det	6	Cytoplasm	-	enzyme	Huntington's Disease Signaling; Mitochondrial Dysfunction; Oxidative Phosphorylation; TCA Cycle II (Eukaryotic)
ATP-citrate synthase	Acly	det	6	Cytoplasm	Y	enzyme	Acetyl-CoA Biosynthesis III (from Citrate); Insulin Receptor Signaling
Fructose-bisphosphate aldolase A	Aldoa	det	5,6	Cytoplasm	Y	enzyme	Gluconeogenesis I; Glycolysis I; Sucrose Degradation V (Mammalian)
ATP synthase subunit beta, mitochondrial	Atp5b	det	5,6	Cytoplasm	Y	transporter	Huntington's Disease Signaling; Mitochondrial Dysfunction; Oxidative Phosphorylation
ATP synthase F(0) complex subunit B1, mitochondrial	Atp5f1	det	-	Cytoplasm	Y	transporter	Mitochondrial Dysfunction; Oxidative Phosphorylation
CAD protein	Cad	det	5,6	Cytoplasm	Y	enzyme	Pyrimidine Ribonucleotides De Novo Biosynthesis; Uridine-5'-phosphate Biosynthesis
T-complex protein 1 subunit gamma	Cct3	det	1,6	Cytoplasm	Y	other	-
Coatomer subunit beta'	Copb2	det	-	Cytoplasm	Y	transporter	Caveolar-mediated Endocytosis Signaling

Coactosin-like protein	Cotl1	det	1	Cytoplasm	Y	other	-
Cleavage and polyadenylation specificity factor subunit 6	Cpsf6	det	-	Nucleus	Y	other	Cleavage and Polyadenylation of Pre-mRNA; Tight Junction Signaling
ATP-dependent RNA helicase DDX3X	Ddx3x	det	6	Cytoplasm	Y	enzyme	Pyrimidine Ribonucleotides De Novo Biosynthesis; Pyrimidine Ribonucleotides Interconversion
DnaJ homolog subfamily B member 1	Dnajb1	det	5	Nucleus	Y	transcription regulator	Aldosterone Signaling in Epithelial Cells; Androgen Signaling; Huntington's Disease Signaling; NRF2-mediated Oxidative Stress Response; Protein Ubiquitination Pathway
Dedicator of cytokinesis protein 2	Dock2	det	-	Cytoplasm	Y	other	-
Dihydropyrimidinase-related protein 2	Dpysl2	det	5	Cytoplasm	Y	enzyme	Axonal Guidance Signaling; Semaphorin Signaling in Neurons; Thymine Degradation; Uracil Degradation II (Reductive)
Elongation factor 1-alpha 1	Eef1a1	det	2,5	Cytoplasm	Y	translation regulator	-
Eukaryotic translation initiation factor 3 subunit F	Eif3f	det	-	Cytoplasm	Y	translation regulator	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
Bifunctional glutamate/proline--tRNA ligase	Eprs	det	1,6	Cytoplasm	Y	enzyme	tRNA Charging
Extended synaptotagmin-1	Esyt1	det	1,6	Cytoplasm	Y	other	-
Fatty acid-binding protein, epidermal	Fabp5	det	6	Cytoplasm	Y	transporter	LPS/IL-1 Mediated Inhibition of RXR Function
Fatty acid synthase	Fasn	det	6	Cytoplasm	Y	enzyme	AMPK Signaling; Fatty Acid Biosynthesis Initiation II; FXR/RXR Activation; LXR/RXR Activation; Palmitate Biosynthesis I (Animals); PPAR α /RXR α Activation; Stearate Biosynthesis I (Animals); TR/RXR Activation
Fermitin family homolog 3	Fermt3	det	-	Cytoplasm	Y	enzyme	-
Ribosomal protein L15	Gm10020	det	-	Other	-	other	-
Guanine nucleotide-binding protein G(i) subunit alpha-2	Gnai2	det	5	Plasma Membrane	-	enzyme	Agranulocyte Adhesion and Diapedesis; Androgen Signaling; Axonal Guidance Signaling; Breast Cancer Regulation by Stathmin1; cAMP-mediated signaling; Cardiac Hypertrophy Signaling; CCR3 Signaling in Eosinophils; CCR5 Signaling in Macrophages; Chemokine Signaling; Corticotropin Releasing Hormone Signaling; CREB Signaling in Neurons; CXCR4 Signaling; Dopamine-DARPP32 Feedback in cAMP Signaling; Endothelin-1 Signaling; Ephrin B Signaling; Ephrin Receptor Signaling; fMLP Signaling in Neutrophils; Gap Junction Signaling; G Beta Gamma Signaling; GNRH Signaling; GPCR-Mediated Integration of Enteroendocrine Signaling Exemplified by an L Cell; GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells; G-Protein Coupled Receptor Signaling; Granulocyte Adhesion and Diapedesis; G α i Signaling; IL-1 Signaling; IL-8 Signaling; Leukocyte Extravasation Signaling; Melatonin Signaling; Molecular Mechanisms of Cancer; P2Y Purigenic Receptor Signaling Pathway; p70S6K Signaling; Protein Kinase A Signaling; Relaxin Signaling; RhoGDI Signaling; Role of NFAT in Cardiac Hypertrophy; Role

							of NFAT in Regulation of the Immune Response; Role of PI3K/AKT Signaling in the Pathogenesis of Influenza; Signaling by Rho Family GTPases; Sphingosine-1-phosphate Signaling; Synaptic Long Term Depression; Tec Kinase Signaling; Thrombin Signaling; α -Adrenergic Signaling
Golgi reassembly-stacking protein 2	Gorasp2	det	6	Cytoplasm	Y	other	-
Histone H3.3	H3f3b	det	6	Nucleus	Y	other	ERK/MAPK Signaling; Estrogen Receptor Signaling; p38 MAPK Signaling; Protein Kinase A Signaling; UVB-Induced MAPK Signaling
Histone H3.3C	H3f3c	det	-	Nucleus	Y	other	-
Host cell factor 1	Hcfc1	det	1,2,3,4,5,6	Nucleus	Y	transcription regulator	-
Histone H1.2	Hist1h1c	det	-	Nucleus	Y	other	Granzyme A Signaling; Protein Kinase A Signaling
Histone H1.3	Hist1h1d	det	6	Nucleus	Y	other	Granzyme A Signaling; Protein Kinase A Signaling
Histone H2A	Hist1h2al	det	-	Other	Y	other	-
Histone H2B type 1-B	Hist1h2bb	det	-	Nucleus	Y	other	-
Histone H2B type 1-C/E/G	Hist1h2bg	det	6	Nucleus	Y	other	-
Histone H2B type 1-H	Hist1h2bh	det	6	Other	Y	other	-
Histone H2B type 1-K	Hist1h2bk	det	-	Nucleus	Y	other	-
Histone H2B type 1-M	Hist1h2bm	det	-	Nucleus	Y	other	-
Histone H2B type 1-F/J/L	Hist1h2bn	det	-	Nucleus	Y	other	-
Histone H2B type 1-P	Hist1h2bp	det	-	Nucleus	Y	other	-
Histone H2B	Hist1h2br	det	-	Nucleus	-	other	-
Histone H2A type 2-A	Hist2h2aa1	det	-	Nucleus	Y	other	-
Histone H2A type 2-A	Hist2h2aa2	det	-	Nucleus	Y	other	-
Histone H2A type 2-C	Hist2h2ac	det	-	Nucleus	Y	other	-
Histone H2B type 2-B	Hist2h2bb	det	-	Nucleus	Y	other	-
Histone H3.2	Hist2h3b	det	-	Nucleus	Y	other	-
Histone H3.2	Hist2h3c2	det	-	Nucleus	Y	other	-

High mobility group protein B1	Hmgb1	det	6	Nucleus	Y	transcription regulator	-
Heterogeneous nuclear ribonucleoprotein A1	Hnrnpa1	det	6	Nucleus	Y	other	-
Heat shock protein HSP 90-alpha	Hsp90aa1	det	2,6	Cytoplasm	Y	enzyme	Aldosterone Signaling in Epithelial Cells; Androgen Signaling; Aryl Hydrocarbon Receptor Signaling; eNOS Signaling; Glucocorticoid Receptor Signaling; HIF1 α Signaling; Hypoxia Signaling in the Cardiovascular System; Mitotic Roles of Polo-Like Kinase; Neuregulin Signaling; Nitric Oxide Signaling in the Cardiovascular System; PI3K/AKT Signaling; PPAR Signaling; PPAR α /RXR α Activation; Prostate Cancer Signaling; Protein Ubiquitination Pathway; Telomerase Signaling; Xenobiotic Metabolism Signaling
Heat shock protein HSP 90-beta	Hsp90ab1	det	2,6	Cytoplasm	Y	enzyme	Aldosterone Signaling in Epithelial Cells; Aryl Hydrocarbon Receptor Signaling; eNOS Signaling; Glucocorticoid Receptor Signaling; Hypoxia Signaling in the Cardiovascular System; Mitotic Roles of Polo-Like Kinase; Neuregulin Signaling; Nitric Oxide Signaling in the Cardiovascular System; PI3K/AKT Signaling; PPAR Signaling; PPAR α /RXR α Activation; Prostate Cancer Signaling; Protein Ubiquitination Pathway; Telomerase Signaling; Xenobiotic Metabolism Signaling
Ras GTPase-activating-like protein IQGAP1	Iqgap1	det	1,6	Cytoplasm	Y	other	Actin Cytoskeleton Signaling; Cdc42 Signaling; Epithelial Adherens Junction Signaling; Germ Cell-Sertoli Cell Junction Signaling; IL-8 Signaling; Rac Signaling; Remodeling of Epithelial Adherens Junctions; Signaling by Rho Family GTPases
Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial	Mccc1	det	6	Cytoplasm	-	enzyme	Leucine Degradation I
DNA replication licensing factor MCM3	Mcm3	det	-	Nucleus	Y	enzyme	Cell Cycle Control of Chromosomal Replication
DNA replication licensing factor MCM5	Mcm5	det	6	Nucleus	Y	enzyme	Cell Cycle Control of Chromosomal Replication
DNA replication licensing factor MCM6	Mcm6	det	-	Nucleus	Y	enzyme	Cell Cycle Control of Chromosomal Replication
DNA replication licensing factor MCM7	Mcm7	det	6	Nucleus	Y	enzyme	Aryl Hydrocarbon Receptor Signaling; Cell Cycle Control of Chromosomal Replication
Myb-binding protein 1A	Mybbp1a	det	6	Nucleus	Y	transcription regulator	
Nucleolin	Ncl	det	6	Nucleus	Y	other	-
Non-POU domain-containing octamer-binding protein	Nono	det	6	Nucleus	Y	other	-
Osteoclast-stimulating factor 1	Ostf1	det	-	Nucleus	Y	transcription regulator	-
Protein disulfide-isomerase	P4hb	det	-	Cytoplasm	-	enzyme	Hypoxia Signaling in the Cardiovascular System; Role of Tissue Factor in Cancer; Unfolded

							protein response
Proliferation-associated protein 2G4	Pa2g4	det	6	Nucleus	Y	transcription regulator	Bladder Cancer Signaling; Cell Cycle: G1/S Checkpoint Regulation; Chronic Myeloid Leukemia Signaling; Cyclins and Cell Cycle Regulation; Glioma Signaling; Molecular Mechanisms of Cancer; Non-Small Cell Lung Cancer Signaling; Ovarian Cancer Signaling; Pancreatic Adenocarcinoma Signaling; Prostate Cancer Signaling; Small Cell Lung Cancer Signaling
Polyadenylate-binding protein 1	Pabpc1	det	5,6	Cytoplasm	Y	translation regulator	Antiproliferative Role of TOB in T Cell Signaling; EIF2 Signaling; Regulation of eIF4 and p70S6K Signaling
Ectonucleotide pyrophosphatase/phosphodiesterase family member 1	Pc	det	-		Y		-
Programmed cell death 6-interacting protein	Pdcd6ip	det	1,6	Cytoplasm	Y	other	14-3-3-mediated Signaling; Mechanisms of Viral Exit from Host Cells
6-phosphogluconate dehydrogenase, decarboxylating	Pgd	det	-	Cytoplasm	Y	enzyme	Pentose Phosphate Pathway; Pentose Phosphate Pathway (Oxidative Branch)
Proteasome subunit alpha type-7	Psm7	det	-	Cytoplasm	Y	peptidase	Protein Ubiquitination Pathway
Proteasome activator complex subunit 2	Psme2	det	1,6	Cytoplasm	Y	peptidase	Huntington's Disease Signaling; Polyamine Regulation in Colon Cancer; Protein Ubiquitination Pathway
MCG22048, isoform CRA_c	PSME2b	det	-		-		-
60S ribosomal protein L15	Rpl15	det	6	Cytoplasm	Y	other	EIF2 Signaling
60S ribosomal protein L18a	Rpl18a	det	6	Cytoplasm	Y	other	EIF2 Signaling
60S ribosomal protein L5	Rpl5	det	6	Cytoplasm	Y	other	Cell Cycle: G1/S Checkpoint Regulation; EIF2 Signaling
60S ribosomal protein L6	Rpl6	det	6	Nucleus	Y	other	EIF2 Signaling
Regulation of nuclear pre-mRNA domain-containing protein 2	Rprd2	det	4,5,6	Nucleus	Y	other	-
40S ribosomal protein S10	Rps10	det	6	Cytoplasm	Y	other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
40S ribosomal protein S15a	Rps15a	det	-	Cytoplasm	-	other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
40S ribosomal protein S2	Rps2	det	6	Cytoplasm	Y	other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
40S ribosomal protein S4, X isoform	Rps4x	det	6	Cytoplasm	-	other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
Serine--tRNA ligase, cytoplasmic	Sars	det	-	Cytoplasm	Y	enzyme	Selenocysteine Biosynthesis II (Archaea and Eukaryotes); tRNA Charging
Histone-lysine N-methyltransferase SETD7	Set	det	6	Nucleus	-	phosphatase	3-phosphoinositide Biosynthesis; 3-phosphoinositide Degradation; D-myo-inositol (1,4,5,6)-Tetrakisphosphate Biosynthesis; D-myo-inositol (3,4,5,6)-tetrakisphosphate Biosynthesis; D-myo-inositol-5-phosphate Metabolism; Granzyme A Signaling; Superpathway of Inositol

							Phosphate Compounds; Transcriptional Regulatory Network in Embryonic Stem Cells
Splicing factor 3A subunit 1	Sf3a1	det	1,6	Nucleus	Y	other	Assembly of RNA Polymerase III Complex; Role of p14/p19ARF in Tumor Suppression
Splicing factor, proline- and glutamine-rich	Sfpq	det	1,5,6	Nucleus	Y	other	-
Small nuclear ribonucleoprotein Sm D1	Snrpd1	det	-	Nucleus	-	other	Systemic Lupus Erythematosus Signaling
TAR DNA-binding protein 43	Tardbp	det	6	Nucleus	Y	transcription regulator	-
Valine--tRNA ligase	Vars	det	6	Cytoplasm	Y	enzyme	tRNA Charging
Voltage-dependent anion-selective channel protein 1	Vdac1	det	1,5	Cytoplasm	-	ion channel	Mitochondrial Dysfunction
Voltage-dependent anion-selective channel protein 2	Vdac2	det	1,6	Cytoplasm	Y	ion channel	Mitochondrial Dysfunction
14-3-3 protein beta/alpha	Ywhab	det	6	Cytoplasm	Y	transcription regulator	14-3-3-mediated Signaling; Cell Cycle: G2/M DNA Damage Checkpoint Regulation; ERK/MAPK Signaling; ERK5 Signaling; HIPPO signaling; IGF-1 Signaling; Myc Mediated Apoptosis Signaling; p70S6K Signaling; PI3K/AKT Signaling; Protein Kinase A Signaling
14-3-3 protein epsilon	Ywhae	det	6	Cytoplasm	Y	other	14-3-3-mediated Signaling; Cell Cycle: G2/M DNA Damage Checkpoint Regulation; ERK5 Signaling; HIPPO signaling; IGF-1 Signaling; Myc Mediated Apoptosis Signaling; Neuroprotective Role of THOP1 in Alzheimer's Disease; p70S6K Signaling; PI3K/AKT Signaling; Protein Kinase A Signaling; VEGF Signaling
14-3-3 protein gamma	Ywhag	det	-	Cytoplasm	Y	other	14-3-3-mediated Signaling; Cell Cycle: G2/M DNA Damage Checkpoint Regulation; ERK/MAPK Signaling; ERK5 Signaling; HIPPO signaling; IGF-1 Signaling; Myc Mediated Apoptosis Signaling; p70S6K Signaling; PI3K/AKT Signaling; Protein Kinase A Signaling
14-3-3 protein eta	Ywhah	det	-	Cytoplasm	Y	transcription regulator	14-3-3-mediated Signaling; Cell Cycle: G2/M DNA Damage Checkpoint Regulation; ERK/MAPK Signaling; ERK5 Signaling; Glucocorticoid Receptor Signaling; HIPPO signaling; IGF-1 Signaling; Myc Mediated Apoptosis Signaling; p70S6K Signaling; PI3K/AKT Signaling; Protein Kinase A Signaling; PTEN Signaling
14-3-3 protein theta	Ywhaq	det	1,6	Cytoplasm	Y	other	14-3-3-mediated Signaling; Cell Cycle: G2/M DNA Damage Checkpoint Regulation; ERK/MAPK Signaling; ERK5 Signaling; HIPPO signaling; IGF-1 Signaling; Myc Mediated Apoptosis Signaling; p70S6K Signaling; PI3K/AKT Signaling; Protein Kinase A Signaling
Zinc finger CCCH domain-containing protein 13	Zc3h13	det	6	Extracellular Space	Y	other	-
BUB3-interacting and GLEBS motif-containing protein ZNF207	Znf207	det	1,6		-		-

S2. Complete list and analysis of singly identified O-GlcNAcylated proteins

Ref = Identification Reference, Phos = Known phosphorylation

Protein	Gene	Ref	Location	Phos	Family	Pathways
Actin-binding LIM protein 3	Ablim3	5	Cytoplasm	Y	other	Axonal Guidance Signaling; Netrin Signaling
3-ketoacyl-CoA thiolase A, peroxisomal	Acaa1a		Cytoplasm		enzyme	Fatty Acid β -oxidation I; PPAR α /RXR α Activation
Acetyl-CoA carboxylase 1	Acaca	5,6	Cytoplasm	Y	enzyme	AMPK Signaling; Biotin-carboxyl Carrier Protein Assembly; LXR/RXR Activation; TR/RXR Activation
Acetyl-CoA acetyltransferase, mitochondrial	Acat1	6	Cytoplasm	Y	enzyme	Glutaryl-CoA Degradation; Isoleucine Degradation I; Ketogenesis; Ketolysis; Mevalonate Pathway I; Superpathway of Cholesterol Biosynthesis; Superpathway of Geranylgeranyldiphosphate Biosynthesis I (via Mevalonate); Tryptophan Degradation III (Eukaryotic)
Actin, cytoplasmic 1	Actb	5,6	Cytoplasm		other	Actin Cytoskeleton Signaling; Agranulocyte Adhesion and Diapedesis; Agrin Interactions at Neuromuscular Junction; AMPK Signaling; Caveolar-mediated Endocytosis Signaling; Cellular Effects of Sildenafil (Viagra); Clathrin-mediated Endocytosis Signaling; Crosstalk between Dendritic Cells and Natural Killer Cells; Death Receptor Signaling; EIF2 Signaling; Epithelial Adherens Junction Signaling; FAK Signaling; Fc γ Receptor-mediated Phagocytosis in Macrophages and Monocytes; Gap Junction Signaling; Germ Cell-Sertoli Cell Junction Signaling; Glucocorticoid Receptor Signaling; Hereditary Breast Cancer Signaling; ILK Signaling; Integrin Signaling; Leukocyte Extravasation Signaling; Mechanisms of Viral Exit from Host Cells; MSP-RON Signaling Pathway; NRF2-mediated Oxidative Stress Response; Paxillin Signaling; RAR Activation; Regulation of Actin-based Motility by Rho; Remodeling of Epithelial Adherens Junctions; RhoA Signaling; RhoGDI Signaling; Role of BRCA1 in DNA Damage Response; Sertoli Cell-Sertoli Cell Junction Signaling; Signaling by Rho Family GTPases; Tec Kinase Signaling; Tight Junction Signaling; VEGF Signaling; Virus Entry via Endocytic Pathways
Actin, cytoplasmic 2	Actg1		Cytoplasm		other	Actin Cytoskeleton Signaling; Agranulocyte Adhesion and Diapedesis; Agrin Interactions at Neuromuscular Junction; Caveolar-mediated Endocytosis Signaling; Cellular Effects of Sildenafil (Viagra); Clathrin-mediated Endocytosis Signaling; Crosstalk between Dendritic Cells and Natural Killer Cells; Death Receptor Signaling; Epithelial Adherens Junction Signaling; FAK Signaling; Fc γ Receptor-mediated Phagocytosis in Macrophages and Monocytes; Gap Junction Signaling; Germ Cell-Sertoli Cell Junction Signaling; ILK Signaling; Integrin Signaling; Leukocyte Extravasation Signaling; Mechanisms of Viral Exit from Host Cells; MSP-RON Signaling Pathway; NRF2-mediated Oxidative Stress Response; Paxillin Signaling; Remodeling of Epithelial Adherens Junctions; RhoA Signaling; RhoGDI Signaling; Sertoli Cell-Sertoli Cell Junction Signaling; Signaling by Rho Family GTPases; Tec Kinase Signaling; Tight Junction Signaling; VEGF Signaling; Virus Entry via Endocytic Pathways
Alpha-actinin-1	Actn1	6	Cytoplasm	Y	transcription regulator	
Alpha-actinin-4	Actn4	6	Cytoplasm	Y	transcription regulator	

Actin-related protein 2	Actr2		Plasma Membrane		other	Actin Cytoskeleton Signaling; Actin Nucleation by ARP-WASP Complex; Axonal Guidance Signaling; CD28 Signaling in T Helper Cells; Cdc42 Signaling; Clathrin-mediated Endocytosis Signaling; Ephrin Receptor Signaling; Epithelial Adherens Junction Signaling; Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes; fMLP Signaling in Neutrophils; Integrin Signaling; Rac Signaling; Regulation of Actin-based Motility by Rho; Remodeling of Epithelial Adherens Junctions; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases
Actin-related protein 3	Actr3		Plasma Membrane		other	Actin Cytoskeleton Signaling; Actin Nucleation by ARP-WASP Complex; Axonal Guidance Signaling; CD28 Signaling in T Helper Cells; Cdc42 Signaling; Clathrin-mediated Endocytosis Signaling; Ephrin Receptor Signaling; Epithelial Adherens Junction Signaling; Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes; fMLP Signaling in Neutrophils; Integrin Signaling; Rac Signaling; Regulation of Actin-based Motility by Rho; Remodeling of Epithelial Adherens Junctions; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases
Alcohol dehydrogenase class-3	Adh5		Cytoplasm	Y	enzyme	Ethanol Degradation II; Formaldehyde Oxidation II (Glutathione-dependent); Noradrenaline and Adrenaline Degradation; Serotonin Degradation
Arf-GAP domain and FG repeat-containing protein 1	Agfg1	1,5,6	Nucleus	Y	other	
AHNAK nucleoprotein (desmoyokin)	Ahnak	1,6	Nucleus		other	Phospholipase C Signaling
A-kinase anchor protein 8-like	Akap8l	6	Nucleus	Y	other	
Ankyrin repeat and KH domain-containing 1	Ankhd1	1,5,6	Other		other	
Annexin A2	Anxa2	6	Plasma Membrane	Y	other	
Annexin A5	Anxa5		Plasma Membrane	Y	transporter	
Annexin A6	Anxa6		Plasma Membrane	Y	ion channel	
AP-2 complex subunit beta	Ap2b1	6	Plasma Membrane	Y	other	Clathrin-mediated Endocytosis Signaling; CTLA4 Signaling in Cytotoxic T Lymphocytes; GABA Receptor Signaling; Lipid Antigen Presentation by CD1; Virus Entry via Endocytic Pathways
Adenine phosphoribosyltransferase	Aprt		Cytoplasm	Y	enzyme	Adenine and Adenosine Salvage I
ADP-ribosylation factor 1	Arf1		Cytoplasm		enzyme	Integrin Signaling; Paxillin Signaling
ADP-ribosylation factor 3	Arf3	6	Cytoplasm		enzyme	Integrin Signaling
ADP-ribosylation factor 5	Arf5		Cytoplasm		enzyme	Integrin Signaling
Rho GDP-dissociation inhibitor 1	Arhgdia	2,6	Cytoplasm	Y	other	Regulation of Actin-based Motility by Rho; RhoGDI Signaling; Sumoylation Pathway
Rho GDP-dissociation inhibitor 2	Arhgdib		Cytoplasm	Y	enzyme	Death Receptor Signaling; RhoGDI Signaling; Sumoylation Pathway

Rho guanine nucleotide exchange factor 10-like protein	Arhgef10l		Cytoplasm		enzyme	
Actin-related protein 2/3 complex subunit 2	Arpc2		Cytoplasm		other	Actin Cytoskeleton Signaling; Actin Nucleation by ARP-WASP Complex; Axonal Guidance Signaling; CD28 Signaling in T Helper Cells; Cdc42 Signaling; Clathrin-mediated Endocytosis Signaling; Ephrin Receptor Signaling; Epithelial Adherens Junction Signaling; Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes; fMLP Signaling in Neutrophils; Integrin Signaling; Rac Signaling; Regulation of Actin-based Motility by Rho; Remodeling of Epithelial Adherens Junctions; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases
Actin-related protein 2/3 complex subunit 3	Arpc3		Cytoplasm	Y	other	Actin Cytoskeleton Signaling; Actin Nucleation by ARP-WASP Complex; Axonal Guidance Signaling; CD28 Signaling in T Helper Cells; Cdc42 Signaling; Clathrin-mediated Endocytosis Signaling; Ephrin Receptor Signaling; Epithelial Adherens Junction Signaling; Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes; fMLP Signaling in Neutrophils; Integrin Signaling; Rac Signaling; Regulation of Actin-based Motility by Rho; Remodeling of Epithelial Adherens Junctions; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases
Actin-related protein 2/3 complex subunit 4	Arpc4		Cytoplasm		other	Actin Cytoskeleton Signaling; Actin Nucleation by ARP-WASP Complex; Axonal Guidance Signaling; CD28 Signaling in T Helper Cells; Cdc42 Signaling; Clathrin-mediated Endocytosis Signaling; Ephrin Receptor Signaling; Epithelial Adherens Junction Signaling; Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes; fMLP Signaling in Neutrophils; Integrin Signaling; Rac Signaling; Regulation of Actin-based Motility by Rho; Remodeling of Epithelial Adherens Junctions; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases
Actin-related protein 2/3 complex subunit 5	Arpc5		Cytoplasm		other	Actin Cytoskeleton Signaling; Actin Nucleation by ARP-WASP Complex; Axonal Guidance Signaling; CD28 Signaling in T Helper Cells; Cdc42 Signaling; Clathrin-mediated Endocytosis Signaling; Corticotropin Releasing Hormone Signaling; Ephrin Receptor Signaling; Epithelial Adherens Junction Signaling; Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes; fMLP Signaling in Neutrophils; Integrin Signaling; Rac Signaling; Regulation of Actin-based Motility by Rho; Remodeling of Epithelial Adherens Junctions; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases
Activating signal cointegrator 1 complex subunit 3	Ascc3		Cytoplasm	Y	enzyme	
Sodium/potassium-transporting ATPase subunit alpha-1	Atp1a1	5	Plasma Membrane	Y	transporter	
Sodium/potassium-transporting ATPase subunit beta-3	Atp1b3		Plasma Membrane		transporter	
ATP synthase subunit alpha, mitochondrial	Atp5a1	5,6,7	Cytoplasm	Y	transporter	
Ataxin-7	Atxn7		Nucleus		peptidase	
Beta-1,4-galactosyltransferase 1	B4galt1		Cytoplasm		enzyme	
Basic leucine zipper and W2 domain-containing protein 1	Bzw1		Cytoplasm	Y	translation regulator	

Calreticulin	Calr	6	Cytoplasm		transcription regulator	
Adenylyl cyclase-associated protein 1	Cap1	6	Plasma Membrane	Y	other	Ephrin B Signaling
F-actin-capping protein subunit alpha-1	Capza1		Cytoplasm	Y	other	
F-actin-capping protein subunit beta	Capzb		Cytoplasm	Y	other	
T-complex protein 1 subunit beta	Cct2	6	Cytoplasm	Y	kinase	
T-complex protein 1 subunit delta	Cct4	6	Cytoplasm	Y	other	
T-complex protein 1 subunit zeta	Cct6a	6	Cytoplasm	Y	other	
T-complex protein 1 subunit eta	Cct7		Cytoplasm		other	NRF2-mediated Oxidative Stress Response
T-complex protein 1 subunit theta	Cct8	6	Cytoplasm	Y	enzyme	
Leukocyte surface antigen CD47	Cd47		Plasma Membrane	Y	transmembrane receptor	
Cell division control protein 42 homolog	Cdc42		Plasma Membrane	Y	enzyme	
Cofilin-1	Cfl1	2,6	Nucleus	Y	other	Actin Cytoskeleton Signaling; Axonal Guidance Signaling; B Cell Receptor Signaling; CCR3 Signaling in Eosinophils; Cdc42 Signaling; Chemokine Signaling; Ephrin A Signaling; Ephrin B Signaling; Ephrin Receptor Signaling; Germ Cell-Sertoli Cell Junction Signaling; ILK Signaling; PAK Signaling; Rac Signaling; Regulation of Actin-based Motility by Rho; RhoA Signaling; RhoGDI Signaling; Role of Tissue Factor in Cancer; Semaphorin Signaling in Neurons; Signaling by Rho Family GTPases
Cytoskeleton-associated protein 5	Ckap5	5,6	Nucleus	Y	transcription regulator	
Clathrin heavy chain 1	Cltc	1,6	Plasma Membrane	Y	other	Clathrin-mediated Endocytosis Signaling; CTLA4 Signaling in Cytotoxic T Lymphocytes; Huntington's Disease Signaling; Virus Entry via Endocytic Pathways
Cytosolic non-specific dipeptidase	Cndp2		Cytoplasm	Y	peptidase	
Coatomer subunit alpha	Copa		Cytoplasm	Y	transporter	
Coronin-1A	Coro1a		Cytoplasm	Y	other	
Coronin-1C	Coro1c	6	Cytoplasm		other	
Coronin-7	Coro7	1,6	Cytoplasm	Y	other	
Calsyntenin-1	Cs		Cytoplasm	Y	enzyme	TCA Cycle II (Eukaryotic)
Exportin-2	Cse11	6	Nucleus	Y	transporter	

Small muscular protein	Csl		Cytoplasm		enzyme	
Casein kinase II subunit alpha	Csnk2a1	2	Nucleus	Y	kinase	Amyloid Processing; Clathrin-mediated Endocytosis Signaling; EGF Signaling; IGF-1 Signaling; IL-2 Signaling; IL-6 Signaling; NF-κB Signaling; PDGF Signaling; PTEN Signaling; RAR Activation; VDR/RXR Activation; Wnt/β-catenin Signaling
Chymotrypsinogen B	Ctrb1		Cytoplasm	Y	peptidase	
Cytoplasmic FMR1-interacting protein 2	Cyfp2	1b,5,6	Cytoplasm		other	Actin Cytoskeleton Signaling; Rac Signaling
Aspartate--tRNA ligase, cytoplasmic	Dars	6	Cytoplasm	Y	enzyme	tRNA Charging
Probable ATP-dependent RNA helicase DDX17	Ddx17	1,6	Nucleus	Y	enzyme	
ATP-dependent RNA helicase DDX39A	Ddx39a	6		Y		
Spliceosome RNA helicase Ddx39b	Ddx39b	6	Nucleus	Y	enzyme	
Probable ATP-dependent RNA helicase DDX5	Ddx5	6	Nucleus	Y	enzyme	Estrogen Receptor Signaling
Pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	Dhx15	1,6	Nucleus	Y	enzyme	
ATP-dependent RNA helicase DHX8	Dhx8		Nucleus	Y	enzyme	
ATP-dependent RNA helicase A	Dhx9	1b	Nucleus	Y	enzyme	
Elongation factor 1-beta	Eef1b			Y		
Elongation factor 1-beta	Eef1b2	6	Cytoplasm		translation regulator	
Elongation factor 1-delta	Eef1d	6	Cytoplasm	Y	translation regulator	
Elongation factor 1-gamma	Eef1g	6	Cytoplasm		translation regulator	
Elongation factor 2	Eef2	6	Cytoplasm	Y	translation regulator	
116 kDa U5 small nuclear ribonucleoprotein component	Eftud2	6	Nucleus	Y	enzyme	Systemic Lupus Erythematosus Signaling
Enolase 1, alpha non-neuron	EG433182					
Eukaryotic translation initiation factor 2 subunit 1	Eif2s1	5	Cytoplasm	Y	translation regulator	

Eukaryotic translation initiation factor 3 subunit A	Eif3a	6	Cytoplasm	Y	other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
Eukaryotic translation initiation factor 3 subunit L	Eif3l	6	Cytoplasm		other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
Eukaryotic initiation factor 4A-I	Eif4a1	2,6	Cytoplasm	Y	translation regulator	
Eukaryotic translation initiation factor 5A-1	Eif5a	6	Cytoplasm		translation regulator	
Eukaryotic translation initiation factor 6	Eif6	6	Cytoplasm	Y	translation regulator	
Alpha-enolase	Eno1	6	Cytoplasm	Y	enzyme	Gluconeogenesis I; Glycolysis I; TR/RXR Activation
E1A-binding protein p400	Ep400	1,5,6	Nucleus	Y	other	
Endoplasmic reticulum aminopeptidase 1	Erap1		Extracellular Space		peptidase	
S-formylglutathione hydrolase	Esd		Cytoplasm		enzyme	Formaldehyde Oxidation II (Glutathione-dependent); Retinol Biosynthesis; Xenobiotic Metabolism Signaling
Ezrin	Ezr	6	Plasma Membrane	Y	other	Actin Cytoskeleton Signaling; Agranulocyte Adhesion and Diapedesis; Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes; Granulocyte Adhesion and Diapedesis; Leukocyte Extravasation Signaling; Regulation of Cellular Mechanics by Calpain Protease; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases
Filamin-A	Flna	6	Cytoplasm	Y	other	Actin Cytoskeleton Signaling; Caveolar-mediated Endocytosis Signaling; ILK Signaling; Protein Kinase A Signaling; Virus Entry via Endocytic Pathways
Forkhead box protein O1	Foxo1	1,6	Nucleus	Y	transcription regulator	
Fibrous sheath-interacting protein 2	Fsip2		Cytoplasm	Y	other	
Glyceraldehyde-3-phosphate dehydrogenase	Gapdh	2,6	Cytoplasm	Y	enzyme	Gluconeogenesis I; Glycolysis I; Maturity Onset Diabetes of Young (MODY) Signaling; NADH Repair
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	2,6		Y		
Glycine--tRNA ligase	Gars	6	Cytoplasm	Y	enzyme	tRNA Charging
Rab GDP dissociation inhibitor beta	Gdi2	6	Cytoplasm	Y	other	RhoGDI Signaling; Sumoylation Pathway
GTPase IMAP family member 4	Gimap4		Nucleus		other	
GTPase, IMAP family member 7	Gimap7		Cytoplasm		enzyme	
Uncharacterized protein	Gm11214		Other		other	

Uncharacterized protein	Gm18025		Other		other	
Nucleoside diphosphate kinase	Gm20390					
Heterogeneous nuclear ribonucleoprotein U	Gm28062					
Ganglioside GM2 activator	Gm2a		Cytoplasm		enzyme	Chondroitin Sulfate Degradation (Metazoa); Dermatan Sulfate Degradation (Metazoa)
Uncharacterized protein	Gm43738					
Uncharacterized protein	Gm5786		Other		other	
Uncharacterized protein	Gm6576		Other		other	
EG627828 protein	Gm6793		Other		other	
Uncharacterized protein	Gm8394		Other		other	
Uncharacterized protein	Gm9242		Other		other	
Uncharacterized protein	Gm9493		Cytoplasm		other	
Guanine nucleotide-binding protein G(k) subunit alpha	Gnai3	5	Cytoplasm		enzyme	Agranulocyte Adhesion and Diapedesis; Androgen Signaling; Axonal Guidance Signaling; Breast Cancer Regulation by Stathmin1; cAMP-mediated signaling; Cardiac Hypertrophy Signaling; CCR3 Signaling in Eosinophils; CCR5 Signaling in Macrophages; Chemokine Signaling; Corticotropin Releasing Hormone Signaling; CREB Signaling in Neurons; CXCR4 Signaling; Dopamine-DARPP32 Feedback in cAMP Signaling; Endothelin-1 Signaling; Ephrin B Signaling; Ephrin Receptor Signaling; fMLP Signaling in Neutrophils; Gap Junction Signaling; G Beta Gamma Signaling; GNRH Signaling; GPCR-Mediated Integration of Enteroendocrine Signaling Exemplified by an L Cell; GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells; G-Protein Coupled Receptor Signaling; Granulocyte Adhesion and Diapedesis; Gαi Signaling; IL-1 Signaling; IL-8 Signaling; Leukocyte Extravasation Signaling; Melatonin Signaling; Molecular Mechanisms of Cancer; P2Y Purigenic Receptor Signaling Pathway; p70S6K Signaling; Protein Kinase A Signaling; Relaxin Signaling; RhoGDI Signaling; Role of NFAT in Cardiac Hypertrophy; Role of NFAT in Regulation of the Immune Response; Role of PI3K/AKT Signaling in the Pathogenesis of Influenza; Signaling by Rho Family GTPases; Sphingosine-1-phosphate Signaling; Synaptic Long Term Depression; Tec Kinase Signaling; Thrombin Signaling; α-Adrenergic Signaling
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	Gnb2		Plasma Membrane	Y	enzyme	Androgen Signaling; Antiproliferative Role of Somatostatin Receptor 2; Axonal Guidance Signaling; Breast Cancer Regulation by Stathmin1; Cardiac Hypertrophy Signaling; Cardiac β-adrenergic Signaling; CCR3 Signaling in Eosinophils; CCR5 Signaling in Macrophages; Colorectal Cancer Metastasis Signaling; CREB Signaling in Neurons; CXCR4 Signaling; Ephrin B Signaling; Ephrin Receptor Signaling; fMLP Signaling in Neutrophils; G Beta Gamma Signaling; G Protein Signaling Mediated by Tubby; Gαi Signaling; Gαq Signaling; Gas Signaling; Huntington's Disease Signaling; IL-1 Signaling; IL-8 Signaling; P2Y Purigenic Receptor Signaling Pathway; Phospholipase C Signaling; Protein Kinase A Signaling; Relaxin

						Signaling; RhoGDI Signaling; Role of NFAT in Cardiac Hypertrophy; Role of NFAT in Regulation of the Immune Response; Signaling by Rho Family GTPases; Tec Kinase Signaling; Thrombin Signaling; α -Adrenergic Signaling
Aspartate aminotransferase, mitochondrial	Got2	6	Cytoplasm	Y	enzyme	Aspartate Biosynthesis; Aspartate Degradation II; Glutamate Degradation II; L-cysteine Degradation I; Phenylalanine Degradation IV (Mammalian, via Side Chain); PPAR α /RXR α Activation; Superpathway of Methionine Degradation
Glucose-6-phosphate isomerase	Gpi	6		Y		
Glutathione S-transferase P 1	Gstp1	1b,6	Cytoplasm	Y	enzyme	Aryl Hydrocarbon Receptor Signaling; Glutathione-mediated Detoxification; Glutathione Redox Reactions I; LPS/IL-1 Mediated Inhibition of RXR Function; NRF2-mediated Oxidative Stress Response; Prostate Cancer Signaling; Xenobiotic Metabolism Signaling
GTP-binding protein 1	Gtpbp1	6	Cytoplasm	Y	enzyme	
Granzyme B(G,H)	Gzmb		Cytoplasm		peptidase	Allograft Rejection Signaling; Autoimmune Thyroid Disease Signaling; Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells; Graft-versus-Host Disease Signaling; Granzyme B Signaling; Tumoricidal Function of Hepatic Natural Killer Cells; Type I Diabetes Mellitus Signaling
H-2 class I histocompatibility antigen, K-B alpha chain	H2-K1		Plasma Membrane	Y	other	Allograft Rejection Signaling; Altered T Cell and B Cell Signaling in Rheumatoid Arthritis; Antigen Presentation Pathway; Autoimmune Thyroid Disease Signaling; B Cell Development; Calcium-induced T Lymphocyte Apoptosis; Caveolar-mediated Endocytosis Signaling; CD28 Signaling in T Helper Cells; Cdc42 Signaling; Communication between Innate and Adaptive Immune Cells; Crosstalk between Dendritic Cells and Natural Killer Cells; CTLA4 Signaling in Cytotoxic T Lymphocytes; Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells; Dendritic Cell Maturation; Graft-versus-Host Disease Signaling; iCOS-iCOSL Signaling in T Helper Cells; IL-4 Signaling; Neuroprotective Role of THOP1 in Alzheimer's Disease; Nur77 Signaling in T Lymphocytes; OX40 Signaling Pathway; Phagosome Maturation; PKC θ Signaling in T Lymphocytes; Protein Ubiquitination Pathway; Role of NFAT in Regulation of the Immune Response; Systemic Lupus Erythematosus Signaling; Th1 and Th2 Activation Pathway; Th1 Pathway; Th2 Pathway; T Helper Cell Differentiation; Type I Diabetes Mellitus Signaling; Virus Entry via Endocytic Pathways
Histone H2A.J	H2afj		Cytoplasm	Y	other	
Histone H3.3	H3f3a		Nucleus	Y	other	ERK/MAPK Signaling; Estrogen Receptor Signaling; p38 MAPK Signaling; Protein Kinase A Signaling; UVB-Induced MAPK Signaling
Trifunctional enzyme subunit alpha, mitochondrial	Hadha	1b	Cytoplasm	Y	enzyme	Fatty Acid β -oxidation I; Glutaryl-CoA Degradation; Isoleucine Degradation I; Ketogenesis; Ketolysis; Mevalonate Pathway I; Superpathway of Cholesterol Biosynthesis; Superpathway of Geranylgeranyldiphosphate Biosynthesis I (via Mevalonate); Tryptophan Degradation III (Eukaryotic); Valine Degradation I
Beta-hexosaminidase subunit beta	Hexb		Cytoplasm		enzyme	Chondroitin Sulfate Degradation (Metazoa); Dermatan Sulfate Degradation (Metazoa)
Histone H1.4	Hist1h1e		Nucleus	Y	other	Granzyme A Signaling; Protein Kinase A Signaling
Histone H2A	Hist1h2		Nucleus		other	

	aa					
Histone H2A type 1	Hist1h2ad		Nucleus	Y	other	
Histone H2A type 1-F	Hist1h2af		Nucleus	Y	other	
Histone H2A type 1-H	Hist1h2ah		Nucleus	Y	other	
Histone H2A type 1-K	Hist1h2ak		Nucleus	Y	other	
Histone H2A type 1	Hist1h2ao		Nucleus	Y	other	
Histone H4	Hist2h4		Nucleus	Y	other	
Histone H2A type 3	Hist3h2a		Nucleus	Y	other	
Histone H4	Hist4h4		Nucleus	Y	other	
Heterogeneous nuclear ribonucleoprotein A0	Hnrnpa0	5,6	Nucleus	Y	other	
Heterogeneous nuclear ribonucleoproteins A2/B1	Hnrnpa2b1	6	Nucleus	Y	other	Systemic Lupus Erythematosus Signaling; Telomere Extension by Telomerase
Heterogeneous nuclear ribonucleoprotein A3	Hnrnpa3	5,6	Nucleus	Y	transporter	
Heterogeneous nuclear ribonucleoprotein A/B	Hnrnpab	6	Nucleus	Y	enzyme	
Heterogeneous nuclear ribonucleoproteins C1/C2	Hnrnpc	6	Nucleus	Y	other	Systemic Lupus Erythematosus Signaling
Heterogeneous nuclear ribonucleoprotein D0	Hnrnpd		Nucleus	Y	transcription regulator	
Heterogeneous nuclear ribonucleoprotein F	Hnrnpf	1,6	Nucleus	Y	other	
Heterogeneous nuclear ribonucleoprotein H	Hnrnph1	6	Nucleus	Y	other	
Heterogeneous nuclear ribonucleoprotein K	Hnrnpk	6	Nucleus	Y	transcription regulator	
Heterogeneous nuclear ribonucleoprotein L	Hnrnpl	1,6	Nucleus	Y	other	
Heterogeneous nuclear ribonucleoprotein M	Hnrnpm	6	Nucleus	Y	other	

Heterogeneous nuclear ribonucleoprotein U	Hnrnpu	6	Nucleus	Y	transporter	
Heterogeneous nuclear ribonucleoprotein U-like protein 2	Hnrnpul2	6	Nucleus	Y	other	
3-hydroxyacyl-CoA dehydrogenase type-2	Hsd17b10	1,6	Cytoplasm		enzyme	Estrogen Biosynthesis; Estrogen-Dependent Breast Cancer Signaling; Ethanol Degradation II; Fatty Acid β -oxidation I; Glutaryl-CoA Degradation; Isoleucine Degradation I; Mitochondrial Dysfunction; Noradrenaline and Adrenaline Degradation; Serotonin Degradation; Tryptophan Degradation III (Eukaryotic)
Heat shock 70 kDa protein 4	Hspa4	6	Cytoplasm	Y	other	Aldosterone Signaling in Epithelial Cells; Androgen Signaling; eNOS Signaling; Glucocorticoid Receptor Signaling; Huntington's Disease Signaling; Protein Ubiquitination Pathway; Unfolded protein response
78 kDa glucose-regulated protein	Hspa5		Cytoplasm	Y	enzyme	Aldosterone Signaling in Epithelial Cells; EIF2 Signaling; Endoplasmic Reticulum Stress Pathway; eNOS Signaling; Glucocorticoid Receptor Signaling; Huntington's Disease Signaling; Protein Ubiquitination Pathway; Unfolded protein response
Heat shock cognate 71 kDa protein	Hspa8	6	Cytoplasm	Y	enzyme	Aldosterone Signaling in Epithelial Cells; Clathrin-mediated Endocytosis Signaling; eNOS Signaling; Glucocorticoid Receptor Signaling; Huntington's Disease Signaling; Protein Ubiquitination Pathway; Unfolded protein response
Stress-70 protein, mitochondrial	Hspa9	6	Cytoplasm	Y	other	Aldosterone Signaling in Epithelial Cells; eNOS Signaling; Glucocorticoid Receptor Signaling; Huntington's Disease Signaling; Protein Ubiquitination Pathway; Unfolded protein response
60 kDa heat shock protein, mitochondrial	Hspd1	6	Cytoplasm	Y	enzyme	Aldosterone Signaling in Epithelial Cells; Protein Ubiquitination Pathway; Type I Diabetes Mellitus Signaling
Isoleucine--tRNA ligase, mitochondrial	Iars2	6	Cytoplasm		enzyme	tRNA Charging
Gamma-interferon-inducible lysosomal thiol reductase	Ifi30		Cytoplasm		enzyme	
Inositol monophosphatase 1	Impa1		Cytoplasm		phosphatase	
Inositol monophosphatase 2	Impa2		Cytoplasm		phosphatase	
Insulinoma-associated protein 1	Insm1		Nucleus		transcription regulator	
Integrin-alpha FG-GAP repeat-containing protein 2	Itfg2		Other	Y	other	
Integrin beta-1	Itgb1		Plasma Membrane	Y	transmembrane receptor	
Integrin beta-2	Itgb2		Plasma Membrane	Y	transmembrane receptor	
Integrin beta-5	Itgb5		Plasma Membrane	Y	other	Caveolar-mediated Endocytosis Signaling; Clathrin-mediated Endocytosis Signaling; Glioma Invasiveness Signaling; HER-2 Signaling in Breast Cancer; IL-8 Signaling; ILK Signaling; Integrin Signaling; Macropinocytosis Signaling; NF- κ B Activation by Viruses; Paxillin Signaling; PPAR α /RXR α Activation; Role of Tissue Factor in Cancer; Virus Entry via Endocytic Pathways

Lysine--tRNA ligase	Kars		Cytoplasm	Y	enzyme	tRNA Charging
Plasma kallikrein	Klkb1		Extracellular Space		peptidase	Acute Phase Response Signaling; Coagulation System; Intrinsic Prothrombin Activation Pathway; MSP-ROn Signaling Pathway
Importin subunit alpha-3	Kpna4		Nucleus	Y	transporter	
Keratin, type I cuticular Ha1	Krt31		Cytoplasm		other	
Keratin, type I cuticular Ha3-I	Krt33a		Cytoplasm		other	
Keratin, type I cuticular Ha3-II	Krt33b		Cytoplasm		other	
Keratin, type I cuticular Ha4	Krt34		Cytoplasm		other	
Keratin, type II cytoskeletal 5	Krt5	5,6	Cytoplasm	Y	other	
Lamin-B receptor	Lbr	1,7	Nucleus	Y	enzyme	Cholesterol Biosynthesis I; Cholesterol Biosynthesis II (via 24,25-dihydrolanosterol); Cholesterol Biosynthesis III (via Desmosterol); Superpathway of Cholesterol Biosynthesis; Zymosterol Biosynthesis
Lamin-B1	Lmnb1	6	Nucleus	Y	other	Granzyme B Signaling; Mechanisms of Viral Exit from Host Cells
Endoplasmic reticulum mannosyl-oligosaccharide 1,2-alpha-mannosidase	Man1b1		Cytoplasm	Y	enzyme	
Matrin-3	Matr3	1,6	Nucleus	Y	other	
DNA replication licensing factor MCM2	Mcm2		Nucleus	Y	enzyme	Cell Cycle Control of Chromosomal Replication
Malate dehydrogenase, cytoplasmic	Mdh1	6	Cytoplasm	Y	enzyme	Aspartate Degradation II; Gluconeogenesis I; TCA Cycle II (Eukaryotic)
Malate dehydrogenase, mitochondrial	Mdh2	5,6,7	Cytoplasm	Y	enzyme	Aspartate Degradation II; Gluconeogenesis I; TCA Cycle II (Eukaryotic)
Transcription factor E2-alpha	Me2		Cytoplasm	Y	enzyme	Gluconeogenesis I
Macrophage migration inhibitory factor	Mif	6	Extracellular Space		cytokine	Eumelanin Biosynthesis; MIF-mediated Glucocorticoid Regulation; MIF Regulation of Innate Immunity; Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis
DNA mismatch repair protein Msh2	Msh2	6	Nucleus	Y	enzyme	Colorectal Cancer Metastasis Signaling; Hereditary Breast Cancer Signaling; Mismatch Repair in Eukaryotes; Ovarian Cancer Signaling; Role of BRCA1 in DNA Damage Response
Moesin	Msn		Plasma Membrane	Y	other	Actin Cytoskeleton Signaling; Agranulocyte Adhesion and Diapedesis; Granulocyte Adhesion and Diapedesis; Leukocyte Extravasation Signaling; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases
C-1-tetrahydrofolate synthase, cytoplasmic	Mthfd1	6	Cytoplasm	Y	enzyme	Folate Polyglutamylatation; Folate Transformations I; Histidine Degradation III; Tetrahydrofolate Salvage from 5,10-methenyltetrahydrofolate
Myosin-binding protein C, fast-type	Mybpc2		Cytoplasm		other	
Myosin-9	Myh9	6	Cytoplasm	Y	enzyme	Actin Cytoskeleton Signaling; Agranulocyte Adhesion and Diapedesis; Calcium Signaling; Cellular Effects

						of Sildenafil (Viagra); Epithelial Adherens Junction Signaling; Hepatic Fibrosis / Hepatic Stellate Cell Activation; ILK Signaling; Tight Junction Signaling
MCG5400	My112a		Cytoplasm		other	Actin Cytoskeleton Signaling; Axonal Guidance Signaling; Cardiac Hypertrophy Signaling; Cdc42 Signaling; Cellular Effects of Sildenafil (Viagra); CXCR4 Signaling; Gα12/13 Signaling; Integrin Signaling; PAK Signaling; Phospholipase C Signaling; Protein Kinase A Signaling; Regulation of Actin-based Motility by Rho; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases; Thrombin Signaling
Myosin regulatory light chain 12B	My112b	6	Cytoplasm	Y	other	Actin Cytoskeleton Signaling; Axonal Guidance Signaling; Cardiac Hypertrophy Signaling; Cdc42 Signaling; Cellular Effects of Sildenafil (Viagra); CXCR4 Signaling; Gα12/13 Signaling; IL-8 Signaling; Integrin Signaling; PAK Signaling; Phospholipase C Signaling; Protein Kinase A Signaling; Regulation of Actin-based Motility by Rho; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases; Thrombin Signaling
Myosin regulatory light polypeptide 9	My19		Cytoplasm	Y	other	Actin Cytoskeleton Signaling; Agranulocyte Adhesion and Diapedesis; Axonal Guidance Signaling; Calcium Signaling; Cardiac Hypertrophy Signaling; Cdc42 Signaling; Cellular Effects of Sildenafil (Viagra); CXCR4 Signaling; Epithelial Adherens Junction Signaling; Gα12/13 Signaling; Hepatic Fibrosis / Hepatic Stellate Cell Activation; IL-8 Signaling; ILK Signaling; Integrin Signaling; PAK Signaling; Phospholipase C Signaling; Protein Kinase A Signaling; Regulation of Actin-based Motility by Rho; RhoA Signaling; RhoGDI Signaling; Signaling by Rho Family GTPases; Thrombin Signaling; Tight Junction Signaling
Nucleosome assembly protein 1-like 1	Nap111	6	Nucleus	Y	other	
Nuclear receptor corepressor 1	Ncor1	1,5,6	Nucleus	Y	transcription regulator	
Nuclear transcription factor Y subunit beta	Nfyb		Nucleus		transcription regulator	
Nipped-B-like protein	Nipbl	5,6	Nucleus	Y	transcription regulator	
Nucleoside diphosphate kinase A	Nme1	6	Cytoplasm	Y	kinase	Granzyme A Signaling; Pyrimidine Deoxyribonucleotides De Novo Biosynthesis I; Pyrimidine Ribonucleotides De Novo Biosynthesis; Pyrimidine Ribonucleotides Interconversion; Remodeling of Epithelial Adherens Junctions; Salvage Pathways of Pyrimidine Ribonucleotides
Nucleoside diphosphate kinase B	Nme2		Nucleus		kinase	Pyrimidine Deoxyribonucleotides De Novo Biosynthesis I; Pyrimidine Ribonucleotides De Novo Biosynthesis; Pyrimidine Ribonucleotides Interconversion; Salvage Pathways of Pyrimidine Ribonucleotides
Nucleolar protein 56	Nop56	6	Nucleus	Y	other	
Nucleolar protein 58	Nop58	1,6	Nucleus	Y	enzyme	
Nitrogen permease regulator 3-like protein	Nprl3		Other	Y	other	
Cytosolic 5'-nucleotidase 3A	Nt5c		Cytoplasm	Y	phosphatase	

Netrin-G2	Ntng2		Plasma Membrane		other	
Nuclear pore complex protein Nup155	Nup155	6,7	Nucleus	Y	transporter	
Nuclear pore complex protein Nup54	Nup54	1,2,6	Nucleus		transporter	
Nuclear pore glycoprotein p62	Nup62	1,2,6,7	Nucleus	Y	transporter	
2-oxoglutarate dehydrogenase, mitochondrial	Ogdh		Cytoplasm	Y	enzyme	2-ketoglutarate Dehydrogenase Complex; Mitochondrial Dysfunction; TCA Cycle II (Eukaryotic)
Protein-arginine deiminase type-2	Padi2		Cytoplasm		enzyme	Protein Citrullination
Platelet-activating factor acetylhydrolase IB subunit alpha	Pafah1b1		Cytoplasm	Y	enzyme	Antioxidant Action of Vitamin C; Atherosclerosis Signaling; Eicosanoid Signaling; Endothelin-1 Signaling; Phospholipases; Reelin Signaling in Neurons; Role of MAPK Signaling in the Pathogenesis of Influenza; Sperm Motility; Synaptic Long Term Depression
Protein DJ-1	Park7	6	Nucleus	Y	enzyme	Glutaryl-CoA Degradation; Mitochondrial Dysfunction; Parkinson's Signaling; Tryptophan Degradation III (Eukaryotic)
Protein piccolo	Pelo	2,5,7	Cytoplasm	Y	transporter	
Protein disulfide-isomerase A3	Pdia3		Cytoplasm	Y	peptidase	14-3-3-mediated Signaling; Aldosterone Signaling in Epithelial Cells; Antigen Presentation Pathway; Antioxidant Action of Vitamin C; Axonal Guidance Signaling; Cardiac Hypertrophy Signaling; Cellular Effects of Sildenafil (Viagra); CREB Signaling in Neurons; Dendritic Cell Maturation; Dopamine-DARPP32 Feedback in cAMP Signaling; Endothelin-1 Signaling; Gap Junction Signaling; Glioblastoma Multiforme Signaling; Glutathione Redox Reactions II; GPCR-Mediated Integration of Enteroendocrine Signaling Exemplified by an L Cell; GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells; Leptin Signaling in Obesity; Lipid Antigen Presentation by CD1; Melatonin Signaling; Neuropathic Pain Signaling In Dorsal Horn Neurons; P2Y Purigenic Receptor Signaling Pathway; p70S6K Signaling; Phagosome Formation; Phospholipases; PI3K Signaling in B Lymphocytes; PPAR α /RXR α Activation; Protein Kinase A Signaling; Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis; Role of NFAT in Cardiac Hypertrophy; Sperm Motility; Sphingosine-1-phosphate Signaling; Synaptic Long Term Depression; Synaptic Long Term Potentiation; Thrombin Signaling; UVA-Induced MAPK Signaling; Wnt/Ca ⁺ pathway
Phosphatidylethanolamine-binding protein 1	Pebp1	6	Cytoplasm	Y	other	Phospholipase C Signaling
ATP-dependent 6-phosphofructokinase, platelet type	Pfkip		Cytoplasm	Y	kinase	AMPK Signaling; Glycolysis I; TR/RXR Activation
Profilin-1	Pfn1	6	Cytoplasm	Y	other	Actin Cytoskeleton Signaling; Axonal Guidance Signaling; Integrin Signaling; PCP pathway; Regulation of Actin-based Motility by Rho; RhoA Signaling
Phosphoglycerate kinase 1	Pgk1	2,6,7	Cytoplasm	Y	kinase	Gluconeogenesis I; Glycolysis I

			sm			
Phosphoglucomutase-2	Pgm1	5	Cytoplasm	Y	enzyme	GDP-glucose Biosynthesis; Glucose and Glucose-1-phosphate Degradation; Glycogen Degradation II; Glycogen Degradation III
Phosphoglucomutase-2	Pgm2	5	Cytoplasm	Y	enzyme	GDP-glucose Biosynthesis; Glucose and Glucose-1-phosphate Degradation; Glycogen Degradation II; Glycogen Degradation III; Purine Ribonucleosides Degradation to Ribose-1-phosphate
Prohibitin-2	Phb		Nucleus	Y	transcription regulator	
Pyruvate kinase PKM	Pkm	2	Cytoplasm	Y	kinase	Glycolysis I; Type II Diabetes Mellitus Signaling
Inorganic pyrophosphatase	Ppa1	6	Cytoplasm	Y	enzyme	
Peptidyl-prolyl cis-trans isomerase B	Ppib	2,6	Cytoplasm		enzyme	Activation of IRF by Cytosolic Pattern Recognition Receptors; NRF2-mediated Oxidative Stress Response
Serine/threonine-protein phosphatase PP1-alpha catalytic subunit	Ppp1ca	6	Cytoplasm	Y	phosphatase	
Protein phosphatase 1 regulatory subunit 7	Ppp1r7		Nucleus	Y	phosphatase	
Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	Ppp2r1a	1b,6	Cytoplasm		phosphatase	
Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	Ppp2r1b	6	Plasma Membrane		phosphatase	
Serine/threonine-protein phosphatase 2A activator	Ppp2r4					
Peroxiredoxin-1	Prdx1	6	Cytoplasm	Y	enzyme	NRF2-mediated Oxidative Stress Response; Phagosome Maturation
Peroxiredoxin-6	Prdx6	6	Cytoplasm	Y	enzyme	Glutathione Redox Reactions I; Heparan Sulfate Biosynthesis; Heparan Sulfate Biosynthesis (Late Stages); Phagosome Maturation; Triacylglycerol Degradation
cAMP-dependent protein kinase type I-alpha regulatory subunit	Prkar1a		Cytoplasm	Y	kinase	AMPK Signaling; Amyloid Processing; Androgen Signaling; Axonal Guidance Signaling; BMP signaling pathway; Breast Cancer Regulation by Stathmin1; Calcium Signaling; cAMP-mediated signaling; Cardiac Hypertrophy Signaling; Cardiac β -adrenergic Signaling; CDK5 Signaling; Cellular Effects of Sildenafil (Viagra); Colorectal Cancer Metastasis Signaling; Corticotropin Releasing Hormone Signaling; CREB Signaling in Neurons; Dopamine-DARPP32 Feedback in cAMP Signaling; Dopamine Receptor Signaling; eNOS Signaling; ERK/MAPK Signaling; Gap Junction Signaling; G Beta Gamma Signaling; GNRH Signaling; GPCR-Mediated Integration of Enteroendocrine Signaling Exemplified by an L Cell; GPCR-Mediated Nutrient Sensing in Enteroendocrine Cells; G-Protein Coupled Receptor

						Signaling; Gustation Pathway; Gai Signaling; Gαs Signaling; Hepatic Cholestasis; IGF-1 Signaling; IL-1 Signaling; Insulin Receptor Signaling; Leptin Signaling in Obesity; Melanocyte Development and Pigmentation Signaling; Melatonin Signaling; Molecular Mechanisms of Cancer; Netrin Signaling; Neuropathic Pain Signaling In Dorsal Horn Neurons; Neuroprotective Role of THOP1 in Alzheimer's Disease; Nitric Oxide Signaling in the Cardiovascular System; Ovarian Cancer Signaling; P2Y Purigenic Receptor Signaling Pathway; Phototransduction Pathway; PPARα/RXRα Activation; Protein Kinase A Signaling; PXR/RXR Activation; RAR Activation; Relaxin Signaling; Renin-Angiotensin Signaling; Role of NFAT in Cardiac Hypertrophy; Sertoli Cell-Sertoli Cell Junction Signaling; Sonic Hedgehog Signaling; Sperm Motility; Synaptic Long Term Potentiation; Tight Junction Signaling; α-Adrenergic Signaling
Pre-mRNA-processing-splicing factor 8	Prpf8		Nucleus	Y	other	Systemic Lupus Erythematosus Signaling
Ribose-phosphate pyrophosphokinase 1	Prps1	6	Cytoplasm		kinase	PRPP Biosynthesis I
Phosphoribosyl pyrophosphate synthetase 1-like 3	Prps113		Other		kinase	PRPP Biosynthesis I
Protein PRRC2C	Prrc2c	1,5,6	Other	Y	other	
MCG124046	Prss1		Extracellular Space		peptidase	Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis
Phosphoserine aminotransferase	Psat1		Cytoplasm	Y	enzyme	Serine Biosynthesis; Superpathway of Serine and Glycine Biosynthesis I
Proteasome subunit alpha type-2	Psm2		Cytoplasm	Y	peptidase	Protein Ubiquitination Pathway
Proteasome subunit alpha type-5	Psm5	6	Cytoplasm	Y	peptidase	Protein Ubiquitination Pathway
Proteasome subunit alpha type-7-like	Psm8		Nucleus		peptidase	Huntington's Disease Signaling; Polyamine Regulation in Colon Cancer
Proteasome subunit beta type-1	Psm1	7	Cytoplasm	Y	peptidase	Protein Ubiquitination Pathway
Proteasome subunit beta type-10	Psm10		Cytoplasm		peptidase	Protein Ubiquitination Pathway
Proteasome subunit beta type-3	Psm3		Cytoplasm		peptidase	Protein Ubiquitination Pathway
Proteasome subunit beta type-4	Psm4		Cytoplasm	Y	peptidase	Protein Ubiquitination Pathway
26S proteasome non-ATPase regulatory subunit 2	Psm2	6	Cytoplasm	Y	other	Protein Ubiquitination Pathway

Proteasome activator complex subunit 1	Psmc1	1,6	Cytoplasm		other	Huntington's Disease Signaling; Polyamine Regulation in Colon Cancer; Protein Ubiquitination Pathway
Serine/threonine-protein phosphatase 2A activator	Ptpa		Cytoplasm		phosphatase	
Tyrosine-protein phosphatase non-receptor type 6	Ptpn6		Cytoplasm	Y	phosphatase	
Poly(U)-binding-splicing factor PUF60	Puf60	1b,6	Nucleus	Y	other	
Pyrin and HIN domain-containing protein 1	Pyhin1					
Ras-related protein Rab-10	Rab10		Cytoplasm		enzyme	
Ras-related protein Rab-1A	Rab1a		Cytoplasm	Y	enzyme	
Ras-related protein Rab-1A	Rab1A			Y		
Ras-related protein Rab-1B	Rab1b		Cytoplasm		other	
Ras-related protein Rab-2A	Rab2a		Cytoplasm		enzyme	
Ras-related C3 botulinum toxin substrate 1	Rac1		Plasma Membrane		enzyme	Actin Cytoskeleton Signaling; Actin Nucleation by ARP-WASP Complex; Agrin Interactions at Neuromuscular Junction; Amyotrophic Lateral Sclerosis Signaling; Axonal Guidance Signaling; B Cell Receptor Signaling; Breast Cancer Regulation by Stathmin1; CCR3 Signaling in Eosinophils; CD28 Signaling in T Helper Cells; Clathrin-mediated Endocytosis Signaling; Colorectal Cancer Metastasis Signaling; CXCR4 Signaling; Ephrin A Signaling; Ephrin B Signaling; Ephrin Receptor Signaling; Epithelial Adherens Junction Signaling; ERK/MAPK Signaling; FAK Signaling; Fc Epsilon RI Signaling; Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes; FGF Signaling; fMLP Signaling in Neutrophils; GDNF Family Ligand-Receptor Interactions; Germ Cell-Sertoli Cell Junction Signaling; Glioblastoma Multiforme Signaling; Glucocorticoid Receptor Signaling; GNRH Signaling; HGF Signaling; HMGB1 Signaling; iCOS-iCOSL Signaling in T Helper Cells; IL-3 Signaling; IL-8 Signaling; Integrin Signaling; Leukocyte Extravasation Signaling; LPS-stimulated MAPK Signaling; Macropinocytosis Signaling; Molecular Mechanisms of Cancer; mTOR Signaling; Natural Killer Cell Signaling; Netrin Signaling; NGF Signaling; PAK Signaling; Pancreatic Adenocarcinoma Signaling; Paxillin Signaling; PCP pathway; PEDF Signaling; Phospholipase C Signaling; PI3K Signaling in B Lymphocytes; PKCθ Signaling in T Lymphocytes; Production of Nitric Oxide and Reactive Oxygen Species in Macrophages; PTEN Signaling; Rac Signaling; RAR Activation; Regulation of Actin-based Motility by Rho; Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes; Renal Cell Carcinoma Signaling; Renin-Angiotensin Signaling; RhoGDI Signaling; Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis; Role of p14/p19ARF in Tumor Suppression; Role of Tissue Factor in Cancer; SAPK/JNK Signaling; Semaphorin Signaling in Neurons; Sertoli Cell-Sertoli Cell

						Junction Signaling; Signaling by Rho Family GTPases; Sphingosine-1-phosphate Signaling; STAT3 Pathway; T Cell Receptor Signaling; Tight Junction Signaling; Virus Entry via Endocytic Pathways
Receptor of activated protein C kinase 1	Rack1		Cytoplasm	Y	enzyme	Androgen Signaling; Antiproliferative Role of Somatostatin Receptor 2; Axonal Guidance Signaling; Breast Cancer Regulation by Stathmin1; Cardiac Hypertrophy Signaling; Cardiac β -adrenergic Signaling; CCR3 Signaling in Eosinophils; CCR5 Signaling in Macrophages; Colorectal Cancer Metastasis Signaling; CREB Signaling in Neurons; CXCR4 Signaling; Ephrin B Signaling; Ephrin Receptor Signaling; fMLP Signaling in Neutrophils; G Beta Gamma Signaling; GM-CSF Signaling; G Protein Signaling Mediated by Tubby; G α i Signaling; G α q Signaling; G α s Signaling; Huntington's Disease Signaling; IL-1 Signaling; IL-8 Signaling; P2Y Purigenic Receptor Signaling Pathway; Phospholipase C Signaling; Protein Kinase A Signaling; Relaxin Signaling; RhoGDI Signaling; Role of NFAT in Cardiac Hypertrophy; Role of NFAT in Regulation of the Immune Response; Signaling by Rho Family GTPases; Tec Kinase Signaling; Thrombin Signaling; α -Adrenergic Signaling
UV excision repair protein RAD23 homolog B	Rad23b	6	Nucleus	Y	other	Nucleotide Excision Repair Pathway
GTP-binding nuclear protein Ran	Ran	2,6	Nucleus	Y	enzyme	RAN Signaling; Sumoylation Pathway
Ras-related protein Rap-1A	Rap1a	6	Cytoplasm		enzyme	Axonal Guidance Signaling; B Cell Receptor Signaling; Calcium Signaling; cAMP-mediated signaling; Corticotropin Releasing Hormone Signaling; Ephrin Receptor Signaling; Epithelial Adherens Junction Signaling; ERK/MAPK Signaling; G-Protein Coupled Receptor Signaling; G α i Signaling; G α s Signaling; HGF Signaling; Integrin Signaling; Leukocyte Extravasation Signaling; Molecular Mechanisms of Cancer; NGF Signaling; Phospholipase C Signaling; Production of Nitric Oxide and Reactive Oxygen Species in Macrophages; Protein Kinase A Signaling; Relaxin Signaling; Renal Cell Carcinoma Signaling; Synaptic Long Term Potentiation
Ras-related protein Rap-1b	Rap1b		Cytoplasm	Y	enzyme	Antiproliferative Role of Somatostatin Receptor 2; Axonal Guidance Signaling; B Cell Receptor Signaling; Calcium Signaling; Corticotropin Releasing Hormone Signaling; Ephrin Receptor Signaling; Epithelial Adherens Junction Signaling; ERK/MAPK Signaling; HGF Signaling; Integrin Signaling; Leukocyte Extravasation Signaling; Molecular Mechanisms of Cancer; NGF Signaling; Phospholipase C Signaling; Production of Nitric Oxide and Reactive Oxygen Species in Macrophages; Protein Kinase A Signaling; Relaxin Signaling; Synaptic Long Term Potentiation
Arginine--tRNA ligase, cytoplasmic	Rars	6	Cytoplasm		enzyme	tRNA Charging
Protein RCC2	Rcc2	1b,6	Nucleus	Y	other	
Transforming protein RhoA	Rhoa		Cytoplasm	Y	enzyme	Actin Cytoskeleton Signaling; Actin Nucleation by ARP-WASP Complex; Axonal Guidance Signaling; Breast Cancer Regulation by Stathmin1; Cardiac Hypertrophy Signaling; CCR3 Signaling in Eosinophils; Chemokine Signaling; Cholecystokinin/Gastrin-mediated Signaling; Colorectal Cancer Metastasis Signaling; CXCR4 Signaling; Ephrin A Signaling; Ephrin B Signaling; Ephrin Receptor Signaling; Epithelial Adherens Junction Signaling; Germ Cell-Sertoli Cell Junction Signaling; Glioblastoma Multiforme Signaling; Glioma Invasiveness Signaling; G α 12/13 Signaling; G α q Signaling; HMGB1 Signaling; IL-8 Signaling; ILK Signaling; Integrin Signaling; Leukocyte Extravasation

						Signaling; Macropinocytosis Signaling; Molecular Mechanisms of Cancer; mTOR Signaling; NGF Signaling; PCP pathway; PEDF Signaling; Phagosome Formation; Phospholipase C Signaling; Production of Nitric Oxide and Reactive Oxygen Species in Macrophages; Protein Kinase A Signaling; Rac Signaling; Regulation of Actin-based Motility by Rho; Regulation of the Epithelial-Mesenchymal Transition Pathway; RhoA Signaling; RhoGDI Signaling; Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis; Semaphorin Signaling in Neurons; Signaling by Rho Family GTPases; Sphingosine-1-phosphate Signaling; Sumoylation Pathway; Tec Kinase Signaling; Thrombin Signaling; Tight Junction Signaling
60S ribosomal protein L10	Rpl10	1b	Cytoplasm		other	EIF2 Signaling
60S ribosomal protein L13	Rpl13		Nucleus	Y	other	EIF2 Signaling
60S ribosomal protein L14	Rpl14		Cytoplasm	Y	other	EIF2 Signaling
60S ribosomal protein L17	Rpl17		Cytoplasm		other	EIF2 Signaling
60S ribosomal protein L18	Rpl18		Cytoplasm	Y	other	EIF2 Signaling
60S ribosomal protein L19	Rpl19	6	Cytoplasm	Y	other	EIF2 Signaling
60S ribosomal protein L21	Rpl21	6	Cytoplasm		other	EIF2 Signaling
60S ribosomal protein L27	Rpl27		Cytoplasm		other	EIF2 Signaling
60S ribosomal protein L27a	Rpl27a	6	Cytoplasm	Y	other	EIF2 Signaling
60S ribosomal protein L34	Rpl34		Cytoplasm	Y	other	EIF2 Signaling
60S ribosomal protein L35a	Rpl35a		Cytoplasm		other	EIF2 Signaling
60S ribosomal protein L37a	Rpl37a		Cytoplasm		other	EIF2 Signaling
60S ribosomal protein L4	Rpl4	6	Cytoplasm	Y	enzyme	EIF2 Signaling
60S ribosomal protein L8	Rpl8	1	Cytoplasm		other	EIF2 Signaling
Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	Rpn2		Cytoplasm		enzyme	

40S ribosomal protein S11	Rps11		Cytoplasm	Y	other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
40S ribosomal protein S13	Rps13		Cytoplasm	Y	other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
40S ribosomal protein S14	Rps14		Cytoplasm	Y	translation regulator	
40S ribosomal protein S26	Rps26		Cytoplasm	Y	other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
Ubiquitin-40S ribosomal protein S27a	Rps27a	6	Cytoplasm	Y	other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
40S ribosomal protein S3	Rps3	6	Cytoplasm	Y	enzyme	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
40S ribosomal protein S7	Rps7		Cytoplasm		other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
40S ribosomal protein S8	Rps8		Cytoplasm	Y	other	EIF2 Signaling; mTOR Signaling; Regulation of eIF4 and p70S6K Signaling
40S ribosomal protein S9	Rps9		Cytoplasm	Y	translation regulator	
RuvB-like 2	Ruvbl2	6	Nucleus	Y	transcription regulator	
SUMO-activating enzyme subunit 1	Sae1	6	Cytoplasm	Y	enzyme	Sumoylation Pathway
Serologically defined colon cancer antigen 3 homolog	Sdccag3	6	Cytoplasm	Y	other	
MKIAA0079 protein	Sec24c	1	Cytoplasm	Y	transporter	
Protein transport protein Sec31A	Sec31a	1,6	Cytoplasm	Y	other	
Septin-1	Sept1		Cytoplasm	Y	other	
Septin-7	Sept7		Cytoplasm	Y	other	RhoA Signaling; Signaling by Rho Family GTPases
Plasminogen activator inhibitor 1 RNA-binding protein	Serbp1	6	Cytoplasm	Y	other	Sumoylation Pathway
Splicing factor 3B subunit 3	Sf3b3	6	Nucleus	Y	other	Spliceosomal Cycle
Calcium-binding mitochondrial carrier protein Aralar1	Slc25a12		Cytoplasm		transporter	
ADP/ATP translocase 2	Slc25a5	1b,5	Cytopla		transporter	

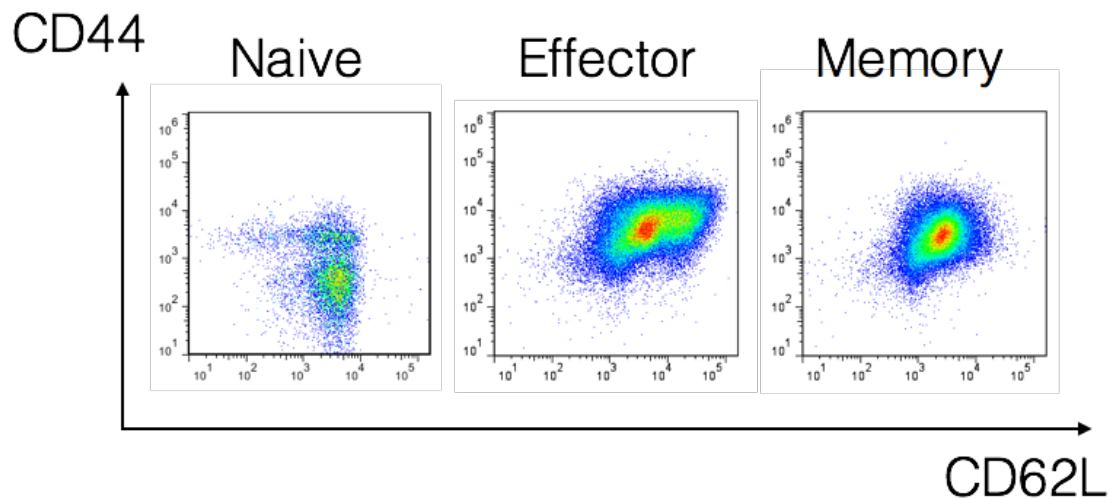
			sm			
Structural maintenance of chromosomes flexible hinge domain-containing protein 1	Smchd1	1b,6	Nucleus	Y	other	
Staphylococcal nuclease domain-containing protein 1	Snd1	6	Nucleus	Y	enzyme	
Spectrin alpha chain, non-erythrocytic 1	Sptan1	6	Plasma Membrane	Y	other	Apoptosis Signaling; Death Receptor Signaling; Sertoli Cell-Sertoli Cell Junction Signaling; Tight Junction Signaling
Tyrosine-protein kinase Srms	Srm		Cytoplasm	Y	enzyme	Spermidine Biosynthesis I
Serine/arginine-rich splicing factor 3	Srsf3	6	Nucleus	Y	other	
SOSS complex subunit B2	Ssb	1b,6	Nucleus		enzyme	
Succinate--CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	Suclg1	6	Cytoplasm		enzyme	TCA Cycle II (Eukaryotic)
Suppressor of Ty16	Supt16		Nucleus		transcription regulator	
FACT complex subunit SPT16	Supt16h	6		Y		
Heterogeneous nuclear ribonucleoprotein Q	Syncrip	6	Nucleus	Y	other	
Transgelin-2	Tagln2	6	Cytoplasm	Y	other	
Transaldolase	Taldo1	6	Cytoplasm	Y	enzyme	Pentose Phosphate Pathway; Pentose Phosphate Pathway (Non-oxidative Branch)
T-complex protein 1 subunit alpha	Tcp1	6	Cytoplasm	Y	other	
THO complex subunit 3	Thoc3	6	Nucleus		other	
Transketolase	Tkt	6	Cytoplasm	Y	enzyme	Pentose Phosphate Pathway; Pentose Phosphate Pathway (Non-oxidative Branch)
Talin-1	Tln1	6	Plasma Membrane	Y	other	Actin Cytoskeleton Signaling; Crosstalk between Dendritic Cells and Natural Killer Cells; ERK/MAPK Signaling; FAK Signaling; Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes; Integrin Signaling; Paxillin Signaling; Regulation of Cellular Mechanics by Calpain Protease
DNA topoisomerase 2-alpha	Top2a		Nucleus	Y	enzyme	Cell Cycle: G2/M DNA Damage Checkpoint Regulation; Cell Cycle Control of Chromosomal Replication
Triosephosphate isomerase	Tpi1	6	Cytopla	Y	enzyme	Glycolysis I; Sucrose Degradation V (Mammalian)

			sm			
Tropomyosin alpha-3 chain	Tpm3		Cytoplasm	Y	other	Calcium Signaling
Uncharacterized protein	Tpm3-rs7		Other		other	
Transformer-2 protein homolog beta	Tra2b	6	Nucleus	Y	other	
Tripartite motif-containing protein 44	Trim44		Cytoplasm	Y	other	
Tubulin alpha-1B chain	Tuba1b		Cytoplasm	Y	other	14-3-3-mediated Signaling; Axonal Guidance Signaling; Breast Cancer Regulation by Stathmin1; Epithelial Adherens Junction Signaling; Gap Junction Signaling; Germ Cell-Sertoli Cell Junction Signaling; Phagosome Maturation; Remodeling of Epithelial Adherens Junctions; Sertoli Cell-Sertoli Cell Junction Signaling
Tubulin beta-4B chain	Tubb4b		Cytoplasm	Y	other	14-3-3-mediated Signaling; Axonal Guidance Signaling; Breast Cancer Regulation by Stathmin1; Epithelial Adherens Junction Signaling; Gap Junction Signaling; Germ Cell-Sertoli Cell Junction Signaling; Phagosome Maturation; Remodeling of Epithelial Adherens Junctions; Sertoli Cell-Sertoli Cell Junction Signaling
Tubulin beta-5 chain	Tubb5		Cytoplasm	Y	other	14-3-3-mediated Signaling; Axonal Guidance Signaling; Breast Cancer Regulation by Stathmin1; Epithelial Adherens Junction Signaling; Gap Junction Signaling; Germ Cell-Sertoli Cell Junction Signaling; Phagosome Maturation; Remodeling of Epithelial Adherens Junctions; Sertoli Cell-Sertoli Cell Junction Signaling
Twinfilin-2	Twf2		Cytoplasm	Y	kinase	
Thioredoxin	Txn	6				
Splicing factor U2AF 65 kDa subunit	U2af2	6	Nucleus	Y	other	
Ubiquitin-like modifier-activating enzyme 1	Uba1	6	Cytoplasm	Y	enzyme	Protein Ubiquitination Pathway
Ubiquitin-associated protein 2-like	Uba2	1,4,5,6	Nucleus	Y	other	
UDP-glucose:glycoprotein glucosyltransferase 1	Uggt1		Cytoplasm	Y	enzyme	
Versican core protein	Vcan		Extracellular Space	Y	other	
Transitional endoplasmic reticulum ATPase	Vcp	6	Cytoplasm	Y	enzyme	NRF2-mediated Oxidative Stress Response; Unfolded protein response

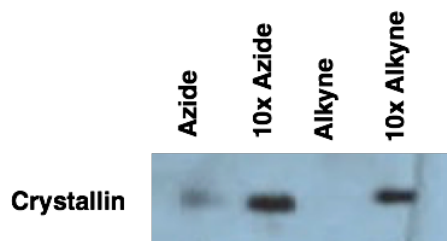
Voltage-dependent anion-selective channel protein 3	Vdac3	1,5,6	Cytoplasm	Y	ion channel	Mitochondrial Dysfunction
WD repeat-containing protein 1	Wdr1		Extracellular Space	Y	other	
Exportin-4	Xpo4		Nucleus	Y	transporter	
14-3-3 protein zeta/delta	Ywhaz	6	Cytoplasm	Y	enzyme	14-3-3-mediated Signaling; Cell Cycle: G2/M DNA Damage Checkpoint Regulation; ERK/MAPK Signaling; ERK5 Signaling; HIPPO signaling; IGF-1 Signaling; Myc Mediated Apoptosis Signaling; p70S6K Signaling; PI3K/AKT Signaling; Protein Kinase A Signaling
Zinc finger MIZ domain-containing protein 1	Zmiz1		Nucleus		other	
E3 ubiquitin-protein ligase Zswim2	Zswim2		Other		enzyme	

Figures

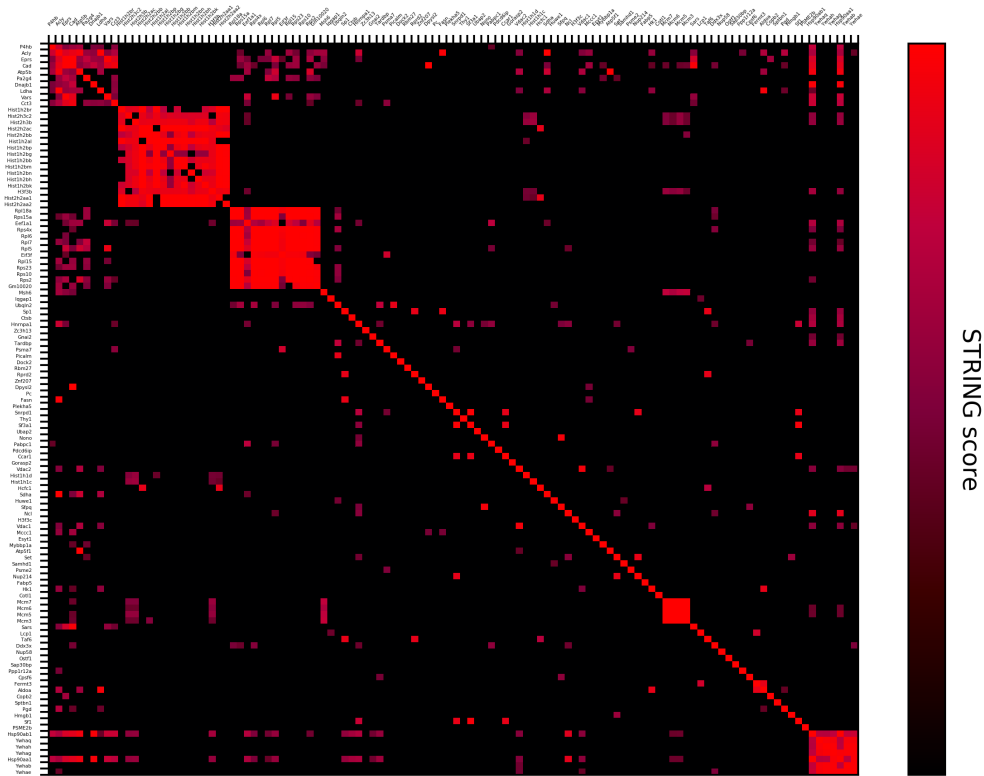
S1. Flow cytometry analysis of *in vitro* differentiated T cells. - Representative dot plot of flow cytometry results showing MFI for CD62L (AlexaFluor 700) vs CD44 (FITC) staining of Naive (total splenocytes), IL-2-treated effector CD8 T cells and IL-15/7-treated memory CD8 T cells.



S2. Comparison between UDP-GalNAz and UDP-GalNAI donors for in solution protein labeling – Western Blot of α -crystallin treated with GalT1 and azide or alkyne-containing UDP donors, followed by reacting with either biotin-accelerated azide or biotin-alkyne via CuAAC.¹



S3. Correlation matrix of all confident proteins. Red to black gradient showing high to low correlation between each protein pair. Data was clustered by K-mean algorithm using $p=15$. Approximately 7 strongly correlated clusters were found.



Materials and Methods

1. General Methods and Materials

All reagents and solvents were acquired from Fisher Scientific unless otherwise stated. PBS and RPMI Media were acquired from Gibco, fluorescently labelled antibodies were obtained from Biolegend, and cytokines were purchased from Gemini. Electrophoresis and Western Blotting were done using BioRad equipment. Primary antibodies were obtained from various sources: O-GlcNAc antibody from Biolegend, GAPDH antibody from R&D Systems, and PARP antibody from TACS. Flow cytometry analysis was performed in an Attune Attune™ NxT Acoustic Focusing Cytometer. FACS data analysis was completed using Flowjo analysis software. Photomicrographs were acquired on a BZ-X700 Keyence Microscope and all images processed using ImageJ software. Statistical analysis and graph generation was completed using GraphPad Prism software.

2. Mice

All animal studies were carried out under a protocol approved by the Institutional Animal Care And Use Committee at The Scripps Research Institute (TSRI). C57BL/6 mice were used for all *in vivo* differentiation studies and OT-1+/- Rag +/- were used for *in vitro* differentiation experiments at 6 to 10 weeks of age. Mice were bred and housed in the vivarium of The Scripps Research Institute.

3. Bacterial infection

Listeria monocytogenes (LM) was grown in Brain Heart Infusion (BHI) Broth from a stock inoculum on day of infection. Bacteria were cultured at 37°C until OD₆₀₀=0.08-0.2, and then diluted for infection in PBS. 10,000 LM were introduced to C57BL/6 mice via Intraperitoneal Injection.

4. T cell isolation and *in vitro* differentiation

Naive mice or mice at peak of infection (7 dpi) were euthanized and the spleen collected. Spleen pieces were suspended in 2 mL culture media (RPMI, 10% FCS, 10 mM HEPES, 1 mM Sodium pyruvate, P/S, 100x non-essential amino acids (Gibco), and 55 μM mercaptoethanol) and passed through a 75 μm strainer. 2 mL of 2x Red Blood Cell lysis buffer was added and cells were incubated at room temperature for 15 min. The cell suspension was centrifuged (5 min-300 x g) and the pellet was suspended in PBS and centrifuged again (5 min-300 x g). Cells were then suspended in culture media and 1 nM of SIINFEKL peptide was added. Cells were grown at 37°C for 3 days after which cells were collected and enriched for CD8+ T cells as follows. Cells were collected by centrifugation (5 min-300 x g), washed with PBS and counted. Cells were diluted to 100 M/mL in PBS containing a cocktail of depletion antibodies prepared in house (5 μg each of PK135 for NK1.1, GK1.5 for CD4, M1/70 for CD11b, RA3-6B2 for B220, MS114 for MHCII, Ter119 (Biolegend) for RBC) and incubated with rotation at 4°C for 1 hr. Cells were collected by centrifugation (5 min-300 x g) and washed once with PBS. Pelleted cells were then diluted to 100 M/mL in PBS containing pre-washed Sheep anti-Rat IgG-Dynabeads (Invitrogen) in a 1 bead/cell concentration and incubated with rotation at 4°C for 1 hr. Dynabeads were then removed by magnetic-based separation and cells were then concentrated by centrifugation (5 min-300 x g). Pelleted cells were suspended in culture media and split in half. IL-2 (10 ng/mL), or IL-15 (20 ng/mL) and IL-7 (10 ng/mL) were added to the media, and cells were then grown for 4 days at 37°C.

5. Flow Cytometry

Cells were washed with PBS and then stained with CD62L-AlexaFluor 700 and CD44-FITC antibodies (1:200 μL in PBS) for 30 min at 4°C. Cells were centrifuged (400 x g for 4 min) and washed with PBS. Cells were suspended in PBS with DAPI and analysed by flow cytometry directly.

6. Cell lysis and subcellular fractionation

Cells were lysed with ThermoFisher's Subcellular Protein Fractionation Kit for Cultured Cells according to manufacturer's protocol. In short, 200 μ L ice cold CEB buffer with protease inhibitors was added to a 20 μ L cell pellet and incubated at 4°C for 10 min. Suspension was centrifuged at 500 x g for 5 min. The supernatant was used as the cytoplasmic fraction and 200 μ L of ice cold MEB buffer with protease inhibitors was added to the pellet. The suspension was vortexed for 5 sec and then incubated at 4°C for 10 min, followed by centrifugation at 3000 x g for 5 mins. The supernatant was used as the membrane fraction and 100 μ L of ice cold NEB buffer with protease inhibitors was added to the pellet. The suspension was vortexed for 15 sec and then incubated at 4°C for 30 min. The suspension was finally centrifuged at 5000 x g for 5 mins and the supernatant used as the nuclear fraction. No further fractionation was done to the pellet.

7. Immunoblotting

Cell lysates were run in 4-12% Bis-Tris gels (Novex) using MES running buffer for 1.5 hrs at 100 V. Gels were then transferred using Novex Blotting buffer onto Nitrocellulose membranes at 300 mA for 2hrs. Membranes were blocked for 1 hr at room temperature using 5% powdered non-fat milk in TBST, and subsequently incubated overnight with primary antibodies (1:100 in 5% milk-TBST) at 4°C with shaking. Membranes were washed 3 x 5 min with TBST at room temperature with shaking and then incubated with HRP-labelled antibody (1:3000 in 5% milk-TBST) for 1 hr under the same conditions. Membranes were then washed 3 x 5 min with TBST and SuperSignal West Pico Chemiluminescent Substrate (ThermoFisher) for 3 min and subjected to X-ray exposure.

8. Histology and O-GlcNAc CHoMP

Mice were euthanized and the spleen was harvested and immediately immersed in Zinc Formalin fixative for 24 hrs. Tissues were paraffin embedded by TSRI's Histology Core Facility, and then sectioned on a microtome at 5 μ m. Sections were deparaffinised and rehydrated according to standard protocols. In summary, the slides were sequentially immersed in coplin jars for 5 min in 40 mL: 2x Histo-Clear II (National Diagnostics), 2x Ethanol, 2x 70% Ethanol, 2x 50% Ethanol and d.H₂O. A hydrophobic barrier was drawn around the tissue samples on the slides using a PAP pen. Tissues were immersed in 50 mL of TBS + 0.1% Tween-20 (TBST) for 10 minutes. Slides were then placed in a humidified chamber and 100 μ L of PNGaseF NEB standard reaction solution was added (1x G1 buffer, 1% NP-40, and 2 μ L PNGaseF). Slides were incubated overnight at 37°C, followed by TBST wash in coplin jars (3 x 5 min). Slides were then placed again in a humidified chamber and 100 μ L of enzyme solution was added. The enzyme solution contained: 25 μ g/mL of GalT1, 500 μ M UDP-Gal-alkyne, 1 mM MnCl₂, 50 mM NaCl, 20 mM HEPES, 2% NP-40, pH7.9). The slides were incubated for 3 hr at 4°C. The slides were then washed with TBST in coplin jars (3 x 5 min). The slides were again placed in a humidified chamber and blocked with avidin/biotin according to standard procedures. In short, 1 drop of avidin solution (Biolegend kit) was added to the tissue and the slides were incubated at room temperature for 10 min, followed by TBST wash (3 x 3 min each). 1 drop of biotin solution was then added to the tissue and the slides were incubated at room temperature for 10 mins, followed by TBST wash (3 x 3 min each). The slides were then placed again in a humidified chamber and 'clicked' to a biotin probe with a solution containing: (50 μ M accelerated azide-biotin probe⁸, 75 μ M CuSO₄, 150 μ M BTTP ligand, 2.5 mM sodium ascorbate) in TBST for 1 hr at RT. Following three washes in TBST (5 min each), the tissues were blocked for 10 min in 0.3% hydrogen peroxide diluted in TBS in coplin jars at RT, and washed with TBST (3 x 5 min) to remove the H₂O₂. The slides were then placed in a humidified chamber and incubated with Neutravidin-HRP (1:100 in TBST) for 1h at RT, then subsequently washed with TBST (3 x 5 min) in coplin jars. The slides were placed in a humidified chamber and incubated with TSA-Plus FITC reagent according to manufacturers protocol (1:50 dilution for 5 min at RT, protected from light), then washed with TBST (3 x 5 min) in coplin jars. Slides were then subjected to antigen retrieval with Vector unmasking solution in a coplin jar heated to 95°C

for 30 min. The coplin jar was removed from heat and allowed to cool at RT for 30 min. Slides then placed in a humidified chamber and incubated with APC-B220 antibody diluted 1:100 TBST with 1% FCS for 1h at RT. Slides were washed with TBST (3 x 5 min) in a coplin jar and mounted with Prolong anti-fade gold with Dapi (Invitrogen).

9. Reagent preparation for chemoenzymatic reactions

9.1. GalT1

Bacteria were grown at 37°C in LB media (2 L) containing 100 µg/ml ampicillin until OD₆₀₀ 0.7. IPTG (1 mM) was then added and the culture was grown for another 4 hrs, and the cells were harvested by centrifugation at 2000 x g for 15 min. The bacterial pellet was suspended in 10 ml PBS and sonicated thoroughly to lyse the cells. The suspension was diluted to 80 ml with PBS and centrifuged at 14,000 x g for 30 min. The inclusion body pellet was washed four times with 25% (w/v) sucrose in PBS, and a final time with PBS alone. Inclusion bodies were dissolved in 10 ml of 5 M guanidine hydrochloride (Gu-HCl) with 0.3 M sodium sulfite at RT. To sulfonate all the free thiols in the protein molecule, 1 ml of 50 mM S-sulfonating agent, 2-nitro- 5-(sulfothio)-benzoate (NTSB)⁹, was added to this solution and stirred vigorously. Completion of sulfonation was judged by the color change of the solution from red to pale yellow. The protein solution was then diluted 10 fold with water in order to precipitate the sulfonated protein. The protein precipitate was collected by centrifugation at 10,000 x g and washed three times by re-suspending in water, followed by centrifugation to remove any remaining sulfonating agent. The sulfonated protein was re-dissolved in 5 M Gu-HCl to a protein concentration of 1 mg/ml which has an absorption of 1.9 to 2.0 OD at 275 nm. The protein solution was diluted ten fold in multiple portions, in a folding solution to give a final concentration of 100 µg/ml protein, 0.5 M GuHCl, 50 mM Tris-HCl, 5 mM EDTA, 4 mM cysteamine and 2 mM cystamine, pH 8.0 at 4°C. The protein was allowed to fold for 48 hrs at 4°C, then dialyzed against 3 times in 4 liters of water containing 50 mM Tris-HCl, pH 8.0, 4 mM cysteamine and 2 mM cystamine at 4°C to remove GuHCl. The protein which precipitated during dialysis was removed by centrifugation and the supernatant was concentrated to obtain working GalT1 which was used without further purification.

9.2 UDP-GalNAI

A reaction mixture (5 mL) containing 10 mM GalNAL, 20 mM ATP, 20 mM UTP, 20 mM MgCl₂, 0.5 mg/mL Agx1¹⁰, and 0.1 mg/mL NahK¹¹ in 100 mM Tris-HCl pH 8.5 was shaken at 36°C and the progress of the reaction was monitored by TLC (6:3:3:1 Ethyl Acetate/MeOH/H₂O/Acetic acid). After overnight incubation, 5 mL ice cold EtOH was added to precipitate the protein. The precipitate was removed by centrifugation and the supernatant was concentrated by rotary evaporation to 1 mL. The crude solution was applied to a Bio-Gel P-2 column, using 50 mM ABC as eluent. The fractions containing product were collected, lyophilized and used without further purification.

9.2 UDP-GalNAz

A reaction mixture (5 mL) containing 10 mM GalNAz, 20 mM ATP, 20 mM UTP, 20 mM MgCl₂, 0.5 mg/mL Agx1¹⁰, and 0.1 mg/mL NahK¹¹ in 100 mM Tris-HCl pH 8.5 was shaken at 36°C and the progress of the reaction was monitored by TLC (6:3:3:1 Ethyl Acetate/MeOH/H₂O/Acetic acid). After overnight incubation, 5 mL ice cold EtOH was added to precipitate the protein. The precipitate was removed by centrifugation and the supernatant was concentrated by rotary evaporation to 1 mL. The crude solution was applied to a Bio-Gel P-2 column, using 50 mM ABC as eluent. The fractions containing product were collected, lyophilized and used without further purification.

10. Chemoenzymatic O-GlcNAc labelling

Cells were lysed in RIPA buffer containing a protease inhibitor cocktail (SIGMA; 2 mL/50 M cells). Lysates were cleared by centrifugation at 5000 x g for 5 min, and then proteins were

precipitated from the supernatant by chloroform/methanol precipitation (add same volume of MeOH, vortex, add half volume of CHCl₃, vortex and centrifuge; discard top layer, add same volume of H₂O, vortex, centrifuge and remove all liquid). Protein pellet was suspended the protein in 500 μ L of 1% SDS in 20 mM HEPES pH 7.9 (heated to 90°C for 5–10 minutes to completely dissolve the proteins). After cooling to RT, enzymatic reaction reagents were added (487 μ L H₂O, 1 mL 2.5 labelling buffer containing 125 mM NaCl, 50 mM HEPES, 5% NP-40, pH7.9, 138 μ L of 100 mM MnCl₂, 125 μ L of 5 mM UDP-GalNAz, and 100 μ L GalT1. The reaction mixture was incubated at 4°C overnight with shaking, after which the proteins were subjected to chloroform/methanol precipitation as described before. The protein pellet was air dried and suspended in 528 μ L of 1% SDS in 50 mM Tris-HCl, pH 8.0. To complete the click chemistry reaction, 15 μ L of 10 mM CuSO₄, 15 μ L of 20 mM BTTP, 15 μ L of 5 mM biotin-PEG₄-alkyne and 30 μ L of 100 mM Sodium ascorbate were added to the solution, and the reaction mixture was shaken at RT for 2 hrs. Excess reagents were removed by chloroform/methanol protein precipitation, and the pellet was suspended in 400 μ L 1% SDS/Tris pH 7.5. PNGase F treatment was completed by the addition of 60 μ L 10% NP-40, 60 μ L NEB Glycobuffer 2, 15 μ L PNGaseF, and 65 μ L H₂O. The reaction mixture was incubated at 37° overnight, after which the proteins were precipitated with CHCl₃/MeOH.

11. Proteomics

11.1 Sample preparation

Chemoenzymatically labelled protein pellets were resuspended in 150 μ L 4%SDS+10mM EDTA and 1.2 mL 1%Brij97+150mM NaCl+50mM triethylamine. pH 7.4. 50 μ L of previously blocked streptavidin-agarose beads (blocked in same buffer + 1% BSA for 3 hrs, and subsequently washed 3 times with PBS). Proteins were allowed to bind to the beads for 3 hrs at RT with gentle rotation, after which the suspension was transferred to a filtering column and the beads were washed sequentially with 2 x 2% SDS in PBS, 2x 8M urea in 250 mM ammonium bicarbonate (ABC), 2x 2.5 M NaCl in PBS, 2 x 0.5 M ABC, 2 x 250 mM ABC, 2 x 50 mM ABC. The beads were then suspended in 120 μ L 8 M urea and 100 mM Tris pH 8.5, and 0.6 μ L of 1M TCEP was added. The reaction was incubated at RT for 20 min, followed by the addition of 13.2 μ L of 500 mM 2-chloro-acetamide. The reaction was further incubated for 15 min at RT in the dark, after which the reaction was diluted with 360 μ L of 100 mM Tris pH 8.5 and 4.8 μ L of 100 mM CaCl₂. Trypsin (sequencing grade, 25 μ g) was added and the reaction was incubated at 37 °C in the dark overnight with gentle shaking. The reaction was then quenched with 27 μ L of 90% formic acid, and the beads were filtered out of the solution.

Dimethyl labeling

For dimethyl labelling, light, medium and heavy 4% (w/v) formaldehyde solution were first prepared by adding 10 μ L of CH₂O (37%), CD₂O or ¹³CD₂O to 80 μ L, 40 μ L and 40 μ L of water respectively. Light and heavy sodium cyanoborohydride solution (0.6 M) were freshly prepared by adding 0.02g of either NaBH₃CN (light, MW = 62.84; Arcos Organics 168550100) or NaBD₃CN (heavy, Sigma-Aldrich 190020-1G) to 500 μ L water. For the light sample of 100 μ L, 4 μ L 4% w/v CH₂O was added, vortexed, spun down, followed by addition 4 μ L of 0.6 M NaBH₃CN, vortexed and spun down. This we labelled as LIGHT, with an expected mass shift of +28.0313. For the medium sample of 100 μ L, 4 μ L 4% w/v CD₂O was added, vortexed, spun down, followed by addition 4 μ L of 0.6 M NaBH₃CN, vortexed and spun down. This we labelled as MEDIUM, with an expected mass shift of +32.0564. For the

heavy sample of 100 μ L, 4 μ l 4% w/v $^{13}\text{CD}_2\text{O}$ was added, vortexed, spun down, followed by addition 4 μ l of 0.6 M NaBD₃CN, vortexed and spun down. This we labelled as HEAVY, with an expected mass shift of +36.0757. All samples were incubated for 1 hour at room temperature with mild shaking. After incubation, 15 μ L of 200 mM ammonium bicarbonate was added to quench the reaction, then vortexed and spun down. To each sample, 7 μ l of 90% FA was added (final conc. of FA = 5%) to further quench the reaction and acidify the samples for mass-spectrometry, vortexed and spun down.

11.2. Proteomics acquisition

For sample acquisition, each sample was first loaded to a C18 loading column and washed with 5% acetonitrile in water to remove salts and other interfering chemicals. The sample was then transferred to a custom made C18 analytical column (25cm) using UltiMate 3000 (Dionex) nano pump. Mass spectrometry data was acquired by an Orbitrap Velos (ThermoFischer) instrument using top-20 ions data-dependent acquisition method. The same UltiMate 3000 (Dionex) nano pump was used to provide a 180 minutes gradient of 10%-90% acetonitrile/water solution.

11.3 . Proteomics data analysis

The acquired data was converted to ms1 and ms2 and then searched by ProLuCID¹² against Uniprot reference proteome database (mus musculus, 2016-02) with common contaminant proteins and reversed sequence added. All identification results were filtered by 1% protein FDR using previously published method.¹²

Protein quantitation

For protein quantitation, total peak area for each identified peptide was extracted from the MS1 data using our in-house script. The extracted ion chromatogram (XIC) was then deconvoluted using exponentially-modified gaussian model and the area under curve (AUC) was calculated using the deconvoluted peak parameters. For singletons, i.e. peptide not identified in all three channels (light-control, medium-memory, heavy-effector), peak area was first tried to recover signal from the corresponding channel, and then discarded if not recoverable. The calculated AUC (cAUC) was used to quantify each peptide. For protein quantitation, a weighted median cAUC by total intensity of all unique peptides was used to calculate the protein ratio among control, memory and effector channel. Proteins that have been consistently identified and quantified from all three replicates were reported for the final result.

Confidence score

For each protein, a confidence score was calculated based on the total spectral count, DeltaCN and XCorr. In short, all peptide identification with an XCorr < average XCorr of background peptide (peptide identified in control and blank) was not used for calculation. With the rest of the peptides, unique peptide was assigned a weight of 1 and shared peptide was assigned a weight of 1/n, n=number of protein sharing this peptide. The weighted sum of DeltaCN (XCorr of the highest identification/second highest identification) by all spectral was then calculated and then divided by total spectral count of each protein to result in a raw confidence score. Raw confidence score was then normalized for all proteins to a scale of 0 to 1.

Gene ontology enrichment analysis

All identified proteins were first converted back to the corresponding gene names from their UniprotID. The gene name was then used as a list with or without the quantitation number for the gene ontology term enrichment analysis by <http://www.enrichnet.org/> (Enrichnet) or <http://geneontology.org/> (PANTHER). The final results were then separated by biological process, molecular function and cellular component to plot into graphs using hierarchical information.

Protein correlation analysis and clustering

For protein correlation analysis, we used STRING database (www.string-db.org) as the reference database protein interaction. A correlation matrix was first generated by calculating the correlation between each protein-protein pair and then clustered by K-mean (As shown in Figure S2). Seven strongly correlated clusters were found from the heatmap, including ATP-binding cluster (P4hb,Acly,Eprs,Cad,Atp5b,Pa2g4,Dnajb1,Ldha,Vars,Cct3), the Histone cluster (Hist1h2br, Hist2h3c2, Hist2h3b, Hist2h2ac, Hist2h2bb, Hist1h2al, Hist1h2bp, Hist1h2bg, Hist1h2bb, Hist1h2bm, Hist1h2bn, Hist1h2bh, Hist1h2bk, H3f3b, Hist2h2aa1, Hist2h2aa2), RNA binding/translation cluster (Rpl18a, Rps15a, Eef1a1, Rps4x, Rpl6, Rpl7, Rpl5, Eif3f, Rpl15, Rps23, Rps10, Rps2, Gm10020), MCM complex cluster (Mcm7, Mcm6, Mcm5, Mcm3) and protein transport/cytoplasmic membrane-bounded vesicle cluster (Hsp90ab1, Ywhaq, Ywhah, Ywhag, Hsp90aa1, Ywhab, Ywhae).

References

- (1) Lopez Aguilar, A., Hou, X., Wen, L., Wang, P. G., and Wu, P. (2017) A chemoenzymatic histology method for O-GlcNAc detection. *ChemBioChem*.
- (2) Lund, P. J., Elias, J. E., and Davis, M. M. (2016) Global Analysis of O-GlcNAc Glycoproteins in Activated Human T Cells. *J. Immunol.* *197*, 3086–3098.
- (3) Wells, L., Vosseller, K., Cole, R. N., Cronshaw, J. M., Matunis, M. J., and Hart, G. W. (2002) Mapping Sites of O-GlcNAc Modification Using Affinity Tags for Serine and Threonine Post-translational Modifications. *Mol. Cell. Proteomics* *1*, 791–804.
- (4) Khidekel, N., Ficarro, S. B., Peters, E. C., and Hsieh-Wilson, L. C. (2004) Exploring the O-GlcNAc proteome: direct identification of O-GlcNAc-modified proteins from the brain. *Proc. Natl. Acad. Sci.* *101*, 13132–13137.
- (5) Nagel, A. K., Schilling, M., Comte-Walters, S., Berkaw, M. N., and Ball, L. E. (2013) Identification of O-Linked N-Acetylglucosamine (O-GlcNAc)-modified Osteoblast Proteins by Electron Transfer Dissociation Tandem Mass Spectrometry Reveals Proteins Critical for Bone Formation. *Mol. Cell. Proteomics* *12*, 945–955.
- (6) Trinidad, J. C., Barkan, D. T., Gulledge, B. F., Thalhammer, A., Sali, A., Schoepfer, R., and Burlingame, A. L. (2012) Global Identification and Characterization of Both O-GlcNAcylation and Phosphorylation at the Murine Synapse. *Mol. Cell. Proteomics* *11*, 215–229.
- (7) Hahne, H., Sobotzki, N., Nyberg, T., Helm, D., Borodkin, V. S., van Aalten, D. M. F., Agnew, B., Kuster, B., Aalten, D. M. F. Van, Agnew, B., and Kuster, B. (2013) Proteome Wide Purification and Identification of O-GlcNAc-Modified Proteins Using Click Chemistry and Mass Spectrometry. *J. Prot. Res* *12*, 927–36.
- (8) Jiang, H., Zheng, T., Lopez-Aguilar, A., Feng, L., Kopp, F., Marlow, F. L., and Wu, P. (2014) Monitoring Dynamic Glycosylation in Vivo Using Supersensitive Click Chemistry. *Bioconjug. Chem.* *25*, 698–706.
- (9) Thannhauser, T. W., Konishi, Y., and Scheraga, H. A. (1984) Sensitive quantitative analysis of disulfide bonds in polypeptides and proteins. *Anal. Biochem.* *138*, 181–188.
- (10) Xue, M., Guan, W., Zou, Y., Fang, J., Liu, X. W., Wang, P. G., and Wang, F. (2012) Investigation of the nucleotide triphosphate substrate specificity of Homo sapiens UDP-N-acetylgalactosamine pyrophosphorylase (AGX1). *Bioorganic Med. Chem. Lett.* *22*, 3957–3961.
- (11) Nishimoto, M., and Kitaoka, M. (2007) Identification of N-acetylhexosamine 1-kinase in the complete lacto-N-biose I/galacto-N-biose metabolic pathway in *Bifidobacterium longum*. *Appl. Environ. Microbiol.* *73*, 6444–6449.
- (12) Xu, T., Park, S. K., Venable, J. D., Wohlschlegel, J. A., Diedrich, J. K., Cociorva, D., Lu, B., Liao, L., Hewel, J., Han, X., Wong, C. C. L., Fonslow, B., Delahunty, C., Gao, Y., Shah, H., and Yates, J. R. (2015) ProLuCID: An improved SEQUEST-like algorithm with enhanced sensitivity and specificity. *J. Proteomics* *129*, 16–24.