SUPPORTING INFORMATION

Extensive metabolic remodeling after limiting mitochondrial lipid burden is consistent with an improved metabolic health profile.

Sujoy Ghosh[1,2], Shawna E. Wicks[3], Bolormaa Vandanmagsar[3], Tamra M. Mendoza[3], David S. Bayless[3], J. Michael Salbaum[4], Stephen P. Dearth[5], Shawn R. Campagna[5], Randall L. Mynatt[3,6], and Robert C. Noland[7]

[1]Laboratory of Computational Biology, Pennington Biomedical Research Center, Baton Rouge, LA, 70808; [2]Program in Cardiovascular and Metabolic Disorders and Center for Computational Biology, Duke-National University of Singapore Medical School, Singapore, 169857; [3]Gene Nutrient Interactions Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA, 70808; [4]Genomics Core Facility, Pennington Biomedical Research Center, Baton Rouge, LA, 70808; [5]Department of Chemistry, University of Tennessee, Knoxville, TN, 37996; [6]Transgenic Core Facility, Pennington Biomedical Research Center, Baton Rouge, LA, 70808; [7]Skeletal Muscle Metabolism Laboratory, Pennington Biomedical Research Center, Baton Rouge, LA, 70808

LIST OF MATERIALS INCLUDED:

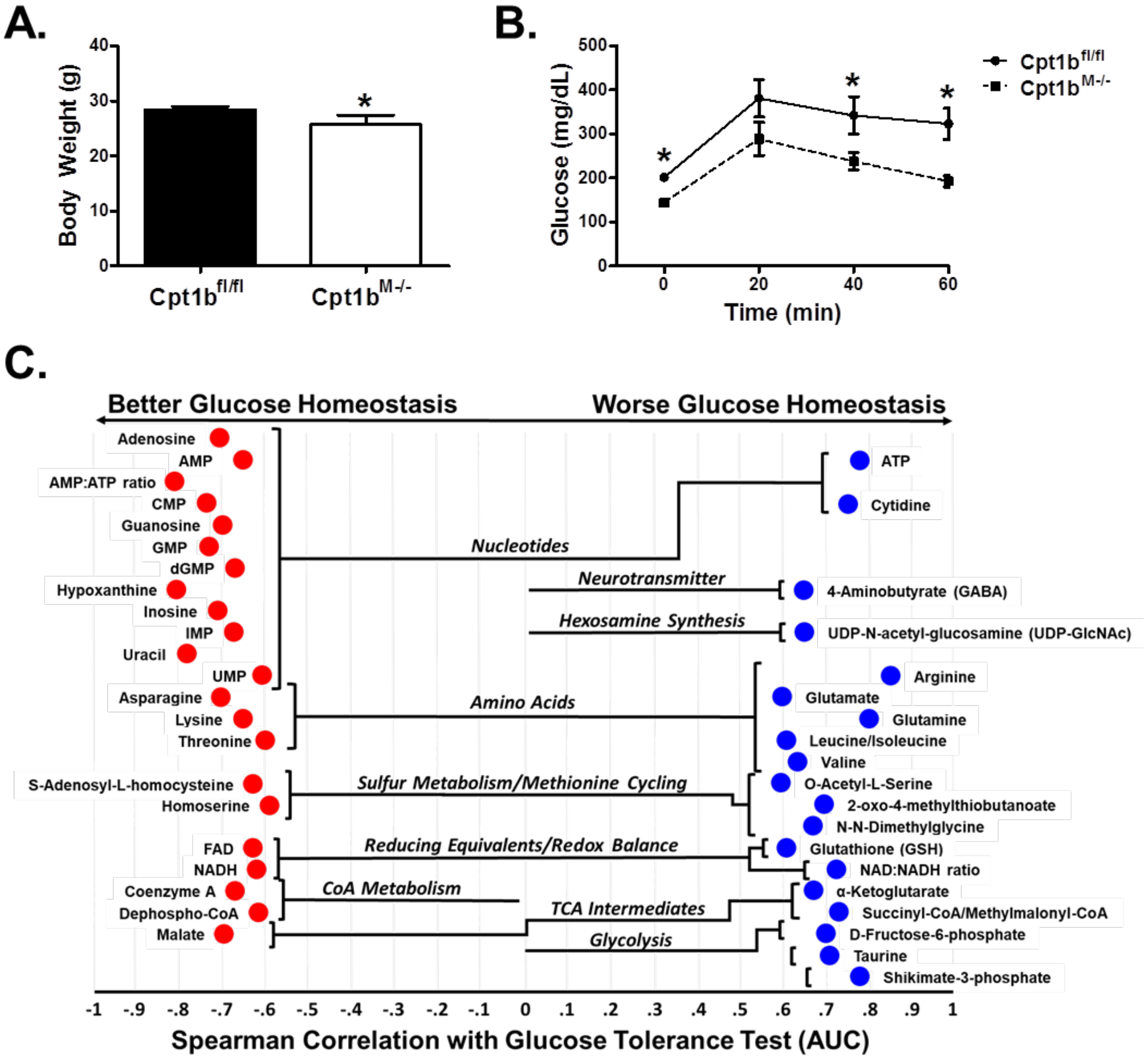
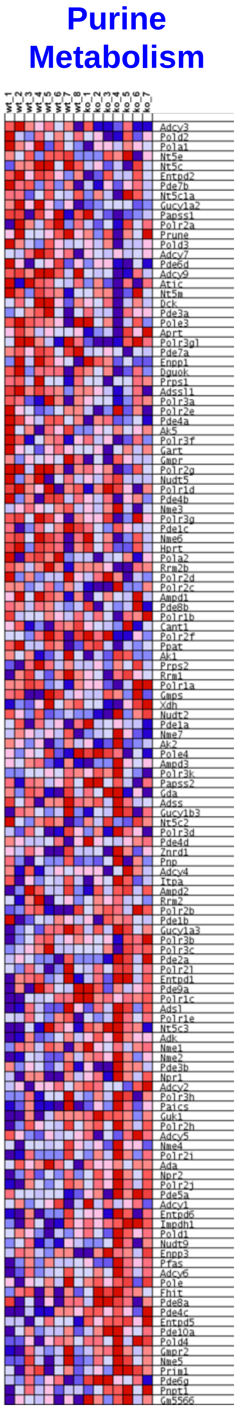
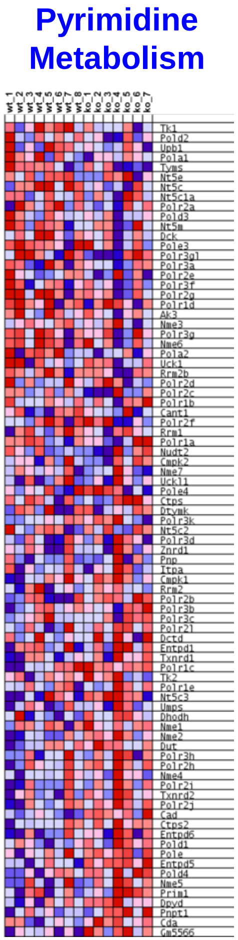


Figure S1. Body weight and glucose tolerance test. Cpt1b$^{M-/-}$ mice (n=8) have lower body weight (panel A) and improved glucose homeostasis vs. Cpt1b$^{fl/fl}$ (n=8) littermate controls. Area under the curve (AUC) calculated for the glucose tolerance test was significantly correlated ($p \leq 0.0251$) with several identified metabolites and only relationships with a false discovery rate <0.1 are shown. Key: Red (negative spearman correlation); Blue (positive spearman correlation); * $p<0.05$



Purine Metabolism



Pyrimidine Metabolism

Figure S2. Purine and pyrimidine metabolism. Heatmaps from SAGE data reveal no core enrichment of genes involved in purine or pyrimidine metabolism in Cpt1b$^{M-/-}$ (KO; n=7) vs. Cpt1b$^{fl/fl}$ (WT; n=8) skeletal muscle. Key: Red (increased); Blue (decreased)

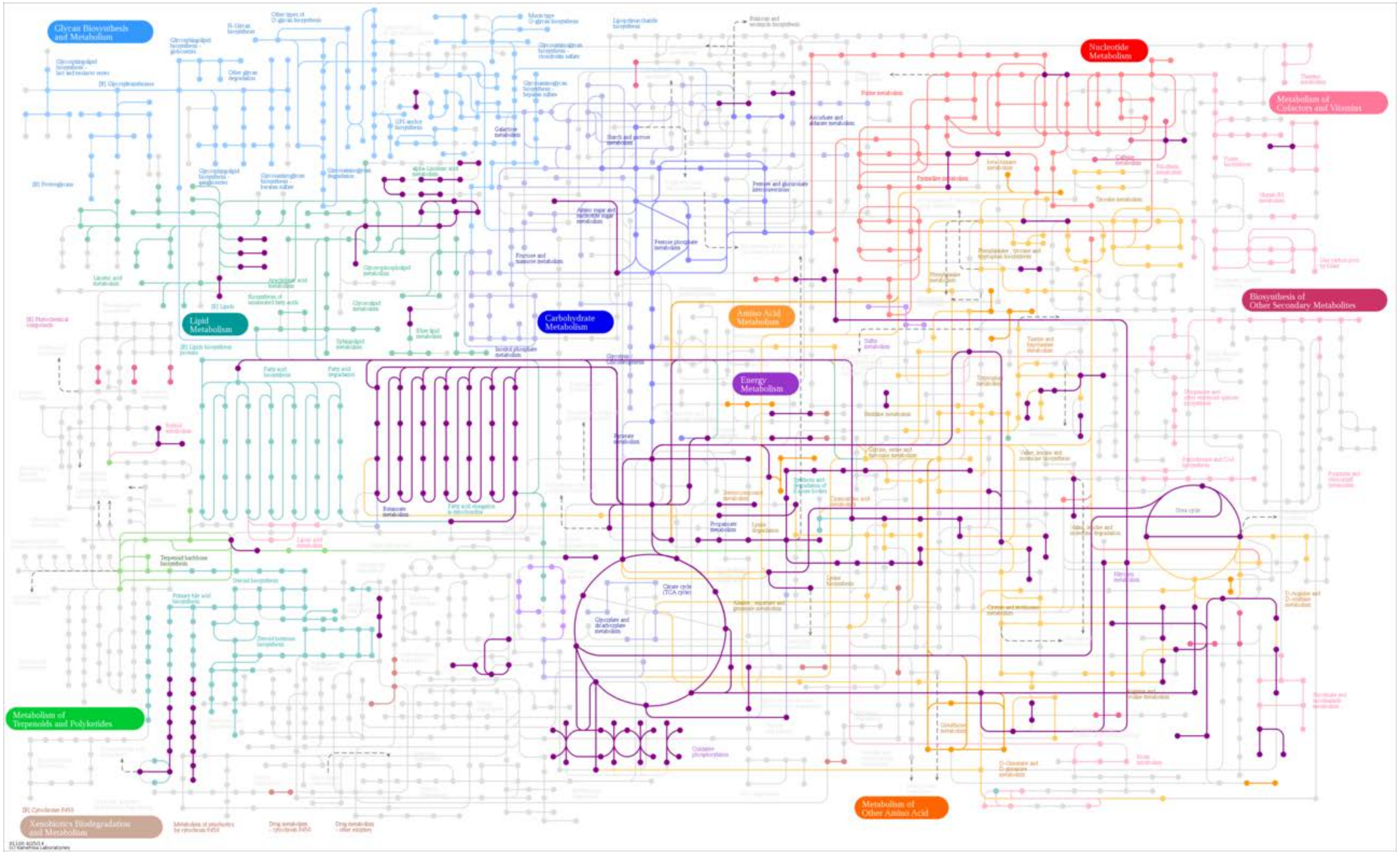


Figure S3. Comprehensive KEGG network map from SAGE. Gene set enrichment analysis (GSEA) of the SAGE data identified numerous metabolic pathways were altered in skeletal muscle from Cpt1b$^{M-/-}$ (n=7) compared to Cpt1b$^{fl/fl}$ (n=8) mice and the results were used to develop a comprehensive KEGG network map that depicts alterations in these metabolic pathways. Significantly regulated pathways are identified in purple.

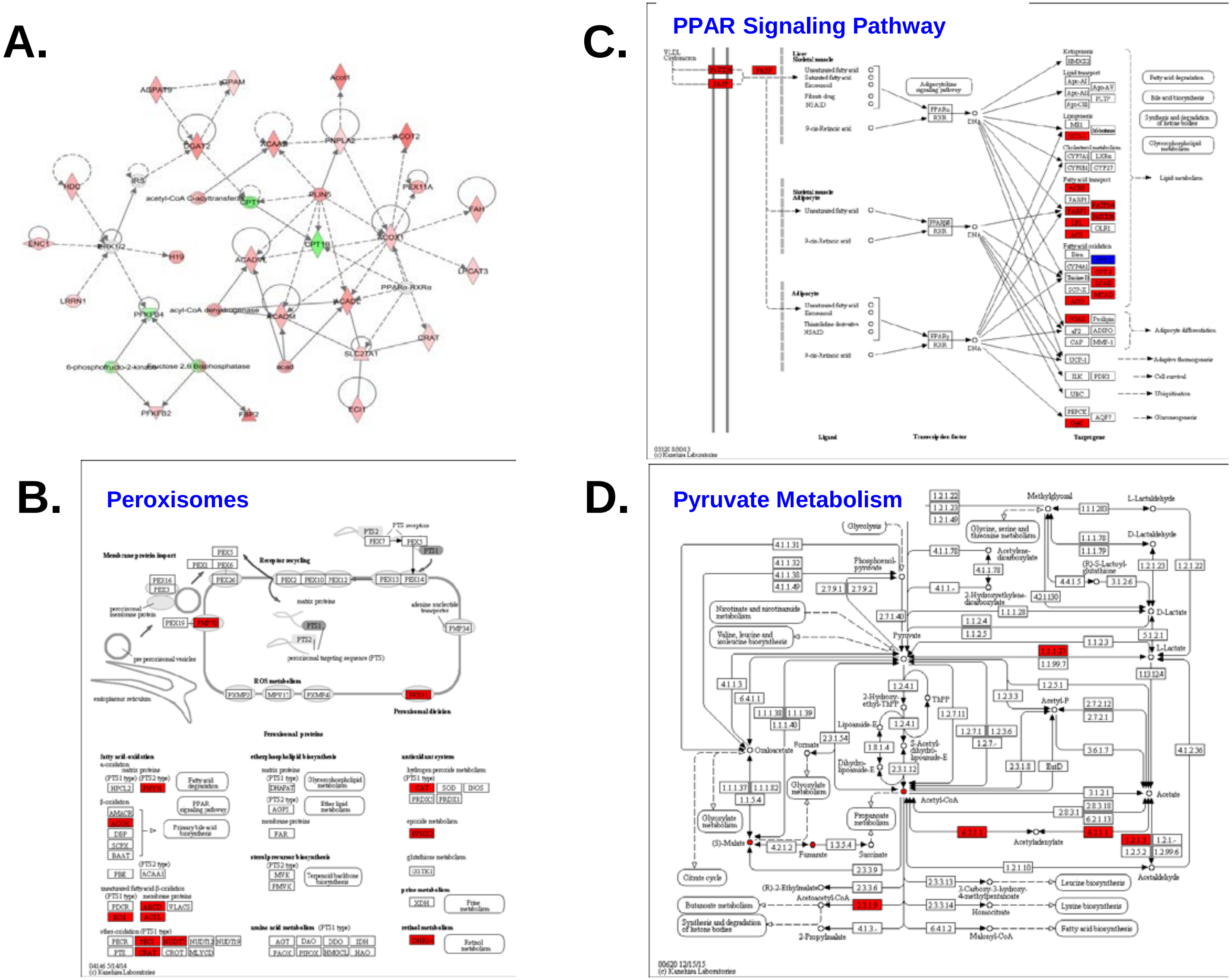


Figure S4. Cpt1b network, peroxisomes, PPAR signaling, and pyruvate handling pathway analysis from SAGE. Integrative Pathway Analysis (IPA) of the SAGE data was used to develop a Network Analysis directly tied to Cpt1b expression (A), while gene set enrichment analysis (GSEA) identified upregulation of peroxisomes (B), PPAR signaling (C) and pyruvate handling (D) pathways in skeletal muscle of Cpt1b$^{M-/-}$ mice. Upregulated genes are identified in red, while downregulated genes are shown in green (A) or blue (B). Key: Red (activation/upregulation); Green (inhibition/downregulation); Multi-colored (multiple isoforms which exhibit opposing changes); Solid Lines (direct relationship); Dashed Lines (indirect relationship); Vertical Diamond (enzyme); Horizontal Diamond (peptidase); Downward Triangle (kinase); Upward Triangle (phosphatase). Data from Cpt1b$^{M-/-}$ (n=7) and Cpt1b$^{fl/fl}$ (n=8) mice were used for the analysis.

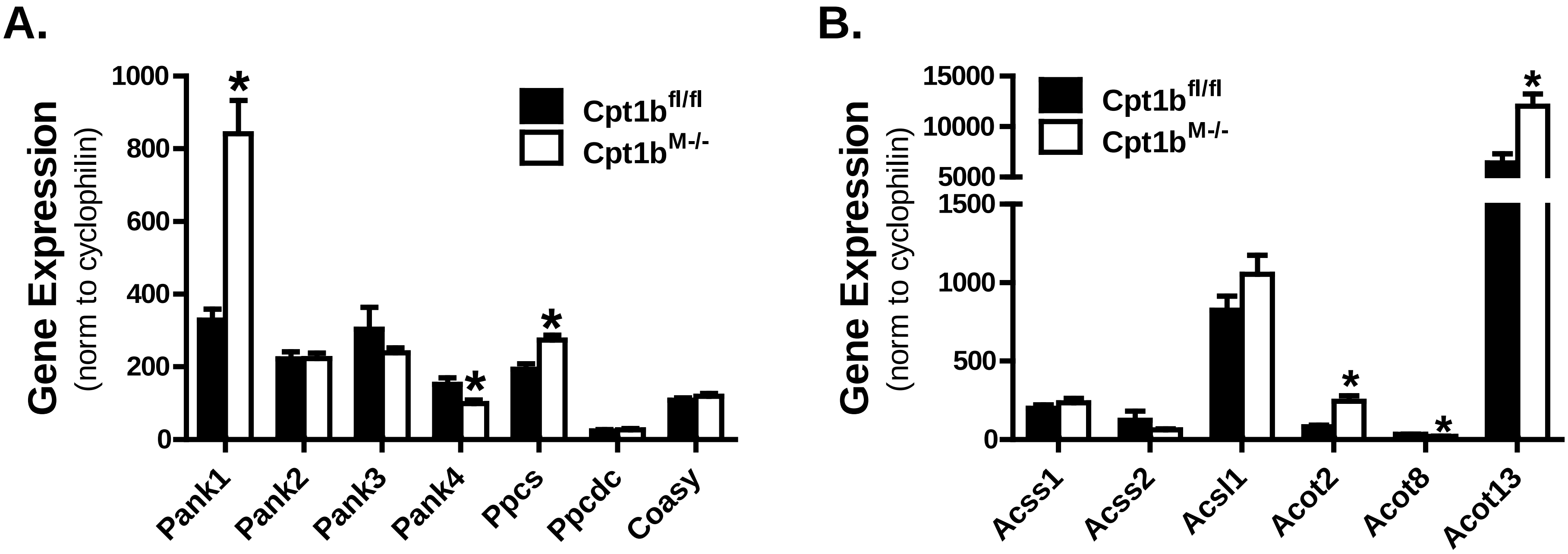
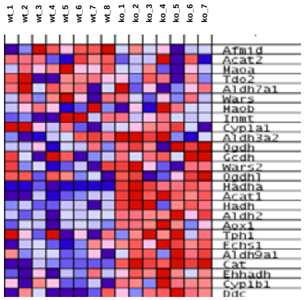
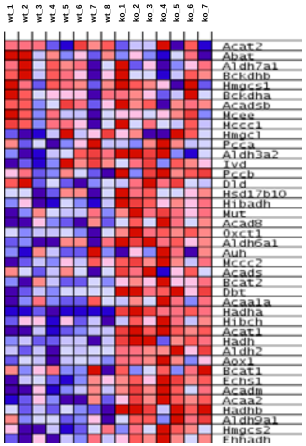


Figure S5. Genes involved in CoA metabolism. Enrichment of key genes involved in CoA biosynthesis (A), as well as acyl-CoA metabolism (B) identified within the SAGE dataset was confirmed via RT-PCR. Data are normalized to cyclophilin. (n=8 per genotype) * $p<0.05$

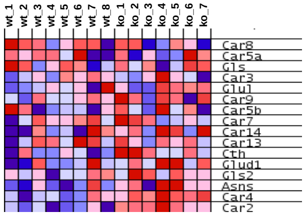
Tryptophan Metabolism



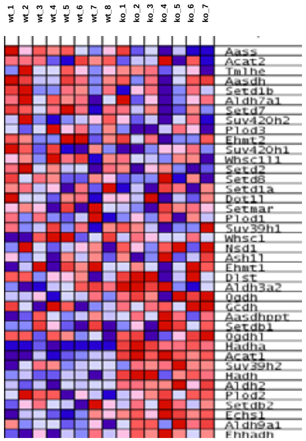
Valine, Leucine and Isoleucine Degradation



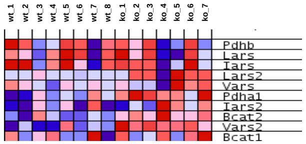
Nitrogen Metabolism



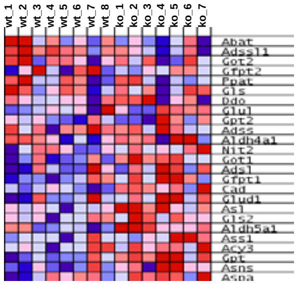
Lysine Degradation



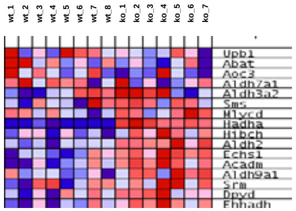
Valine, Leucine and Isoleucine Biosynthesis



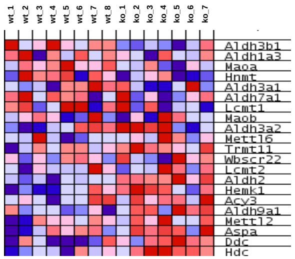
Alanine, Aspartate and Glutamate Metabolism



Beta-Alanine Metabolism



Histidine Metabolism



Arginine and Proline Metabolism

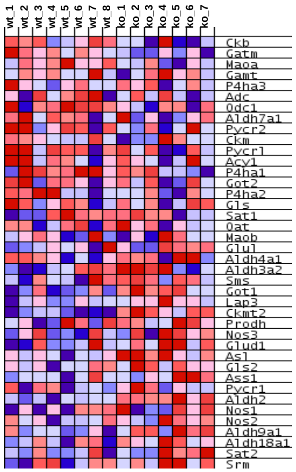


Figure S6. Alterations in amino acid metabolism pathways in Cpt1b$^{M-/-}$ mice. Heatmaps from SAGE data reveal substantial remodeling of genes involved in several pathways of amino acid metabolism in Cpt1b$^{M-/-}$ (KO; n=7) vs. Cpt1b$^{fl/fl}$ (WT; n=8) skeletal muscle. Key: Red (increased); Blue (decreased)

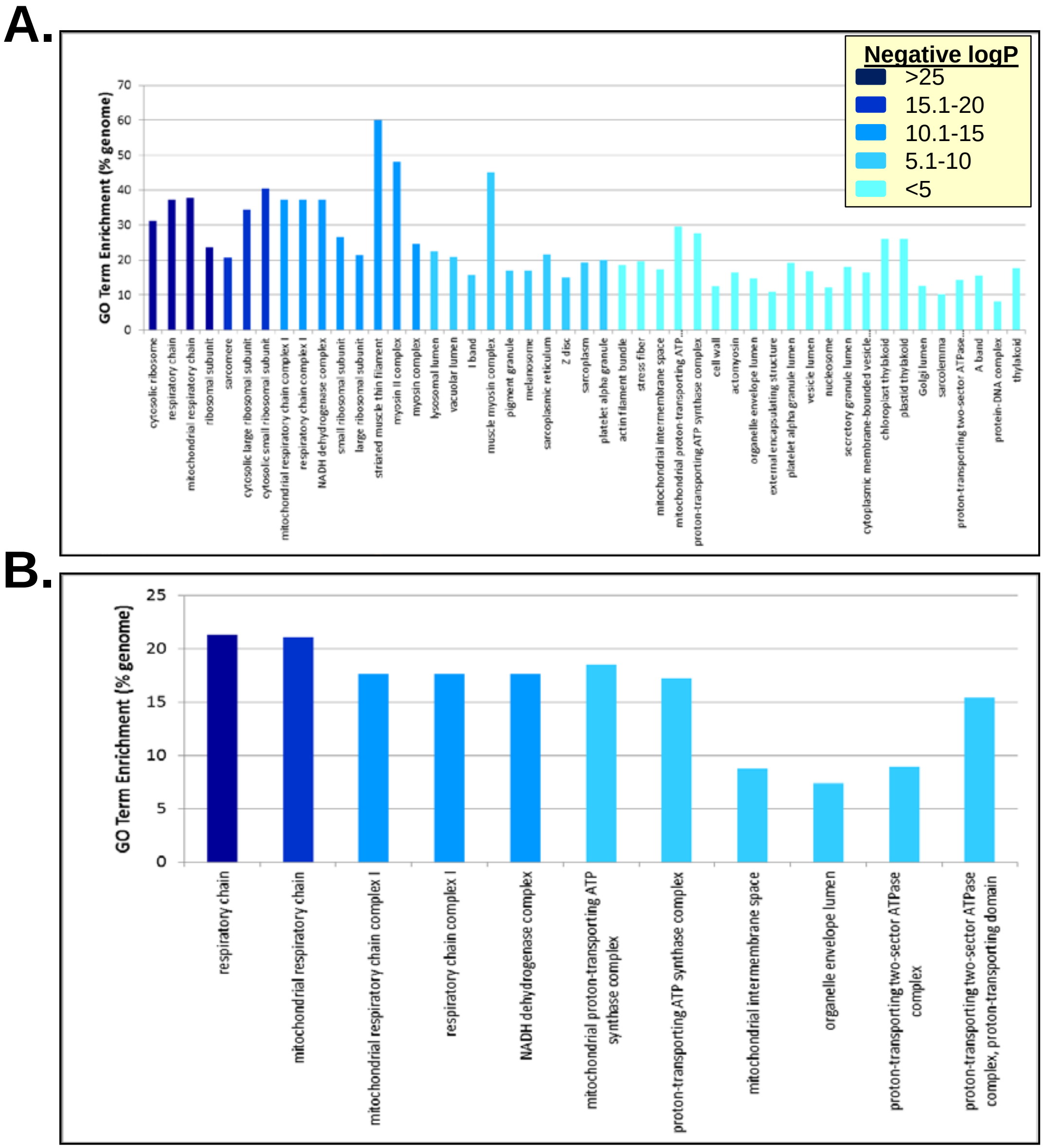


Figure S7. Cellular localization of proteins identified to be regulated in Cpt1b$^{M-/-}$ mice. Gene ontology analysis of the proteomics dataset was used to identify the cellular compartmentalization of differentially expressed proteins (A). Several of the most robustly regulated factors were localized to the mitochondrion (B). Data from Cpt1b$^{M-/-}$ (n=7) and Cpt1b$^{fl/fl}$ (n=8) mice were used for the analysis.

KEGG Pathway	SIZE	NES	NOM p-val	FDR q-val	Regulation
Valine, Leucine and Isoleucine Degradation	41	-2.04	0.00	0.00	Up_KO
Peroxisome	69	-1.97	0.00	0.01	Up_KO
Propanoate Metabolism	28	-1.89	0.00	0.01	Up_KO
Tryptophan Metabolism	26	-1.92	0.00	0.01	Up_KO
Fatty Acid Metabolism	32	-1.89	0.00	0.01	Up_KO
PPAR Signaling Pathway	51	-1.85	0.00	0.01	Up_KO
Beta Alanine Metabolism	16	-1.79	0.01	0.02	Up_KO
Alanine, Aspartate and Glutamate Metabolism	25	-1.72	0.01	0.04	Up_KO
Oxidative Phosphorylation	95	-1.69	0.00	0.05	Up_KO
Nitrogen Metabolism	16	-1.70	0.01	0.05	Up_KO
Terpenoid Backbone Biosynthesis	14	-1.59	0.04	0.09	Up_KO
Glycerolipid Metabolism	34	-1.61	0.01	0.09	Up_KO
Arginine and Proline Metabolism	40	-1.58	0.02	0.09	Up_KO
TCA Cycle	29	-1.60	0.01	0.09	Up_KO
Proximal Tubule Bicarbonate Reclamation	19	-1.55	0.03	0.11	Up_KO
Histidine Metabolism	21	-1.54	0.05	0.11	Up_KO
Lysine Degradation	40	-1.45	0.04	0.17	Up_KO
Pyruvate Metabolism	31	-1.45	0.03	0.17	Up_KO
Retinol Metabolism	15	-1.47	0.06	0.17	Up_KO
Valine, Leucine and Isoleucine Biosynthesis	10	-1.46	0.06	0.17	Up_KO
Drug Metabolism Other Enzymes	16	-1.42	0.09	0.19	Up_KO
O Glycan Biosynthesis	18	1.74	0.01	0.16	Dn_KO
ECM Receptor Interaction	69	1.67	0.00	0.22	Dn_KO
Natural Killer Cell-Mediated Cytotoxicity	75	1.64	0.00	0.24	Dn_KO
Systemic Lupus Erythematosus	44	1.77	0.00	0.25	Dn_KO

Table S1: GSEA of KEGG Pathway Data

Category	Biofunction	Predicted Activation State	Activation z-score	p-Value	# of Molecules
Lipid Metabolism	Metabolism of Triacylglycerol	Increased	2.587	2.45E-13	14
Lipid Metabolism	Metabolism of Acylglycerol	Increased	2.581	2.72E-12	15
Lipid Metabolism	Transport of Fatty Acid	Increased	2.563	1.31E-08	9
Lipid Metabolism	Cleavage of Lipid	Increased	2.405	1.30E-03	8
Lipid Metabolism	Synthesis of Acylglycerol	Increased	2.397	4.09E-07	9
Lipid Metabolism	Synthesis of Triacylglycerol	Increased	2.386	7.98E-08	8
Carbohydrate Metabolism	Concentration of D-glucose	Increased	2.248	2.18E-14	25
Molecular Transport	Transport of Carboxylic Acid	Increased	2.734	9.97E-08	10
Organismal Functions	Thermoregulation	Increased	2.345	1.35E-05	10
Cell Morphology	Size of Muscle Cells	Increased	2.393	1.07E-03	6
Organismal Development	Size of Body	Increased	2.347	1.95E-03	21
Metabolic Disease	Hyperglycemia	Decreased	-2.121	4.03E-05	9
Metabolic Disease	Hypoglycemia	Decreased	-2.906	6.77E-12	14
Metabolic Disease	Glucose Metabolism Disorder	Decreased	-3.185	8.22E-13	46
Gastrointestinal Disease	Hepatic Steatosis	Decreased	-2.377	2.56E-12	20
Organismal Injury and Abnormalities	Fatigue	Decreased	-2.236	9.28E-06	6
Cardiovascular Disease	Hypertrophy of Heart	Decreased	-2.654	3.54E-07	14
Organismal Survival	Perinatal Death	Decreased	-2.92	9.62E-04	17
Organismal Survival	Organismal Death	Decreased	-3.892	7.29E-06	53

Table S2. Biofunctions

Gene symbol	Ref.Seq ID	Forward Primer	Reverse Primer
Pank1	NM_001114339.2	CCATGCACTTGTTCATCCAG	CAAACAGTCCAGCTCATCCA
Pank2	NM_153501.2	TGGAAACATGATGAGCAAGG	CCGACAAATACGACCTGGTT
Pank3	NM_145962.2	TCAGATTTCCAACCCAGGAC	GTTTGTGCAGGTGGAGGTTT
Pank4	NM_001305804.1	GCAAGCAGTTTTGGGAAGTC	CCCAAAGTAGACCCTGTCCA
Ppcs	NM_026494.3	CGAAATGCCTGAACACAAGA	ATGATGTCCGGGTCTGTCTC
Ppcdc	NM_176831.4	GATGCCAACACTCTGGGGAA	CAGGGAATCTCCACGTAGCC
Coasy	NM_001305982.1	TATGCTGCTTGAAGCTGGCT	ACCACGTTGCTCTGCTCTAC
Dcakd	NM_026551.3	GGTATTCCAACAGCTGGGCT	ATGTCGCCATTCTCCAGCAA
Acss1	NM_080575.2	ATCCCGCTGTTCCAGAGACT	CAGCACAATGAAGGCAAATG
Acss2	NM_019811.3	ACTTGGCGACAAAGTTGCTT	GCTGAACTGACACACCTGGA
Acsl1	NM_001302163.1	AAGCCACCATGTGACCTCTC	TCAAGGACTGCTGATCTTCG
Acot2	NM_134188.3	CCCCAAGAGCATAGAAACCA	AGCCCAATTCCAGGTCCTTT
Acot8	NM_133240.2	AGAAGACCTGCTGGATCACG	TCAGCCCCACTCGATACTTC
Acot13	NM_025790.2	AGCATGACCCAGAACCTACG	GCTGCTCTTCCACCTTCATC

Table S3. Primers