YMTHE, Volume *26*

Supplemental Information

Therapeutic Benefit of Autophagy Modulation in Pompe Disease

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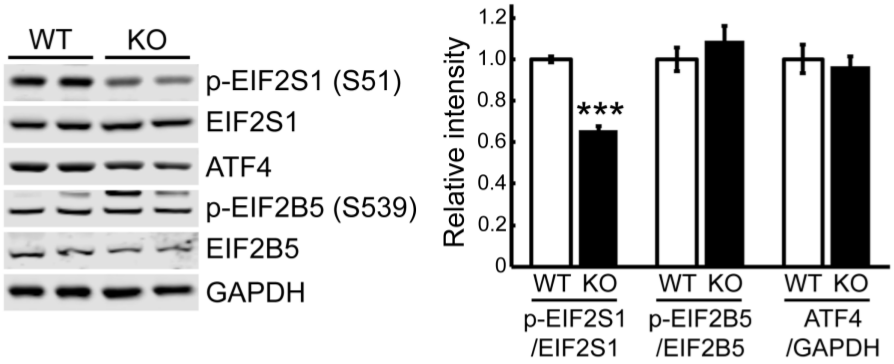
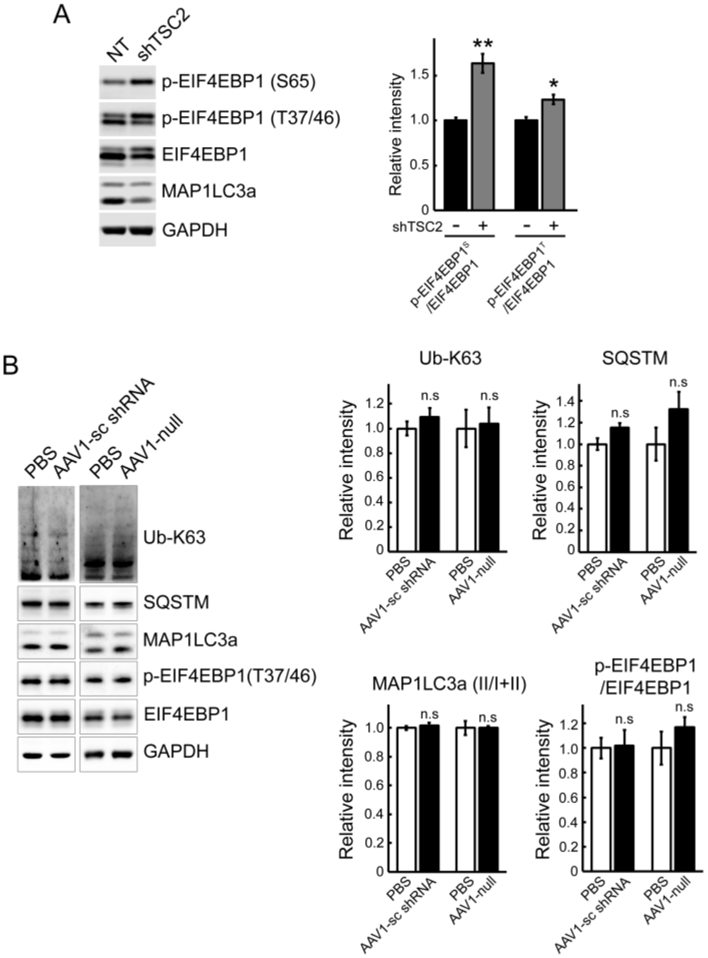
Figure S1



Decreased levels of phosphorylated EIF2S1 (p-EIF2S1^{S51}) in muscle of the KO mice.

Western blot analysis of muscle lysates from WT and KO mice with the indicated antibodies. The levels of ATF4 (n=5) and p-EIF2B/EIF2B5 ratio (n=6) are not altered in the KO muscle, but the level of p-EIF2S1^{S51} (n=6) is significantly decreased in the diseased muscle compared to the WT control. When EIF2S1 is less phosphorylated, it cannot inhibit the guanine nucleotide exchange factor EIF2B5, thus allowing the recycling of EIF2S1 between successive rounds of protein synthesis. A combination of decreased phosphorylation of EIF2S1 without changes in the EIF2B levels results in the upregulation of the overall rate of protein synthesis in the KO muscle. Graph represent mean ± SE. ***$P < 0.001$. Student’s t-test.

Figure S2

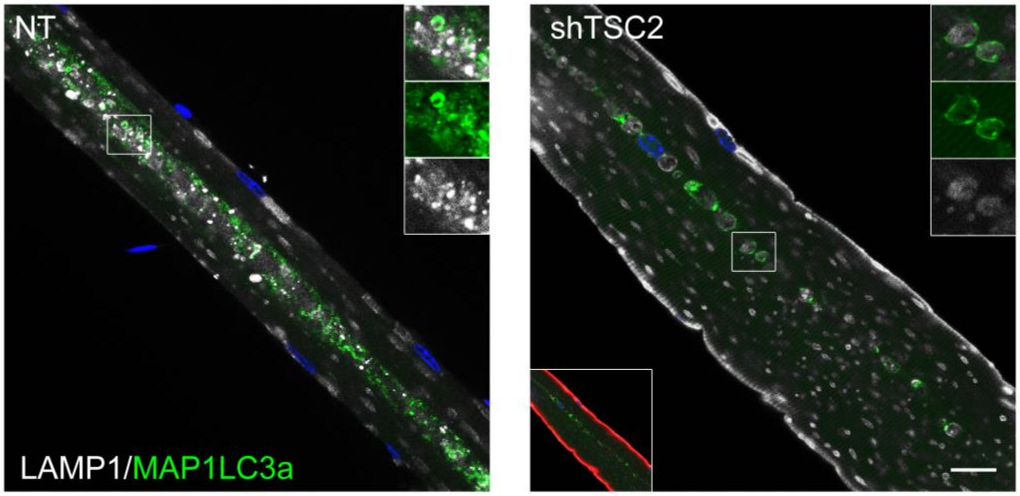


Effects of AAV-mediated TSC2 inhibition on mTOR and autophagy in muscle of the KO mice.

A. Representative image of western blot analysis of whole muscle lysates from sham-treated (NT) left gastrocnemius and AAV1-shRNA-TSC2 (shTSC2)-infected right gastrocnemius muscle from KO mice with indicated antibodies. Increased phosphorylation of a downstream mTOR target, EIF4EBP1, and a decrease in the autophagosomal marker MAP1LC3a-II are observed following TSC knockdown (consistent with our previously reported data[1]). **B.** Western blot analysis of muscle lysates from PBS-treated and AAV1-null and AAV1-scrambled shRNA (AAV1-sc shRNA)-infected KO mice with the indicated antibodies. 3-month-old KO mice were injected with a total dose of 10^{11} GC of AAV1-sc shRNA or AAV1-null into three sites of the right gastrocnemius muscle; PBS was injected into the contralateral muscle. Animals were sacrificed 6 weeks after the procedure. White part of gastrocnemius muscle was used for western blots. GAPDH was used as a loading control. No changes in the levels of Ub-proteins, SQSTM/p62, MAP1LC3a or EIF4EBP1 were seen following AAV1-null or AAV1-sc-shRNA injections. Graphs represent mean ± SE. n=3; **P* < 0.05, ***P* < 0.01. n.s.: not significant. Student's t-test.

1. Lim, JA, Li, L, Shirihai, OS, Trudeau, KM, Puertollano, R, and Raben, N (2017). Modulation of mTOR signaling as a strategy for the treatment of Pompe disease. *EMBO Mol Med*. 9(3):353-370.

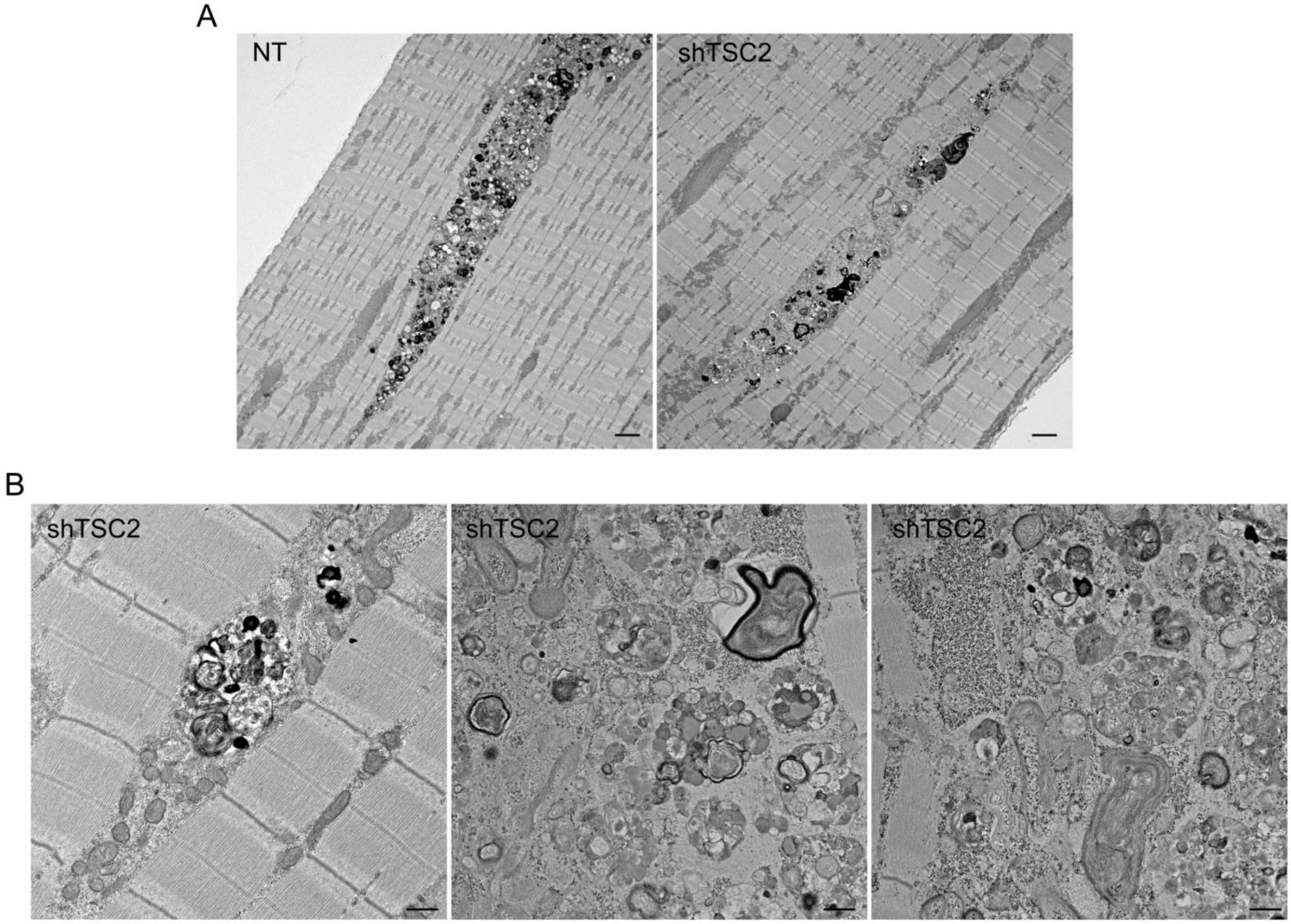
Figure S3



Efficient lysosomal-autophagosomal fusion in muscle of the KO mice following TSC2 knockdown.

Immunostaining of sham-infected (NT; left) and AAV1-RFP-shTSC (shTSC2; right)-infected single fibers from GFP-LC3:KO mice with anti-LC3 antibody (MAP1LC3a; green) and anti-LAMP1 (white) antibodies. The experiments were performed in GFP-LC3:KO, in which autophagosomes are labeled with GFP-LC3. 3-month-old mice were injected with AAV1-RFP-shRNA TSC2 vector preparation into the right gastrocnemius muscle; PBS was injected into the contralateral muscle. Animals were sacrificed 6 weeks after the procedure. White part of gastrocnemius muscle was used for analysis. A typical autophagic buildup is seen in the sham-infected fiber: the buildup occupies a sizeable portion of the fiber, and the co-localization of LC3- and LAMP1-positive structures are observed only occasionally. In contrast, the infected fiber (red) is largely free from typical buildup, and the ring-shaped MAP1LC3a-positive autophagosomes in the core of the fiber colocalize with LAMP1-positive lysosomes. Bar: 20 µm

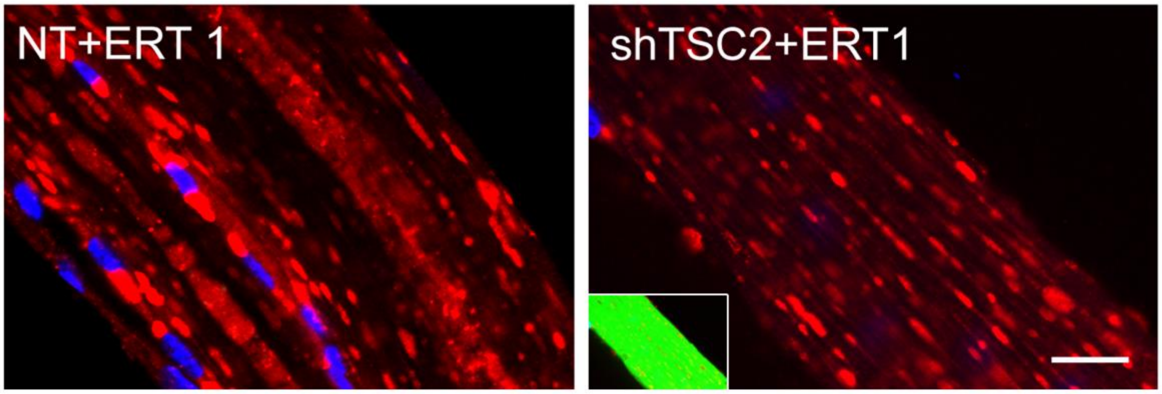
Figure S4



Effect of AAV-mediated TSC2 inhibition on autophagic buildup in muscle of the KO mice.

Additional electron micrograph images of sham-treated and AAV-shTSC-infected (shTSC2) muscle of the KO mice (see Figure 3B in the main text) showing reduced total area of the autophagic buildup following TSC knockdown. **A.** Bar: 2 µm. A higher magnification images (lower panels) of the AAV-shTSC-infected KO muscle show dispersed clusters of vesicles separated by vesicle-free areas. **B.** Bar: 500 nm. 3-month-old KO mice were injected with AAV1- shTSC into three sites of the right gastrocnemius muscle (shTSC2); PBS was injected into the contralateral muscle. Animals were sacrificed 5.5 weeks after the procedure. White part of gastrocnemius muscle was used for analysis.

Figure S5



Effect of ERT in skeletal muscle of KO mice following TSC knockdown.

4-month-old KO mice were infected with AAV1-GFP-shRNA TSC2 vector preparation into the right gastrocnemius muscle; PBS was injected into the contralateral muscle; after 4 weeks, animals received a single i.v. injections of rhGAA (at a dose of 100 mg/kg); the mice were sacrificed 5 days after the drug injection. Immunostaining of single fibers from sham-infected (NT+ERT1; left) and AAV1-GFP-shRNA TSC2-infected (shTSC2+ERT1; green; right) muscle with LAMP1. Comparison of the two panels shows the absence of autophagic buildup and shrinking of lysosomes following TSC knockdown. Bar: 20 µm.

Supplemental Videos

Supplemental video 1. mCherry-LAMP-transfected fiber derived from a GFP-LC3:KO mouse.
Time-lapse confocal microscopy images were taken every 5 minutes. There is little (if any) interaction between lysosomes (red) and autophagosomes (green).

Supplemental video 2. mCherry-LAMP-transfected fiber derived from a GFP-LC3:KO mouse; the fiber was cultured in the presence of bvPLA2 (10 µg/mL). Time-lapse confocal microscopy images were taken every 5 minutes. PLA2 stimulates the fusion between autophagosomes and lysosomes.

Table S1. Ubiquitin-related upregulated genes

Gene Symbol	Name	Transcript	p-value	Adjusted p-value (q-value)	FC
Neurl1a	neuralized E3 ubiquitin protein ligase 1A	NM_021360	2E-05	6.21E-04	38.53
Tspo	translocator protein	NM_009775	4E-08	1.40E-05	7.99
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	NM_009397	0.0026	1.73E-02	4.39
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	NM_001166402	7E-05	1.46E-03	7.26
Faap20	Fanconi anemia core complex associated protein 20	NM_001190445	0.0011	8.89E-03	6.91
Park2	Parkinson disease (autosomal recessive, juvenile) 2, parkin	NM_016694	8E-10	1.52E-06	6.33
Fbxo39	F-box protein 39	NM_001037713	0.0005	4.92E-03	6.31
Fbxl22	F-box and leucine-rich repeat protein 22	NM_175206	1E-05	4.00E-04	4.85
Cd300lh	CD300 antigen like family member H	NM_199201	0.0006	6.14E-03	4.67
Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	NM_007669	0.0002	2.42E-03	4.52
Uchl1	ubiquitin carboxy-terminal hydrolase L1	NM_011670	1E-04	1.77E-03	3.64
Otub2	OTU domain, ubiquitin aldehyde binding 2	NR_033587	0.0203	7.51E-02	3.24
Klhl40	kelch-like 40	NM_028202	1E-06	1.15E-04	2.80
Usp20	ubiquitin specific peptidase 20	NM_028846	2E-07	3.74E-05	2.65
Dcaf4	DDB1 and CUL4 associated factor 4	NM_001165256	0.0082	3.93E-02	2.61
Map1lc3a	microtubule-associated protein 1 light chain 3 alpha	NM_025735	7E-07	7.71E-05	2.59
Trp53	transformation related protein 53	NM_001127233	0.0113	4.98E-02	2.59
Tnip1	TNFAIP3 interacting protein 1	NM_001199276	0.0012	9.50E-03	2.57
H2-Ab1	histocompatibility 2, class II antigen A, beta 1	NM_207105	3E-05	7.72E-04	2.55
Zfand2a	zinc finger, AN1-type domain 2A	NM_133349	0.0008	7.02E-03	2.54
Trim72	tripartite motif-containing 72	NM_001079932	2E-07	3.90E-05	2.52

Table S2. Proteasome-related upregulated genes

Input	Name	Transcript	p-value	Adjusted p-value (q-value)	FC
Park2	Parkinson disease (autosomal recessive, juvenile) 2, parkin	NM_016694	7.94E-10	1.52E-06	6.33
Xaf1	F-box protein 39	NM_001037713	0.000462	4.92E-03	6.31
Fbxl22	F-box and leucine-rich repeat protein 22	NM_175206	9.51E-06	4.00E-04	4.85
Kbtbd5	kelch-like 40	NM_028202	1.31E-06	1.15E-04	2.80
Agt	alanine-glyoxylate aminotransferase	NM_007428	1.56E-05	5.57E-04	2.60
Zfand2a	zinc finger, AN1-type domain 2A	NM_133349	0.000777	7.02E-03	2.54
Trim72	tripartite motif-containing 72	NM_001079932	2.21E-07	3.90E-05	2.52

Table S3. Proteolysis-related upregulated genes

Input	Name	Transcript	p-value	Adjusted p-value (q-value)	FC
Timp1	tissue inhibitor of metalloproteinase 1	NM_011593	1.17E-05	4.56E-04	28.02
Timp1	tissue inhibitor of metalloproteinase 1	NM_001044384	0.000158	2.42E-03	565.23
Klk1	kallikrein 1-related peptidase b1	NM_010639	0.000169	2.52E-03	389.04
Perp	PERP, TP53 apoptosis effector	NM_022032	2.63E-08	1.17E-05	13.91
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	NM_009397	0.002631	1.73E-02	4.39
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	NM_001166402	7.19E-05	1.46E-03	7.26
Cst6	cystatin B	NM_028623	6.68E-08	2.01E-05	4.23
Uchl1	ubiquitin carboxy-terminal hydrolase L1	NM_011670	9.62E-05	1.77E-03	3.64
Ctss	cathepsin S	NM_021281	0.000192	2.72E-03	3.56
Lgmn	legumain	NM_011175	1.17E-07	2.65E-05	3.44
Otub2	OTU domain, ubiquitin aldehyde binding 2	NR_033587	0.020306	7.51E-02	3.24
Hk1	kallikrein 1	NM_001146100	2.59E-05	7.55E-04	3.20
Casp4	caspase 1	NM_007609	0.000128	2.13E-03	2.83
Usp20	ubiquitin specific peptidase 20	NM_028846	2.07E-07	3.74E-05	2.65
Cpe	carboxypeptidase E	NM_013494	4.48E-09	4.61E-06	2.64
Trp53	transformation related protein 53	NM_001127233	0.011323	4.98E-02	2.59
Tnip1	TNFAIP3 interacting protein 1	NM_001199276	0.001174	9.50E-03	2.57
Atpif1	ATPase inhibitory factor 1	NM_007512	4.58E-07	6.12E-05	2.55

Table S4. Protease-related upregulated genes

Input	Name	Transcript	p-value	Adjusted p-value (q-value)	FC
Timp1	tissue inhibitor of metalloproteinase 1	NM_011593	1.17E-05	4.56E-04	28.02
Timp1	tissue inhibitor of metalloproteinase 1	NM_001044384	0.000158	2.42E-03	565.23
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	NM_009397	0.002631	1.73E-02	4.39
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	NM_001166402	7.19E-05	1.46E-03	7.26
Park2	Parkinson disease (autosomal recessive, juvenile) 2, parkin	NM_016694	7.94E-10	1.52E-06	6.33
Cst6	cystatin B	NM_028623	6.68E-08	2.01E-05	4.23
Panx1	pannexin 1	NM_019482	4.14E-05	1.01E-03	4.01
Uchl1	ubiquitin carboxy-terminal hydrolase L1	NM_011670	9.62E-05	1.77E-03	3.64
Otub2	OTU domain, ubiquitin aldehyde binding 2	NR_033587	0.020306	7.51E-02	3.24
Anxa2	annexin A2	NM_007585	7.25E-08	2.09E-05	3.15
Serpinb6a	serine (or cysteine) peptidase inhibitor, clade B, member 6a	NM_001164118	3.88E-07	5.63E-05	2.48
Serpinb6a	serine (or cysteine) peptidase inhibitor, clade B, member 6a	NM_009254	2.53E-07	4.25E-05	3.11
Serpinb6a	serine (or cysteine) peptidase inhibitor, clade B, member 6a	NM_001164117	7.02E-06	3.40E-04	2.62
Trp53	transformation related protein 53	NM_001127233	0.011323	4.98E-02	2.59
Tnip1	TNFAIP3 interacting protein 1	NM_001199276	0.001174	9.50E-03	2.57

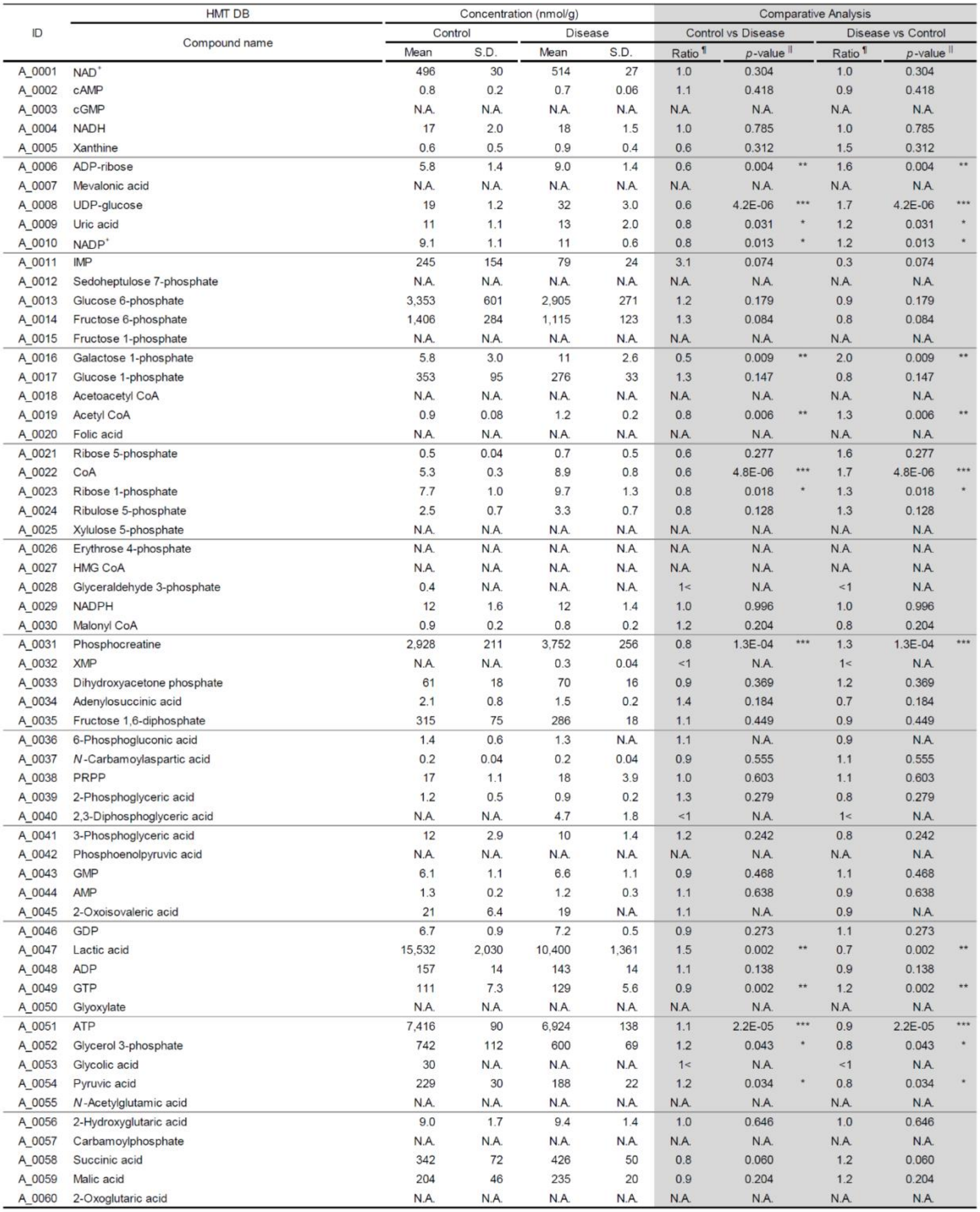
Table S5. Peptidase-related upregulated genes

Input	Name	Transcript	p-value	Adjusted p-value (q-value)	FC
Mt4	protease, serine 21	NM_008631	5.82E-12	6.89E-08	9063.46
Timp1	tissue inhibitor of metalloproteinase 1	NM_011593	1.17E-05	4.56E-04	28.02
Timp1	tissue inhibitor of metalloproteinase 1	NM_001044384	0.000158	2.42E-03	565.23
Fetub	fetuin beta	NM_021564	0.001012	8.53E-03	237.85
Fetub	fetuin beta	NM_001083904	0.000438	4.75E-03	404.75
Klk1	kallikrein 1-related peptidase b1	NM_010639	0.000169	2.52E-03	389.04
Serpinb1a	serine (or cysteine) peptidase inhibitor, clade B, member 1a	NM_025429	2.74E-09	3.35E-06	18.61
Perp	PERP, TP53 apoptosis effector	NM_022032	2.63E-08	1.17E-05	13.91
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	NM_009397	0.002631	1.73E-02	4.39
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	NM_001166402	7.19E-05	1.46E-03	7.26
Serpinb8	serine (or cysteine) peptidase inhibitor, clade B, member 8	NM_011459	4.97E-06	2.78E-04	6.98
Bok	BCL2-related ovarian killer	NM_016778	1.1E-06	1.04E-04	5.80
Arc	nucleolar protein 3 (apoptosis repressor with CARD domain)	NM_018790	0.004423	2.53E-02	5.56
Cst6	cystatin B	NM_028623	6.68E-08	2.01E-05	4.23
Uchl1	ubiquitin carboxy-terminal hydrolase L1	NM_011670	9.62E-05	1.77E-03	3.64
Serpina3n	serine (or cysteine) peptidase inhibitor, clade A, member 3C	NM_009252	3.95E-08	1.42E-05	3.62
Ctss	cathepsin S	NM_021281	0.000192	2.72E-03	3.56
Lgmn	legumain	NM_011175	1.17E-07	2.65E-05	3.44
Serpina3m	serine (or cysteine) peptidase inhibitor, clade A, member 3M	NM_009253	2.8E-05	7.94E-04	3.36
Otub2	OTU domain, ubiquitin aldehyde binding 2	NR_033587	0.020306	7.51E-02	3.24
Hk1	kallikrein 1	NM_001146100	2.59E-05	7.55E-04	3.20
Serpinb6a	serine (or cysteine) peptidase inhibitor, clade B, member 6a	NM_001164118	3.88E-07	5.63E-05	2.48
Serpinb6a	serine (or cysteine) peptidase inhibitor, clade B, member 6a	NM_009254	2.53E-07	4.25E-05	3.11
Serpinb6a	serine (or cysteine) peptidase inhibitor, clade B, member 6a	NM_001164117	7.02E-06	3.40E-04	2.62
Casp4	caspase 1	NM_007609	0.000128	2.13E-03	2.83
Arl6ip5	ADP-ribosylation factor-like 6 interacting protein 5	NM_022992	6.7E-07	7.59E-05	2.81
Usp20	ubiquitin specific peptidase 20	NM_028846	2.07E-07	3.74E-05	2.65
Cpe	carboxypeptidase E	NM_013494	4.48E-09	4.61E-06	2.64
Agt	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	NM_007428	1.56E-05	5.57E-04	2.60

Table S6. Autophagy-related upregulated genes

Input	Name	Transcript	p-value	Adjusted p-value (q-value)	FC
Mt3	metallothionein 3	NM_013603	3.03E-08	1.23E-05	395.68
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	NM_009397	0.002631	1.73E-02	4.39
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	NM_001166402	7.19E-05	1.46E-03	7.26
Park2	Parkinson disease (autosomal recessive, juvenile) 2, parkin	NM_016694	7.94E-10	1.52E-06	6.33
Qsox1	quiescin Q6 sulfhydryl oxidase 1	NM_001024945	1.42E-09	2.10E-06	2.90
Casp4	caspase 1	NM_007609	0.000128	2.13E-03	2.83
Map1lc3a	microtubule-associated protein 1 light chain 3 alpha	NM_025735	7.04E-07	7.71E-05	2.59

Table S7. Metabolome analysis of muscle biopsies from 3-month old WT and KO female mice; absolute concentration of 116 compounds.



ID	HMT DB	Concentration (nmol/g)				Comparative Analysis					
	Compound name	Control		Disease		Control vs Disease			Disease vs Control		
		Mean	S.D.	Mean	S.D.	Ratio [¶]	*p*-value [‖]		Ratio [¶]	*p*-value [‖]	
A_0001	NAD$^+$	496	30	514	27	1.0	0.304		1.0	0.304	
A_0002	cAMP	0.8	0.2	0.7	0.06	1.1	0.418		0.9	0.418	
A_0003	cGMP	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0004	NADH	17	2.0	18	1.5	1.0	0.785		1.0	0.785	
A_0005	Xanthine	0.6	0.5	0.9	0.4	0.6	0.312		1.5	0.312	
A_0006	ADP-ribose	5.8	1.4	9.0	1.4	0.6	0.004	**	1.6	0.004	**
A_0007	Mevalonic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0008	UDP-glucose	19	1.2	32	3.0	0.6	4.2E-06	***	1.7	4.2E-06	***
A_0009	Uric acid	11	1.1	13	2.0	0.8	0.031	*	1.2	0.031	*
A_0010	NADP$^+$	9.1	1.1	11	0.6	0.8	0.013	*	1.2	0.013	*
A_0011	IMP	245	154	79	24	3.1	0.074		0.3	0.074	
A_0012	Sedoheptulose 7-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0013	Glucose 6-phosphate	3,353	601	2,905	271	1.2	0.179		0.9	0.179	
A_0014	Fructose 6-phosphate	1,406	284	1,115	123	1.3	0.084		0.8	0.084	
A_0015	Fructose 1-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0016	Galactose 1-phosphate	5.8	3.0	11	2.6	0.5	0.009	**	2.0	0.009	**
A_0017	Glucose 1-phosphate	353	95	276	33	1.3	0.147		0.8	0.147	
A_0018	Acetoacetyl CoA	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0019	Acetyl CoA	0.9	0.08	1.2	0.2	0.8	0.006	**	1.3	0.006	**
A_0020	Folic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0021	Ribose 5-phosphate	0.5	0.04	0.7	0.5	0.6	0.277		1.6	0.277	
A_0022	CoA	5.3	0.3	8.9	0.8	0.6	4.8E-06	***	1.7	4.8E-06	***
A_0023	Ribose 1-phosphate	7.7	1.0	9.7	1.3	0.8	0.018	*	1.3	0.018	*
A_0024	Ribulose 5-phosphate	2.5	0.7	3.3	0.7	0.8	0.128		1.3	0.128	
A_0025	Xylulose 5-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0026	Erythrose 4-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0027	HMG CoA	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0028	Glyceraldehyde 3-phosphate	0.4	N.A.	N.A.	N.A.	1<	N.A.		<1	N.A.	
A_0029	NADPH	12	1.6	12	1.4	1.0	0.996		1.0	0.996	
A_0030	Malonyl CoA	0.9	0.2	0.8	0.2	1.2	0.204		0.8	0.204	
A_0031	Phosphocreatine	2,928	211	3,752	256	0.8	1.3E-04	***	1.3	1.3E-04	***
A_0032	XMP	N.A.	N.A.	0.3	0.04	<1	N.A.		1<	N.A.	
A_0033	Dihydroxyacetone phosphate	61	18	70	16	0.9	0.369		1.2	0.369	
A_0034	Adenylosuccinic acid	2.1	0.8	1.5	0.2	1.4	0.184		0.7	0.184	
A_0035	Fructose 1,6-diphosphate	315	75	286	18	1.1	0.449		0.9	0.449	
A_0036	6-Phosphogluconic acid	1.4	0.6	1.3	N.A.	1.1	N.A.		0.9	N.A.	
A_0037	*N*-Carbamoylaspartic acid	0.2	0.04	0.2	0.04	0.9	0.555		1.1	0.555	
A_0038	PRPP	17	1.1	18	3.9	1.0	0.603		1.1	0.603	
A_0039	2-Phosphoglyceric acid	1.2	0.5	0.9	0.2	1.3	0.279		0.8	0.279	
A_0040	2,3-Diphosphoglyceric acid	N.A.	N.A.	4.7	1.8	<1	N.A.		1<	N.A.	
A_0041	3-Phosphoglyceric acid	12	2.9	10	1.4	1.2	0.242		0.8	0.242	
A_0042	Phosphoenolpyruvic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0043	GMP	6.1	1.1	6.6	1.1	0.9	0.468		1.1	0.468	
A_0044	AMP	1.3	0.2	1.2	0.3	1.1	0.638		0.9	0.638	
A_0045	2-Oxoisovaleric acid	21	6.4	19	N.A.	1.1	N.A.		0.9	N.A.	
A_0046	GDP	6.7	0.9	7.2	0.5	0.9	0.273		1.1	0.273	
A_0047	Lactic acid	15,532	2,030	10,400	1,361	1.5	0.002	**	0.7	0.002	**
A_0048	ADP	157	14	143	14	1.1	0.138		0.9	0.138	
A_0049	GTP	111	7.3	129	5.6	0.9	0.002	**	1.2	0.002	**
A_0050	Glyoxylate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0051	ATP	7,416	90	6,924	138	1.1	2.2E-05	***	0.9	2.2E-05	***
A_0052	Glycerol 3-phosphate	742	112	600	69	1.2	0.043	*	0.8	0.043	*
A_0053	Glycolic acid	30	N.A.	N.A.	N.A.	1<	N.A.		<1	N.A.	
A_0054	Pyruvic acid	229	30	188	22	1.2	0.034	*	0.8	0.034	*
A_0055	*N*-Acetylglutamic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0056	2-Hydroxyglutaric acid	9.0	1.7	9.4	1.4	1.0	0.646		1.0	0.646	
A_0057	Carbamoylphosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
A_0058	Succinic acid	342	72	426	50	0.8	0.060		1.2	0.060	
A_0059	Malic acid	204	46	235	20	0.9	0.204		1.2	0.204	
A_0060	2-Oxoglutaric acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	

Table S7 (continued). Metabolome analysis of muscle biopsies from 3-month old WT and KO female mice; absolute concentration of 116 compounds

ID	HMT DB	Concentration (nmol/g)				Comparative Analysis					
	Compound name	Control		Disease		Control vs Disease			Disease vs Control		
		Mean	S.D.	Mean	S.D.	Ratio ¶	*p*-value ‖		Ratio ¶	*p*-value ‖	
A_0061	Fumaric acid	81	20	118	10	0.7	0.010	**	1.5	0.010	**
A_0062	Citric acid	114	11	191	4.8	0.6	3.0E-05	***	1.7	3.0E-05	***
A_0063	*cis*-Aconitic acid	0.05	N.A.	0.6	0.6	0.08	N.A.		13	N.A.	
A_0064	Isocitric acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0001	Urea	4,169	303	5,184	784	0.8	0.014	*	1.2	0.014	*
C_0002	Gly	2,658	549	2,324	206	1.1	0.254		0.9	0.254	
C_0003	Putrescine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0004	Ala	2,209	354	3,113	233	0.7	0.002	**	1.4	0.002	**
C_0005	β-Ala	1.9	2.6	1.4	N.A.	1.3	N.A.		0.8	N.A.	
C_0006	Sarcosine	3.0	2.5	4.9	N.A.	0.6	N.A.		1.7	N.A.	
C_0007	γ-Aminobutyric acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0008	*N*,*N*-Dimethylglycine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0009	Choline	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0010	Ser	345	76	499	44	0.7	0.007	**	1.4	0.007	**
C_0011	Carnosine	882	144	764	48	1.2	0.142		0.9	0.142	
C_0012	Creatinine	71	3.4	72	5.9	1.0	0.815		1.0	0.815	
C_0013	Pro	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0014	Betaine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0015	Val	108	45	81	31	1.3	0.294		0.8	0.294	
C_0016	Homoserine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0017	Thr	270	55	359	29	0.8	0.017	*	1.3	0.017	*
C_0018	Betaine aldehyde	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0019	Cys	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0020	Hydroxyproline	265	51	188	21	1.4	0.024	*	0.7	0.024	*
C_0021	Creatine	21,035	1,846	18,204	875	1.2	0.023	*	0.9	0.023	*
C_0022	Leu	120	25	126	16	0.9	0.612		1.1	0.612	
C_0023	Ile	45	18	44	8.6	1.0	0.946		1.0	0.946	
C_0024	Asn	28	15	40	13	0.7	0.183		1.4	0.183	
C_0025	Ornithine	41	5.8	55	5.6	0.7	0.003	**	1.3	0.003	**
C_0026	Asp	179	35	275	7.9	0.6	0.003	**	1.5	0.003	**
C_0027	Homocysteine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0028	Adenine	4.4	0.9	4.8	0.9	0.9	0.478		1.1	0.478	
C_0029	Hypoxanthine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0030	Spermidine	2.7	0.5	4.8	1.4	0.6	0.008	**	1.7	0.008	**
C_0031	Gln	2,067	231	2,442	125	0.8	0.018	*	1.2	0.018	*
C_0032	Lys	1,679	424	3,056	679	0.5	0.002	**	1.8	0.002	**
C_0033	Glu	934	190	857	127	1.1	0.460		0.9	0.460	
C_0034	Met	89	12	92	8.1	1.0	0.589		1.0	0.589	
C_0035	Guanine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0036	His	118	25	159	9.7	0.7	0.018	*	1.3	0.018	*
C_0037	Carnitine	205	19	385	33	0.5	4.2E-07	***	1.9	4.2E-07	***
C_0038	Phe	80	9.4	82	4.2	1.0	0.668		1.0	0.668	
C_0039	Arg	692	139	790	189	0.9	0.326		1.1	0.326	
C_0040	Citrulline	105	16	145	15	0.7	0.002	**	1.4	0.002	**
C_0041	Tyr	75	8.0	78	8.6	1.0	0.602		1.0	0.602	
C_0042	*S*-Adenosylhomocysteine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0043	Spermine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0044	Trp	29	2.5	33	3.3	0.9	0.083		1.1	0.083	
C_0045	Cystathionine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0046	Adenosine	12	1.6	12	1.4	1.0	0.760		1.0	0.760	
C_0047	Inosine	5.2	N.A.	6.5	N.A.	0.8	N.A.		1.2	N.A.	
C_0048	Guanosine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.		N.A.	N.A.	
C_0049	Argininosuccinic acid	11	N.A.	13	2.9	0.9	N.A.		1.1	N.A.	
C_0050	Glutathione (GSSG)	47	21	59	4.0	0.8	0.254		1.3	0.254	
C_0051	Glutathione (GSH)	552	68	868	93	0.6	4.8E-05	***	1.6	4.8E-05	***
C_0052	*S*-Adenosylmethionine	30	2.0	35	2.2	0.8	0.002	**	1.2	0.002	**

ID represents analysis mode and number. 'C' and 'A' showed cation and anion modes, respectively.
N.D. (Not Detected): The target peak or metabolite was below detection limits.
N.A. (Not Available): The calculation was impossible because of insufficiency of the data.
¶ The ratio is computed by using averaged detection values. The latter was used as denominator.
‖ The p-value is computed by Welch's t-test. (*<0.05, **<0.01, ***<0.001)
The data are sorted by ID in ascending order.

Table S7A. Metabolome analysis of muscle biopsies from 5-6-month-old WT and KO male mice; absolute concentration of 116 compounds.

	HMT DB	Concentration (nmol/g)				Comparative Analysis		
ID	Compound name	WT		KO		KO vs WT		
		Mean	S.D.	Mean	S.D.	Ratio ¶	*p*-value ‖	
A_0001	NAD^{+}	441	14	446	13	1.0	0.579	
A_0002	cAMP	0.6	0.10	0.7	0.09	1.0	0.713	
A_0003	cGMP	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
A_0004	NADH	11	0.4	12	0.6	1.1	0.009	**
A_0005	Xanthine	1.1	0.4	2.4	0.6	2.2	0.001	**
A_0006	ADP-ribose	6.7	3.6	6.1	1.2	0.9	0.769	
A_0007	Mevalonic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
A_0008	UDP-glucose	12	1.1	20	1.0	1.6	9.2E-07	***
A_0009	Uric acid	3.0	0.9	7.5	0.9	2.5	2.5E-05	***
A_0010	NADP^{+}	8.0	0.7	13	0.5	1.6	2.5E-06	***
A_0011	IMP	190	81	75	15	0.4	0.033	*
A_0012	Sedoheptulose 7-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
A_0013	Glucose 6-phosphate	2,690	182	1,866	76	0.7	2.1E-04	***
A_0014	Fructose 6-phosphate	1,020	60	686	39	0.7	2.1E-05	***
A_0015	Fructose 1-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
A_0016	Galactose 1-phosphate	5.3	3.8	9.0	1.5	1.7	0.094	
A_0017	Glucose 1-phosphate	337	43	195	15	0.6	0.001	**
A_0018	Acetoacetyl CoA	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
A_0019	Acetyl CoA	0.7	0.14	1.0	0.2	1.4	0.019	*
A_0020	Folic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
A_0021	Ribose 5-phosphate	1.3	0.8	2.2	0.6	1.6	0.095	
A_0022	CoA	4.4	0.3	7.5	0.6	1.7	8.1E-07	***
A_0023	Ribose 1-phosphate	3.1	0.7	4.6	1.6	1.5	0.069	
A_0024	Ribulose 5-phosphate	4.7	1.4	7.6	2.8	1.6	0.047	*
A_0025	Xylulose 5-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
A_0026	Erythrose 4-phosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
A_0027	HMG CoA	0.5	N.A.	0.5	0.03	1.0	N.A.	
A_0028	Glyceraldehyde 3-phosphate	3.2	2.6	2.3	2.4	0.7	0.649	
A_0029	NADPH	8.4	0.6	5.8	0.6	0.7	3.0E-05	***
A_0030	Malonyl CoA	1.1	0.10	1.4	0.14	1.3	0.002	**
A_0031	Phosphocreatine	2,265	140	2,143	226	0.9	0.280	
A_0032	XMP	0.14	N.A.	0.3	0.06	2.3	N.A.	
A_0033	Dihydroxyacetone phosphate	216	47	193	38	0.9	0.395	
A_0034	Adenylosuccinic acid	1.4	0.3	1.7	0.3	1.2	0.136	
A_0035	Fructose 1,6-diphosphate	417	102	214	33	0.5	0.009	**
A_0036	6-Phosphogluconic acid	2.5	0.5	1.4	0.7	0.6	0.010	**
A_0037	*N*-Carbamoylaspartic acid	0.2	0.07	0.2	0.07	1.1	0.671	
A_0038	PRPP	29	5.7	19	4.8	0.6	0.011	*
A_0039	2-Phosphoglyceric acid	3.6	0.3	2.2	0.3	0.6	8.0E-05	***
A_0040	2,3-Diphosphoglyceric acid	2.4	1.3	2.1	0.4	0.9	0.588	
A_0041	3-Phosphoglyceric acid	32	3.0	19	2.0	0.6	9.1E-05	***
A_0042	Phosphoenolpyruvic acid	4.8	0.6	1.0	0.8	0.2	6.2E-06	***
A_0043	GMP	3.1	0.4	3.2	0.4	1.0	0.709	
A_0044	AMP	1.4	0.8	0.9	0.3	0.7	0.272	
A_0045	2-Oxoisovaleric acid	19	1.7	12	0.6	0.6	3.5E-04	***
A_0046	GDP	3.5	0.5	3.9	0.6	1.1	0.168	
A_0047	Lactic acid	12,142	1,398	8,113	545	0.7	0.002	**
A_0048	ADP	85	9.1	96	9.3	1.1	0.066	
A_0049	GTP	120	6.4	175	5.3	1.5	4.8E-07	***
A_0050	Glyoxylate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
A_0051	ATP	7,273	196	7,158	212	1.0	0.357	
A_0052	Glycerol 3-phosphate	504	76	492	34	1.0	0.764	
A_0053	Glycolic acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
A_0054	Pyruvic acid	195	12	151	16	0.8	2.9E-04	***
A_0055	*N*-Acetylglutamic acid	N.A.	N.A.	0.07	0.09	1<	N.A.	
A_0056	2-Hydroxyglutaric acid	26	1.5	8.3	1.0	0.3	1.8E-07	***
A_0057	Carbamoylphosphate	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
A_0058	Succinic acid	206	51	265	34	1.3	0.061	
A_0059	Malic acid	125	27	202	19	1.6	0.001	**
A_0060	2-Oxoglutaric acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	

Table S7A (continued). Metabolome analysis of muscle biopsies from 5-6-month-old WT and KO male mice; absolute concentration of 116 compounds.

	HMT DB	Concentration (nmol/g)				Comparative Analysis		
ID	Compound name	WT		KO		KO vs WT		
		Mean	S.D.	Mean	S.D.	Ratio ¶	*p*-value ||	
A_0061	Fumaric acid	38	16	74	13	1.9	0.004	**
A_0062	Citric acid	209	17	240	18	1.1	0.013	*
A_0063	*cis*-Aconitic acid	0.6	0.4	1.0	0.2	1.8	0.048	*
A_0064	Isocitric acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0001	Urea	6,895	500	6,038	850	0.9	0.054	
C_0002	Gly	3,117	282	2,903	232	0.9	0.204	
C_0003	Putrescine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0004	β-Ala	77	15	30	3.2	0.4	0.002	**
C_0005	Sarcosine	2.1	0.8	2.2	1.8	1.1	0.883	
C_0006	Ala	2,534	247	3,380	328	1.3	4.8E-04	***
C_0007	γ-Aminobutyric acid	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0008	*N*,*N*-Dimethylglycine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0009	Choline	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0010	Ser	273	44	436	48	1.6	1.7E-04	***
C_0011	Carnosine	4,361	300	1,570	192	0.4	1.0E-06	***
C_0012	Creatinine	79	4.9	53	3.0	0.7	4.1E-05	***
C_0013	Pro	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0014	Betaine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0015	Val	93	13	107	40	1.1	0.429	
C_0016	Thr	231	17	316	37	1.4	4.7E-04	***
C_0017	Homoserine	2.8	N.A.	3.4	0.3	1.2	N.A.	
C_0018	Betaine aldehyde	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0019	Cys	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0020	Hydroxyproline	476	91	258	48	0.5	0.003	**
C_0021	Creatine	16,950	744	15,621	953	0.9	0.022	*
C_0022	Leu	102	6.4	157	19	1.5	1.1E-04	***
C_0023	Ile	35	2.0	58	11	1.6	0.002	**
C_0024	Asn	26	6.3	55	14	2.1	8.4E-04	***
C_0025	Ornithine	4.5	2.4	34	11	7.5	3.0E-04	***
C_0026	Asp	104	12	272	19	2.6	4.9E-09	***
C_0027	Homocysteine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0028	Adenine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0029	Hypoxanthine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0030	Spermidine	9.8	0.6	10	0.9	1.0	0.581	
C_0031	Gln	1,618	137	2,180	101	1.3	1.1E-04	***
C_0032	Lys	400	91	1,431	100	3.6	1.2E-08	***
C_0033	Glu	681	164	1,150	86	1.7	0.001	**
C_0034	Met	55	5.4	63	11	1.1	0.135	
C_0035	Guanine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0036	His	131	12	135	8.0	1.0	0.523	
C_0037	Carnitine	235	15	360	20	1.5	2.3E-07	***
C_0038	Phe	73	7.5	74	8.0	1.0	0.863	
C_0039	Arg	186	45	418	34	2.2	2.4E-05	***
C_0040	Citrulline	102	8.9	137	11	1.3	1.3E-04	***
C_0041	Tyr	105	24	71	11	0.7	0.032	*
C_0042	*S*-Adenosylhomocysteine	4.1	0.6	4.3	0.5	1.1	0.424	
C_0043	Spermine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0044	Trp	26	2.3	22	1.8	0.9	0.016	*
C_0045	Cystathionine	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	
C_0046	Adenosine	1.7	2.0	5.4	3.5	3.2	0.077	
C_0047	Inosine	9.4	2.6	20	4.5	2.2	3.6E-04	***
C_0048	Guanosine	N.A.	N.A.	1.6	0.7	1<	N.A.	
C_0049	Argininosuccinic acid	3.6	N.A.	7.4	0.3	2.1	N.A.	
C_0050	Glutathione (GSSG)	92	6.2	92	13	1.0	0.952	
C_0051	Glutathione (GSH)	875	78	1,569	65	1.8	3.1E-07	***
C_0052	*S*-Adenosylmethionine	14	0.8	24	2.2	1.7	5.3E-06	***

ID consists of analysis mode and number. 'C' and 'A' showed cation and anion modes, respectively.
N.D. (Not Detected): The target peak or metabolite was below detection limits.
N.A. (Not Available): The calculation was impossible because of insufficiency of the data.
¶ The ratio is computed by using averaged detection values. The latter was used as denominator.
| | The p-value is computed by Welch's t-test. (*<0.05, **<0.01, ***<0.001)
The data are sorted by ID in ascending order.

Table S8. The effect of ERT on glycogen levels (μg glucose/hr/mg protein) in KO and DKO strains.

Genotype	Tissue	Untreated	Treated	% Reduction*
KO	Gastroc	44.2 ± 12.9 (n=7)	28.0 ± 20.3 (n=6)	41
	Quad	69.0 ± 34.1 (n=7)	25.9 ± 6.6 (n=6)	67
	Heart	153.5 ± 41.2 (n=2)	14.2 ± 10.0 (n=2)	93
DKO	Gastroc	19.8 ± 19.2 (n=5)	5.0 ± 6.8 (n=6)	98
	Quad	27.7 ± 27.6 (n=5)	5.5 ± 10.5 (n=9)	98
	Heart	165.0 ± 31.8 (n=2)	7.9 ± 8.1 (n=2)	98

Each value is the mean ± SD from the indicated number of animals. The WT levels of glycogen in gastrocnemius/quadriceps of 8.5-month-old mice are 3.6 ± 3.0 μg glucose/hr/mg protein. [*]The amount of residual glycogen (minus the WT level) in treated animals was compared to the level of glycogen in the respective tissue of KO mice.