Supporting Information

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SI Methods

Histology and Immunohistochemistry. Succinic dehydrogenase (SDH) staining on cryosections was carried out using a 0.2-M phosphate buffer at pH 7.6. For SDH staining, 270 mg of succinic acid and 10 mg of nitro blue tetrazolium were freshly dissolved in 10 mL phosphate buffer and incubated with sections for 1 h. For immunohistochemistry, cryosections of tibialis anterior (TA) or soleus were fixed in 4% paraformaldehyde in PBS for 10 min at 4 °C and washed in PBS. Tissue sections were incubated for 30 min with 3% goat serum (Sigma) in PBS, followed by overnight incubation with primary antibody at 4 °C (1:100 dilution of rabbit polyclonal anti-laminin antibody, Sigma, or 1:100 dilution of rabbit polyclonal anti-SQSTM1, H-290 Santa Cruz). Primary antibodies were detected by Alexa Fluor 488 (Invitrogen) diluted at 1:800 in 3% goat serum in PBS.

Western Blot Analysis. Antibodies against LC3 (rabbit, polyclonal, 1:1,000 in 2.5% milk in Tris-Buffered Saline Tween 20 (TBST); Novus Biologicals), p62 (mouse, monoclonal, 1:1,000 in 2.5% milk in TBST; BD Biosciences), phosphorylated AMP-activated protein kinase (P-AMPK) (rabbit, polyclonal, 1:1,000 in 5% BSA; Cell Signaling), AMPK (rabbit, polyclonal, 1:1,000 in TBST; Cell Signaling), histone deacetylase 1 (HDAC1) and HDAC2 (rabbit, polyclonal, 1:1,000 in 2.5% milk; Sigma), and α-tubulin (mouse, monoclonal, 1:1,000 in 2.5% milk TBST; Sigma) were used. The ECL Advance Western Blotting Detection Kit (Amersham Biosciences) was used for signal detection.

DNA Delivery by Electroporation. Experiments were performed on 4-wk-old mice. In some experiments, GFP-LC3 plasmid was used in a 1:1 ratio of either a control plasmid or a combination of HDAC1- and HDAC2-expressing plasmids. Myofibers expressing GFP-LC3 were analyzed by fluorescence microscopy. Autophagosomes were quantified by counting GFP-LC3–positive dots in the fibers by normalizing for the number of GFP-positive fibers. As a positive control for the ubiquitin-proteasome activity assay, mice cotransfected with the dsRED and Ub-G76V-GFP reporter plasmid were treated for 4 d with a daily i.p. injection of 150 μL of MG262 (5 mmol/kg) before killing.

Probes and Primers for RT-PCR. Taqman probes were as follows: *Ppargc1a*: Mm00447183_m1; *Ppargc1b*: Mm01258518_m1; *Myh7*: Mm00600555_m1; *Myh1*: m01332488_g1; *Myh4*: Mm01332518_m1; *Myh2*: Mm01332564_m1; *p62*: Mm_00448091_m1; *Gapdh*: Mm99999915_g1.

SYBR green primers were as follows: *Atg5* for: ggagagaagaggagccaggt, rev: gctgggggacaatgctaata; *Atg7* for: gcctaacacagatgctgcaa, rev: tgctcttaaaccgaggctgt; *Gabarapl1* for: catcgtggagaaggctccta, rev: atacagctggcccatggtag; *LC3b* for: cactgctctgtcttgtgtaggttg, rev: tcgttgtgcctttattagtgcatc; *Ucp2* for: tgcccgtaatgccattgtc, rev: agtggcaagggaggtcatct; *Acadm* for: ggaaatgatcaacaaaaaaaagaagtattt, rev: atggccgccacatcaga; *β-actin* for: ctggctcctagcaccatgaagat, rev: ggtggacagtgaggccaggat.

Microarray Analysis. Samples were labeled using the Illumina TotalPrep RNA Amplification Kit (Ambion). Fold change was calculated according to signal intensity ratios between double knockout (dKO) and control samples. By setting a threshold of twofold induction, the list of transcripts altered between control and dKO mice was used for a gene ontology analysis by the DAVID bioinformatics browser (http://david.abcc.ncifcrf.gov/).

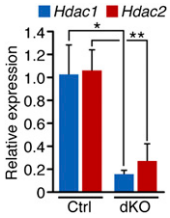

Fig. S1. Deletion of *Hdac1* and *Hdac2* alleles in dKO mice. Real-time RT-PCR analysis of *Hdac1* and *Hdac2* expression using primers spanning the deleted regions, using RNA isolated from skeletal muscle of control and dKO mice at postnatal day 1. Data are expressed as mean ± SEM $n = 4$. $^{*}P < 0.05$; $^{**}P < 0.005$.

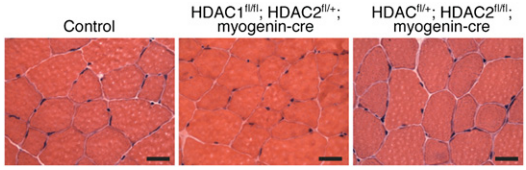

Fig. S2. Normal skeletal muscle architecture in mice with one *Hdac1* or *Hdac2* allele. H&E sections of TA muscle of 3-mo-old mice of indicated genotypes. (Scale bars, 40 μm.)

Time of analysis	Total mice	Expected dKO	Obtained dKO	X^2
P10	703	257	149 (~58%)	45.38
E18.5	71	35	29 (~83%)	1.03

Fig. S3. A portion of dKO mice die perinatally. Number of dKO mice expected and obtained at 10 d after birth (P10) or at embryonic day 18.5 (E18.5). $\chi^2 > 5.99$ indicates that the difference between expected and observed dKO mice is statistically significant.

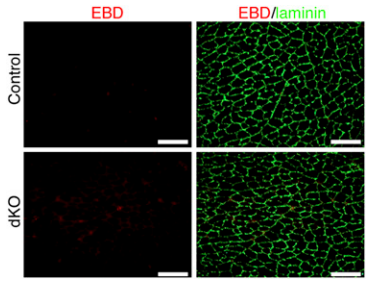

Fig. S4. Histological sections of 4-wk-old control and dKO TA harvested after i.p. administration of Evans blue dye (EBD) (10 mg/mL at 0.1 mL per 10 g body mass). EBD stains red, and immunostain of laminin is green. (Scale bars, 100 μm.) No evidence of myofiber leakage is seen in dKO mice.

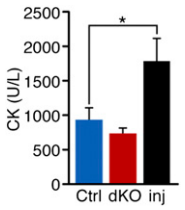

Fig. S5. dKO mice do not show signs of skeletal muscle damage. Quantification of creatine kinase (CK) from serum of control (Ctrl) and dKO mice at 4 wk of age. Serum from mice 24 h after cardiotoxin injury (inj) were used as a positive control for the assay. Values are mean ± SEM. $n = 6$. $^{*}P < 0.05$.

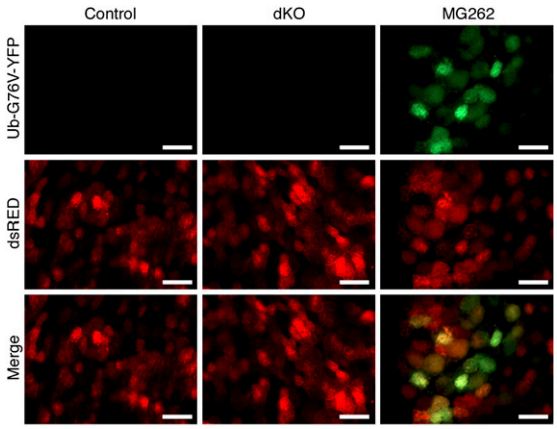

Fig. S6. dKO mice do not show inhibition of proteasome activity in vivo. Four-week-old TA muscles were coelectroporated with Ub-G76V-YFP (green), which is a ubiquitin-proteasome pathway activity reporter, and dsRED plasmid (red) for monitoring the efficiency of electroporation. Fluorescence in transfected muscles was monitored 12 d after electroporation by confocal microscopy. Mice treated with MG262 were used as a positive control. (Scale bars, 100 μm.)

Gene Symbol	Fold Induction vs. Control
Rbm13	4.51
Myh8	4.46
Chac1	3.84
Gfpt1	3.24
Lin7B	3.09
Klk1b22	2.86
Slc25a25	2.79
Dbp	2.52
Tpmt	2.24
Mbp	2.22
Fut10	2.22
Ttbk1	2.20
Mpz	2.15
Sgk1	2.12
Kcnh2	2.09
Coq10B	2.08
Dusp26	2.05
Cdh4	2.04

Fig. S7. List of the transcripts up-regulated in dKO mice as identified by gene array analysis. Threshold was set at twofold induction.

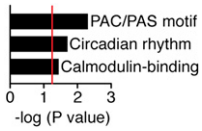

Fig. S8. Gene ontology analysis was performed with the DAVID browser on RNA isolated from TA of 4-wk-old control and dKO mice. Significantly ($P < 0.05$) enriched biological processes are shown. Plotted is the −log (*P* value) with the threshold set to 1.3 [log (0.05)].

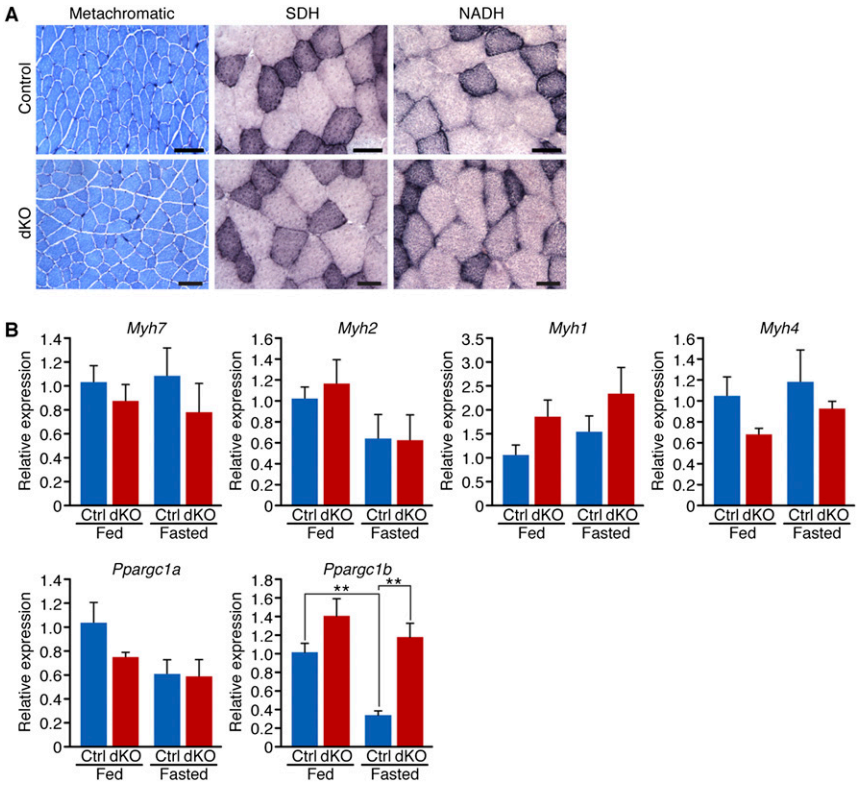

Fig. S9. dKO mice do not show changes in metabolism at baseline before histological onset of myopathy. (*A*) Histological sections of TA muscle isolated from 4-wk-old control and dKO mice were analyzed by metachromatic ATPase, SDH, and NADH staining. (Scale bars, 100 μm for metachromatic ATPase, 40 μm for SDH and NADH staining.) (*B*) Real-time RT-PCR analysis of different myosin heavy chain genes, *Ppargc1a*, and *Ppargc1b* in control (Ctrl) and dKO mice, fed or fasted for 48 h, at 4 wk of age. Data are expressed as mean ± SEM. $n = 4$. $^{**}P < 0.005$.

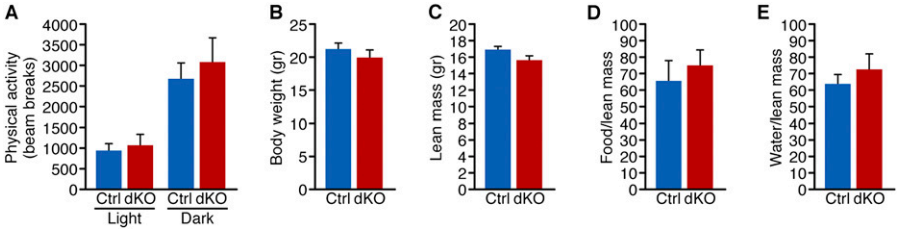

Fig. S10. dKO mice have higher energy expenditure without differences in other metabolic parameters. No significant differences were seen in (*A*) physical activity, (*B*) body weight, (*C*) lean mass, (*D*) food, or (*E*) water consumption between control (Ctrl) and dKO mice, between 5 and 7 wk of age. Values are mean ± SEM. $n = 7$.

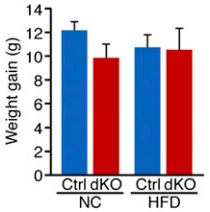

Fig. S11. Eight weeks of high-fat diet (HFD) does not significantly alter body mass of female control or dKO mice. Body weight of control (Ctrl) or dKO mice on normal chow (NC) or HFD, expressed as the difference between the final and the starting body weight in grams. Values are mean ± SEM. $n = 6$.

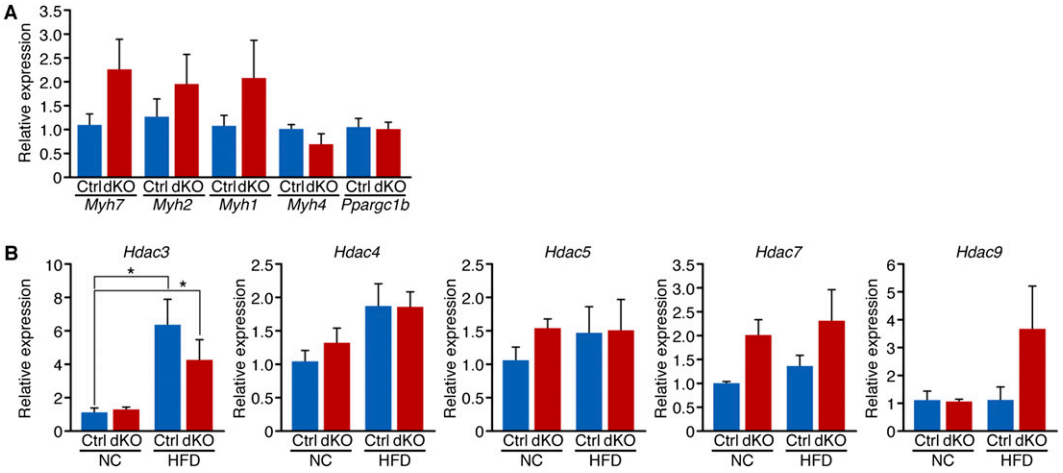

Fig. S12. High-fat diet (HFD) prevents significant changes of metabolic genes in dKO mice, without modifying HDAC expression between control and dKO mice. Real-time RT-PCR analysis of (*A*) different myosin heavy chain genes and *Ppargc1b* and (*B*) different *Hdac* genes in control (Ctrl) and dKO mice after 8 wk of normal chow (NC) or HFD. Data are expressed as mean ± SEM. $n = 4$.

Time of analysis	Total mice	Expected dKO	Obtained dKO	X^2
P10	45	22	19 (~86%)	0.41

Fig. S13. High-fat diet (HFD) prevents perinatal lethality of dKO mice. Number of total and dKO mice expected and obtained at postnatal day 10 from mothers fed HFD. $\chi^2 < 5.99$ indicates that the difference between expected and observed dKO mice is not significant.