Supporting Information

O'Neill et al. 10.1073/pnas.1105062108

SI Materials and Methods

Generation of AMPK β1 and β2 Floxed Mck-Cre Mice. β1 and β2 floxed mice were generated on a pure C57BL/6 background by OZGENE (Perth, Australia) using standard homologous recombination techniques and the targeting strategy illustrated in Fig. S1*A*. MCK-Cre mice were backcrossed for at least 10 generations onto a C57BL/6 background (1). All experiments were approved by the St. Vincent's Hospital and McMaster University Animal Ethics Committees. Mice were housed in SPF microisolators and maintained under control environment conditions (12 h/12 h light-dark cycle with lights on at 07:00 and temperature of 23 °C). Mice received chow food (18% kcal fat; Diet 8664, Harlan Teklad, Madison, WI) and water ad libitum. Metabolic monitoring was performed in a Comprehensive Lab Animal Monitoring System (Columbus Instruments, Columbus, OH) as described (2). Insulin tolerance was tested in mice fasted for 6 h after i.p. injection of 0.6 U/kg of insulin (3). Fed and fasting serum analyses were performed as described (2).

Treadmill Running and Contraction Experiments. Before treadmill running experiments, mice were acclimatized as described (4). For exercise capacity testing, mice ran at a 5-degree gradient at 10 m/min for 2 min before intensity (running speed) was increased by 1 m/min every 2 min until mice could not be prompted to continue running by electric shockers at the back of the treadmill. The following week, VO_2, VCO_2, RER, and % substrate used were determined by running mice at 50% of maximal running speed in an enclosed Oxymax treadmill (Columbus Instruments) (4) for 20 min. In vivo glucose clearance in gastrocnemius and soleus during treadmill running was determined as described (5). Ex vivo 2-DG uptake was determined in extensor digitorum longus (EDL) and soleus muscles as described (3, 4, 6).

Analytical Methods. ***In vivo glucose clearance.*** In vivo glucose clearance in gastrocnemius and soleus during treadmill running was determined as described (7). Briefly, immediately before the commencement of rest/exercise protocol, mice were given an i.p. injection of saline (800 μL/100 g body wt) containing 0.1 mM 2-deoxyglucose and 60 μCi/mL 2-[^{3}H]deoxyglucose corresponding to ∼12 μCi/mouse. Blood samples (∼30 μL) were collected from the tail at −2, 10, and 20 min time points during the exercise/rest period. After 20 min, mice were euthanized by cervical dislocation and muscles snap-frozen in liquid nitrogen. Plasma lactate (Biovison) and NEFA (Wako Chemicals, Japan) were determined using the manufacturers' recommendations.

Ex vivo 2-deoxyglucose (2DG) uptake. Glucose uptake experiments in isolated extensor digitorum longus (EDL) and soleus muscles dissected from anesthetized mice were performed as recently described (8, 9). For matching of muscle force between WT and AMPK β1β2M-KO mice, EDL muscles were stimulated at 100 Hz, 40 V, 350 ms pulse duration, 3 tetani/min for 5 min and soleus muscles at 30 Hz, 40 V, 600-ms pulse duration, 12 tetani/min for 10 min with 2DG uptake being measured over the duration of the contraction protocol.

Muscle analyses. Muscles lysates were generated as described (7, 10). For validation of AS160 T642 phospho antibody and determination of TBC1D1 PAS phosphorylation, Protein A Sepharose beads (Invitrogen) were incubated with TBC1D1 antibody (1:50) (Cell Signaling Technology) for 2 h at 4 °C on a rotating wheel. Beads plus antibody were centrifuged at 6,000 rpm for 4 min; supernatant removed and further washed 3 times (PBS, PBST, PBS) with 30 s in between each wash. PBS was added to make up a final volume that was double that of the bead volume (i.e., 1:1; beads+Ab: PBS), and 50 μL of this suspension was added to each sample tube containing 60 μg of muscle lysate. Suspensions were incubated overnight at 4 °C on a rotating wheel and washed as described above. Immunoprecipitates were heated for 5 min at 95 °C in 4× sample buffer (200 mM Tris, pH 6.8/8% SDS/0.25% bromophenol blue/25% glycerol/20 mM DTT) and centrifuged (6,000 rpm, 1 min) to clarify. Protein phosphorylation and expression levels were determined by SDS/PAGE followed by immunoblotting using muscle lysates that were adjusted to equal protein concentration as described (7, 10). For adequate separation of TBC1D1 and AS160, immunoprecipitates and lysates were run on 4.5% Tris·HCl gels, and for verification of equal protein concentration in total AS160 and TBC1D1 blots, proteins were also run on 7.5% gels and blotted for GAPDH. Membranes were blocked in 5% skim-milk/PBST for 1 h at room temperature before incubation with primary antibodies overnight at 4 °C. Antibodies for determination of phosphorylation status and total expression of various proteins are as follows: AMPK α1 and α2 (8), AMPK β1, β2, and phospho- αT172, Akt S473, AS160 T642, phospho-(Ser/Thr) Akt substrate (PAS) and total Akt, AS160 and TBC1D1 (Cell Signaling Technology), hexokinase II (Alpha Diagnostic International), MitoProfile OXPHOS (MitoSciences), and GLUT4 (Affinity Bioreagents). GAPDH or β-tubulin (AbCam) was used as a protein loading control. Membranes were washed 3 × 10 min with PBST and incubated with an appropriate HRP-conjugated secondary antibody (1:10,000) for 1 h at room temperature. Protein bands were visualized using a Fusion Image Dock Station (Vilber Lourmat, Germany) and enhanced chemiluminescence (ECL$^+$). Bands were quantified using ImageJ software and protein content was expressed in relative units in comparison with control samples loaded on each gel or in the case of phosphorylated proteins as a ratio of the phosphorylated to total protein (e.g., pAkt S473/Akt). Membranes used for detection of phosphorylated ACC, Akt, AS160 or TBC1D1 were stripped with a buffer containing 100 mM 2-mercaptoetahnol, 2% SDS, and 62.5 mM Tris·HCl (pH 7.8). Membranes were reprobed with the corresponding total antibody. For determination of TBC1D1 expression in phospho AS160 T642 and TBC1D1 PAS blots, a separate blot was run using the same samples.

Myosin heavy chain isoforms (MyHC). MyHC isoforms were resolved using polyacrylamide gel electrophoresis (PAGE) as described (11). Briefly, 2 μg of EDL or soleus muscle proteins were resolved using a SDS/PAGE gel consisting of a stacking gel with 4% bis-acrylamide and a separating gel with 8% bis-acrylamide (final concentration separating: 30% glycerol, 0.2 M Tris·HCl pH 8.8, 0.1 M glycine, 0.4% SDS, 0.1% ammonium persulfate, 0.05% TEMED; stacking: 30% glycerol, 0.07 M Tris·HCl pH 6.8, 40 mM EDTA pH 7.0; 0.4% SDS, 0.1% ammonium persulfate, 0.05% TEMED). The electrophoresis buffer consisted of 300 mM Tris, 450 mM glycine, 0.1% SDS and 0.08% mercaptoethanol (12). The gels were run on a Minigel electrophoresis system (Biorad Laboratories, Rockford, IL) at 4 °C at constant current of 20 mA until proteins entered the stacking gel and then at 10mA for a further 20 h. Following electrophoresis, gels were silver stained using SilverSNAP Stain kit following manufacturers' recommendations (Pierce, Rockford, IL). Bands were identified as described (11, 13). The bottom band in soleus is referred to as MyHC I whereas in EDL the bottom band is referred to as MyHC IIb. The upper bands are combined MyHC IIa and x and

referred to as IIa/x. Bands were scanned and quantified using ImageJ software.

AMPK activity. AMPK α-isoform-specific AMPK activity was measured in immunoprecipitates from 500 μg of quadriceps muscle lysates using anti-α1 and anti-α2 antibodies. In brief, muscle lysates were incubated with AMPK-α1- and -α2-bound Protein A Sepharose beads overnight at 4 °C. Immunoprecipitates were washed in cell lysis- and Tris (50 mM) buffers and supernatant drawn off to remove nonspecific binding of antibodies. The immunoprecipitates were then resuspensed in TDGA (50 mM Tris, pH 7.5/1 mM DTT/10% glycerol/0.1% TritonX-100) buffer. AMPK activities were measured by in vitro kinase assay using a buffer containing AMP (10 μU), DTT (1 mM), SAMS peptide (100 μM), ATP (200 μM) and [γ-^{32}P]-ATP. AMPK-α1 and α2 activities were measured using liquid scintillation counting (Tri-Carb 2000; Packard Instrument), and data were expressed as picomoles of phosphate transferred to the SAMS peptide per minute per milligram of total protein.

Mitochondrial enzymatic activities: Citrate synthase (CS), cytochrome (cyt) c oxidase, and succinate oxidoreductase. Freeze-clamped quadriceps were chipped and weighed, homogenized in Mito-Hepes buffer (100 mM KCl, 200mM mannitol, 70 mM sucrose, 5 mM Hepes, pH 7.4, 1 mM EGTA) and centrifuged at 3000 rpm for 15 min. Supernatant was collected and snap frozen in liquid nitrogen. BCA protein assay was performed for determination of protein concentration. CS activity was determined by measuring the formation of thionitrobenzoate anion. Briefly, 15 μL of homogenate per sample was added to a cuvettete containing 810 μL prewarmed potassium phosphate (Kpi) buffer (0.05 M, pH 7.4, 30 °C), 10 μL acetyl-CoA (7.5 mM in 0.1 M Tris·HCL buffer, pH 8.0) and 100 μL of 0.1 mM dithionitrobenzoic acid. The reaction was started by addition of 50 μL of 9.0 mM oxaloacetate and absorbance was read at 412 nm every 30 s for 2 min at 37 °C for determination of CS activity.

Cyt *c* oxidase (Complex IV) activity was determined by measuring the rate of oxidation of reduced cyt *c* (14). Briefly, stock cyt *c* (oxidized) was reduced by sodium ascorbate in Kpi (0.05 M, pH 7.4). 10 μL of reduced cyt *c* was added to a cuvettete containing 1 mL prewarmed Kpi buffer (0.05 M, pH 7.4, 30 °C) and after the addition of 15 μL of homogenate per sample, cyt *c* activity was determined by recording absorbance at 550 nm every 30 s for 2 min at 37 °C.

Succinate oxidoreductase (Complex II) activity was determined by measuring the rate of reduction of coenzyme Q1 (ubiquinone, ε = 12 mM^{-1} cm^{-1}) at 280 nm over 2 min. Briefly, 60 μL of homogenate per sample was preincubated with Kpi (0.05 mM, pH 7.4) containing succinate (0.01 M) for 10 min at 37 °C to activate complex II. Rotenone and antimycin A were added and baseline absorbance was recorded. 5 μL of coenzyme Q1 (10 mM in ethanol) was added and a absorbance was recorded over 2 min. All samples were analyzed in duplicate on a spectrophotometer (Cary Bio-300, Varion, Palo Alto, CA).

RNA isolation and real-time quantitative PCR (RT-qPCR). Total RNA was isolated from ∼10–15 mg quadriceps muscle as described (4). RT-qPCR on nuclear (*Cs, Pgc1-α*, *Cpt-1*, *β-had, Nd1*) and mitochondrial encoded (*Sdhd*) mitochondrial transcripts was performed using a Rotor-Gene 6000 (Corbett Research, Mortlake, Australia) and gene expression was analyzed using TaqMan assay-on-demand expression kits (Applied Biosystems, Foster City, CA) or SYBR Green chemistry (PerfeC$_T$a SYBR Green Supermix with ROX, Quanta Biosciences, Gaithersburg, MD) as described (4). Primer sequences for primers (*Nd1* and *Sdhd*) used with SYBRgreen are as follows: *Nd1*: forward: 5′-GTGGCTCATCTACTCCACTGA-3′, reverse: 5′-TCGAGCGATCCATAACAATAA-3′ and *Sdhd*: forward: 5′-TGTTCCCTCCTGAGTTAGA-3′, reverse: 5′-CTAGAAGCAGAGCCATCAA-3′. Melt-curve analysis was performed to verify a single transcript was produced. RT-qPCR relative gene expression was calculated using the comparative *Ct* ($2^{-\Delta\Delta Ct}$) method where expression was normalized to TATA box binding protein.

Mitochondrial DNA copy number. Total DNA was extracted from whole quadriceps muscle using the QIAmp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNase was added to remove any contaminating RNA. DNA concentration and quality was assessed using the Nanodrop 2000 (Thermoscientific, Wilmington, DE). RTqPCR was performed in duplicate using the 7300 Real-time PCR system (Applied Biosystems) and SYBR Green chemistry (PerfeC$_T$a SYBR Green Supermix with ROX, Quanta Biosciences) under standard thermocycling conditions using primers specific to a nuclear gene (*Mapk1*) and a portion of the mitochondrial genome (*CoxII*) to determine copies of mtDNA per diploid nuclear genome. Primers used are as follows: Mapk1, forward: 5′-GCTTATGATAATCTCAACAAAGTTCG-3′, reverse: 5′-ATGTTCTCATGTCTGAAGCG-3′; COXII, forward: 5′-GCCGACTAAATCAAGCAACA-3′, reverse: 5′-CAATGGGCATAAAGCTATGG-3′. All primers were found to have >90% efficiencies using a standardized serial dilution curve and a melt-curve analysis was performed to verify a single transcript was produced.

Electron microscopy. Transmission electron microscopy was used to determine mitochondrial morphology and distribution as described (15, 16). In brief, tibialis anterior muscle was cut into longitudinal sections and fixed in 1% osmium tetroxide, dehydrated with ethanol, and embedded in Spurr's resin. Seventy-nanometer sections were cut using an ultramicrotome (Ultracut E; Reichert), before being placed on Cu/Pd grids (200 mesh size) and stained (5 min with uranylacetate and 2 min with lead acetate). The preparation, fixation, and sectioning of all samples were performed by the electron microscopy group at McMaster University Medical Center. Samples were viewed using a transmission electron microscope (TEM) (Jeol 1200 EX: Jeol, Tokyo, Japan). The images were taken using a 4 megapixel TEM imaging system (AMT XR41-S, AMT, Woburn, MA). Two representative images per muscle fiber were obtained for a total of eight images per sample (four images of the subsarcolemmal region near the nucleus and four images of the opposite side of the fiber, away from a nucleus; all images contained the intermyofibrillar region). Images were analyzed for mitochondrial number and area using a computerized image analysis program (Image Pro Plus, ver. 4.5, Media Cybernetics, Silver Spring, MD), as described (16). For mitochondrial area distribution, data expressed was determined by calculating the upper and lower 95% confidence intervals of the geometric mean (GM) given data were not normally distributed, significant differences were illustrated by no overlap between group GMs.

Enzymatic metabolite assays. Resting and treadmill exercised quadriceps were chipped under liquid nitrogen and freeze-dried overnight. Freeze-dried samples were powdered and weighed (∼4 mg) and metabolites were extracted using 0.5 M Perchloric acid followed by neutralization with 2.3 M $KHC0_3$.

Muscle lactate was determined using 10 μL of muscle extract/sample, standard or blank and 185 μL reaction mix (100 mM hydrazine, 100 mM glycine, 0.5 mM NAD+, pH 10.0) added to a 96-well black plate (ex 340 nm em 460 nm; TECAN). Lactate dehydrogenase (8 U/mL) (Roche, ON, Canada) was added and fluorescence was again measured after 1 h.

Muscle glycogen content was determined as glycosyl units after acid hydrolysis as described (17). In short, 10 mg wt/wt muscle was hydrolyzed in 1 M HCl at 98 °C for 2 h and analyzed using an automatic analyzer (Hitachi automatic analyzer 912; Boehringer Mannheim, Ingelheim, Germany).

PCr and nucleotides. For determination of muscle ATP and PCr, 10 μL of muscle extract/sample, standard or blank was added in triplicate to the appropriate wells of a 96-well black plate. 185 μL

of buffer (50 mM Tris, 1 mM $MgCl_2$, 0.5 mM DTT, 0.1 mM glucose, 50 μM NADP, G6PDH 0.02 U/mL, pH 8.1) was added to each well and allowed to incubated for 15 min before background florescence was measured (ex 340nm em 460nm). Dilute hexokinase (0.015 U/mL final concentration) was then added to all wells and plate was incubated for 1 h at room temperature before fluorescence was read for determination of ATP. For the second reaction used to determine PCr levels, dilute creatine kinase (324 U/mg CK plus 5 mg ADP per 5 mL buffer) was added to all wells and plate was incubated for 1 h at room temperature before final fluorescence was read. ADP and AMP were calculated as described (18). Muscle creatine levels were determined by adding 4 μL of either extract/sample, blank or standard in triplicate to appropriate wells of a 96-well black plate. 185 μL of buffer (50 mM Imidazole, 5 mM $MgCl_2$, 30 mM KCl, 25 μM PEP, 200 μM ATP, 45 μM NADH, 0.24 U/mL, 0.75 U/mL) was added to all wells and plate was incubated for 15 min before background fluorescence was determined (ex 340 nm, em 460 nm). Dilute creatine kinase (2.3 μg/well) was added to all wells of the plate and incubated for 1 h at room temperature before final fluorescence was determined. Total muscle creatine was calculated by adding PCr and Cr whereas PCr/Cr was determined by dividing PCr by Total creatine.

Statistics. Unless other wise noted data were expressed as means ± SEM. Results were analyzed using Student *t* test, paired *t* test, or analysis of variance (ANOVA) procedures where appropriate using Graphpad Prism software. Significance was accepted at $P < 0.05$.

1. Chen S, Wasserman DH, MacKintosh C, Sakamoto K (2011) Mice with AS160/TBC1D4-Thr649Ala knockin mutation are glucose intolerant with reduced insulin sensitivity and altered GLUT4 trafficking. *Cell Metab* 13:68–79.
2. Jørgensen SB, et al. (2004) Knockout of the alpha2 but not alpha1 5′-AMP-activated protein kinase isoform abolishes 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranosidebut not contraction-induced glucose uptake in skeletal muscle. *J Biol Chem* 279:1070–1079.
3. Morino K, Petersen KF, Shulman GI (2006) Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes* 55 (Suppl 2):S9–S15.
4. Watt MJ, et al. (2006) CNTF reverses obesity-induced insulin resistance by activating skeletal muscle AMPK. *Nat Med* 12:541–548.
5. Jørgensen SB, Jensen TE, Richter EA (2007) Role of AMPK in skeletal muscle gene adaptation in relation to exercise. *Appl Physiol Nutr Metab* 32:904–911.
6. Barnes BR, et al. (2004) The 5′-AMP-activated protein kinase gamma3 isoform has a key role in carbohydrate and lipid metabolism in glycolytic skeletal muscle. *J Biol Chem* 279:38441–38447.
7. Maarbjerg SJ, et al. (2009) Genetic impairment of {alpha}2-AMPK signaling does not reduce muscle glucose uptake during treadmill exercise in mice. *Am J Physiol Endocrinol Metab* 297:E924–E934.
8. Steinberg GR, et al. (2010) Whole body deletion of AMP-activated protein kinase beta2 reduces muscle AMPK activity and exercise capacity. *J Biol Chem* 285: 37198–37209.
9. Dzamko N, et al. (2008) AMPK-independent pathways regulate skeletal muscle fatty acid oxidation. *J Physiol* 586:5819–5831.
10. Beck Jorgensen S, et al. (2009) Reduced AMP-activated protein kinase activity in mouse skeletal muscle does not exacerbate the development of insulin resistance with obesity. *Diabetologia* 52:2395–2404.
11. Talmadge RJ, Roy RR (1993) Electrophoretic separation of rat skeletal muscle myosin heavy-chain isoforms. *J Appl Physiol* 75:2337–2340.
12. Schertzer JD, Gehrig SM, Ryall JG, Lynch GS (2007) Modulation of insulin-like growth factor (IGF)-I and IGF-binding protein interactions enhances skeletal muscle regeneration and ameliorates the dystrophic pathology in mdx mice. *Am J Pathol* 171:1180–1188.
13. Agbulut O, Noirez P, Beaumont F, Butler-Browne G (2003) Myosin heavy chain isoforms in postnatal muscle development of mice. *Biol Cell* 95:399–406.
14. Gianni P, Jan KJ, Douglas MJ, Stuart PM, Tarnopolsky MA (2004) Oxidative stress and the mitochondrial theory of aging in human skeletal muscle. *Exp Gerontol* 39:1391–1400.
15. Safdar A, et al. (2011) Endurance exercise rescues progeroid aging and induces systemic mitochondrial rejuvenation in mtDNA mutator mice. *Proc Natl Acad Sci USA* 108:4135–4140.
16. Trifunovic A, et al. (2004) Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* 429:417–423.
17. Passonneau JV, Gatfield PD, Schulz DW, Lowry OH (1967) An enzymic method for measurement of glycogen. *Anal Biochem* 19:315–326.
18. Dudley GA, Tullson PC, Terjung RL (1987) Influence of mitochondrial content on the sensitivity of respiratory control. *J Biol Chem* 262:9109–9114.

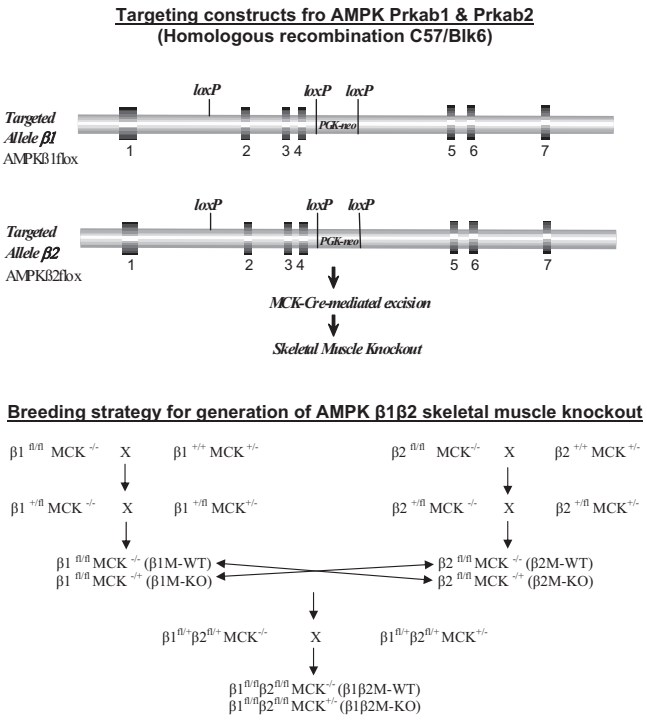

Fig. S1. Generation of β1β2M-KO mice. Gene targeting strategy for generation of AMPK β1 and β2 floxed (fl/fl) mice and breeding strategy for generation of β1, β2 and β1β2 M-KO mice.

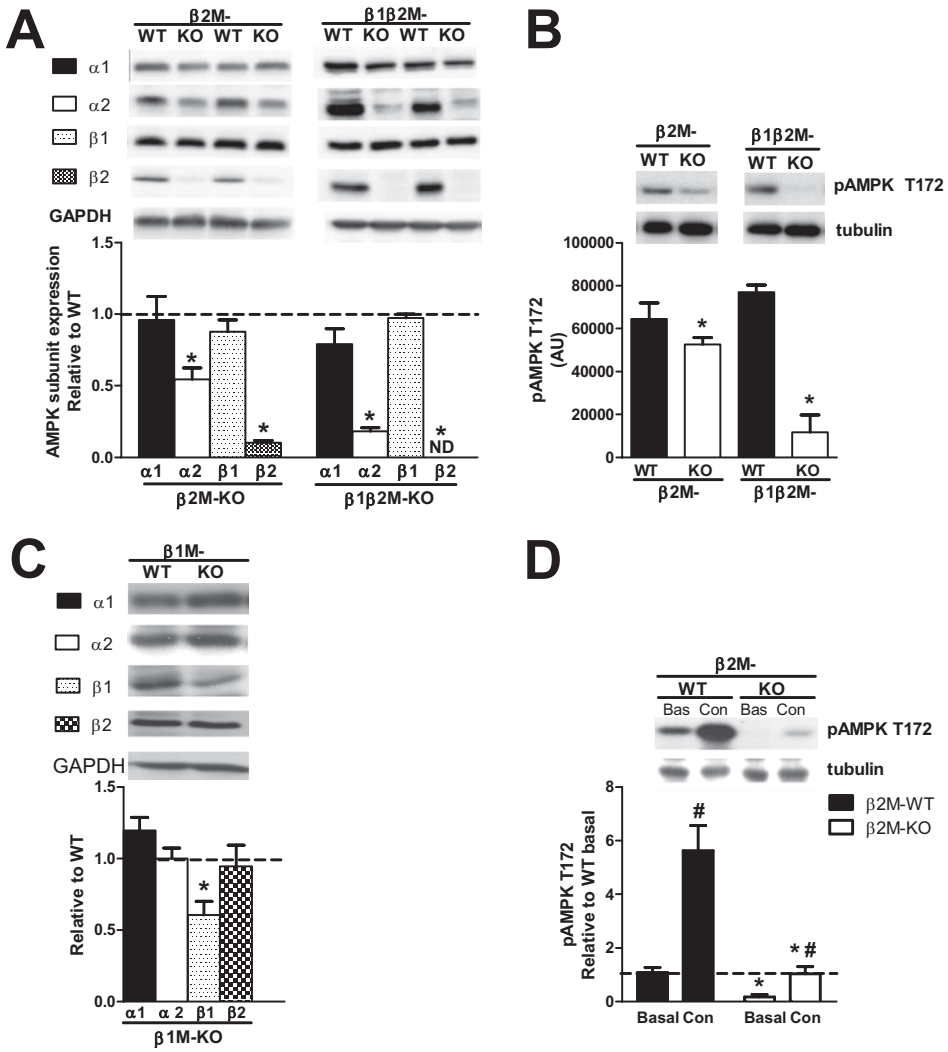

Fig. S2. AMPK subunit expression and T172 phosphorylation in heart and skeletal muscle muscle. (*A*) Protein expression of AMPK α1, α2, β1, β2 in heart muscle from β2 and β1β2 M-KO mice relative to WT littermates. (*B*) Basal AMPK T172 phosphorylation in heart muscle from β2 and β1β2 M-KO mice. (*C*) Expression of AMPK α1, α2, β1, β2 in extensor digitorium longus (EDL) muscle of β1M-KO mice relative to WT. (*D*) AMPK T172 phosphorylation in resting (basal) and electrically stimulated (Con = contraction, 50 Hz, 6 tetani/min, 350 ms pulse duration, 20 min) β2M-KO EDL muscles. Data are means ± SEM, $n = 4–8$. $^{*}P < 0.05$ compared with WT littermate. $^{\#}P < 0.05$ compared with basal, same genotype. Representative blots are shown above, and densitometry is shown below. Values were corrected for equal protein loading using GAPDH or tubulin.

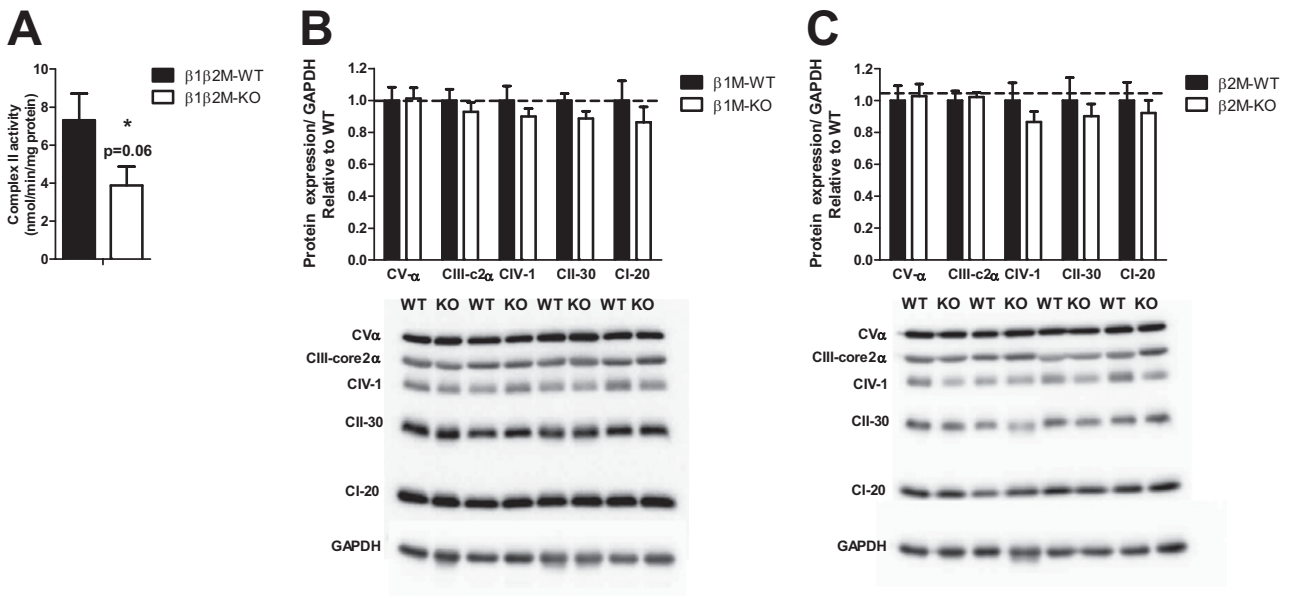

Fig. S3. Mitochondrial biogenesis is reduced in β1β2M-KO mice but not β1 or β2 M-KO. (*A*) Complex II (*succinate dehydrogenase oxidoreductase*) activity in quadriceps from β1β2M-KO mice. (*B* and *C*) OXPHOS protein expression in extensor digitroum longus (EDL) from β1M-KO (*B*) and β2M-KO (*C*) mice and wild-type (WT) littermates. Data are means ± SEM, $n = 5–8$. *$P < 0.05$ compared with WT littermate. For protein expression, representative blots are shown above and densitometry below. Values were corrected for equal protein loading using GAPDH.

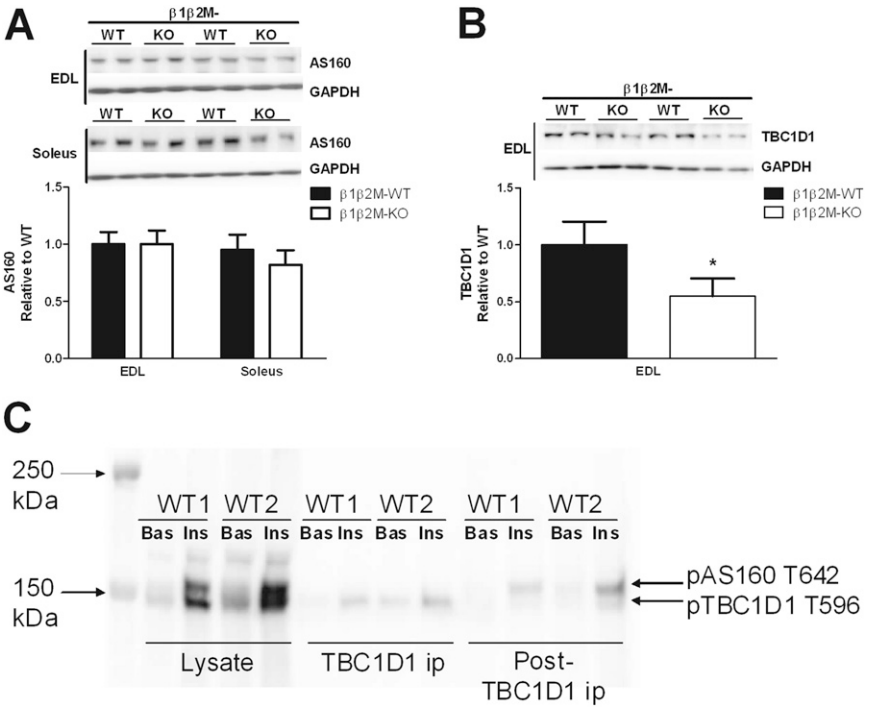

Fig. S4. (*A*) Akt substrate of 160 kDa (AS160) expression is normal in extensor digitroum longus (EDL) and soleus from β1β2M-KO mice. (*B*) TBC1D1 expression is reduced by ∼50% in EDL muscle from β1β2M-KO mice compared with wild type (WT). Data are means ± SEM, $n = 4$. *$P < 0.05$ compared with WT littermates. Values were corrected for equal protein loading using GAPDH. (*C*) Basal and insulin-stimulated EDL muscle lysates from wild-type (WT) mice were immunoprecipitated (ip) with TBC1D1 antibody, proteins separated by SDS/PAGE and immunblotted with phospho AS160 T642 (pAS160 T642) antibody to determine whether this antibody also detected paralogous T596 site on TBC1D1. Immunoblot reveals that in: (*i*) muscle lysate 2 bands are visible (∼150 and 160 kDa); (*ii*) phospho detection of TBC1D1 ip samples with AS160p T642 antibody reveals phospho signal ∼150kD, which confirms AS160p T642 antibody also detects TBC1D1p T596; and (*iii*) lysate from post-TBC1D1 ip showing no band around ∼150 kDa (TBC1D1) but a higher band at the expected size of ∼160kDa (AS160), showing sufficient ip of TBC1D1 from muscle lysates. $n = 2$.

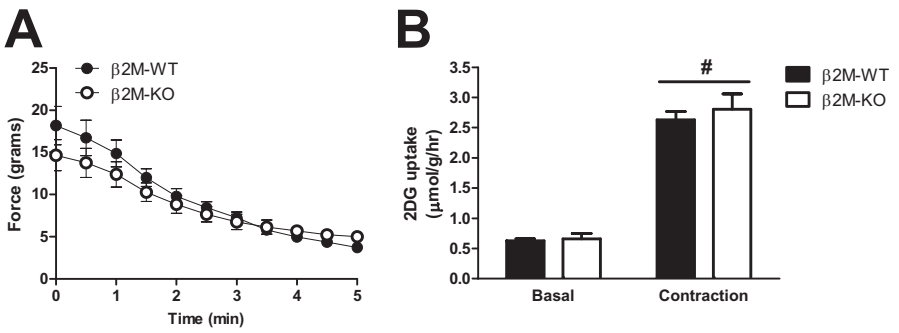

Fig. S5. AMPK β2M-KO mice have normal glucose uptake during muscle contractions. β2M-KO extensor digitroum longus (EDL) was isolated and contracted by electrical stimulation (50 Hz, 6 tetani/min, 350-ms pulse duration, 20 min). Force fatigue curves (*A*) and 2-deoxyglucose uptake (*B*) during muscle contractions. Data are means ± SEM, $n = 8$, $^{\#}P < 0.05$ compared with basal, same genotype. $^{*}P < 0.05$ compared with wild-type (WT) littermates.

Table S1. Physical characteristics of AMPK β1M-KO, β2M-KO, and β1β2M-KO mice

Characteristic	β1M-WT	β1M-KO	β2M-WT	β2M-KO	β1β2M-WT	β1β2M-KO
Litter size	5.2 ± 0.6		4.1 ± 0.4		4.9 ± 0.5	
Genotype	2.5 ± 0.4	2.4 ± 0.4	2.6 ± 0.3	2.6 ± 0.3	2.9 ± 0.5	3.4 ± 0.5
Body mass (g)	23.7 ± 0.4	24.7 ± 0.6	23.0 ± 0.6	23.5 ± 0.8	25.4 ± 0.4	23.8 ± 0.7
% adiposity	2.19 ± 0.08	2.36 ± 0.34	1.72 ± 0.22	2.65 ± 0.12*	1.73 ± 0.18	1.84 ± 0.23
Heart mass (mg/g body mass)	5.26 ± 0.20	5.52 ± 0.35	5.43 ± 0.27	4.68 ± 0.27	5.45 ± 0.28	5.54 ± 0.27
EDL mass (mg/g body mass)	0.45 ± 0.01	0.46 ± 0.01	0.48 ± 0.02	0.51 ± 0.01	0.47 ± 0.01	0.46 ± 0.01
SOL mass (mg/g body mass)	0.40 ± 0.01	0.44 ± 0.1	0.35 ± 0.02	0.39 ± 0.19	0.42 ± 0.01	0.43 ± 0.02

Average litter size and genotype. Adiposity was determined by dividing white epididymal fat pad weight by body weight × 100. Heart, extensor digitorum longus (EDL) and soleus (SOL) muscle weights expressed relative to body weight (mg/g). Data are expressed as means ± SEM for 14–18 wk old male mice. $n = 8$–10 mice. $^{*}P < 0.05$ compared with wild-type (WT) littermates.

Table S2. Resting metabolic parameters and activity levels

Variable	β2M-WT	β2M-KO	β1β2M-WT	β1β2M-KO
VO_2 (ml/kg/hr)				
Dark	2724 ± 50.08	2657 ± 36.13	2860 ± 55.18	2704 ± 46.68
Light	1854 ± 33.29	1813 ± 22.48	2427 ± 26.98	2288 ± 26.61
VCO_2 (ml/kg/hr)				
Dark	2531 ± 44.34	2472 ± 32.19	2720 ± 54.87	2514 ± 48.77
Light	1694 ± 32.04	1641 ± 22.56	2258 ± 27.65	2097 ± 29.61
Activity (beam breaks)				
Dark	671.64 ± 48.74	594.07 ± 12.45	461.65 ± 47.77	383.22 ± 38.97
Light	96.96 ± 23.32	117.16 ± 32.80	167.56 ± 13.21	117.25 ± 10.21
Food intake (g)	3.11 ± 0.09	2.87 ± 0.10	3.57 ± 0.20	3.00 ± 0.2*
Water intake (ml)	4.02 ± 0.38	3.13 ± 0.20	3.08 ± 0.19	2.71 ± 0.10

VO_2, VCO_2, activity levels, and food and water intake in AMPK β2M-KO and β1β2M-KO mice and WT littermates. Monitoring was conducted over 72 h and data were analyzed for light (7am-7pm) and dark cycles (7pm-7am) or cumulative over 24 h if not indicated. $^{*}P < 0.05$ compared with WT littermates, data are expressed as means ± SEM, $n = 4$ β2M-WT and KO and $n = 8$ β1β2M-WT and KO.

Table S3. Blood glucose and serum insulin and adipokine levels in AMPK β1β2M-KO mice

Variable	β1β2M-WT	β1β2M-KO
Body weight (g)	28.31 ± 2.99	26.91 ± 3.73
Glucose (mM)	8.03 ± 0.38	8.43 ± 0.50
Insulin (ng/mL)	0.59 ± 0.09	0.51 ± 0.09
Leptin (pg/mL)	4145 ± 1167	4265 ± 1547
IL-6 (pg/mL)	75.62 ± 34.85	49.07 ± 12.27
TNF-α (pg/mL)	10.50 ± 0.73	10.52 ± 1.16
MCP-1 (pg/mL)	93.30 ± 31.47	94.88 ± 31.90

Overnight fasted blood was collected from AMPK β1β2M-KO and WT littermates for determination of blood glucose and serum insulin and adipokine levels (leptin, IL-6, TNF-α, and MCP-1). Data are expressed as means ± SEM; n = 8–10 mice.

Table S4. Plasma and muscle analyses during treadmill running

	β1β2M-WT		β1β2M-KO	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Plasma				
Glucose (mM)	6.7 ± 0.37	12.8 ± 0.76#	7.0 ± 0.75	16.4 ± 1.30*#
Lactate (mM)	6.31 ± 0.60	6.54 ± 0.54	6.40 ± 0.24	5.56 ± 0.58
NEFA (mM)	0.81 ± 0.17	1.32 ± 0.08	0.59 ± 0.14	1.54 ± 0.28
Muscle				
ATP (μmol/g)	23.81 ± 2.02	29.52 ± 1.66	21.21 ± 6.87	26.88 ± 2.11
ADP (nmol/g)	58.50 ± 19.46	86.81 ± 13.97	42.52 ± 14.78	88.32 ± 19.89
AMP (nmol/g)	0.10 ± 0.04	0.36 ± 0.11	0.10 ± 0.05	0.33 ± 0.12
PCr/ Cr	0.67 ± 0.05	0.59 ± 0.07	0.78 ± 0.04	0.66 ± 0.04 P = 0.058
Lactate (μmol/g)	18.32 ± 1.16	18.61 ± 1.82	16.71 ± 2.32	22.88 ± 5.36
Glycogen (μmol/g)	16.55 ± 1.21	9.96 ± 0.70#	14.61 ± 0.63	8.08 ± 1.41#

AMPK β1β2M-KO mice and WT littermates were either rested or exercised on a treadmill at the same relative intensity (50% max running speed) for 20 min. Blood was collected for analysis of plasma lactate, nonesterified fatty acids (NEFA) and glucose. Quadriceps muscles were quickly dissected, snap-frozen and prepared for analyses of nucleotides (ATP, AMP, ADP), phosphocreatine/creatine (PCr/Cr), lactate and glycogen relative to muscle dry weight. Data are expressed as means ± SEM, n = 6–9 for each group. *$P < 0.05$ genotype difference; #$P < 0.05$ effect of exercise, same genotype.