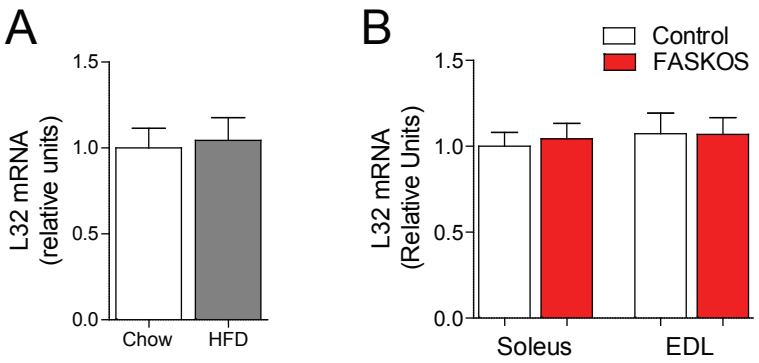
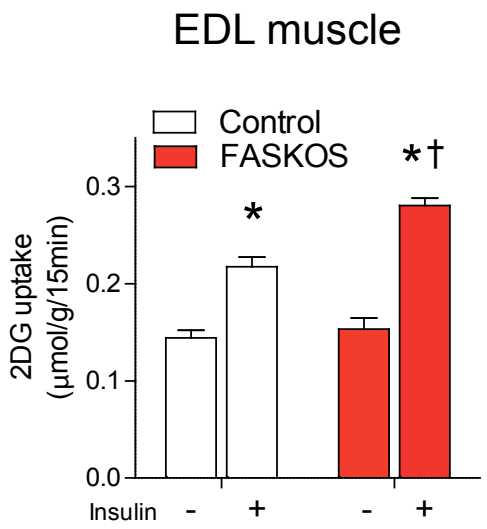
Supplemental Figure 1



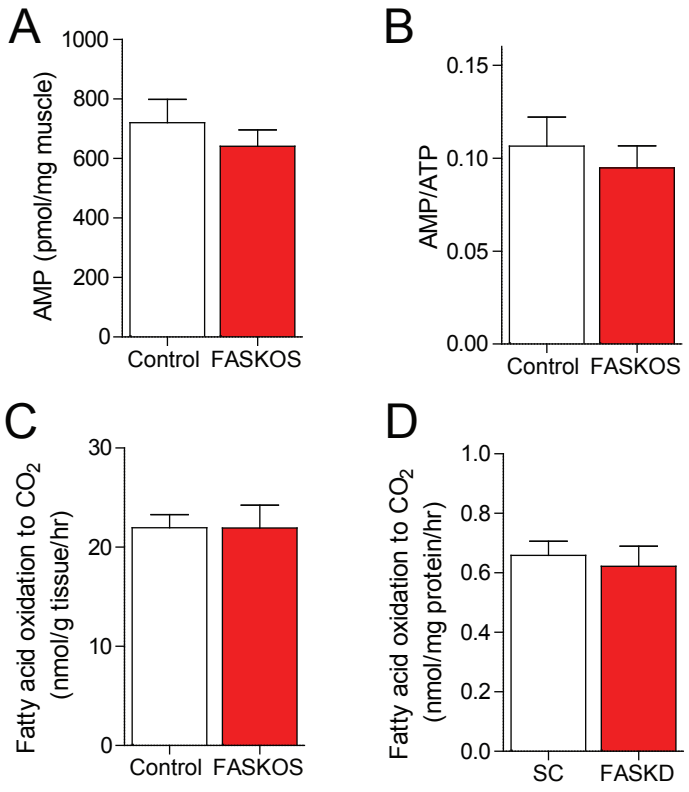
Supplemental Figure 1
Control message levels. (A) L32 mRNA levels of soleus muscles from mice fed with chow or HFD (n=7). (B) L32 mRNA levels of soleus and EDL muscles from HFD-fed control and FASKOS mice (n=6). Data are expressed as mean ± SEM.

Supplemental Figure 2



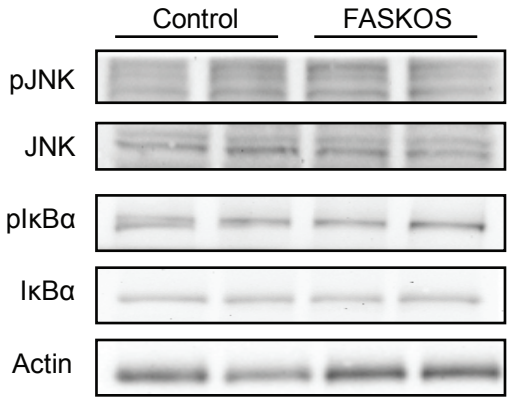
Supplemental Figure 2
Insulin-stimulated 2DG uptake in EDL muscles from HFD-fed control or FASKOS mice. *Effects of insulin, P=0.0002 for control muscles, P<0.0001 for FASKOS muscles. †Effect of genotype, P=0.0006 (n=6). Data are expressed as mean ± SEM.

Supplemental Figure 3

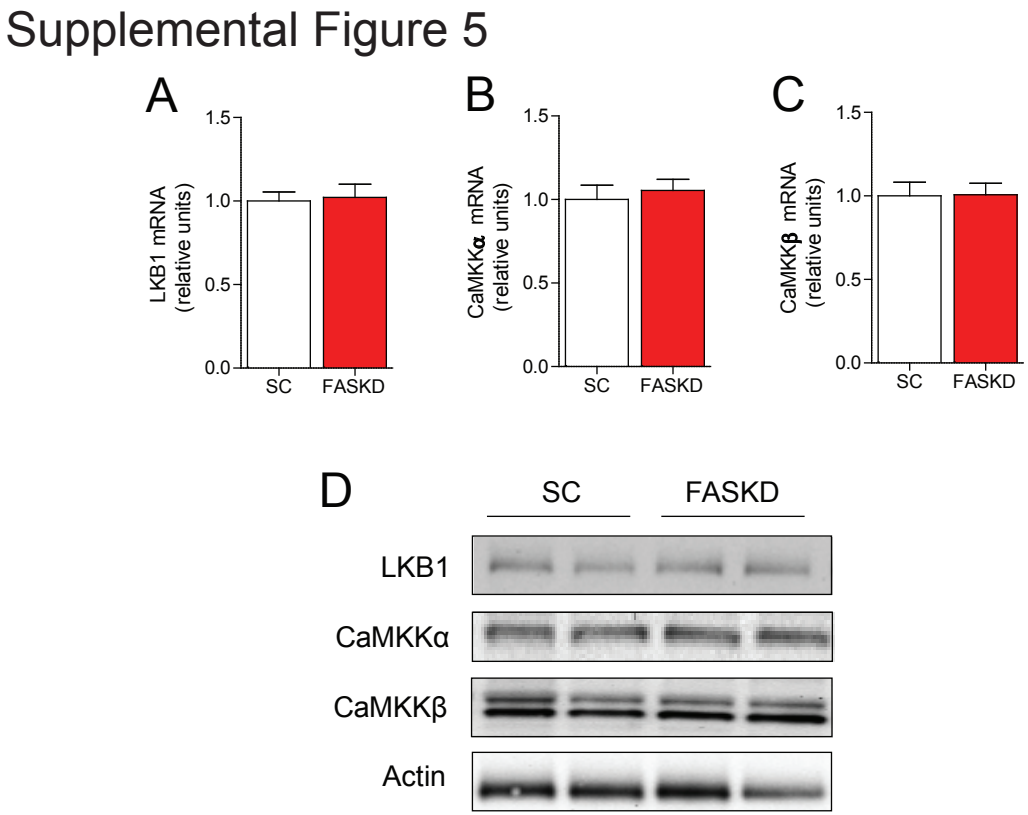


Supplemental Figure 3
AMP and fatty acid oxidation. (A) AMP levels in muscles from HFD-fed control or FASKOS mice (n=5). (B) AMP/ATP ratios in muscles from HFD-fed control or FASKOS mice (n=the same 5 mice per group as in panel A). (C) Rate of fatty oxidation in muscle homogenates from HFD-fed control or FASKOS mice (n=6). (D) Rate of fatty acid oxidation in lysates from scrambled or FASKD C2C12 myocytes (n=6). Data are expressed as mean ± SEM.

Supplemental Figure 4

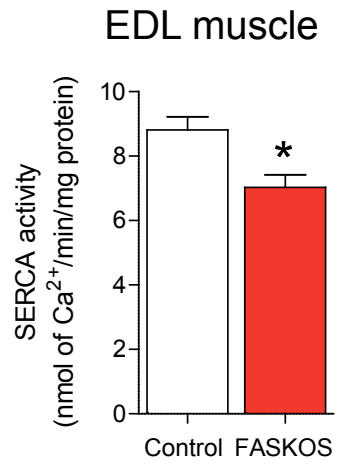


Supplemental Figure 4
Western blotting of inflammation markers in muscles from HFD-fed control or FASKOS mice.



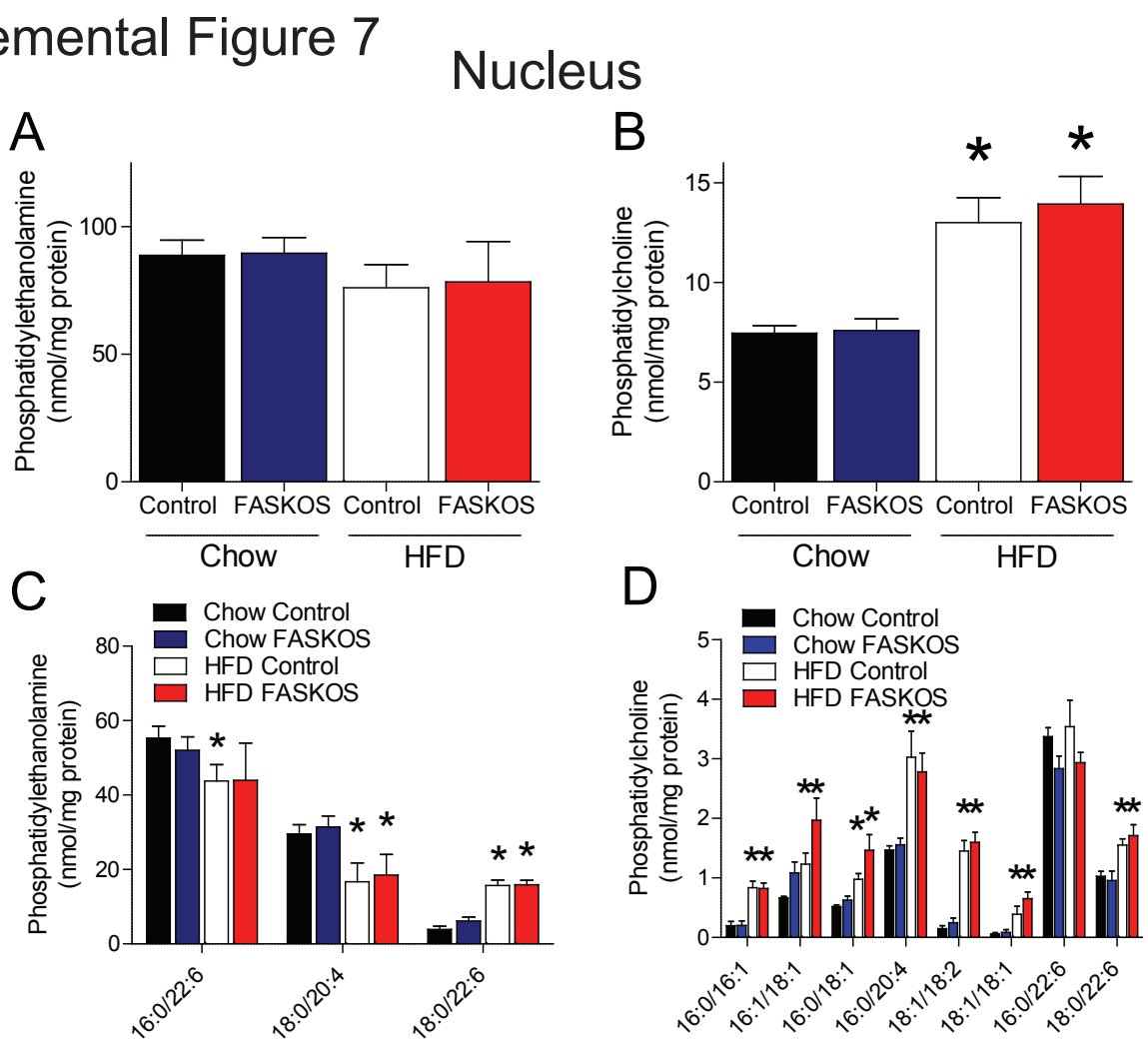
Supplemental Figure 5
FAS deletion does not alter mRNA levels or protein abundance of potential AMPK kinases in C2C12 myocytes. (A) LKB1 mRNA. (B) CaMKKα mRNA. (C) CaMKKβ mRNA. (D) Western blotting of LKB1, CaMKKα and CaMKKβ. Data are expressed as mean ± SEM.

Supplemental Figure 6



Supplemental Figure 6
SERCA activity in EDL muscles from HFD-fed control or FASKOS mice. *Effect of genotype, *P*=0.0098 (n=6). Data are expressed as mean ± SEM.

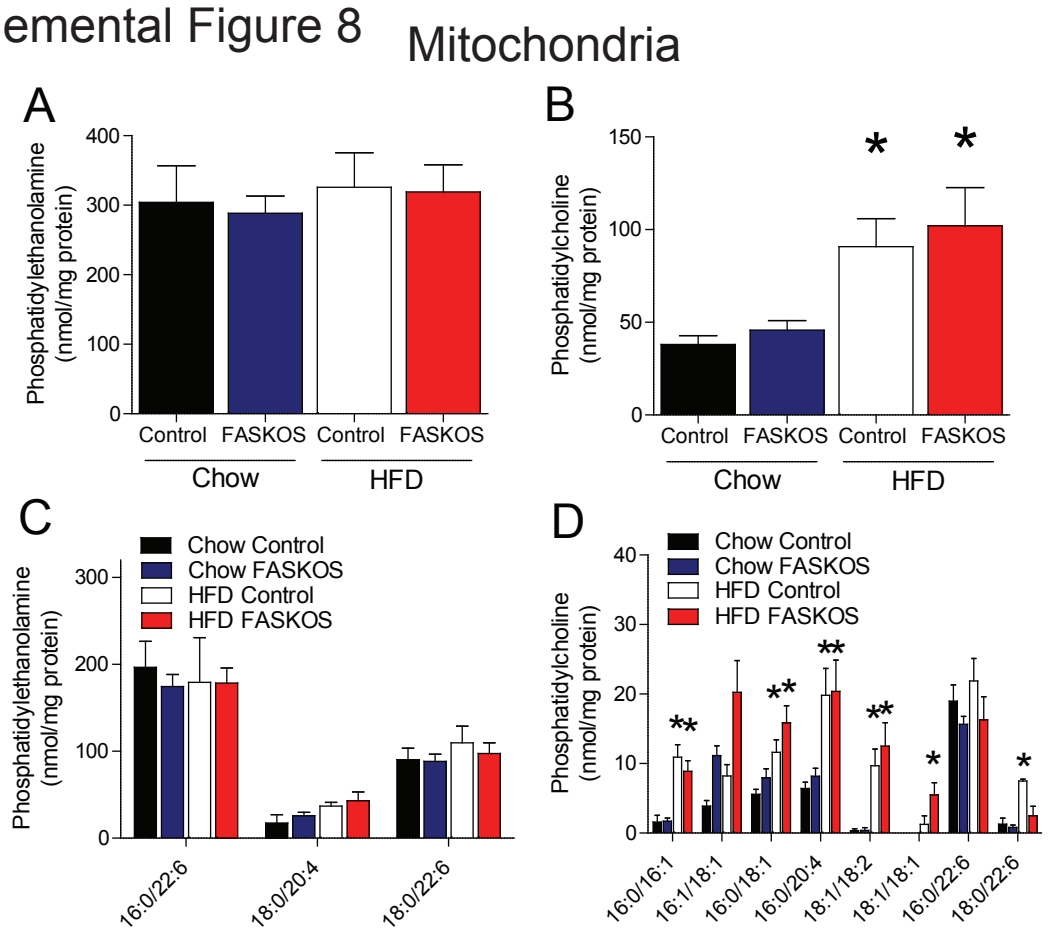
Supplemental Figure 7



Supplemental Figure 7

Mass spec quantification of PE and PC species in isolated nucleus from hindlimb muscles of control or FASKOS mice fed with chow or HFD (n=6). (A) Total PE. (B) Total PC. *Effects of HFD, P=0.002 for control muscles, *P*=0.002 for FASKOS muscles. (C) PE species. *Effects of HFD, *P*=0.050 for 16:0/22:6 in control muscles, *P*=0.047 for 18:0/20:4 in control muscles, *P*=0.047 for 18:0/20:4 in FASKOS muscles, *P*<0.0001 for 18:0/22:6 in control muscles, *P*=0.0001 for 18:0/22:6 in FASKOS muscles. (D) PC species. *Effects of HFD, *P*=0.0006 for 16:0/16:1 in control muscles, *P*=0.0003 for 16:0/16:1 in FASKOS muscles, *P*=0.012 for 16:1/18:1 in control muscles, *P*=0.050 for 16:1/18:1 in FASKOS muscles, *P*=0.001 for 16:0/18:1 in control muscles, *P*=0.011 for 16:0/18:1 in FASKOS muscles, *P*=0.005 for 16:0/20:4 in control muscles, *P*=0.004 for 16:0/20:4 in FASKOS muscles, *P*<0.0001 for 18:1/18:2 in control muscles, *P*<0.0001 for 18:1/18:2 in FASKOS muscles, *P*=0.034 for 18:1/18:1 in control muscles, *P*=0.0008 for 18:1/18:1 in FASKOS muscles, *P*=0.003 for 18:0/22:6 in control muscles, *P*=0.012 for 18:0/22:6 in FASKOS muscles. Data are expressed as mean ± SEM.

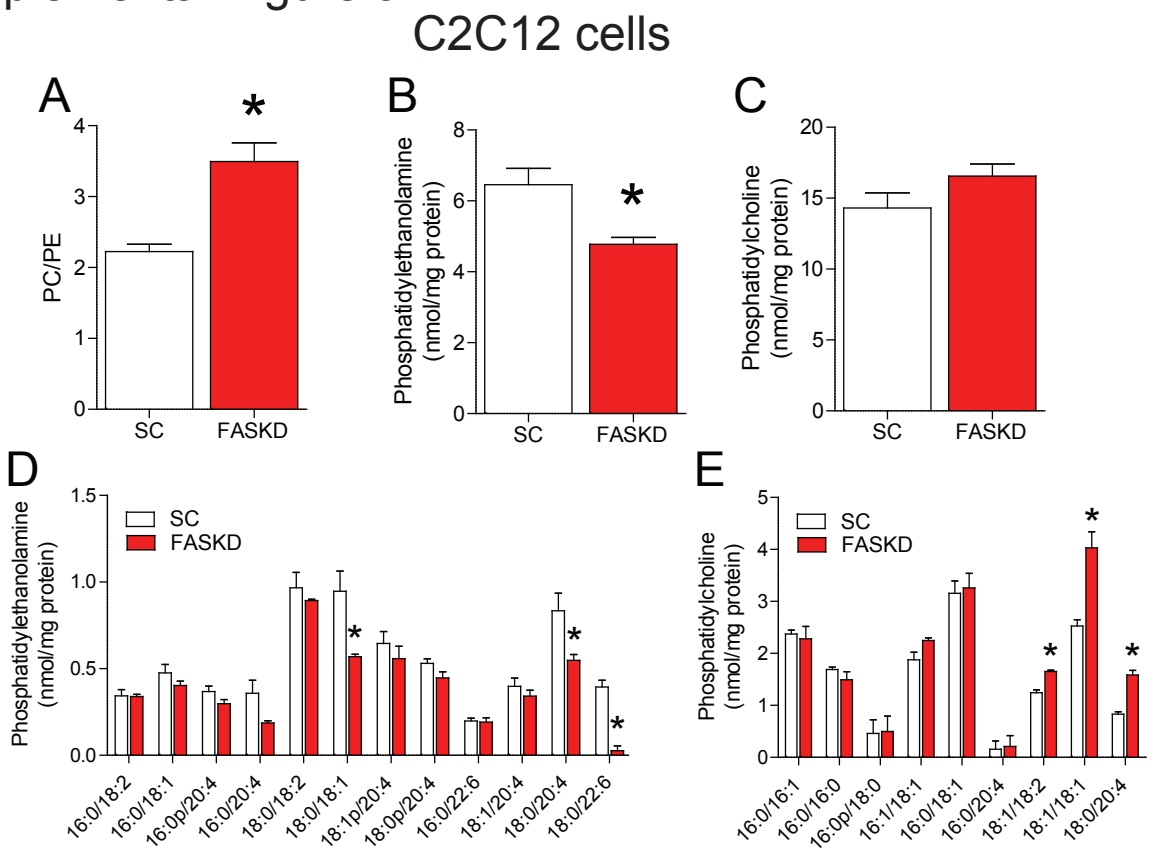
Supplemental Figure 8



Supplemental Figure 8

Mass spec quantification of PE and PC species in isolated mitochondria from hindlimb muscles of control or FASKOS mice fed with chow or HFD (n=3). (A) Total PE. (B) Total PC. *Effects of HFD, *P*=0.029 for control muscles, *P*=0.050 for FASKOS muscles. (C) PE species. (D) PC species. *Effects of HFD, *P*=0.010 for 16:0/16:1 in control muscles, *P*=0.011 for 16:0/16:1 in FASKOS muscles, *P*=0.037 for 16:0/18:1 in control muscles, *P*=0.047 for 16:0/18:1 in FASKOS muscles, *P*=0.028 for 16:0/20:4 in control muscles, *P*=0.049 for 16:0/20:4 in FASKOS muscles, *P*=0.018 for 18:1/18:2 in control muscles, *P*=0.023 for 18:1/18:2 in FASKOS muscles, *P*=0.038 for 18:1/18:1 in FASKOS muscles, *P*=0.0027 for 18:0/22:6 in control muscles. Data are expressed as mean ± SEM.

Supplemental Figure 9



Supplemental Figure 9

Mass spec quantification of PE and PC species in isolated SR from SC and FASKD C2C12 myocytes (n=4). (A) Relative abundance of PC and PE. **P*=0.0043. (B) Total PE. **P*=0.015. (C) Total PC. (D) PE species. **P*=0.018 for 18:0/18:1, *P*=0.038 for 18:0/20:4, *P*<0.0001 for 18:0/22:6. (E) PC species. **P*=0.0005 for 18:1/18:2, *P*=0.004 for 18:1/18:1, *P*=0.0002 for 18:0/20:4. Data are expressed as mean ± SEM.

Gene Abbreviation	Gene Name	Score	Queries matched	emPAI
G3P	Glyceraldehyde-3-phosphate dehydrogenase	23012	604	1034.86
AT2A1	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	9978	290	12.33
PYGM	Glycogen phosphorylase, muscle form	8792	388	40.50
KCRM	Creatine kinase M-type	6967	231	33.12
K6PF	6-phosphofructokinase, muscle type	6873	208	10.36
ATPA	ATP synthase subunit alpha	6089	176	19.02
ALDOA	Fructose-bisphosphate aldolase A	5328	186	27.75
UGPA	UTP--glucose-1-phosphate uridylyltransferase	4327	151	13.68
AMPD1	AMP deaminase 1	4042	153	6.15
KPB1	Phosphorylase b kinase regulatory subunit alpha	3386	109	2.17
ENOB	Beta-enolase	3374	116	14.88
GYS1	Glycogen synthase, muscle	3277	111	4.32
AT2A2	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	3028	114	1.64
PGK1	Phosphoglycerate kinase 1	2866	93	13.78
KPBB	Phosphorylase b kinase regulatory subunit beta	2859	138	2.73
MDHM	Malate dehydrogenase	2755	81	8
ACON	Aconitate hydratase	2634	87	2.56
KCRS	Creatine kinase S-type	2546	82	5.44
GPDA	Glycerol-3-phosphate dehydrogenase [NAD+]	2539	80	8.44
LDHA	L-lactate dehydrogenase A chain	2409	99	19.34
PURA1	Adenylosuccinate synthetase isozyme 1	2286	81	4.87
HS90B	Heat shock protein HSP 90-beta	2242	97	3.15
SRCA	Sarcalumenin	2217	86	1.91
ATPB	ATP synthase subunit beta	2122	77	3.91
THIM	3-ketoacyl-coA thiolase	2052	55	6.07
SUCB1	Succinyl-CoA ligase [ADP-forming] subunit beta	1860	70	4.89
CAND2	Cullin-associated NEDD8-dissociated protein 2	1814	64	0.93
IDH3A	Isocitrate dehydrogenase [NAD] subunit alpha	1699	65	3.89
TPIS	Triosephosphate isomerase	1632	53	4.71
EF2	Elongation factor 2	1588	78	1.73
KAD1	Adenylate kinase isoenzyme 1	1571	49	12.38
PGAM2	Phosphoglycerate mutase 2	1563	63	9.95
AT2A3	Sarcoplasmic/endoplasmic reticulum calcium ATPase 3	1549	62	0.75
ODPB	Pyruvate dehydrogenase E1 component subunit beta	1527	42	2.36
PYGB	Glycogen phosphorylase, brain form	1525	78	1.36
VDAC1	Voltage-dependent anion-selective channel protein 1	1470	31	2.9
TCPB	T-complex protein 1 subunit beta	1409	39	1.29
QCR2	Cytochrome b-c1 complex subunit 2	1327	42	2.51
IDHP	Isocitrate dehydrogenase [NADP]	1261	41	2.94
ALBU	Serum albumin	1258	58	0.37
PSMD2	26S proteasome non-ATPase regulatory subunit 2	1237	47	1.15
CAH3	Carbonic anhydrase 3	1201	57	3.93
UN45B	Protein unc-45 homolog B	1195	40	0.74
PGK2	Phosphoglycerate kinase 2	1183	44	2.31
ADT1	ADP/ATP translocase 1	1161	58	7.2
HBB1	Hemoglobin subunit beta-1	1153	35	4.65
PHKG1	Phosphorylase b kinase gamma catalytic chain	1152	52	3.68
TERA	Transitional endoplasmic reticulum ATPase	1074	40	0.84
HS90A	Heat shock protein HSP 90-alpha	1054	49	1.13
CASQ1	Calsequestrin-1	1021	42	1.66
EF1A2	Elongation factor 1-alpha 2	1001	46	2.11

Supplemental Table 1

Mass spectrometry analyses of proteins immunoprecipitated with FAS antibody from muscle SR fraction. SR fraction was isolated from mouse hindlimb muscles and proteins immunoprecipitated with FAS antibody were identified by proteomic analyses. Proteins with total scores of more than 1000 were listed. FAS, keratin and cytoskeletal proteins (myosin, actin, tubulin, etc.) were excluded from the list. emPAI: exponentially modified protein abundance index.

Supplemental Movie 1. A control (left lane) and a FASKOS (right lane) mouse were subjected to sprinting after a period of high fat diet feeding. Images are from a sprint interval at 35 m/min. The control mouse completed the 1 min interval, but the FASKOS mouse was unable to sustain running at this intensity.

Supplemental Methods

Body composition measurements. Body composition was determined using an EchoMRI 3-in-1 instrument (Echo Medical Systems). Free water mass was less than 0.1 g for all mice and results were not different between control and FASKOS mice.

Glucose and insulin tolerance tests. Mice were fasted for 6 h and injected with 10% D-glucose (1 g/kg) or human regular insulin (0.75 U/kg body weight, Eli Lilly) in procedures separated by at least 1 week. Tail vein blood was assayed for glucose using a blood glucose meter.

Cell culture experiments. C2C12 cells were maintained in DMEM + 10% FBS. At ~80% confluence, media was changed to DMEM + 2% horse serum to induce differentiation. HEK 293 and HEK 293T cells were maintained in DMEM + 10% FBS.

Hyperinsulinemic euglycemic clamp studies. A jugular catheter was implanted, tunneled subcutaneously, and exteriorized at the back of the neck. Five days after surgery, animals were fasted overnight and glucose turnover was measured in the basal state and during a hyperinsulinemic euglycemic clamp in conscious mice. For the basal phase, blood samples were obtained then 3-[^{3}H]D-glucose (American Radiolabeled Chemicals) was infused (0.05 μCi/min) with a Y-connector. One hour after the start of the tracer infusion, a second basal blood sample was taken for measurement of glucose concentration and tracer specific activity to estimate rate of appearance (R_a) or rate of disappearance (R_d) ($R_a = R_d$ for the basal phase). For the clamp phase, infusion of 3-[^{3}H]D-glucose was replaced with a solution that contained 3-[^{3}H]D-glucose (0.05 μCi/min) and regular human insulin (Humulin R, Eli Lilly) at 2.5 mU/kg/min (with 50 mU/kg prime). Through a second port on the Y-connector, 20% D-glucose was infused at an adjustable rate to maintain blood glucose at 120 mg/dl. After 75 min of steady state blood

glucose at 120 mg/dl, a final blood sample was taken to estimate R_a and R_d during the clamp phase ($R_a \neq R_d$ for the clamp phase). Insulin-stimulated GDR (IS-GDR) was calculated by subtracting R_d-basal from R_d-clamp. Percent hepatic glucose production (HGP) suppression, an indicator of liver insulin sensitivity, was calculated from R_a-clamp and R_a-basal values ([R_a-basal – R_a-clamp] / R_a-basal). Free fatty acids in serum during basal and clamp phases were measured in order to indirectly assess adipose insulin sensitivity. Mice were euthanized with 160 mg/kg ketamine and 20 mg/kg xylazine then muscles were harvested and quickly frozen in liquid nitrogen for subsequent Western blot analyses.

Isolated skeletal muscle incubation studies. Mice were fasted for 6 h, then paired soleus muscles from anesthetized mice were excised and incubated using a 2-step incubation protocol. For all incubation steps, vials were continuously gassed with 95% O_2/5% CO_2 and shaken in a heated water bath. For all incubation steps, one muscle from each mouse was incubated in solution supplemented with 100 μU/ml of insulin (Humulin R), and the contralateral muscle was incubated in solution without insulin (basal). For the 1st incubation step, muscles were incubated in Krebs-Henseleit buffer (KHB) + 0.1% bovine serum albumin (BSA) + 2 mM sodium pyruvate + 6 mM mannitol for 15 min in a water bath at 35°C. After the 1st incubation step, muscles were transferred to a 2nd vial with KHB + 0.1% BSA + 1 mM 2-deoxyglucose (2DG, containing 2-deoxy-[^{3}H]glucose, 6 mCi/mmol), + 9 mM mannitol (containing [^{14}C]mannitol, 0.053 mCi/mmol) for 15 min at 35°C (American Radiolabeled Chemicals). After incubation with 2DG for 15 min, the muscles were rapidly blotted on filter paper dampened with incubation medium, trimmed, freeze-clamped, and stored at -80°C. Muscles were homogenized as described below, then 2DG uptake was determined and Western blot analyses were performed.

Adenoviral expression of PPARα. Ad-GFP was obtained from Vector Biolabs. Ad-FLAG-PPARα is described in reference 17. Particles were amplified in HEK 293 cells and purified in cesium chloride gradients.

PPARα luciferase reporter assay. Scrambled or FASKD C2C12 myocytes were infected with Ad-FLAG-PPARα 48 h after cells were switched to differentiation media. After an additional 48 h, C2C12 myocytes were transfected using Lipofectamine 2000 (Invitrogen) with 3X-PPRE-luciferase (firefly) and *Renilla* luciferase, and were incubated with vehicle (DMSO) or 15 μM of WY14643 (Sigma-Aldrich) in media with charcoal-stripped serum for 24 h. Cells were harvested and luciferase reporter assays were performed using the Dual-Glo®Luciferase Assay (Promega) and a Synergy 4 plate reader (BioTek instruments). The firefly luciferase readings were normalized to the *Renilla* luciferase readings.

Lentiviral knockdowns. Plasmids encoding shRNA for mouse FAS (TRCN0000075703) and CaMKKβ (TRCN0000028761) were obtained from Open Biosystems. Packaging vector psPAX2 (ID #12260), envelope vector pMD2.G (ID #12259) and scrambled shRNA plasmid (ID #1864) were obtained from Addgene. 293T cells in 10 cm dishes were transfected using Lipofectamine 2000 (Invitrogen) with 2.66 μg psPAX2, 0.75 μg pMD2.G, and 3 μg shRNA plasmid. After 48 h, media were collected, filtered using 0.45 μm syringe filters, and used to treat undifferentiated C2C12 cells. After 36 h, target cells were selected with puromycin. After an additional 48 h, cells were differentiated.

Measurement of cytosolic calcium content. CalciFluor^{TM} fluo-8 AM (Santa Cruz Biotechnology) was used to detect cytosolic calcium. Scrambled or FAS knockdown (FASKD) myocytes were briefly washed in HBSS and incubated in HBSS containing 5 μM of fluo-8 AM for 30 min at room temperature. Cells were then washed in HBSS twice to remove excess probe

and then visualized using a Nikon TE300 fluorescence microscope at 490/520 nm. Fluorescence per cell was calculated by dividing total fluorescence by the number of cells.

Assays for protein S-palmitoylation. Biotin switch assays were performed to detect S-acylation of SERCA1 and SERCA2. Cells were homogenized in lysis buffer (150 mM NaCl, 50 mM Tris [pH 7.4], 5 mM EDTA) in the presence of 20 mM *N*-ethylmaleimide (NEM). After sonication on ice, Triton X-100 (1.7%) was added and the mixture was rotated in a cold room. Samples were treated with chloroform/methanol (1:4), solubilized in 4% SDS with 20 mM NEM at 37°C for 3 h, and subjected to serial protein precipitations to remove residual NEM. Each sample was then aliquoted to two tubes and resuspended in buffer containing *N*-(6-(biotinamido)hexyl)-3'-(2'-pyridyldithio)-propionamide-biotin (1 mM) and Triton X-100 (0.2%) in the presence or absence of hydroxylamine. After 1 h, proteins were precipitated twice with chloroform/methanol and solubilized in buffer containing 0.1% SDS. After 1.5 h incubation (room temperature) with streptavidin-agarose beads, biotin-labeled proteins were detected by Western blotting.

Quantitative RT-PCR gene expression. Total RNA was extracted from samples with TRIzol reagent (Invitrogen). One μg of total RNA was reverse transcribed using the IScript™ cDNA synthesis kit (BioRad). Quantitative PCR was performed with an ABI Prism 7700 PCR instrument using SYBR® Green reagent (Applied Biosystems). Pre-validated primers spanning exon-exon boundaries were used for amplifications (FAS forward primer: 5'-CTC GCT TGT CGT CTG CCT-3', FAS reverse primer: 5'-TTG GCC CAG AAC TCC TGT AG-3', CaMKKα forward primer: 5'-CAG AGA CTG TCG CCA GGT G-3'; CaMKKα reverse primer: 5'-GAG GGT CTT GGC AGC AGA C-3', CaMKKβ forward primer: 5'-CAC GTC TCC ATT ACC GGT TT-3', CaMKKβ reverse primer: 5'-TTC ATT GTA GGC CAG CTT GA-3', LKB1

forward primer: 5'-GCG GTC AAG ATC CTC AAG AA-3', LKB1 reverse primer: 5'-GGA TCA CAT TCC GAT GCC-3', L32 forward primer: 5'-AAG CGA AAC TGG CGG AAA C-3', L32 reverse primer: 5'-GAT CTG GCC CTT GAA CCT TCT-3'). Results were normalized to ribosomal protein L32 mRNA levels, which were unaffected by interventions.

Differential centrifugation. Samples were homogenized in hypotonic buffer (250 mM sucrose, 10 mM $NaHCO_3$, 5 mM NaN_3, 0.1 mM PMSF and 1X Halt^{TM} protease and phosphatase inhibitor cocktail [Thermo Fisher]) at 4°C. Homogenates were rotated for 1 h at 4°C, and then subjected to differential centrifugations at 1,300*g* for 10 min, 10,000*g* for 10 min, 20,000*g* for 15 min, 30,000*g* for 15 min, 50,000*g* for 30 min, 100,000*g* for 60 min and 179,000*g* for 90 min.

Tissue and cell homogenization. Tissues and cells were homogenized (glass homogenization tube with a motor-Teflon pestle for tissue, pipetting up and down 20 times for cells) in 50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.1% sodium deoxycholate and 1X Halt^{TM} protease and phosphatase inhibitor cocktail (Thermo Fisher,) at 4°C. Homogenates were rotated for 1 h at 4°C prior to centrifugation at 12,000*g* for 10 min at 4°C. Supernatants were collected and protein concentrations determined using the bicinchoninic acid assay (Thermo Fisher).

Western blotting. Proteins were resolved on 4-20% gradient SDS-polyacrylamide gels, and membranes were blocked in 5% BSA for 1 h at room temperature. Primary antibodies were: anti-FAS [ab22759], anti-SERCA1 [ab2818], anti-SERCA2 [ab3625], anti-SERCA3 [ab54876], anti-Histone H3 [ab1791], and anti-pIκBα [ab5682] from Abcam; anti-COXIV [#4844], anti-AMPKα [#2532], anti-phospho-AMPKα^{Thr172} [#2531], anti-ACC [#3662], anti-phospho-ACC^{Ser79} [#3661], anti-Akt [#9272], anti-phospho-Akt^{Thr308} [#9275], anti-phospho-Akt^{Ser473} [#9271], anti-GSK3α [#9338], anti-phospho-GSK3α/β$^{Ser21/9}$ [#9331], anti-AS160 [#2447], anti-

phospho-AS160^{Thr642} [#4228], anti-CaMKII [#3362], anti-phospho-CaMKII^{Thr286} [#3361], anti-GM130 [#2296], anti-p70 [#9202], anti-pJNK [#9251], anti-JNK [#9258], and anti-LKB1 [#3050] from Cell Signaling; anti-CaMKI [sc-33165], anti-phospho-CaMKI^{Thr177} [sc-28438], anti-myc [sc-789], anti-calnexin [sc-6465], anti-IκBα [sc-371], anti-CaMKKα [sc-11370], and anti-CaMKKβ [sc-50341] from Santa Cruz Biotechnology; anti-PLB [A010-14], anti-phospho-PLB^{Thr17} [A010-13], anti-phospho-PLB^{Ser16} [A010-12] from Badrilla; anti-GLUT4 [GT41-A] from Alpha Diagnostic International Inc.; anti-actin [A2066] from Sigma-Aldrich; anti-Na^+/K^+-ATPase [a6F] developed by Douglas M. Fambrough from the Developmental Studies Hybridoma Bank [NICHD/ University of Iowa]. Incubations were performed in 5% BSA overnight at 4°C, and secondary antibodies were incubated in 5% non-fat dry milk for 1 h at room temperature. Protein bands were detected by chemiluminescence (Western Lightning® Plus-ECL, PerkinElmer) and quantified by densitometry.

Proteomic analyses. Gel slices were subjected to trypsin digestion. Mass spectrometric analyses were performed on a Thermo LTQ-Obitrap (Thermo Fisher) instrument. Samples were loaded with an Eksigent autosampler onto a 15 cm Magic C18 column (5 μm particles, 300 Å pores, Michrom Bioresources) packed into a PicoFrit tip (New Objective) and analyzed on a nanoLC-2D HPLC. Analytical gradients were from 0-80% organic phase (98% acetonitrile, 0.1% formic acid in water, Sigma-Aldrich) over 60 min. The LTQ-Orbitrap was operated in a data-dependent mode with preview scanning over the range *m/z* 350-2000. For tandem MS, the LTQ isolation width was 2 Da, the normalized collision energy 30%, and the activation time 10 ms. Raw data were submitted through the mascot daemon client program to Mascot Server 2.0 and searched against the NCBInr database.

Lipid analyses by Electrospray Ionization (ESI) MS/MS. Samples were reconstituted in 1.8 ml ddH_2O. Four ml of extraction buffer [2:2(v/v) chloroform/methanol] was added in the presence of the following internal standards: 5 µg 14:0-PC ($[M+Li]^+$ *m/z* 684.58), 5 µg 14:0-PE ($[M-H]^-$ m/z 678.62), 500 ng 8:0 ceramide ($[M+Li]^+$ *m/z* 432.34), or 500 ng 15:0/15:0-DAG ($[M+Li]^+$ *m/z* 547.43). After vortex-mixing and centrifugation (800*g*), the organic (lower) layer was collected, concentrated to dryness under nitrogen, and reconstituted in 200 µl methanol. A 20 µl aliquot was removed, diluted with 180 µL methanol containing 0.6% LiCl, and analyzed by direct injection ESI-MS on a Thermo Vantage triple-quadruple mass spectrometer in positive mode with different neutral loss scans for the analysis of PC (neutral loss of 183), ceramide (neutral loss of 48), and DAG (neutral loss of 18) species. Another aliquot of 20 µl was diluted in 200 µl methanol and analyzed in negative mode for PE species. The relative intensity ratio of each individual species to its internal standard was calculated, and then read against a standard curve to obtain its absolute quantity. The final result was calculated by normalizing to the total protein in post-nuclear supernatant and reported as nmol/mg protein.

Immunofluorescence studies. C2C12 cells were fixed with 4% formaldehyde, neutralized with 100 mM glycine, permeabilized with 0.1% Triton X-100, and then incubated with anti-FAS (ab22759, Abcam), anti-SERCA1 (ab2818, Abcam), anti-calnexin (sc-6465, Santa Cruz), and anti-GM130 (#610822, BD Biosciences) antibodies. Following incubation with secondary antibody and counterstaining with DAPI, cells were imaged by confocal microscopy (Zeiss LSM 700).

Forelimb strength measurements. Mouse forelimb strength was determined using a Rodent Grip Strength Meter (Harvard Apparatus). Mice grasped a bar attached to a force transducer, and then were pulled backwards. Force applied just before the grip is lost was

recorded. Ten measurements were made with ten minutes rest between each grip. The two highest and two lowest numbers were discarded, and strength was reported as the mean of the remaining six values.