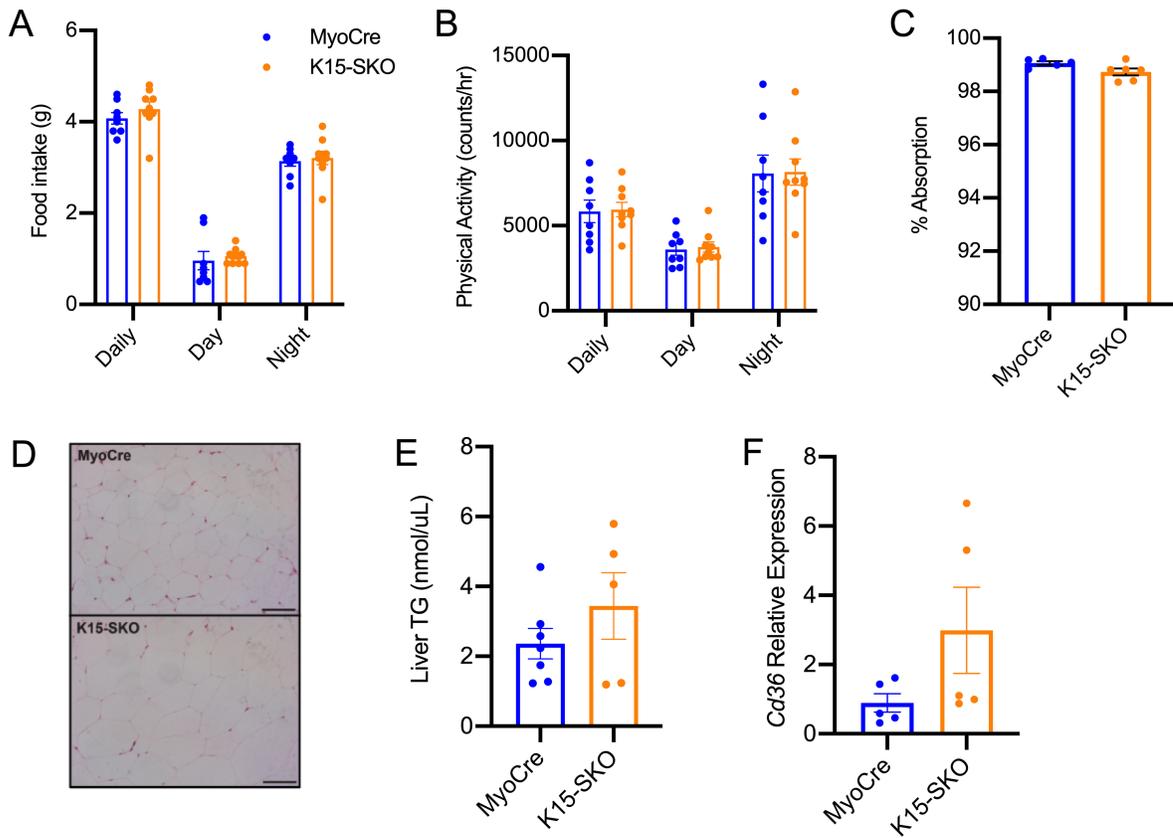
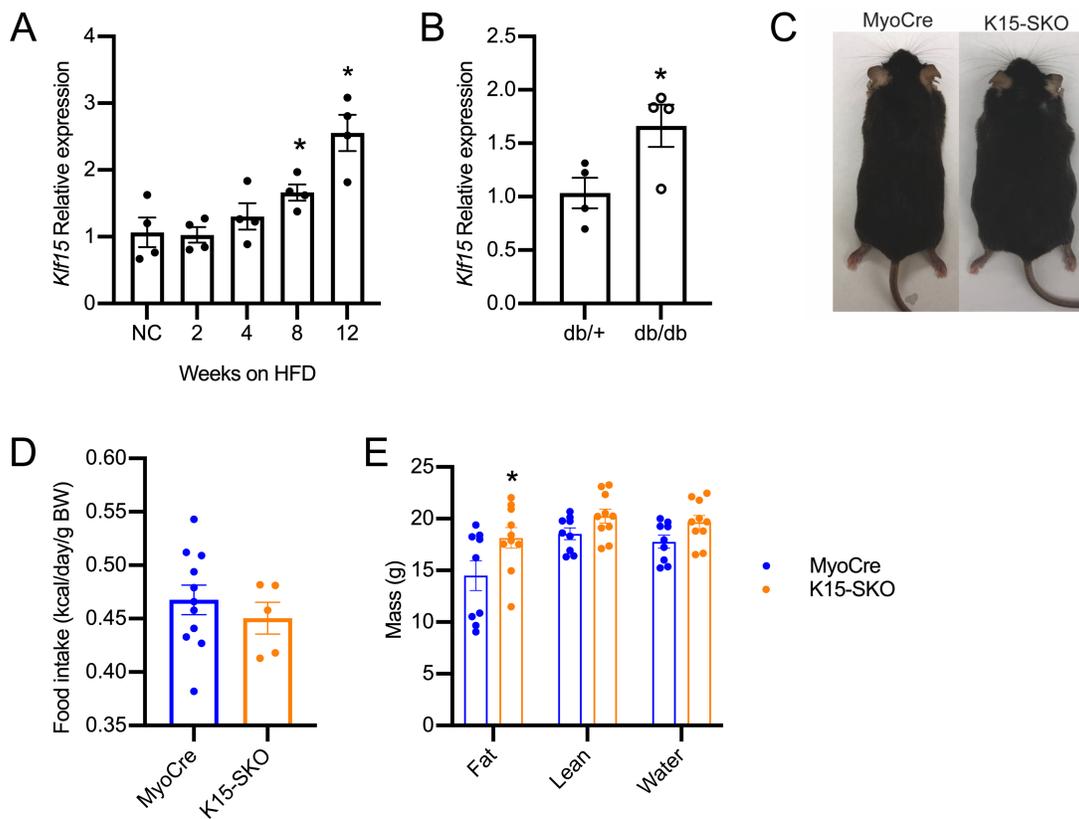


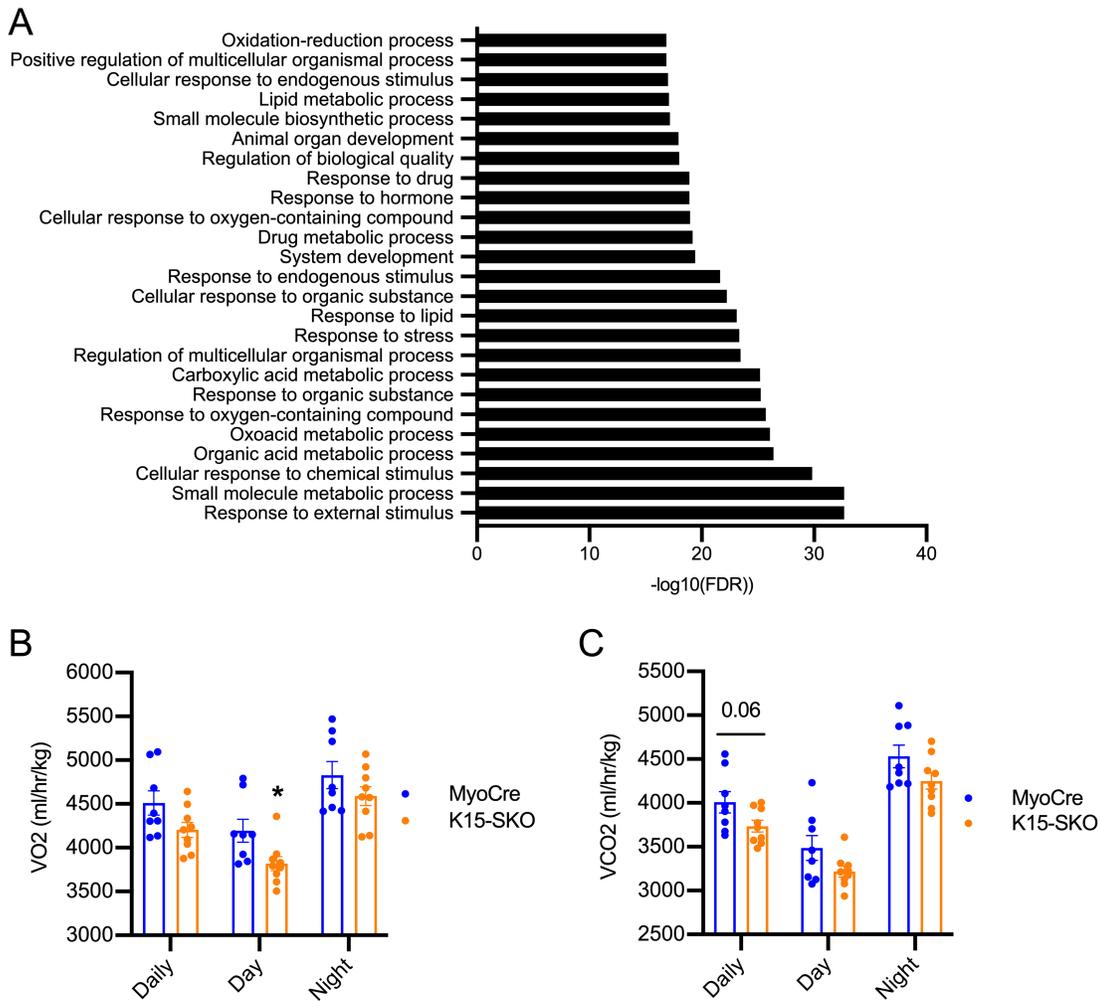
Supplemental Figure 1. (A) Relative *Kif15* gene expression across different organs (n = 4). WAT = white adipose tissue, BAT = brown adipose tissue, Gastroc = gastrocnemius, Quad = quadriceps. **(B)** Relative *Kif15* gene expression in different hindlimb skeletal muscle beds in WT mice (n = 5). Representation of previously published data on percentage of fiber types within each muscle bed (Bloemberg and Quadrilatero, 2012). GR = gastrocnemius red, GW = gastrocnemius white, VI = vastus intermedius, VL = vastus lateralis, TA = tibialis anterior, EDL = extensor digitorum longus. **(C)** *Kif15* gene expression qPCR in different organs in MyoCre vs K15-SKO mice confirming skeletal muscle specificity of *Kif15* deletion (n = 4). **(D)** Relative gene expression of various skeletal muscle contractile genes in MyoCre vs K15-SKO soleus (n = 4). **(E)** Average cross-sectional area (CSA) of 29,551 muscle fibers in MyoCre vs K15-SKO soleus (n = 7). **(F)** Representative images of fiber type distribution in MyoCre vs K15-SKO as determined by immunofluorescence MHC staining, top panel: blue = Type I, green = Type IIA, red = Type IIX, green/red = Type IIAX, green/blue = Type I/II; quantification of fiber type as percentage of total number of fibers per section. Scale bars are 75uM (n = 4-9) **(G)** Representative images of electron microscopy of soleus demonstrating normal skeletal muscle architecture in both genotypes. Scale bars in top panels are 1uM, bottom panels are 2uM (n = 4). Data represent mean \pm SEM. Comparisons between groups were performed using an unpaired, 2-tailed Student's *t* test. * $p < 0.05$, **** $p < 0.0001$.



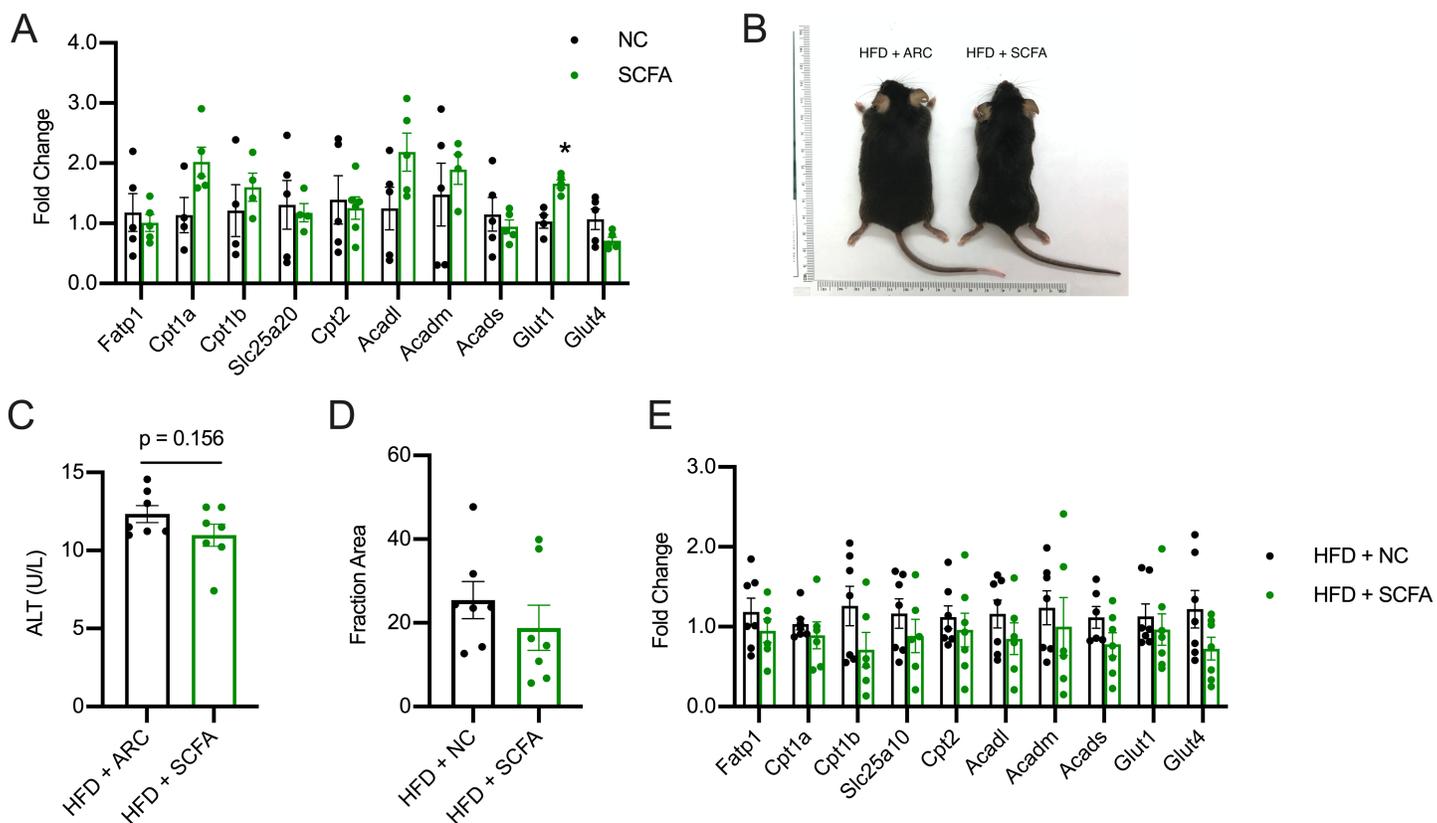
Supplemental Figure 2. Daily, day, and night average metabolic cage data of MyoCre vs K15-SKO for **(A)** Food intake ($n = 8-9$) and **(B)** Physical activity ($n = 8-9$) over three days. **(C)** Percent intestinal lipid absorption calculated from fecal concentration of behenic acid to other fatty acids ($n = 5-6$). **(D)** Representative images of hematoxylin and eosin (H&E) stained sections of white adipose tissue (WAT). **(E)** Liver tissue triglycerides (TG) ($n = 6-7$). **(F)** *Cd36* expression in MyoCre vs K15-SKO liver ($n = 5$). Data represent mean \pm SEM. Comparisons between groups were performed using an unpaired, 2-tailed Student's *t* test.



Supplemental Figure 3. (A) Relative *Klf15* expression in skeletal muscle of WT mice on normal chow (NC) and throughout 12 weeks of high fat diet (HFD) (n = 4). (B) Relative *Klf15* expression in skeletal muscle of db/+ controls compared to db/db (n = 4). (C) Representative picture of gross morphology of MyoCre vs K15-SKO after 10 weeks HFD. (D) Average HFD food intake (n = 5-11). (E) Body composition analysis of fat, lean, and water mass by quantitative magnetic resonance imaging after 10 weeks HFD (n = 8-9). Data represent mean \pm SEM. Comparisons between groups were performed using an unpaired, 2-tailed Student's *t* test, *p < 0.05, **p < 0.01, ***p < 0.001.



Supplemental Figure 4. (A) Gene-set enrichment analysis using Gene Ontology Biological Processes (GO BP) showing top 25 most significantly enriched pathways in quadricep muscle of MyoCre vs K15-SKO ($n = 3$). Daily, day, and night average metabolic cage data of MyoCre vs K15-SKO for **(B)** Food intake ($n = 8-9$) and **(C)** Physical activity ($n = 8-9$). Data represent mean \pm SEM. Comparisons between groups were performed using an unpaired, 2-tailed Student's t test, * $p < 0.05$.



Supplemental Figure 5. (A) Soleus qPCR of various lipid and glucose metabolism genes after 8 weeks NC or SCFA (n = 5). **(B)** Representative picture of gross morphology of K15-SKO after 5 weeks of HFD followed by either 8 weeks of NC or SCFA diet. **(C)** Plasma alanine transaminase (ALT) levels in K15-SKO after 5 weeks of HFD followed by either 8 weeks of NC or SCFA diet (n = 7). **(D)** Quantification of proportional area of positive staining on ORO stain (n = 7). **(E)** Soleus qPCR of various lipid and glucose metabolism genes after 5 weeks HFD followed by either 8 weeks of NC or SCFA (n = 7). Data represent mean \pm SEM. Comparisons between groups were performed using an unpaired, 2-tailed Student's *t* test, * $p < 0.05$.

SUPPLEMENTAL FIGURE LEGENDS

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SUPPLEMENTAL METHODS

Mice. The skeletal muscle specific KLF15 deletion (K15-SKO) was generated by mating the Myogenin-Cre (MyoCre) mouse line to the $Klf15^{flox/flox}$ mouse line. The MyoCre mouse line was a generous gift from Eric Olson's laboratory (University of Texas Southwestern) and backcrossed >6 generations to C57BL/6J [1]. The $Klf15^{flox/flox}$ ($Klf15^{tm2Jain}$) mouse line was generated by Ozgene by inserting *LoxP* sites flanking exon 2 of the *Klf15* gene and maintained at our facilities as an inbred strain [2]. All K15-SKO mice used have been backcrossed to $Klf15^{flox/flox}$ for >6 generations, and each experimental animal is genotyped for homozygous floxed alleles and positive for Cre. Age matched, male, non-littermate MyoCre mice were used as controls to K15-SKO. Mice are kept on a daily 12 hr light/dark schedule, fed with tap water ad libitum and either standard chow, control diet (Research Diets, D12450J), or high fat diet (Research Diets, D12492) for 10 weeks starting at 8 weeks of age. Where indicated, mice were fed a short chain fatty acid enriched diet (SCFA: Harlan Teklad TD.09849) for 8 weeks either starting at 8 weeks of age or following 5 weeks HFD. Animals were weighed weekly for each diet experiment. Food intake was measured daily by multiplying grams consumed by each diet's caloric density and averaged across the diet regimen. Eight week old mice heterozygous (db/+) and homozygous (db/db) for the diabetes spontaneous mutation ($Lepr^{db}$) were obtained from Jackson Laboratories. All experiments involving animals were conducted under protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Case Western Reserve University.

Intraperitoneal Glucose and Insulin Tolerance Tests (IPGTT and IPITT). Intraperitoneal injection of glucose (2 g/kg body weight) or insulin (0.5 U/kg body weight)

was performed following 5 hr fast. Blood glucose was measured at baseline, 15 min, 30 min, 60 min, 90 min, and 120 min by tail bleed by glucometer reading. IPGTT and IPITT were performed at 8 weeks of age for baseline measurements; following 10 weeks of HFD; following 8 weeks of SCFA diet; and following 5 weeks of HFD and then again after an additional 8 weeks of SCFA diet.

Body Composition. Absolute amounts of body fat, lean tissue, and body water were determined by quantitative magnetic resonance imaging of mice at University of Cincinnati, Mouse Metabolic Phenotyping Center.

RNA Isolation and Quantitative Real-Time PCR. Tissue samples were disrupted in PureZOL in a Tissue-lyzer (Qiagen) using stainless steel beads (30Hz for total 2 minutes). Total RNA was isolated using Bio-Rad Aurum Total RNA Fatty and Fibrous Tissue Kit using manufacturer's instructions and transcribed to complementary DNA using iScript (Bio-Rad). Quantitative real-time PCR was performed using Taqman method and appropriate probes from Roche Universal Probe Library System. Gene expression was normalized to cyclophilin B and compared using $\Delta\Delta C_t$ method. All primers were efficiency tested and validated. Primers used in this study include: *Klf15* (Forward: ACAGGCGAGAAGCCCTTT, Reverse: CATCTGAGCGGGAAAACCT), *Cyclophilin B* (Forward: TTCTTCATAACCACAGTCAAGACC, Reverse: ACCTTCCGTACCACATCCAT), *Fatp1* (Forward: GACAAGCTGGATCAGGCAAG, Reverse: GAGGCCACAGAGGCTGTTC), *CD36* (Forward: TTGTACCTATACTGTGGCTAAATGAGA, Reverse: CTTGTGTTTTGAACATTTCTGCTT), *Fabp3* (Forward: CTTTGTCGGTACCTGGAAGC, Reverse: TGGTCATGCTAGCCACCTG), *Cpt1a* (Forward:

GACGAATCGGAACAGGGATA, Reverse: TGGCATAGCTGTCAATAGATGC), *Cpt1b*
(Forward: GAGTGACTIONGGTGGGAAGAATATG, Reverse:
GCTGCTTGCACATTTGTGTT), *Slc25a20* (Forward: TGAAGGCCCTGTACTCA,
Reverse: CCTCCAGAGAGTCAGCCATC), *Cpt2* (Forward:
CCAAAGAAGCAGCGATGG, Reverse: TAGAGCTCAGGCAGGGTGA), *Acads*
(Forward: TCTTCCCCACAGCTCAGGT, Reverse: GTAATCCAAGCCTGCACCA),
Acadm (Forward: AGTACCCTGTGGAGAAGCTGAT, Reverse:
TCAATGTGCTCACGAGCTATG), *Acadl* (Forward: GCTTATGAATGTGTGCAACTCC,
Reverse: CCGAGCATCCACGTAAGC), *Hadh* (Forward: CTTGCGCTCCATGTCCTC,
Reverse: ACTACTGTATGGCCAGTTGCTG), *Ccl2* (Forward:
GATCATCTTGCTGGTGAATGAGT, Reverse: CATCCACGTGTTGGCTCA), *Cd64*
(Forward: AGGTTCTCAATGCCAAGTGA, Reverse: GCGACCTCCGAATCTGAAGA),
Cd11b (Forward: GAGGCCCCAGGACTTTAAC, Reverse:
CTTCTTGGTGAGCGGGTTCT), *Glut1* (Forward: ATGGATCCCAGCAGCAAG,
Reverse: CCAGTGTTATAGCCGAAGTGC), *Glut4* (Forward:
GACGGACACTCCATCTGTTG, Reverse: GCCACGATGGAGACATAGC), *Fabp1*
(Forward: CCATGACTGGGGAAAAAGTC, Reverse: GCCTTTGAAAGTTGTCACCAT),
Pdk4 (Forward: CGCTTAGTGAACACTCCTTCG, Reverse:
CTTCTGGGCTCTTCTCATGG), *Pepck1* (Forward: CAGCCGTCTGGCTAAGGA,
Reverse: GCCTTCGGGGTTAGTTATGC), *Ppargc1a* (Forward:
AATTTTCAAGTCTAACTATGCAGACC, Reverse:
CAAATCCAGAGAGTCATACTTGC), *Myh7* (Forward: CGCATCAAGGAGCTCACC,
Reverse: CTGCAGCCGCAGTAGGTT), *Tnni1* (Forward:

GAATGTGGAGGCTATGTCTGG, Reverse: TGTCATACAGCAAGCCAACC), *Tnnc1*
(Forward: CGACAGCAAAGGGAAGTCTG, Reverse: TGTAGCCATCAGCGTTTTTTG),
Tnnt1 (Forward: GTGCTCTACAACCGCATCAG, Reverse:
GCTCCACACAGCAGGTCAT), *Tnni2* (Forward: GAGATGAGGACAAGCGCAAC,
Reverse: CGCTATCTGGAGCATCACAC), *Tnnc2* (Forward:
GAGTGCGGAGGAGACAACC, Reverse: AGCCTGTTGGTCCGTCAT), *Tnnt3* (Forward:
TTGACCAAGCCCAGAAGC, Reverse: GCAGTGACCTCTCTGCTCT)

RNA-sequencing. Soleus muscles (n=4) and quadriceps muscles (n=3) were isolated from MyoCre vs K15-SKO. RNA isolation was performed, as described above. cDNA sequencing libraries were prepared by MacroGen NGS using the TruSeq Stranded Total RNA with Ribo-Zero Gold Human/Mouse/Rat kit and sequenced on a Illumina platform. Reads from each sample were aligned to the mouse genome (UCSC mm10) using TopHat2 [3]. Gene expression was quantitated as fragments per kilobase of transcript per million mapped reads (FPKM) using Cufflinks [4]. A total of 496 differentially expressed genes (DEGs) were identified with an adjusted p-value cutoff $q < 0.05$. DEGs were input into shinyheatmap to generate heat map clustering [5]. Significantly enriched Gene Ontology Biological Processes (GO BP) were generated by iDEP [6].

Histology. Liver tissue were harvested and fixed using 10% neutral buffered formalin for 24 h. For cryosections, tissues were transferred to 15% sucrose/PBS solution for 24 h at 4°C and subsequently transferred to 30% sucrose/PBS solution for 24 h at 4°C. Livers were then frozen in Optimal Cutting Temperature (OCT) compound (Fisher Scientific, Hampton, NH) and sectioned at 7µM thick sections. Triglycerides and lipids were stained using Oil Red O (American MasterTech, Lodi, CA), imaged, and quantified by ImageJ

software (NIH). White adipose tissue were fixed, embedded with paraffin, and cut into 6µM thick sections. Adipocyte size was measured on two independent H&E-stained sections per animal and 50 adipocytes were quantified per section. For all histological analyses, including imaging, the researcher was blinded.

Cross-sectional area analysis and fiber-type staining. Soleus muscles were harvested into 15% sucrose/PBS for 4 hours at room temperature and then transferred to 30% sucrose/PBS overnight at 4°C. Tissues were then dipped in isopentane cooled in liquid nitrogen followed by freezing in OCT. The soleus muscles were cut in 5µM thick sections and prepared for fiber type staining (protocol modified from Bloemberg and Quadrilatero [7] and Bergmeister et al. [8]). Slides were washed in phosphate buffered saline (PBS) + 0.05% Triton X (PBST) for 10 minutes and then 5 minutes. Slides were then blocked for 1 hour at room temperature with PBST containing 10% goat serum. The sections were incubated in PBST containing 10% goat serum with MHC I antibody (BA-F8, 1:50 dilution), MHC IIa antibody (SC-71, 1:600 dilution), MHC IIx (6H1, 1:50 dilution) for 1 hour at room temperature. All primary antibodies were purchased from Developmental Studies Hybridoma Bank. Slides were then washed in PBST for 10 minutes, followed by 5 minutes. The slides were then incubated in PBST containing 10% goat serum with a cocktail of Alexa Fluor 350 IgG_{2b} (ThermoScientific), Alexa Fluor 488 IgG₁ (BioLegend), and Alexa Fluor 555 IgM (ThermoScientific) for 1 hour at room temperature followed by another PBST wash for 10 minutes and then 5 minutes. Coverslips were mounted with Prolong Gold antifade reagent. Individual images of tissue sections were taken on a Leica DFC7000 T microscope and then assembled using Leica Application Suite X software. Muscle cross-sectional area was determined using ImageJ

software by randomly selecting at least 200 fibers across 4-5 sections per animal and measuring area for each fiber. Total muscle fibers for each fiber type were counted and averaged for 3-4 muscle sections per animal. For all cross-sectional area and fiber type analyses, including imaging, the researcher was blinded.

Electron Microscopy – Following euthanasia, MyoCre and K15-SKO mice were perfused with $\frac{1}{4}$ strength Karnovsky's fixative. Soleus muscles were immediately harvested and fixed for one hour in a 1:1 mixture of solution A (25% glutaraldehyde, 20% paraformaldehyde, 0.2M buffer (pH 7.4), and deuterium-depleted water) and solution B (5% DMSO). Fixative was replaced with fresh solution and tissues were fixed for another hour at room temperature. Samples were then washed with PBS three times for 5 minutes each and then stored in PBS at 4°C until processing and imaging. All tissue embedding, processing, and imaging was done at the Electron Microscopy Core Facility, Case Western Reserve University.

Plasma Chemistry. Blood was rapidly harvested in heparin from MyoCre and K15-SKO mice after 5 hr fast. Samples were spun at 2000rpm at 4°C for 20 minutes, and the plasma was collected for analysis. Free fatty acids were quantified by thin-layer chromatography by University of Cincinnati, MMPC. Plasma triglycerides were quantified using the colorimetric assay kit from ThermoFisher Scientific (TR22421). Insulin was measured using the Ultra Sensitive Mouse Insulin ELISA kit from Crystal Chem (90080). Alanine transaminase was measured using Alanine Transaminase Colorimetric Activity Assay Kit from Cayman Chemicals (700260). All analyses were performed according to manufacturers' instructions.

Acylcarnitine measurements. Following a 5 hour fast, soleus tissue from MyoCre and K15-SKO mice were rapidly harvested and flash frozen. Tissue homogenates (50mg/mL) were prepared and homogenized in 50% acetonitrile and 0.3% formic acid. Acylcarnitine species were analyzed by tandem mass spectrometry using sample preparation methods described previously [9], [10]. The data were acquired using a Waters TQD mass spectrometer equipped with Acquity™ UPLC system and controlled by MassLynx 4.1 operating system (Waters, Milford, MA).

Non-invasive Intestinal fat absorption. Dietary fat containing 5% sucrose polybehenate was fed to individually housed mice in a semi-synthetic diet containing absorbable fat ad lib for 3 days. Six to eight fecal pellets were collected on days 3 and 4 for each mouse. Fat absorption was measured and analyzed by gas chromatography of fatty acid methyl esters at the University of Cincinnati MMPC. Fat absorption was calculated from the ratios of behenic acid to other fatty acids in the diet and in the feces.

Indirect Calorimetry. Measurements were performed at the University of Massachusetts MMPC using a TSE PhenoMaster/LabMaster Metabolic Cage system. Body composition of mice was assessed prior to metabolic cage measurement in order to calculate energy expenditure. Mice were individually housed in metabolic chambers with food and tap water ad libitum. VO_2 and VCO_2 of individual mice were measured over three consecutive days, and RER was calculated by VCO_2/VO_2 .

Lipid Tracing. Experiments were performed at the University of Massachusetts MMPC. In brief, an indwelling catheter was placed 5-6 days before the experiment. After collecting a baseline plasma sample, mice received a bolus of 30 uCi of [9,10- $^3H(N)$]-

palmitic acid, and blood sampling was performed at 1 ,2 ,3 ,4 , and 5 minutes post-injection. Euthanization and tissue-collection followed.

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