Supplemental Materials

Expanded Methods

Animal Model

ACC2 flox/flox-MerCreMer+ ($ACC2^{-f/f-MCM+}$) mice were mated with $ACC2^{f/f}$ to produce both study and control littermates on the C57BL/6 background carrying homozygous Nnt mutations.[20] The alpha myosin heavy chain-MerCreMer (αMHC-MCM) mice is applied as another control for $ACC2^{f/f-MCM+}$ mice. A transgenic mouse expressing mitochondria targeted Keima (designated as mt-Keima) was generated on an FVB background as described previously.[21] Mt-Keima was crossed with $ACC2^{-f/f-MCM+}$ four times to generate $ACC2^{f/f}$, mt-Keima and $ACC2^{f/f-MCM+}$, mt-Keima bigenic mice on a mixed background carrying homozygous Nnt mutations. At 8 weeks of age, all of the mice received an intraperitoneal injection of tamoxifen (20mg/kg) for 5 days, which was sufficient to induce ACC2 deletion in $ACC2^{f/f-MCM+}$. Four weeks after the last injection of tamoxifen, the alpha myosin heavy chain-MerCreMer (designated as αMHC-MCM), $ACC2^{f/f}$ (designated as Con), $ACC2^{f/f-MCM+}$ (designated as ACC2 iKO), $ACC2^{f/f}$, mt-Keima (designated as Con/mt-Keima) and $ACC2^{f/f-MCM+}$, mt-Keima (designated as ACC2 iKO/mt-Keima) mice were subjected to high fat diet (Research Diets, D12492) feeding for 24 weeks. In a previous study, the MerCreMer (MCM) mice showed the similar obese and cardiac phenotypes compared with wild type (WT) mice after 25 weeks of high fat diet feeding, similar feeding period as discussed above.[47] Similarly, we found that both αMHC-MCM and $ACC2^{f/f}$ developed cardiac dysfunction after HFD, and $ACC2^{f/f}$ was used as controls in majority of the comparisons. All the mice were housed at 22°C with a 12-hour light, 12-hour dark cycle with free access to water and standard chow. The experiments included in this study were performed with male mice. All protocols concerning animal use were approved by the Institutional Animal Care and Use Committee at University of Washington.

Transthoracic Echocardiography

The mice were anesthetized and maintained with 1-2% isofluorane in 95% oxygen. Transthoracic echocardiography was conducted with Vevo 2100 high-frequency, high-resolution digital imaging system (VisualSonics) equipped with a MS400 MicroScan Transducer. A parasternal short axis view was used to obtain M-mode images for analysis of fractional shortening, ejection fraction, and other cardiac functional parameters. Diastolic function was assessed via Tissue Doppler Imaging.

Isolated Heart Perfusion Experiments and Nuclear Magnetic Resonance (NMR) Spectroscopy

Isolated mouse hearts were perfused in Langendorff-mode at 37°C and a constant pressure as previously described.[10, 20] The perfusate contained (in mmol/L): NaCl 118, NaHCO3 25, KCl 5.3, CaCl2 2, MgSO4 1.2, EDTA 0.5, glucose 5.5, mixed long-chain fatty acids 0.4 (bound to 1.2% albumin), lactate 1.2, and insulin 50 μU/mL, equilibrated with 95% O2 and 5% CO2 (pH 7.4). After equilibration, hearts were perfused with ^{13}C-labeled substrates (1,6-^{13}C glucose and U-^{13}C fatty acids) to determine substrate utilization.[10, 20] To ensure the comparability, all the groups were perfused in the same batch under identical conditions. Changes in cardiac high-energy phosphate content were monitored by 31P nuclear magnetic resonance (NMR) spectroscopy simultaneously with a continuous recording of LV function via the PowerLab data acquisition system (ADInstruments). The phosophocreatine (PCr) to ATP ratio was calculated by dividing the peak area of PCr by the average of the peak areas from γ-ATP and β-ATP.[48]

^{13}C NMR Spectroscopy of Tissue Extracts

At the end of the perfusion protocol with ^{13}C labeled substrates, hearts were freeze-clamped with Wollenberger tongs precooled in liquid nitrogen. The1,6-^{13}C glucose and U-^{13}C fatty acids, are converted to 2-^{13}C acetyl CoA and 1,2-^{13}C acetyl CoA, respectively, and their individual contributions to the TCA cycle can be determined by the appearance of ^{13}C labeled carbons in the glutamate pool. Frozen heart tissues were extracted with perchloric acid and neutralized by

potassium hydroxide. Lyophilized extracts were dissolved in deuterium oxide (D_2O) and loaded into a 3-mm NMR tube. Proton-decoupled ^{13}C NMR spectra of tissue extracts were obtained using a Bruker Avance (14T) spectrometer. Spectra were generated by Fourier transformation following multiplication of the free-induction decays (FIDs) by an exponential function. The resonance signals from the C3 and C4 carbons of glutamate were quantified using Lorenztian fitting with the ACD/NMR Processor (ACD Labs). Isotopomer analysis was performed by modeling the TCA cycle metabolism using the peak areas of the C3 and C4-^{13}C resonances of glutamate in the NMR spectra (TCACALC).[49] The values obtained from the modeling software determined the contributions of labeled substrates (glucose and fatty acids) and unlabeled substrates (lactate, glycogen, triglycerides) to oxidative metabolism.

Mouse Heart Mitochondria Isolation

Hearts removed from anesthetized animals were rinsed with ice-cold mitochondria isolation buffer (MIB; 70 mM Sucrose, 200 mM D-Mannitol, 5mM MOPS, 2mM Taurine, 1.6 mM Carnitine Hydrochloride, 1 mM EDTA, 0.025% BSA) to remove the blood, and finely minced. The minced heart tissue was incubated in 5 ml of MIB plus 0.1mg/ml trypsin for 10 min on ice. 5 ml of MIB containing 0.2% BSA and 0.5mg/ml trypsin inhibitor was added to neutralize the trypsin. After removing and discarding the supernatant, heart tissue was resuspended in 4ml of MIB containing 0.2% BSA and homogenized on ice with a Teflon pestle (10mL) with six strokes at 1200 rpm using the Overhead Stirrer (Wheaton). Mitochondria were isolated by differential centrifugation. Briefly, the homogenized tissue was centrifuged at 600*g* for 5 min at 4°C. The supernatant was centrifuged at 8000*g* for 10 min at 4°C. The resulting pellet was resuspended in 2mL of MIB and centrifuged at 8000*g* for 10 min at 4°C. The final pellet was rinsed and gently resuspended in 150 μL of MIB. Concentration of mitochondria was determined by the Pierce BCA Protein Assay Kit (Thermo Scientific).

Measurement of Mitochondria Respiration

Mitochondrial respiration was calculated from oxygen consumption rates (OCRs) under specific conditions using an XF24 Extracellular Flux Analyzer (Seahorse Bioscience). Mitochondria isolated from heart tissue were suspended in the mitochondrial assay buffer (MAS; 70 mM sucrose, 220 mM mannitol, 10 mM KH_2PO_4, 5 mM $MgCl_2$, 2 mM HEPES, 1 mM EGTA, and 0.2% fatty acid free BSA; pH 7.2 at room temperature). 1-2 μg mitochondria was loaded in the assay plate in a volume of 50 μl MAS with pyruvate (10 mM)/malate (2 mM), palmitoyl-L-carnitine (50 μM)/Malate (2mM) or succinate (10 mM)/rotenone (2 μM) as substrates for coupling assays. After centrifugation at 2000*g* for 20 min at 4°C, 450 μl of MSA containing indicated substrates was added to each well, and the plate was incubated in a 37 °C non-CO_2 incubator for 10 min. Then, ADP (4mM) and oligomycin (2.5 mg/ml) were sequentially injected into each well to assess state 3 and state 4 respiration, respectively.[28]

Measurement of Mitochondrial DNA

DNA from frozen hearts was extracted and purified using the DNeasy Tissue kit (Qiagen) according to the supplier's protocol. The quantification of nuclear and mitochondrial DNA was determined by quantitative real-time PCR using β1-globin and cytochrome b, respectively. All reactions were performed in triplicate. Relative quantification was performed by interpolating crossing point data on an independent standard curve, thereby accounting for any difference in amplification efficiency. Mitochondrial DNA was expressed relative to nuclear DNA.

Measurement of Fatty Acid Oxidation

FAO was calculated from OCR under specific conditions using an XF24 Extracellular Flux Analyzer. For quantifying FAO, neonatal rat cardiomyocytes were plated at a density of 75K in the XF24 cell culture microplates in growth medium. One hour before the assay, cells were washed and incubated in FAO assay media (XF assay medium supplemented with 2.5 mM glucose and 500 μM carnitine) at 37°C in a CO_2-free atmosphere. Etomoxir (100 μM) was

added 20 minutes before the assay. For measurement of FAO, BSA or BSA conjugated FAs (0.1 mM) was added right before starting the assay and changes to OCR were measured in real time. Oligomycin (2.5 μg/ml), carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) (3μM), antimycin A (1μM), and rotenone (1μM), were sequentially injected into each well to assess basal respiration, coupling of respiratory chain, proton leak, and mitochondrial spare respiratory capacity. FAO was determined by the difference of OCR between vehicle and Etomoxir treated conditions.[50] The OCR values were normalized to total protein amount in individual wells.

Immunoblot Analysis

Heart homogenates, isolated mouse heart mitochondria or cell lysates were prepared using RIPA buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1% deoxycholic acid, 1 mM EDTA, 1 mM Na_3VO_4, 1 mM NaF, 0.5 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride, 0.5 μg/ml aprotinin, and 0.5 μg/ml leupeptin. Equal amounts of proteins (10–20 μg) were subjected to SDS-PAGE. After proteins were transferred to a PVDF membrane, immunoblots were probed with the indicated antibodies. The protein abundance was analyzed densitometrically and normalized by the level of α-tubulin, GAPDH or SDHA. Antibodies used for immunoblots were purchased from the indicated companies. Primary antibodies against CD36 (1:1000, ab133625), p62 (1:2000, ab109012) and SDHA (1:20000, ab14715) were from Abcam. Primary antibodies against p-ACC (Ser 79) (1: 1000, 11818), ACC (1: 1000, 3662), BiP (1:1000, 3177), Caspase 12 (1:2000, 2202), CHOP (1:1000, 2895), p-eIF2α (Ser 51) (1:1000, 3398), eIF2α (1:2000, 5324), GAPDH (1: 10000, 5174), parkin (1:1000, 2132), p-PERK (Thr 980) (1:1000, 3179) and PERK (1:2000, 5683) were from Cell Signaling Technology. Primary antibody against LC3 (1:2000, M186-3) was from MBL International. Primary antibody against Pink1 (1:2000, BC100-494) was from Novus Biologicals. Primary antibody against α-tubulin (1: 5000, T6199) was from Sigma.

Cell Cultures

HEK 293 cells were purchased from American Type Culture Collection (ATCC) and were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 5.5 mM Glucose (Life Technology) with 10% FBS.

Primary cultures of ventricular cardiac myocytes were prepared from 1-day-old Crl: (WI) BR-Wistar rats (Harlan Laboratories). A cardiac myocyte-rich fraction was obtained by centrifugation through a discontinuous Percoll gradient. Cells were cultured in complete medium (CM) containing DMEM/F-12 supplemented with 5% horse serum, 4 µg/ml transferrin, 0.7 ng/ml sodium selenite (Life Technologies), 2 g/L bovine serum albumin (fraction V), 3 mM pyruvic acid, 15 mM HEPES, 100 µM ascorbic acid, 100 µg/ml ampicillin, 5 µg/ml linoleic acid and 100 µM 5-bromo-2'-deoxyuridine (Sigma). Myocytes were switched to serum-free medium for 24 h before experiments.

Mouse ventricular myocytes were isolated enzymatically as previously described.[51] Briefly, mouse was anesthetized by intraperitoneal injection of 150 mg/kg pentobarbital. The heart was quickly removed and cannulated via aorta and mounted on a perfusion system. The heart was perfused with oxygenated myocyte isolation solution (113 mM NaCl, 4.7 mM KCl, 0.6 mM KH_2PO_4, 0.6 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 11 mM HEPES and 20 mM glucose) supplemented with collagenase II (300 U/ml, Worthington), hyaluronidase (0.5 mg/ml, Sigma), 50 µM $CaCl_2$, and 10 µM blebbistatin at 37°C for 10 min. The heart was disaggregated with forceps, and gently triturated five to six times and incubated in enzyme solution for 5 min at room temperature to allow further digestion. The dissociated myocytes were transferred to fresh solution, followed by stepwise increasing Ca^{2+} concentration to 0.5 mM. Myocytes were collected and cultured in serum-free M199 medium (Sigma) supplemented with 10 mM glutathione, 26.2 mM sodium bicarbonate, 0.02% bovine serum albumin and 50 U ml^{-1} penicillin/streptomycin. The experiments were carried out at room temperature and cells were used within 5-6 h after isolation.

Measurement of Triglyceride Content

Myocardial lipids were extracted from frozen heart tissues with 2:1 chloroform: methanol. The chloroform layer was dried under a nitrogen stream. The residue was resuspended in 0.5% Triton X/isopropanol. TG content was measured using Triglyceride Quantification Assay (Wako Chemicals) according to the supplier's protocol. Liver and serum TG level was measured using Triglyceride Colorimetric Assay (Cayman Chemical) according to the supplier's protocol. Values were normalized to frozen tissue weight.

Measurements of Cell Viability

Viability of the cells was measured by CellTiter-Blue (CTB) assays (Promega) according to the supplier's protocol. In brief, cardiac myocytes ($1X10^5$ per 100 µl) were seeded onto 96-well plates. 72 hours after transfection with indicated adenovirus, the cells were incubated in DMEM containing BSA or BSA conjugated palmitate (0.4 mM) for 12 h before the CTB assay.

Measurement of Mitochondrial Protein Carbonylation

Protein carbonylation was measured by OxyBlot Protein Oxidation Detection Kit (Millipore) according to the supplier's protocol. Briefly, 15 µg of mitochondria protein were derivatized to their 2,4-dinitrophenylhydrazones by reaction with 2,4-dinitrophenylhydrazine for 15 min in 3% (w/v) SDS. The samples were subjected to SDS-PAGE. After proteins were transferred to a PVDF membrane, immunoblots were probed with the primary antibody specific to the dinitrophenylhydrazone moiety attached to the derivatized proteins. This step was followed by incubation with a horseradish peroxidase antibody conjugate directed against the primary antibody. The level of protein carbonylation was analyzed densitometrically and normalized to the level of total mitochondria protein by Image J.

Construction of Adenoviral Expression Vectors

Briefly, pBHGloxΔE1,3Cre (Microbix), including the ΔE1 adenoviral genome, was co-transfected with pDC shuttle vector containing the gene of interest into HEK-293 cells using Lipofectamine 2000 (Invitrogen). Through homologous recombination, the test genes were integrated into the

E1-deleted adenoviral genome. The viruses were propagated in HEK-293 cells as described.[52] Mitochondria targeted GFP (mt-GFP) was generated by inserting the first 25 amino acids of the human ornithine transcarbamylase leader sequence to the amino terminus of GFP.[53] The cDNA clone for parkin was a gift from Dr. Richard Youle (Addgene plasmid #23956).[54] Adenovirus harboring β-galactosidase (Ad-LacZ) was used as a control.

Construction of Short Hairpin RNA (shRNA) Adenoviral Expression Vectors

Adenoviruses harboring shRNA for ACC2 (Ad-sh-ACC2) and parkin (Ad-sh-parkin) were generated using the following hairpin forming oligos:

ACC2:(5'-

GCTGAAGGACATACGGCTTCTTTCAAGAGAAGAAGCCGTATGTCCTTCAGCTTTTTT-3')

parkin:(5'-

GCACGACCTCATGGGAAACACTTCAAGAGAGTGTTTCCCATGAGGTCGTGCTTTTTT-3')

These oligos and their corresponding antisense oligos with ApaI and Hind III overhangs were synthesized, annealed, and subcloned into the pDC311 vector. The loop sequences are underlined. Recombinant adenoviruses were generated using homologous recombination in HEK-293 cells as described above.

***In vivo* treatment with CCCP (Carbonyl cyanide *m*-chlorophenyl hydrazine) or CQ (chloroquine)**

Mice were intraperitoneally injected with CCCP or DMSO diluted in co-solvent solution (vehicle). CCCP was first dissolved in DMSO. The stock was further diluted in the co-solvent solution (10% Ethanol, 40% Propylene glycol, 5% Benzyl alcohol and 45% PBS) to a final concentration 5 mg/kg. To inhibit autophagic flux in vivo, mice were intraperitoneally injected with chloroquine (CQ, 10 mg/kg) or PBS (pH7.4, vehicle). 12 hours after CCCP injection or 4 hours after CQ injection, animals were heparinized (5000 U/kg), anesthetized with sodium pentobarbital (100 mg/kg), and checked to ensure the absence of movement, flexor, and pedal reflexes. Hearts

were removed and subsequently used for mitochondria isolation, electron microscope imaging and cardiomyocyte isolation.

Live Cell Imaging

Isolated cardiomyocytes were plated on the coverslip and loaded with 5 μM MitoSOX Red (Life Technology) in Dulbecco's modified Eagle's medium for 10 min at 37°C, followed by two PBS washes. Confocal images were obtained at 20 second intervals by excitation at 488 nm and measuring emission at 585 nm. MitoSOX Red fluorescence was measured at each time point and the mean of the measurements were performed in three replicates. The signal from each cell was normalized to that at the start of the experiment, which was set to 1.

Isolated cardiomyocytes were plated on the coverslip and loaded with 20 nM TMRE (Life Technology) in Dulbecco's modified Eagle's medium for 15 min at 37°C, followed by two PBS washes. Confocal images of TMRM fluorescence were obtained by excitation at 568 nm and emission at 585 nm.

Assessing Cardiac Mitophagy using the mt-Keima Transgenic Mice

Cardiomyocytes isolated from mice harboring mt-Keima transgene were plated on a glass slide and analyzed using a Leica TCS SP8 confocal laser scanning microscope. Fluorescence of mt-Keima was imaged in two channels via two sequential excitations (458 nm, green; 561 nm, red) and using a 570 to 695-nm emission range. Laser power was set at the lowest output that would allow clear visualization of the mt-Keima signal. Imaging settings were maintained with the same parameters for comparison between different experimental conditions. Images were acquired from random microscopic fields. Ratio (561/458) images were created using the Leica Application Suite X software. High (561/458) ratio areas were segmented and quantified with the Analyze Particles plugin in ImageJ. The parameter (high [561/458] ratio area/total cell area) was used as an index of mitophagy, as described.[22]

Electron Microscopy

Mouse hearts were harvested and immediately rinsed in ice-cold Krebs Henseleit Buffer supplemented with 30mM KCl. Small sections (1mm x 1mm) were dissected from the apex of the heart and fixed overnight in 0.1 M Cacodylate buffer, 2.5% Glutaraldehyde and 4% Formaldehyde, post fixed in osmium tetroxide, dehydrated in ethanol and then embedded in epon araldite resin. Thin sections of 70nm were cut on an ultramicrotome (Leica Microsystems) and imaged with an electron microscope (JEOL). 15-20 fields were randomly chosen from each heart and images were captured at 10000x or 50000x magnification. For mitochondrial density, the total mitochondrial area was determined in each field and divided by the total tissue area using Image J software.

Histological Analyses

Mouse heart tissues were rinsed with PBS and fixed in 10% neutral buffered formalin overnight. Fixed samples were dehydrated, embedded in paraffin wax and sectioned for Wheat Germ Agglutinin staining. The outline of cross-sectional areas for 100–150 myocytes was traced and quantified with ImageJ software in each section. Suitable cross sections of the myocardium were defined as having nearly circular capillary profiles and circular-to-oval myocyte sections. Picrosirius Red (PSR) staining was performed to examine cardiac fibrosis. The images of sections were acquired by Hamamatsu Nanozoomer Whole Slide Scanner and viewed by NDP.view2 imaging software (Hamamatsu Photonics). The obtained images were analyzed with ImageJ software. The fibrosis content in the interstitial area was calculated as the mean ratio of the fibrotic tissue area to the total tissue area of all measurements of the section.

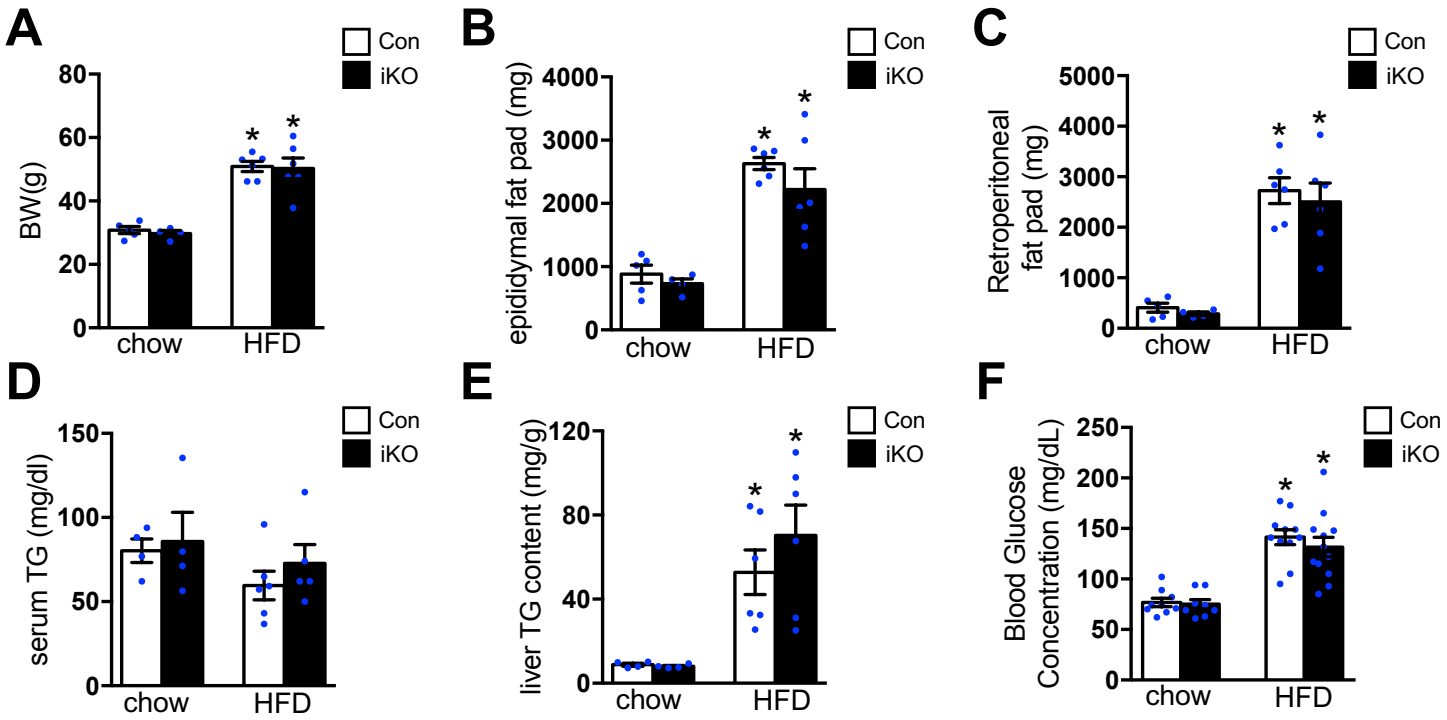
RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from frozen LV tissue using the RNeasy Fibrous Tissue Mini Kit (Qiagen) according to the manufacturer's instructions. Total RNA was reverse-transcribed into the first-strand cDNA using the Superscript First-Strand Synthesis Kit (Invitrogen). cDNA transcripts were quantified by Rotor Gene Real-Time PCR System (Qiagen) using SYBR Green

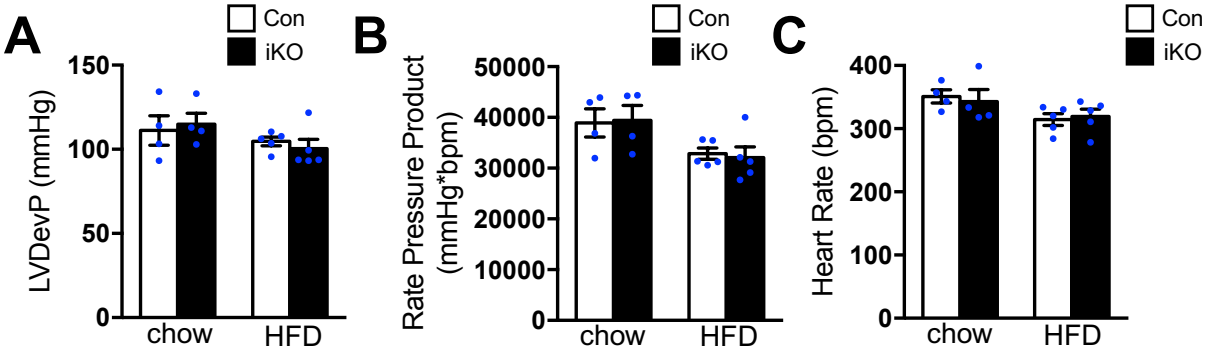
(Biorad). Results of mRNA levels were normalized to 18S rRNA levels and reported as fold-change over Control. All primer sequences are listed in Table VI in the Supplement.

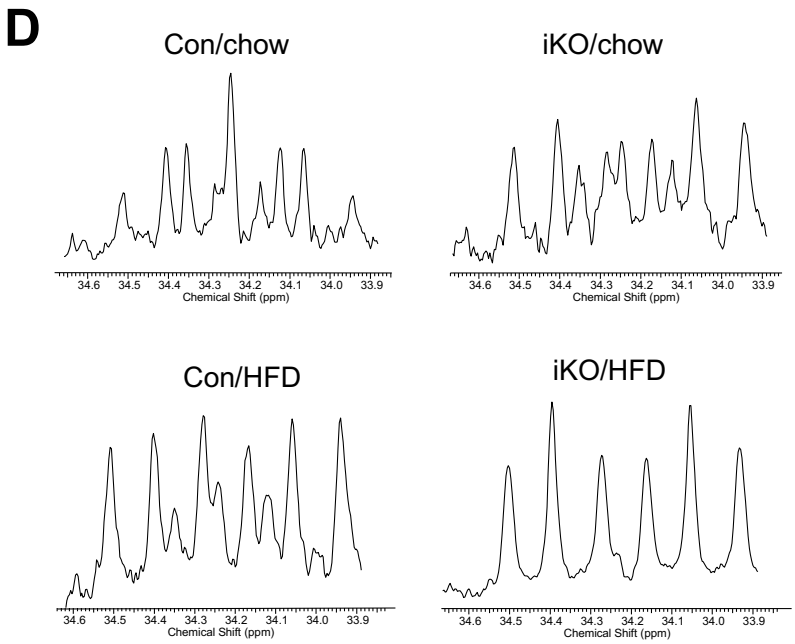
Statistical Analysis

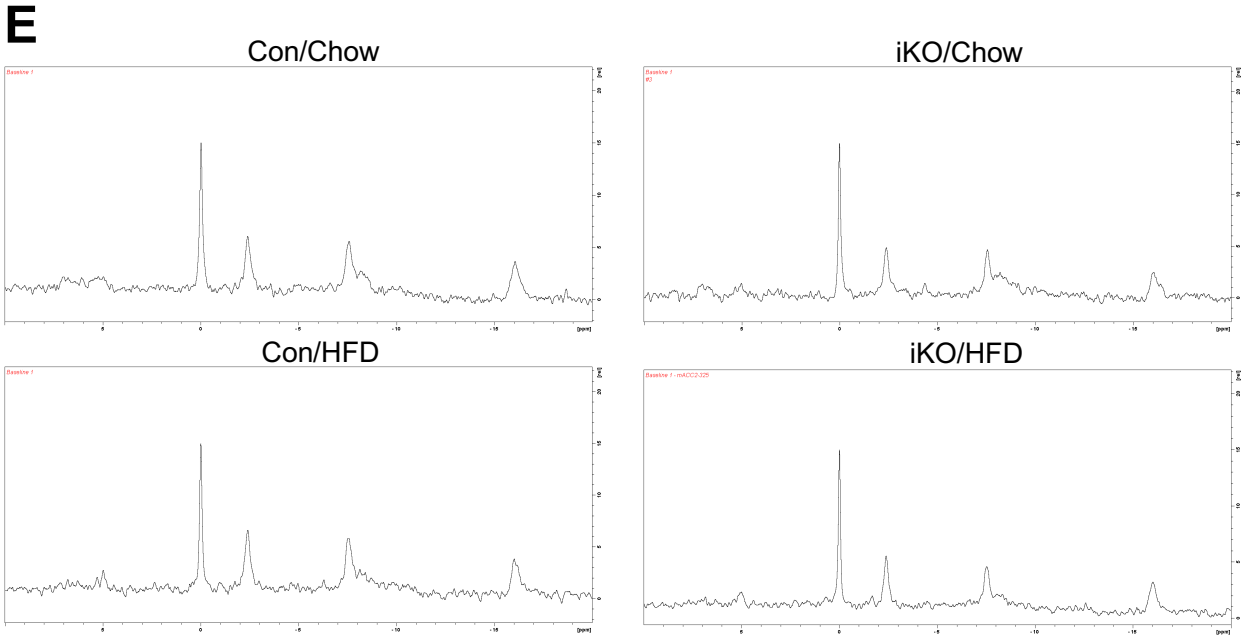
The numbers of independent experiments are specified in the relevant figure legends. Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed with a one-way analysis of variance (ANOVA) or two-way ANOVA with Tukey test for multiple comparisons. Two group comparisons were made using Student's t-test. All analyses were performed using Prism 7.0 (GraphPad). The value of $p<0.05$ was considered to be significant.



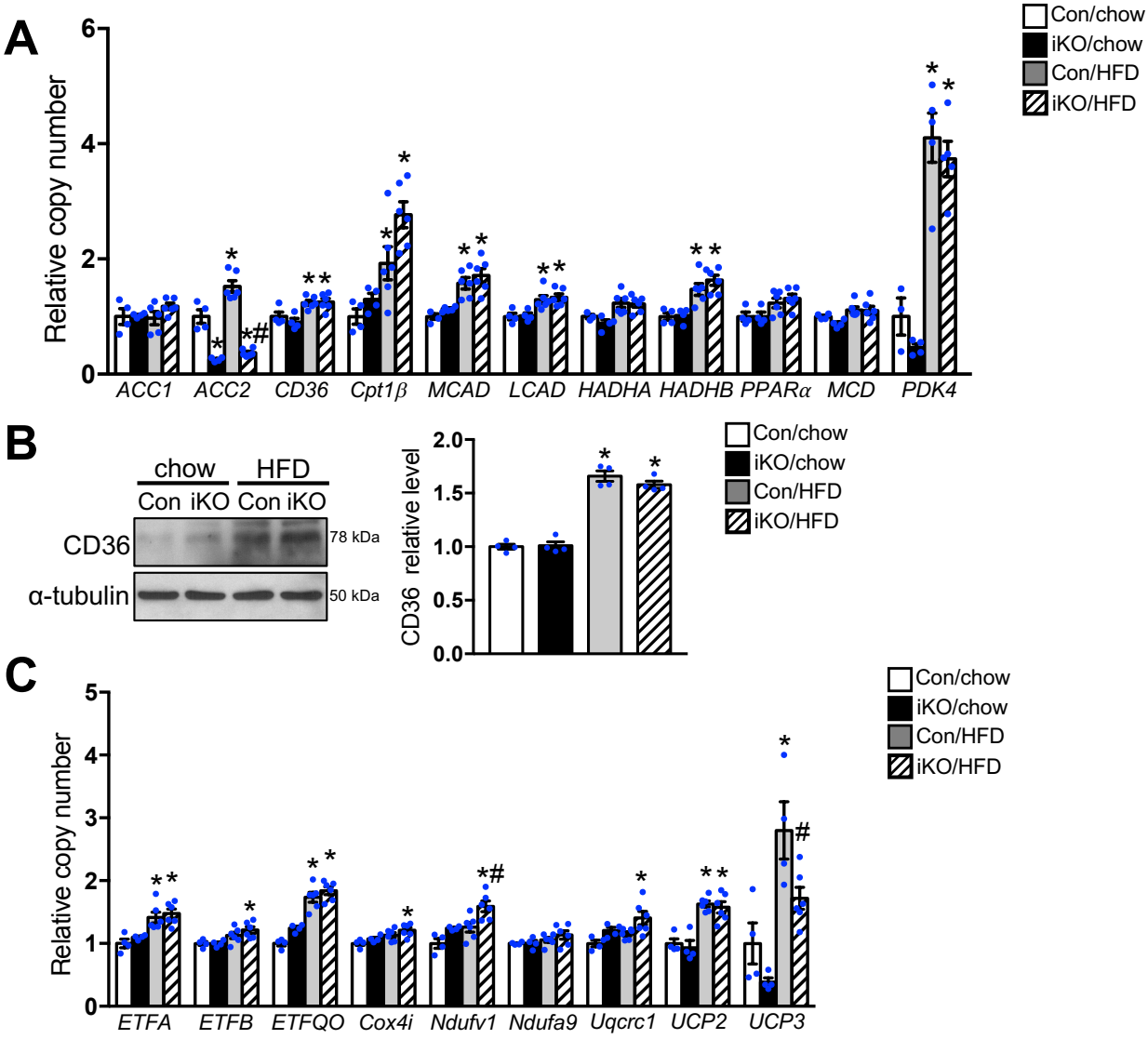
Supplemental Figure I. (A-F) Con and ACC2 iKO mice were subjected to chow or HFD feeding for 24 weeks. Body weight (A), epididymal fat pad (B), retroperitoneal fat pad (C), serum TG level (D) and liver TG content (E) were measured (*p<0.05 vs. Con/chow, n=4-6). (F) After 18 hours fasting, blood glucose level was measured (*p<0.05 vs. Con/chow, n=8-12).



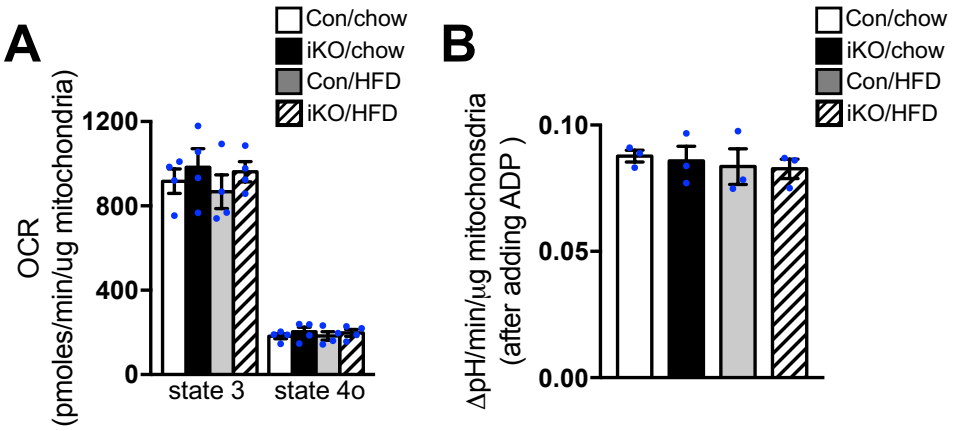




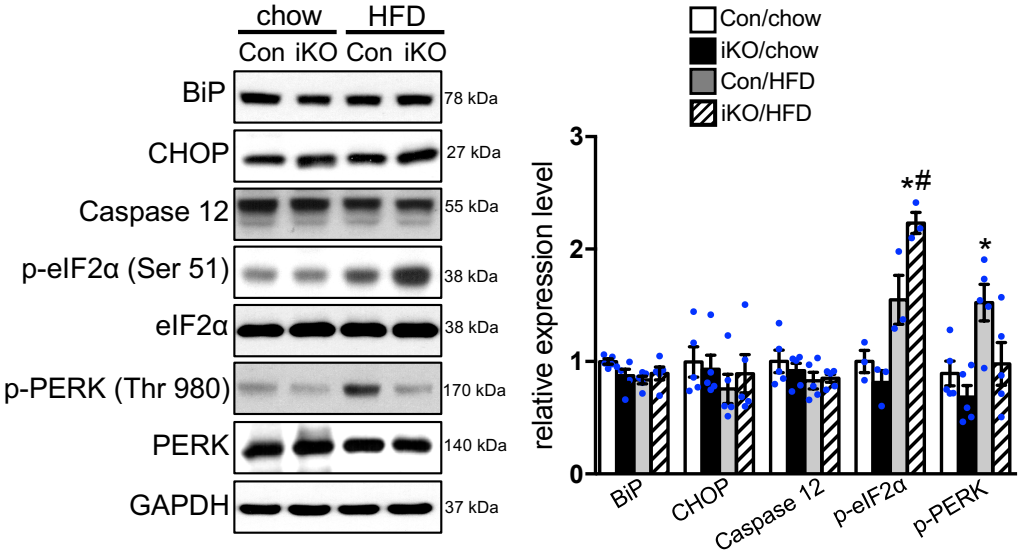
Supplemental Figure II. Con and ACC2 iKO mice were subjected to chow or HFD feeding for 24 weeks. (A-C) Ex vivo cardiac function was assessed in Langendorff isolated heart preparations. (A) LVDevP, left ventricular developed pressure (the difference between systolic and diastolic pressure), (B) RPP, rate pressure product (the product of LVDevP and heart rate) and (C) Heart Rate were measured (n=4-5). (D) Representative 13C NMR spectra of C4-glutamate from heart extracts and (E) representative ^{31}P NMR spectra collected from hearts in the indicated group.



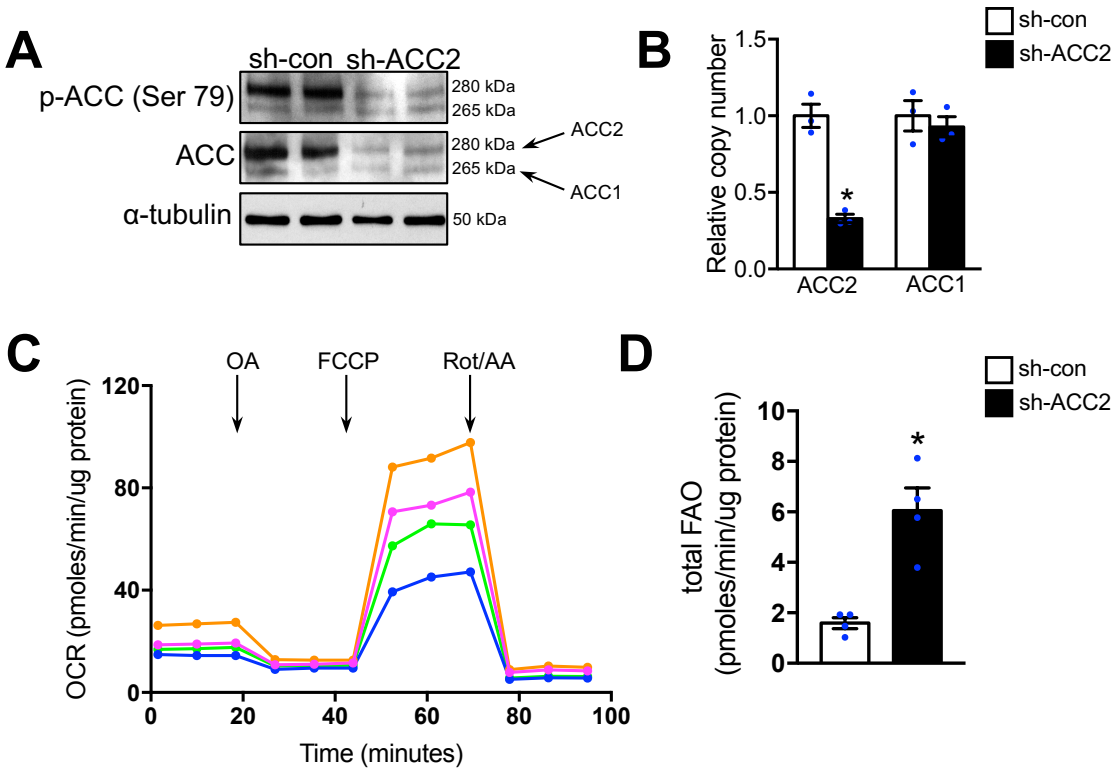
Supplemental Figure III. Con and ACC2 iKO mice were subjected to chow or HFD feeding for 24 weeks. qRT-PCR measurements of indicated genes involved in FAO and glucose oxidation (A), electron transport and mitochondria uncoupling proteins (C) (*p<0.05 vs. Con/chow, #p<0.05 vs. Con/HFD, n=4-6). (B) Representative immunoblots of CD36 and α-tubulin in heart lysates (left) and statistical analysis of densitometric measurement of CD36 (right) is shown. (*p<0.05 vs. Con/chow, n=4).



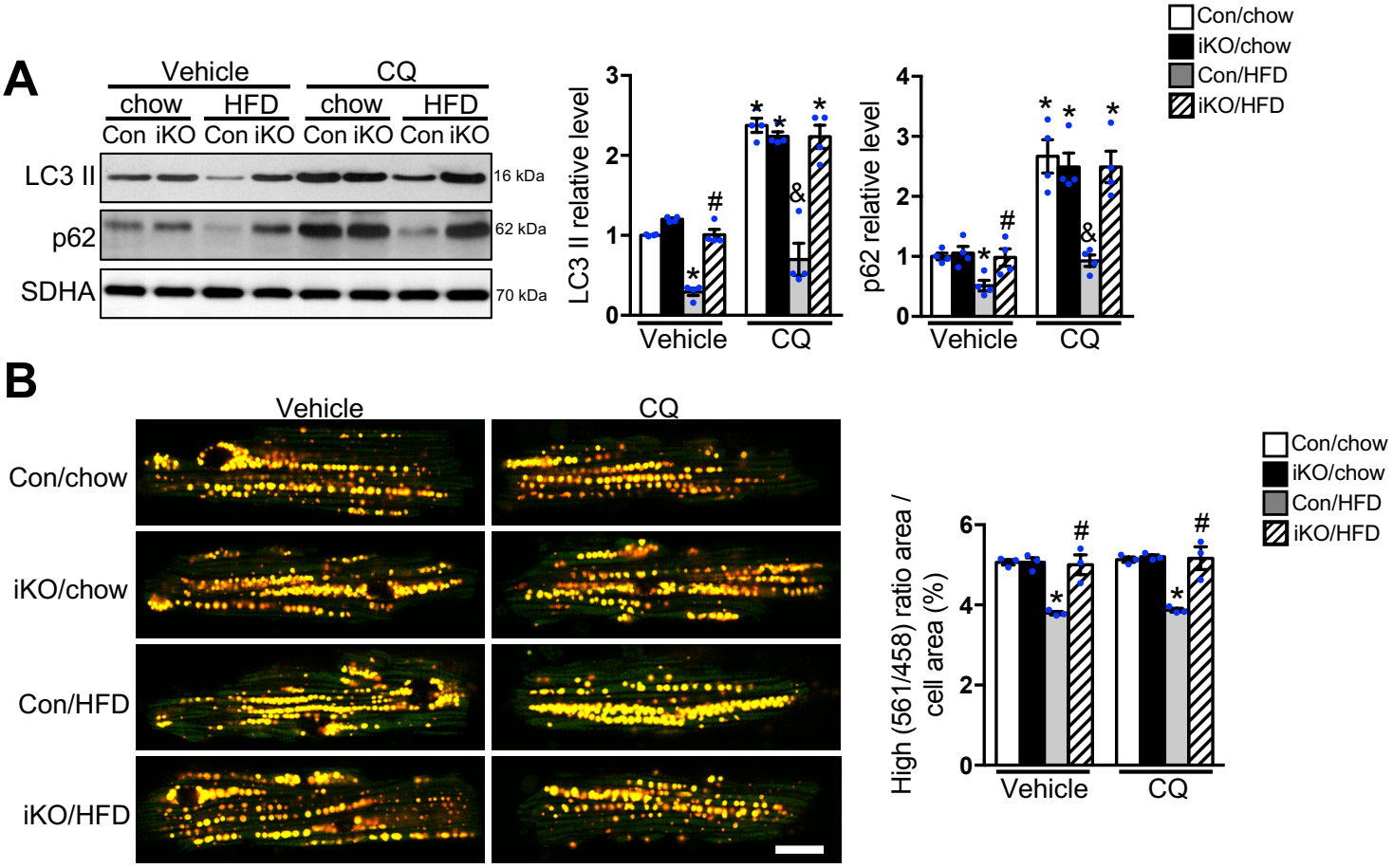
Supplemental Figure IV. Mitochondria isolated from Con and ACC2 iKO mouse hearts after 24 weeks' chow or HFD feeding. (A) Succinate/rotenone driven respiration was measured by Seahorse XF24 Analyzer. State 3 (+ADP), State 4o (+oligomycin) (n=4). (B) Absolute pH level change per minute after adding ADP in the presence of succinate/rotenone as substrates (n=3).



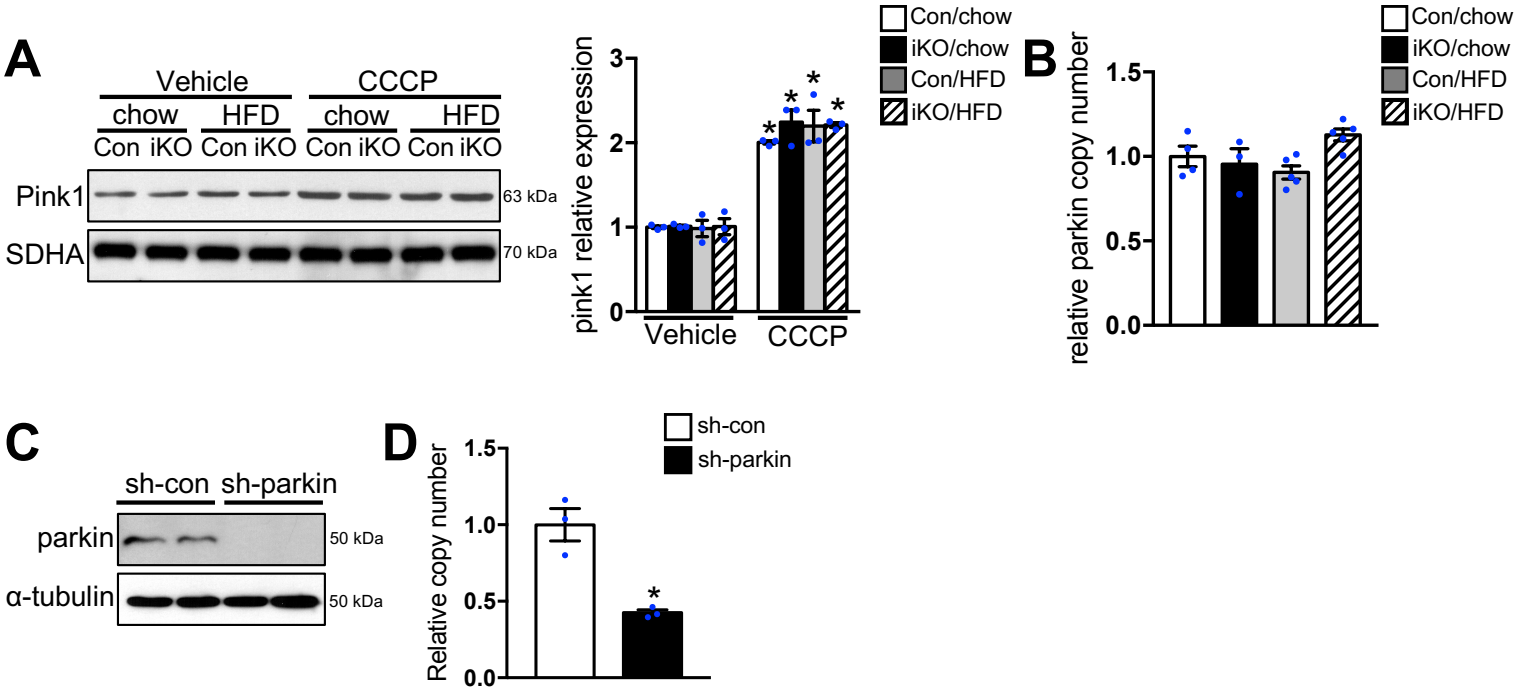
Supplemental Figure V. Con and ACC2 iKO mice were subjected to chow or HFD feeding for 24 weeks. Representative immunoblots of BiP, CHOP, Caspase 12, p-eIF2α (Ser 51), eIF2α, p-PERK (Thr 980), PERK and GAPDH in total heart lysates (left) and statistical analyses of densitometric measurements of BiP, CHOP, Caspase 12, p-eIF2α (Ser 51) and p-PERK (Thr 980) are shown (*p<0.05 vs. Con/chow, #p<0.05 vs. Con/HFD, n=3-5).



Supplemental Figure VI. (A-B) The protein and mRNA levels of ACC1 and ACC2 in NRCMs 72 hours after the transduction of indicated adenovirus (*p<0.05 vs. sh-con, n=3). (C-D) NRCMs were transfected with indicated adenovirus for 72h and mitochondria OCR was measured by Seahorse XF24 Analyzer. Mitochondrial respiration reflected by OCR levels was detected in NRCMs incubated with basal media containing exogenous FAs with or without Etomoxir (100 μM) treatment under basal condition and following the addition of oligomycin (2.5 μg/ml), the uncoupler FCCP (3 μM) and the electron transport inhibitor Rotenone/Antimycin (1 μM). (D) Quantification of total (endogenous and exogenous) FAO in ACC2 deficient cells (*p<0.05 vs. sh-con, n=4).



Supplemental Figure VII. (A) Con and ACC2 iKO mice after 24 weeks' chow or HFD feeding were subjected to chloroquine (CQ, 10 mg/kg) injection for 4 hours. Representative immunoblots of LC3 II, p62 and SDHA in mitochondria homogenates (left) and statistical analyses of densitometric measurements of LC3 II and p62 (right) are shown (*p<0.05 vs. Con/chow/Vehicle, #p<0.05 vs. Con/HFD/Vehicle, &p<0.05 vs. Con/chow/CQ, n=4). (B) Con/mt-Keima and ACC2 iKO/mt-Keima bigenic mice after 24 weeks' chow or HFD feeding were subjected to chloroquine (CQ, 10 mg/kg) injection for 4 hours. Cardiomyocytes from these groups were isolated and high (561/458) ratio area/total cell area was quantified as an index of mitophagy. About 25 to 35 cardiomyocytes per heart were analyzed (*p<0.05 vs. Con/chow/Vehicle or Con/chow/CQ, #p<0.05 vs. Con/HFD/Vehicle or Con/HFD/CQ, n=3), Scale bar 15μm.



Supplemental Figure VIII. (A) Con and ACC2 iKO mice after 24 weeks' chow or HFD feeding were subjected to CCCP (5mg/kg) injection for 12 hours. Representative immunoblots of Pink1 and SDHA in mitochondria homogenates (left) and statistical analysis of densitomeric measurement of Pink1 (right) are shown (*p<0.05 vs. Con/chow/Vehicle, n=3). (B) Con and ACC2 iKO mice were subjected to chow or HFD feeding for 24 weeks. mRNA expression of parkin is measured by qRT-PCR. (C-D) The protein and mRNA levels of parkin in NRCMs 72 hours after the transduction of indicated adenovirus (*p<0.05 vs. sh-con, n=3).

	chow		HFD	
	Con	iKO	Con	iKO
IVS;d (mm)	1.00±0.07	0.96±0.07	1.09±0.06	1.04±0.04
IVS;s (mm)	1.42±0.07	1.45±0.06	1.36±0.09	1.49±0.04
LVID;d (mm)	3.37±0.12	3.18±0.11	3.45±0.09	3.41±0.07
LVID;s (mm)	2.08±0.09	1.79±0.11	2.31±0.10	2.11±0.07
LVPW;d (mm)	0.90±0.11	0.69±0.02	0.83±0.07	0.88±0.05
LVPW;s (mm)	1.35±0.10	1.24±0.07	1.26±0.04	1.39±0.06
EF (%)	68.78±3.43	76.23±2.41	58.35±0.86*	69.38±1.40#
FS (%)	37.99±2.86	43.93±2.10	30.01±0.60*	38.13±1.10#
LV Mass (mg)	116.08±11.82	84.9±3.57	119.9±6.87	117.62±4.90
LV Mass (Corrected, mg)	92.86±9.45	67.95±2.86	95.92±5.50	94.10±3.92
LV Vol;d (ml)	46.84±3.89	40.69±3.33	49.29±2.93	47.86±2.34
LV Vol;s (ml)	14.28±1.46	9.80±1.39	18.72±1.92	14.77±1.23
HR (bpm)	528±23	528±28	543±27	548±25

Supplemental Table I. Systolic function measurements of ACC2 iKO mice and their control littermates after 24 weeks' HFD feeding by echocardiography (*p<0.05 vs. Con/chow, #p<0.05 vs. Con/HFD, n=6-8). IVS;d, Interventricular septum thickness at end–diastole; IVS;s, Interventricular septum thickness at end-systole; LVID;d, Left ventricular internal dimension at end-diastole; LVID;s, Left ventricular internal dimension at end–systole; LVPW;d, Left ventricular posterior wall thickness at end-diastole; LVPW;s, Left ventricular posterior wall thickness at end-systole; EF, Ejection fraction; FS, Fractional shortening; LV, Left ventricle; LV Vol;d, Left ventricular diastolic volume; LV Vol;s, Left ventricular systolic volume; HR, Heart rate.

	chow		HFD	
	Con	iKO	Con	iKO
A' (m/s)	14.94±0.74	15.86±1.38	15.25±2.13	16.31±1.46
E' (m/s)	15.93±0.50	17.61±1.33	13.09±1.26	17.64±1.48
IVCT (ms)	20.90±1.96	19.67±3.54	20.56±0.77	22.95±2.10
IVRT (ms)	22.36±2.57	20.22±2.86	32.22±1.57*	23.77±1.21#
MV A (mm/s)	374.2±22.4	370.2±26.3	359.8±51.4	335.6±26.7
MV E (mm/s)	618.9±31.8	582.6±25.3	460.7±38.6*	575.4±21.3#
E'/A'	1.08±0.07	1.12±0.02	0.85±0.08*	1.09±0.04#
MV E/A	1.66±0.04	1.59±0.06	1.36±0.14	1.76±0.10#
MV E/E'	38.95±1.96	33.81±2.71	36.25±3.66	33.72±2.14
HR (bpm)	415±19	411±21	400±11	398±9

Supplemental Table II. Diastolic function measurements of ACC2 iKO mice and their control littermates after 24 weeks' HFD feeding by echocardiography (*p<0.05 vs. Con/chow, #p<0.05 vs. Con/HFD, n=6-8). A', Peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler; E', Peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler; IVCT, Isovolumic contraction time; IVRT, Isovolumic relaxation time; MV, Mitral valve; A, Peak velocity of late transmitral flow; E, Peak velocity of early diastolic transmitral flow; E/A, Ratio of E to A; E/E', Ratio of E to E'; HR, Heart rate.

	MCM-chow	MCM-HFD
IVS;d (mm)	0.97±0.13	0.95±0.07
IVS;s (mm)	1.44±0.12	1.33±0.07
LVID;d (mm)	4.32±0.02	4.29±0.22
LVID;s (mm)	2.90±0.05	3.16±0.14
LVPW;d (mm)	0.91±0.08	0.88±0.06
LVPW;s (mm)	1.23±0.09	1.23±0.08
EF (%)	61.53±1.28	51.11±3.21*
FS (%)	32.81±0.92	26.03±2.10*
LV Mass (mg)	168.2±23.44	161.0±17.74
LV Mass (Corrected, mg)	134.59±18.76	128.83±14.19
LV Vol;d (ml)	84.10±0.89	84.34±10.55
LV Vol;s (ml)	32.37±1.24	32.37±1.24
HR (bpm)	516±10	511±10

Supplemental Table III. One month after last tamoxifen injection, αMHC-MCM mice were subjected to chow or HFD feeding for 24 weeks and the systolic function was measured by echocardiography (*$p<0.05$ vs. MCM/chow, n=4-6). IVS;d, Interventricular septum thickness at end–diastole; IVS;s, Interventricular septum thickness at end-systole; LVID;d, Left ventricular internal dimension at end-diastole; LVID;s, Left ventricular internal dimension at end–systole; LVPW;d, Left ventricular posterior wall thickness at end-diastole; LVPW;s, Left ventricular posterior wall thickness at end-systole; EF, Ejection fraction; FS, Fractional shortening; LV, Left ventricle; LV Vol;d, Left ventricular diastolic volume; LV Vol;s, Left ventricular systolic volume; HR, Heart rate.

	MCM-chow	MCM-HFD
A' (m/s)	12.58±2.09	15.12±1.17
E' (m/s)	16.13±1.72	13.53±0.83
IVCT (ms)	18.75±0.29	20.60±1.45
IVRT (ms)	21.94±2.74	33.38±2.37*
MV A (mm/s)	287.8±77.8	377.8±29.1
MV E (mm/s)	673.1±30.6	504.9±18.4*
E'/A'	1.39±0.30	0.92±0.09
MV E/A	2.08±0.47	1.37±0.09
MV E/E'	42.86±4.05	37.68±1.41
HR (bpm)	376±15	370±14

Supplemental Table IV. One month after last tamoxifen injection, αMHC-MCM mice were subjected to chow or HFD feeding for 24 weeks and the diastolic function was measured by echocardiography. (*$p<0.05$ vs. MCM/chow, n=4-6). A', Peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler; E', Peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler; IVCT, Isovolumic contraction time; IVRT, Isovolumic relaxation time; MV, Mitral valve; A, Peak velocity of late transmitral flow; E, Peak velocity of early diastolic transmitral flow; E/A, Ratio of E to A; E/E', Ratio of E to E'; HR, Heart rate.

	chow		HFD	
	Con	iKO	Con	iKO
Body Weight (g)	30.82±1.10	29.78±0.90	50.9±1.59*	50.28±3.25*
Heart (mg)	112.4±1.67	112.68±2.40	134.38±4.14*	122.95±5.18
Left Ventricle (mg)	89.2±1.55	89.88±2.08	101.92±1.36*	93.32±3.78#
Lung (mg)	162.62±6.78	151.4±6.93	160.07±11.77	160.32±3.55
Liver (mg)	1382.3±60.0	1496.8±54.3	1610.5±80.4	1694.7±187.9
Epididymal fat pad (mg)	880.6±142.2	728.9±76.3	2631.1±93.5*	2218.9±331.3*
Retroperitoneal fat pad (mg)	408.4±88.0	287.73±35.2	2723.2±256.5*	2500.9±374.1*
Tibia length (mm)	18.84±0.14	19.0±0.08	18.6±0.15	19.0±0.13
HW/TL(mg/mm)	5.98±0.08	5.93±0.13	7.19±0.22*	6.47±0.28
LVW/TL(mg/mm)	4.74±0.07	4.73±0.11	5.45±0.05*	4.91±0.21#
Lung/TL (mg/mm)	8.63±0.37	7.96±0.33	8.63±0.67	8.44±0.16
Liver/TL(mg/mm)	73.33±2.89	78.76±2.66	86.56±3.80	89.19±9.86

Supplemental Table V. Measurement of organ weight parameters in ACC2 iKO mice and their control littermates after 24 weeks' HFD feeding (*p<0.05 vs. Con/chow, #p<0.05 vs. Con/HFD, n=6-8).

Gene	Species	Forward sequence	Reverse sequence
ACC1	Mouse	TAACAGAATCGACACTGGCTGGCT	ATGCTGTTCCTCAGGCTCACATCT
ACC2	Mouse	ACTCACGGAGCCACTCCAAATGTA	ATCCACTGGGACAAACCCTGAAGT
CD36	Mouse	ATGGGCTGTGATCGGAACTG	GTCTTCCCAATAAGCATGTCTCC
Cpt1b	Mouse	TCTAGGCAATGCCGTTCAC	GAGCACATGGGCACCATAC
MCAD	Mouse	AGGGTTTAGTTTTGAGTTGACGG	CCCCGCTTTTGTCATATTCCG
LCAD	Mouse	ACTTGGGAAGAGCAAGCGTA	TCGCAATATAGGGCATGACA
HADHA	Mouse	TGCATTTGCCGCAGCTTTAC	GTTGGCCCAGATTTCGTTCA
HADHB	Mouse	ACTACATCAAAATGGGCTCTCAG	AGCAGAAATGGAATGCGGACC
PPARα	Mouse	CATACTCGCGGGAAAGACCA	CGTCTTCTCGGCCATACACA
MCD	Mouse	GCACGTCCGGGAAATGAAC	GCCTCACACTCGCTGATCTT
PDK4	Mouse	AGTGACTCAAAGACGGGAAAC	GTGTGAGGTTTAATTCTGGCG
ETFA	Mouse	TCTATGCAGGAAATGCGTTGT	AGCACTACCTCCACTTGTTGC
ETFB	Mouse	GACTGTAACCAGACAGGTCAGA	CCCGTCAATTTCCCGTTCCA
ETFQO	Mouse	CCTCTGTGGCTTTGAGTGGT	TCGAAATCCATCACCTTGTTC
Cox4i	Mouse	CGCTGAAGGAGAAGGAGAAG	GCAGTGAAGCCAATGAAGAA
Ndufv1	Mouse	TTTCTCGGCGGGTTGGTTC	GGTTGGTAAAGATCCGGTCTTC
Ndufa9	Mouse	GTCCGCTTTCGGGTTGTTAGA	CCTCCTTTCCCGTGAGGTA
Uqcrc1	Mouse	AGACCCAGGTCAGCATCTTG	GCCGATTCTTTGTTCCCTTGA
UCP2	Mouse	GCGTTCTGGGTACCATCCTA	GCTCTGAGCCCTTGGTGTAG
UCP3	Mouse	TGCTGAGATGGTGACCTACGA	CCAAAGGCAGAGACAAAGTGA
PGC1α	Mouse	GTAAATCTGCGGGATGATGG	AGCAGGGTCAAAATCGTCTG
ERRα	Mouse	CCAGGCTTCTCCTCACTGTC	GCCCCCTCTTCATCTAGGAC
NRF1	Mouse	AGAAACGGAAACGGCCTCAT	ACTCATCCAACGTGGCTCTG
PGC1b	Mouse	TCTGACGTGGACGAGCTTTCA	CCCGTCCTTCAGAGCGTCAG
TFAM	Mouse	CCCGGCAGAGACGGTTAAAA	TCCCTGAGCCGAATCATCCT
Drp1	Mouse	ACAACAGGAGAAGAAAATGGAGT	GATGGATTGGCTCAGGGCTT
Fis1	Mouse	GGTTGCGTGGTAAGGGATGA	TCCTTTCAAAATTCCTTGCAGCTT
mff	Mouse	GCCCAGGGGATAAAGGTGAC	AGTGGGAGAAGGAAATGCCG
OPA1	Mouse	ATTTCGCTCCTGACCTGGAC	GGTGTACCCGCAGTGAAGAA
mfn1	Mouse	TGACTTGGACTACTCGTGCG	TGGCCATTTCTTGCTGGACT
mfn2	Mouse	ATCTGGACCTGAATCGGCAC	AGAGCAGGGACATCTCGTTTC
18S	Mouse	GGACAGGATTGACAGATTGATAG	ATCGCTCCACCAACTAAGAA
cytochrome b	Mouse	ATTCCTTCATGTCGGACGAG	ACTGAGAAGCCCCCTCAAAT
β1-globin	Mouse	GCACCTGACTGATGCTGAGAA	TTCATCGGCGTTCACCTTTCC
parkin	Mouse	GGGATTCAGAAGCAGCCAGA	CAGCAAGATGGGCCCTGG
ACC1	Rat	GGACAGGATTGACAGATTGATAG	ATCGCTCCACCAACTAAGAA
ACC2	Rat	GGACAGGATTGACAGATTGATAG	ATCGCTCCACCAACTAAGAA
parkin	Rat	GGACAGGATTGACAGATTGATAG	ATCGCTCCACCAACTAAGAA
18S	Rat	GGACAGGATTGACAGATTGATAG	ATCGCTCCACCAACTAAGAA

Supplemental Table VI. Table of RT-PCR primer sequences.