Cell Metabolism, Volume *24*

Supplemental Information

Lack of Adipocyte AMPK Exacerbates Insulin Resistance and Hepatic Steatosis through Brown and Beige Adipose Tissue Function

Emilio P. Mottillo, Eric M. Desjardins, Justin D. Crane, Brennan K. Smith, Alex E. Green, Serge Ducommun, Tora I. Henriksen, Irena A. Rebalka, Aida Razi, Kei Sakamoto, Camilla Scheele, Bruce E. Kemp, Thomas J. Hawke, Joaquin Ortega, James G. Granneman, and Gregory R. Steinberg

SUPPLEMENTAL INFORMATION

Supplemental Figures

Figure S1, Related to Figure 1

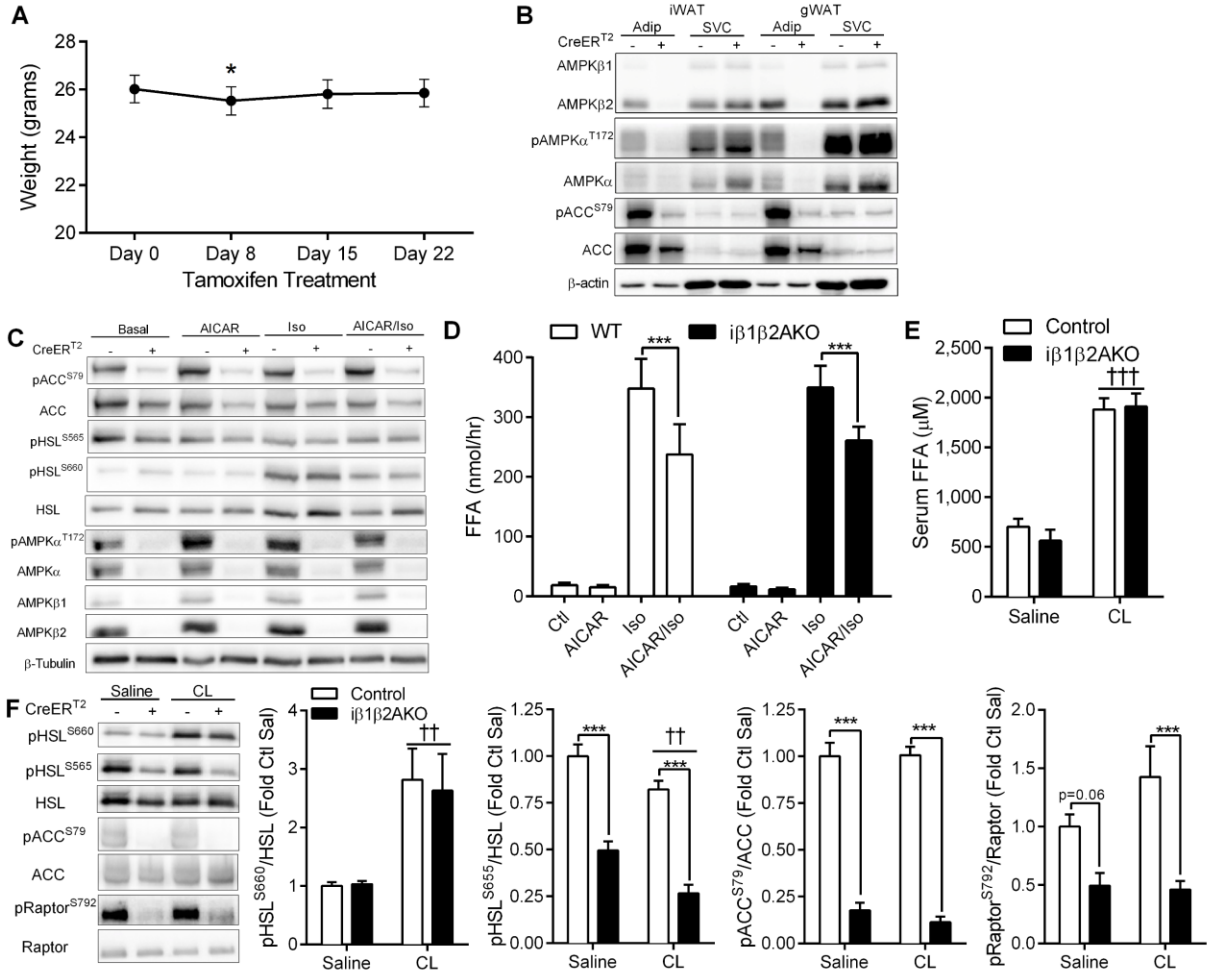

Figure S1. **Tamoxifen effects on body weight and recombination in adipocytes, AICAR reduces lipolysis independent of AMPK, and deletion of adipocyte AMPK does not alter lipolysis *in vivo*.** (A) Tamoxifen induced, adipocyte specific deletion of AMPK β1 and β2 in CreERT2 negative (-, Control) or positive (+, iβ1β2AKO) mice. iWAT and gWAT was enzymatically digested and separated into adipocytes (Adip) or stromal vascular fraction (SVC) and probed with the indicated antibodies (n=3 mice per genotype, representative of two independent experiments). (B) Tamoxifen effects on body weight during daily tamoxifen oral gavage (day 0 to day 5) and recovery period. *P <0.05, denotes an effect of tamoxifen on body weight as determined by repeated-measures one-way ANOVA and Bonferroni post hoc test. (C and D) Control and iβ1β2AKO adipocytes from Figure 1C were pretreated with 500 μM AICAR for 45 min followed by 10 μM isoproterenol (Iso) for an additional 45 min and immunoblotted for indicated antibodies (C) and the release of FFA was quantified (D) (n = 3 mice per genotype, from three independent experiments). (E and F) Control (n = 4 per treatment) and iβ1β2AKO mice (n = 5 per treatment) were treated with CL (1 mg/kg for 20 min) followed by blood and tissue collection. Plasma FFA were quantified (E) and BAT was immunoblotted for total and phosphorylated levels of HSL, ACC and Raptor and the ratio of phospho/total levels were normalized to fold Control Saline (Fold Ctl Sal). (E). ***P <0.001, denotes an effect of AICAR as determined by two-way ANOVA and Bonferroni post hoc test (D). ***P <0.001, denotes a genotype effect within groups and ††P <0.01, †††P <0.001 denotes a CL effect within genotype as determined by two-way ANOVA and Bonferroni post hoc test (E and F).

Figure S2, Related to Figure 2

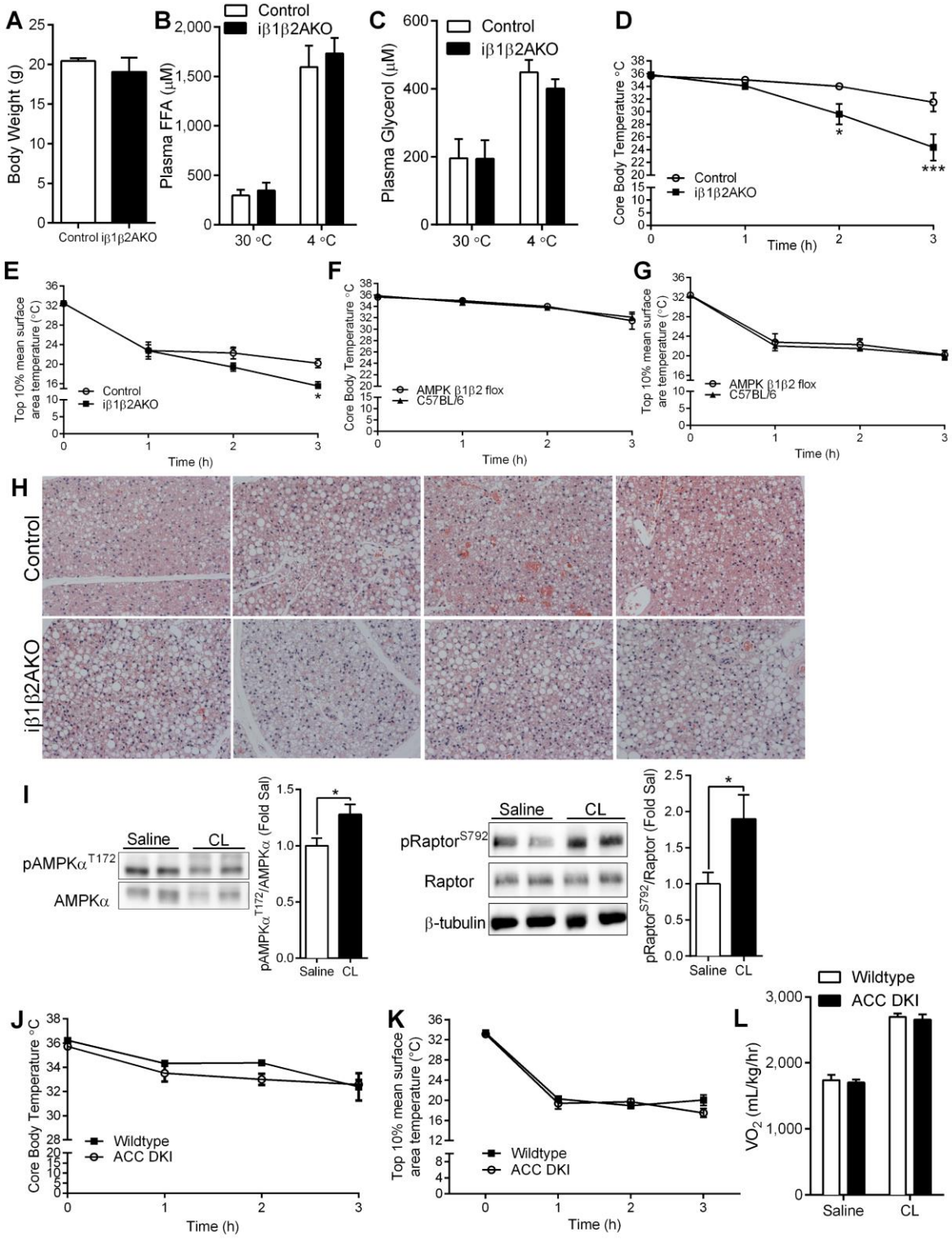

Figure S2. **Plasma FFA and glycerol values during cold exposure, cold exposure in Control, iβ1β2AKO and C57BL/6 housed at 23 °C and AMPK inhibition of ACC is not required for BAT-mediated thermogenesis.** (A-C) Body weights (A, n = 10 per group), plasma FFA (B) and glycerol (C) values in Control and iβ1β2AKO mice

that were maintained at thermoneutrality for 1 week ,then exposed to cold for 3 h (n = 4-9 per group). (D-H) Control, iβ1β2AKO and C57BL/6 male mice were housed at 23 °C and exposed to cold (4 °C) for 3 h and the core body temperature (D and F) and interscapular BAT temperature (E and G) was monitored (n = 4 per group). (H) Hematoxylin and eosin (H&E) stained sections from BAT of Control and iβ1β2AKO mice exposed to 4 °C for 3 h. *P <0.05 and ***P <0.001 denotes a genotype effect within groups as determined by a repeated-measures two-way ANOVA and Bonferroni post hoc test. (I) Wildtype mice (n = 6 per treatment) were treated with saline or the β3-AR agonist CL 316,243 for 20 min (0.033 nmol/g body weight), BAT was collected and proteins blotted for phosphorylated ($pAMPK\alpha^{T172}$, $pRaptor^{S792}$) and total AMPKα and Raptor. *P <0.05, denotes a CL effect as determined by a Student's *t*-test. (J and K) Core body temperature (J) and interscapular BAT temperature (K) in wildtype and ACC DKI mice. (L) Oxygen consumption (VO_2) in response to a single injection of saline or CL in wildtype and ACC DKI mice (n = 8-10).

Figure S3, Related to Figure 3

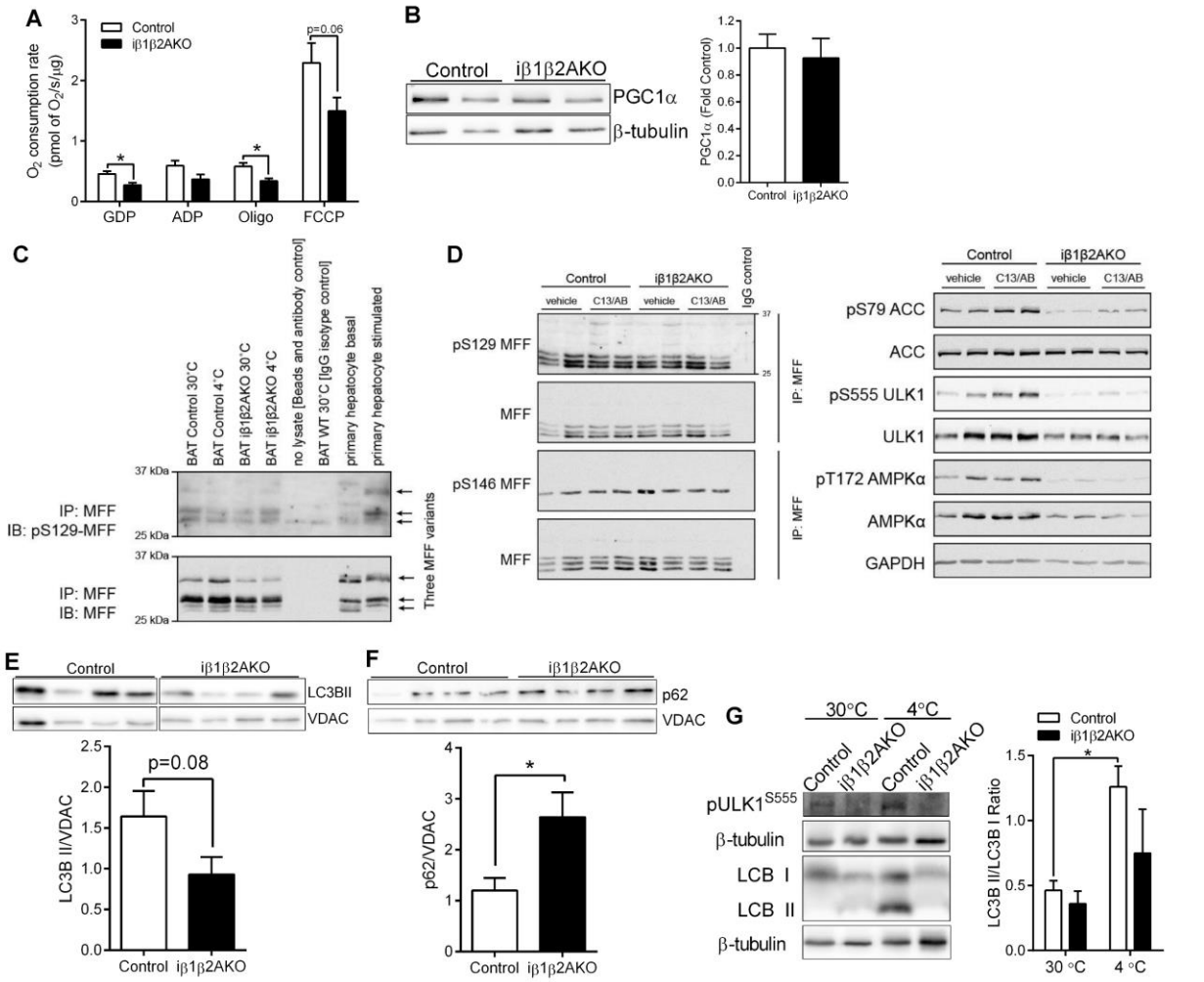

Figure S3. Measurements of PGC1α, MFF and mitochondrial autophagy markers in Control and iβ1β2AKO mice. (A) Respiration in isolated BAT mitochondria in response to sequential additions of GDP (2 mM), ADP (450 μM), oligomycin (2 mg/ml) and FCCP (1 μM). (n = 7 BAT mitochondrial isolations per genotype). (B) PGC1α protein levels (n = 5 per group) in Control and iβ1β2AKO mice under chow fed conditions. (C) immunoblot analysis for phospho MFF S129 (pS129-MFF) in BAT of Control and iβ1β2AKO mice maintained at 30 °C or exposed to 4 °C for 3 h. Total lysates were immunoprecipitated for MFF and blotted for pS129-MFF or total MFF. Sample with no lysates and IgG isotype served as negative controls and hepatocytes stimulated with AMPK activators (0.3 mM AICAR and 10μM A769662 for 45 min) served as positive controls. (D) Immunoblot analysis in Control brown adipocytes and brown adipocytes deficient for AMPK (iβ1β2AKO), treated with vehicle (DMSO) or compound 13/A769662 (C13/AB; 30 μM each), for 4 h. Lysates were immunoprecipitated for MFF and blotted for phospho-MFF S129 and S146 (pS219 MFF and pS146 MFF) and total MFF levels. IgG isotype served as a negative control for immunoprecipitations and immunoblotting. Blots were also probed for phosphorylated and total levels of ACC, ULK1 and AMPKα as indicated, and GAPDH served as a loading control. (E and F) Immunoblot analysis of lipidated LC3B (LCB II) (E) and p62 (F) levels in isolated BAT mitochondria from chow-fed Control and iβ1β2AKO mice normalized to total VDAC protein levels (n = 6-7 per genotype). (G) phospho-ULK1 S555, β-tubulin, and LC3B levels in Control and iβ1β2AKO mice maintained at 30 °C or exposed to cold for 3h (4 °C) (n = 3 mice per group) *P <0.05, denotes an effect of cold (4 °C) by two-way ANOVA and Bonferroni post hoc test.

Figure S4, Related to Figure 4

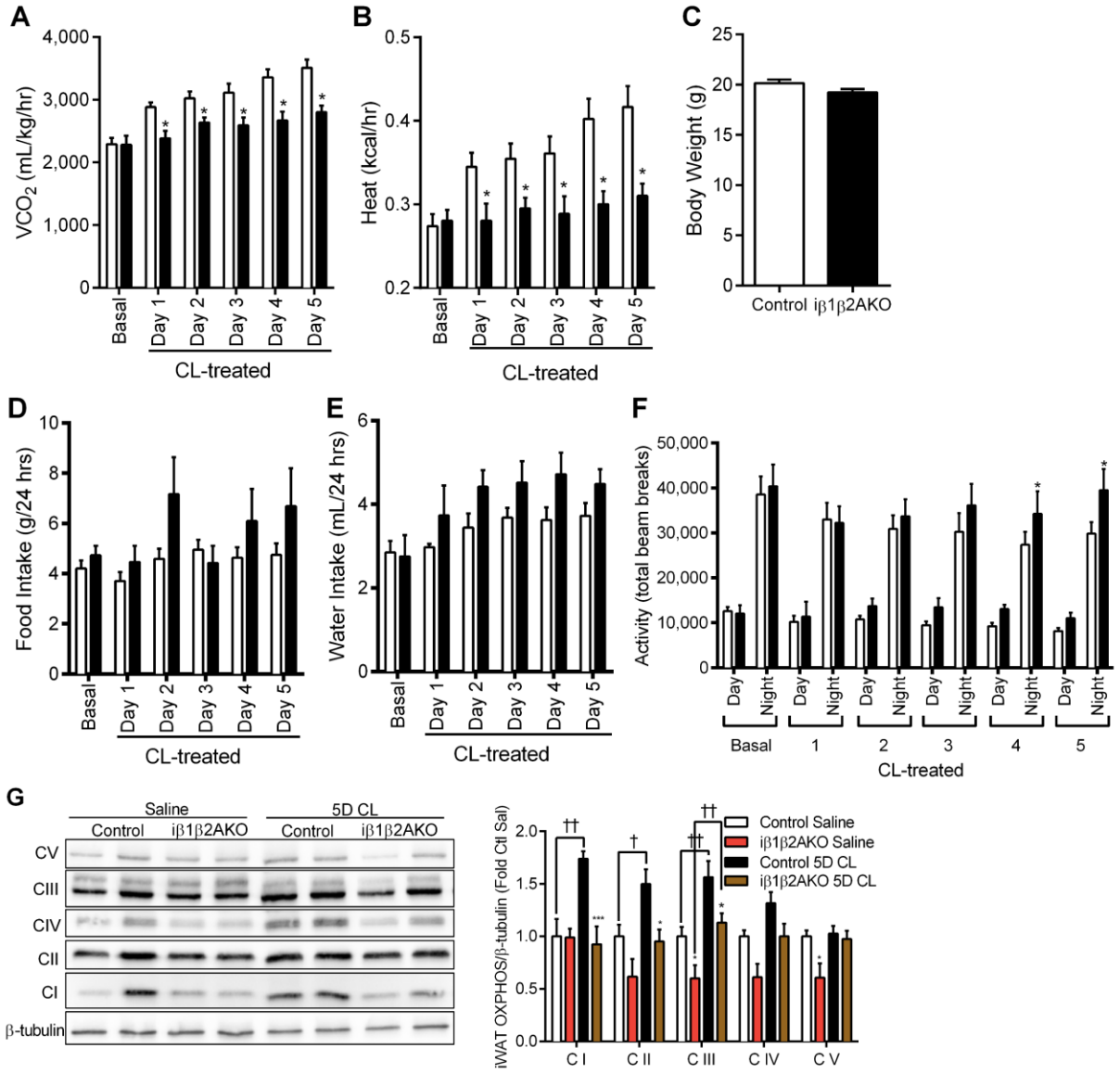

Figure S4. **Metabolic parameters in mice treated with CL for five days and OXPHOS subunit immunoblotting in iWAT.** (A) CO_2 production (VCO_2), (B) energy expenditure (heat), (D) daily food intake, (E) daily water intake and (F) total activity levels (Activity) in Control and iβ1β2AKO mice untreated (Basal) or treated daily with CL on indicated days (n = 6-9 per group). (C) Initial body weights in Control and iβ1β2AKO mice prior to five days of CL treatment. *P <0.05, denotes a genotype effect within groups as determined by two-way ANOVA and Bonferroni post hoc test. (G) Representative OXPHOS subunit immunoblotting with quantification (n = 8-10 per group) in iWAT of Control and iβ1β2AKO mice treated with saline or CL for 5 days (5D CL). *P <0.05 denotes a genotype effect within groups and †P <0.05, ††P <0.01, denotes a CL effect within genotype as determined by two-way ANOVA and Bonferroni post hoc test.

Figure S5, Related to Figure 5

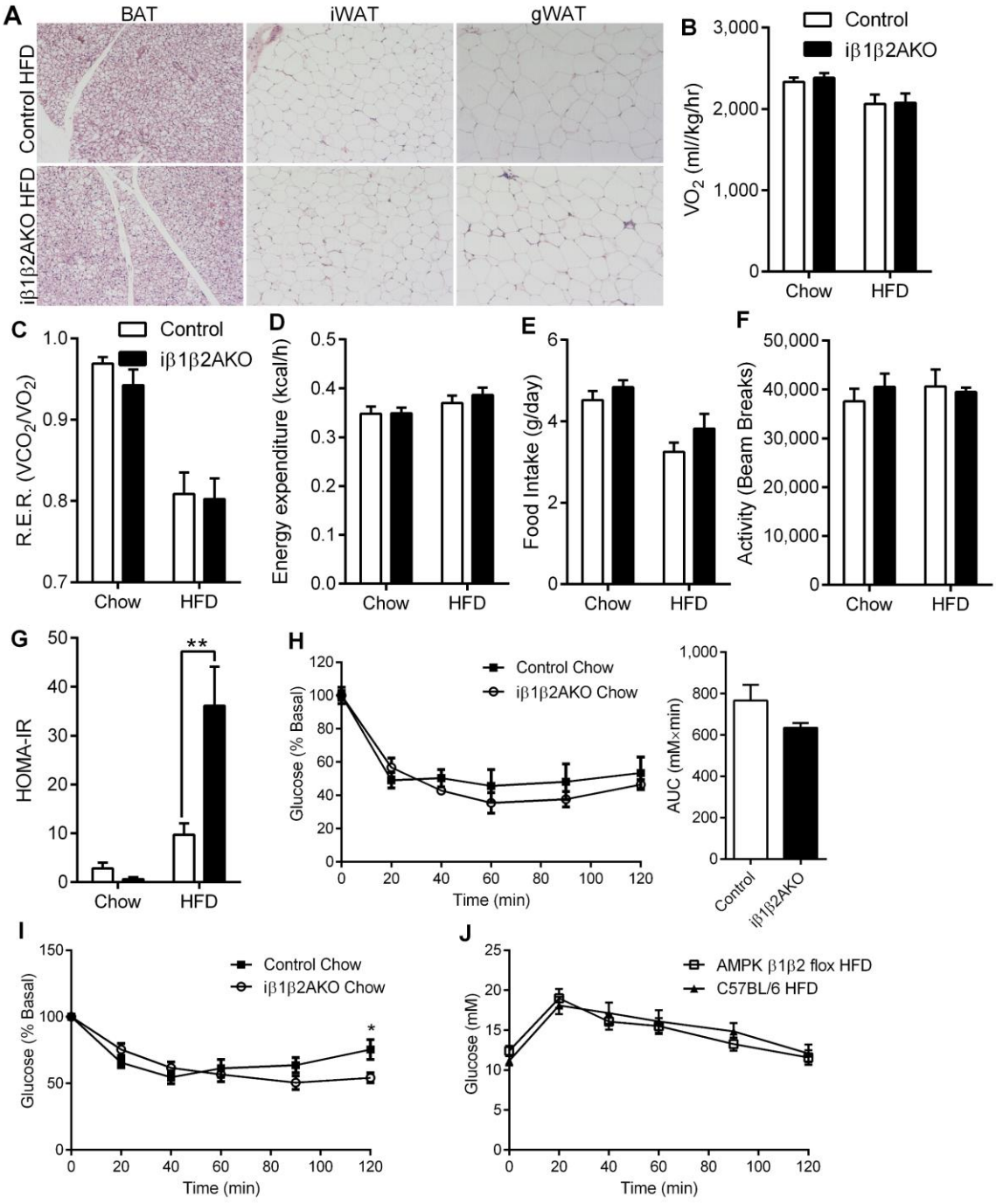

Figure S5. Metabolic parameters in male mice on chow and HFD, HOMA-IR levels, and insulin tolerance test in chow fed animals. (A) Representative histological images (10×) of BAT, iWAT and gWAT in Control and iβ1β2AKO mice fed HFD for 12 weeks. (B) Oxygen consumption (VO_2), (C) respiratory exchange quotient (RER), (D) energy expenditure (heat), (E) daily food intake and (F) activity levels in Control and iβ1β2AKO mice on chow or HFD for 11 weeks (n = 8-10 per group) averaged over three days. (G) HOMA-IR levels in Control and iβ1β2AKO mice on chow or HFD for 11 weeks. (H) Insulin tolerance test in Control and iβ1β2AKO mice on chow

prior to diet intervention, AUC; area under the curve (n = 5 per group). (I) Insulin tolerance test in Control and iβ1β2AKO mice on chow diet for 11 weeks (n = 9 per group). (J) Glucose tolerance test in Control and C56BL/6 mice on HFD for 11 weeks (n = 11 Control and n = 5 C57BL/6). *P <0.05, **P <0.01, denotes a genotype effect within groups as determined using a two-way ANOVA and Bonferroni post hoc test.

Figure S6, Related to Figure 6

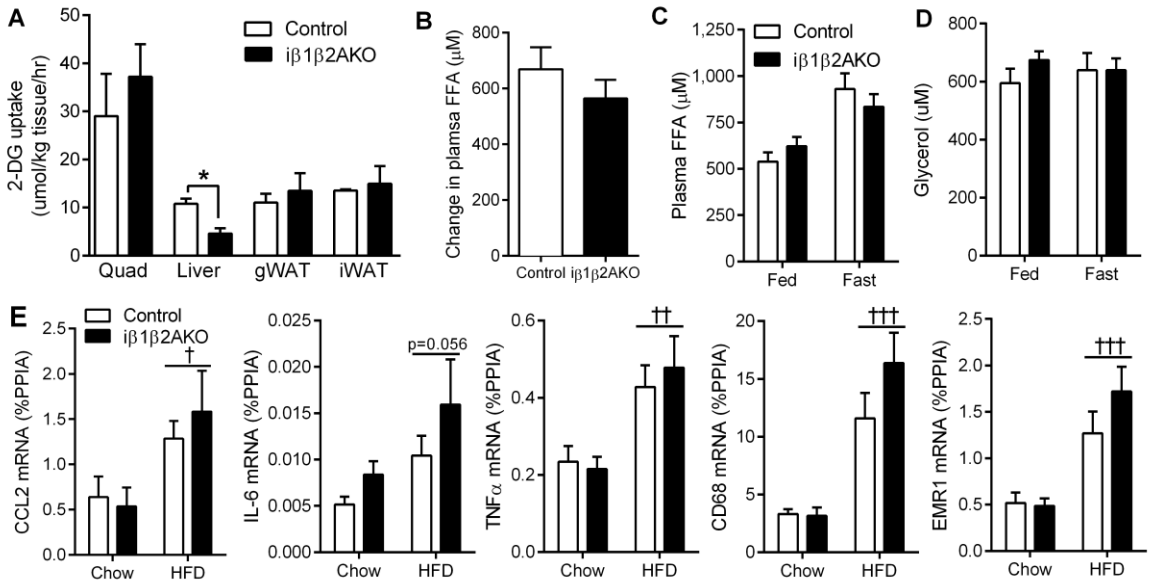

Figure S6. **Tissue 2-DG uptake, plasma FFA and glycerol measurements in mice on HFD and expression of inflammatory markers in gWAT.** (A) Tissue ^{3}H-2-deoxy-D-glucose (2-DG) uptake in quadriceps muscle (Quad), Liver, gonadal WAT (gWAT) and inguinal WAT (iWAT) from male Control and iβ1β2AKO mice on HFD that were fasted for 6 h and injected with 0.7 U/kg insulin (n = 3-4). (B) Change in plasma FFA 20 min after an acute injection of CL (0.033 nmol/g body weight) in Control and iβ1β2AKO mice fed HFD for 12 weeks (n = 7-8). (C-D) plasma FFA (C) and glycerol values (D) of Control and iβ1β2AKO mice on HFD for 12 weeks in the fed or fasted (10 h) state (n = 8-12). (E) Levels of gonadal WAT (gWAT) inflammatory and immune markers in male Control and iβ1β2AKO mice fed HFD for 12 weeks (n = 9-11 for chow, n = 15-16 for HFD). *P <0.05, denotes a genotype effect as determined using a Student's *t*-test. †P <0.05, ††P <0.01, †††P <0.001 denotes an overall HFD effect as determined by two-way ANOVA and Bonferroni post hoc test.

Supplemental Experimental Procedures

Animals.
The first generation (AMPK $\beta1^{wt/flox}\beta2^{wt/flox}$, $CreER^{T2}$ +) was backcrossed onto AMPK $\beta1^{flox/flox}\beta2^{flox/flox}$ mice to generate founder mice ($CreER^{T2}$ +, AMPK $\beta1^{flox/flox}\beta2^{flox/flox}$). Deletion of AMPK β1 β2 was performed in adult mice (8-10 weeks of age) by administering tamoxifen (Cayman Chemical, MI, USA) via oral gavage as previously described (Mottillo et al., 2014). Littermates were used for all experiments and all mice received tamoxifen treatment. All in vivo manipulations were performed the third week or later, following the initial tamoxifen treatment. Mice were maintained under controlled environment conditions (12 h/12 h light-dark cycle with lights on at 07:00 and temperature of 23 °C) unless stated otherwise. Starting at 10 weeks of age, male mice received either a chow diet (17% kcal fat; Diet 8640, Harlan Teklad, Madison, WI) or high-fat diet (HFD, 45% kcal fat, D12451, Research Diets; New Brunswick, NJ) and water ad libitum for 12 weeks. Female ACC1-S79A and ACC2-S212A knock-in mutation mice (ACC DKI) were as previously described and were used at 3-4 months of age (Fullerton et al., 2013). For cold exposure experiments, mice were maintained at thermoneutrality (29-30 °C) for one week prior to cold challenge to reduce basal sympathetic tone, unless other wise indicated. Mice had ad libitum access to food before the experiment, which was conducted midway through the dark cycle. Food was removed for the short period of time when the animals were placed at 4 °C and no differences were observed in shivering behavior between genotypes**.**

Metabolic and blood measurements.
For chronic CL treatment, mice were injected intraperitoneally with saline or CL (4 days of 0.5 mg/kg and 1 mg/kg on last day) at 09:00 and measurements for VO_2, VCO_2 and heat were calculated 6 h post-injection. Glucose tolerance was performed in 6 h fasted mice on HFD diet for 10 weeks, after an i.p. injection of glucose (0.75 g/kg) (Steinberg et al., 2010). Insulin tolerance was tested with 6 h fasted mice at 11 weeks of diet, after i.p. injection of 0.6 or 1.0 U/kg of insulin for chow and HFD fed mice, respectively (Steinberg et al., 2010). Blood glucose levels were determined using a One Touch Ultra Glucometer (LifeScan, Canada). Plasma insulin measurements, and fasting plasma analyses were performed on 10 h overnight-fasted mice as described (Crane et al., 2014a). Fed plasma analyses were performed on mice at 07:00. Plasma FFA (NEFA-HR 2, Wako, USA), glycerol (glycerol reagent, Sigma, ON), triglyceride (Triglyceride Colorimetric Assay Kit, Cayman Chemical, MI), and ALT (BIOO Scientific, TX, USA) levels were determined by biochemical assays. Small-animal micro-computed tomography (CT) (Gamma Medica-Ideas Xspect System, NorthRidge, CA) was performed as previously described (Crane et al., 2014a).
In vivo glucose clearance. Insulin-stimulated glucose uptake was assessed by tail vein injection of 2 μCi of 3H-2-deoxy-D-glucose (2-DG) immediately following administration of i.p. injection 1.0 U/kg of insulin for 15 min. Blood samples were taken at baseline, 5, 10, and 15 minutes and subsequently analyzed for 2-DG radioactivity as was tissue specific uptake as previously described (Henriksbo et al., 2014). Briefly, tissues were analyzed for 2-DG radioactivity with and without deproteinization (0.3 mM BaOH and 0.3 mM ZnSO4) to determine the rates of tissue-specific glucose uptake.

Adipocyte isolations and lipolysis.
Adipose tissue depots from three mice per genotype were pooled and digested with collagenase Type II (Sigma, ON, Canada) as previously described (Rodbell, 1964). For lipolysis assays, a 10% packed volume of inguinal adipocytes was incubated in 500 μl of HKRB + 1% BSA. Where indicated, adipocytes were treated with 500 μM AICAR for 45 min (Toronto Research Chemical, ON, Canada), followed by 10 μM isoproterenol for 45 min (Sigma, ON, Canada), at which point the supernatant was collected. Cells were washed with 1 ml PBS and prepared for immunoblot analysis.

Immunoblotting and Immunoprecipitation.
Tissue and cell lysates were prepared in lysis buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 100 mM NaF, 10 Na-pyrophosphate, 5 EDTA mM , 250 mM sucrose, 1 mM DTT, and 1 mM Na-orthovanadate, 1% Triton X and Complete protease inhibitor cocktail (Roche)). Samples (1 μg/μL) were prepared in 4x SDS sample buffer and boiled at 95 °C for 5 min, with the exception of OXPHOS immunoblotting, which was prepared at room temperature to avoid the degradation of complexes. 20 μg of protein was loaded except 5 for BAT UCP1 and total OXPHOS. Immunoblotting was performed as previously described (Galic et al., 2011; O'Neill et al., 2011), using antibodies from Cell Signaling Technology (ACC, #3662; pACC Ser79/221, #3661; AMPKα, #2532; pAMPKα Thr172,

#2535; pHSL S660, #4126; pHSL S565, #4137; total HSL, #4107; AMPK β1/β2, #4150; AMPK β1, #4178; AMPK β2, #4148; β-actin, #4970; AKT, #9272; pAKT Ser473, #4058; GAPDH, LC3B, #2775; p62, #5114; VDAC, #4661; anti-Rabbit IgG horseradish peroxidase (HRP)-linked, #7074; and anti-Mouse IgG HRP-linked; #7076), Invitrogen, (β-tubulin, #32-2600), Alpha Diagnostic International (UCP1, #UCP11-A), Abcam (total OXPHOS #ab110413) and Millipore (PGC-1α, #ab3242). Antibodies against ATGL an phospho-ATGL S406 were previously described (Pagnon et al., 2012). For detecting UCP1 in human brown adipocytes, an antibody from Sigma (#U6382-100) was incubated over night (1:1000 dilution) in 1% FSG (Sigma G7765-IL). Briefly, membranes were blocked for 1 h in 10 mM Tris (pH 7.6), 137 mM NaCl, 0.1% (vol/vol) Tween 20 (TBST) containing 5% (wt/vol) skimmed milk powder or BSA. Membranes were incubated in primary antibody (TBST containing 5% (wt/vol) BSA or skimmed milk overnight at 4°C as recommended by the manufacturer. Detection was performed with HRP-conjugated secondary antibodies and enhanced chemiluminescence reagent. For determination of phospho/total protein levels, immunoblots were first probed for phospho levels, then stripped at 37°C for 30 min using Restore Plus (Thermo Scientific, ON, Canada) and re-probed overnight to detect total levels unless otherwise stated. In some instances, phospho and total levels were determined separately and were normalized first to loading control (β-actin or β-tubulin). Densitometry was performed using Image J Software (NIH, MD, USA).

Analysis of MFF phosphorylation: For immunoprecipitation of MFF, total MFF antibody (Santa Cruz Biotechnology, sc-168593) was cross-linked to NHS-activated Sepharose according to the manufacturer's instructions (1 μg antibody per μl beads). Total cell lysates (200 μg for brown adipose tissue, 500 μg for brown adipocyte cell lysate) were incubated overnight with 2 μl cross-linked Sepharose. Immunoprecipitates were eluted by boiling in Laemmli buffer and the resulting samples were analysed by immunoblotting using the indicated antibodies. Antibodies against pS129 and pS146 MFF were described previously (Ducommun et al., 2015), total MFF for immunoblotting was from Proteintech (17090-1-AP).

Tissue Triglyceride determination.
20-50 mg of tissue was homogenized in chloroform: methanol (2:1) (Folch et al., 1957) and a portion of the organic phase was dried down and resuspended in isopropanol and assayed for triglyceride amount using a Triglyceride Colorimetric Assay Kit (Cayman Chemical, MI).

Cytochrome *c* oxidase (COX) activity assay.
Reduced cytochrome c (2 mg/mL; Sigma-Aldrich C2506) was prepared in 10 mM KPO4 (KH2PO4 Sigma P5655, K2HPO4 Sigma P8281) and 0.08 mg/mL sodium dithionite (Sigma 157953). The cytochrome c solution was protected from light, pre-heated to 30 °C and used within 30 min. Briefly, 10 μL of BAT lysate was added to a 96-well plate, in duplicate, and mixed with 250 μL of fully reduced cytochrome c one column at a time. A kinetic reading was made at a 550 nm wavelength, set to read every 10 seconds for a total of 9 times in a Synergy H4 microplate reader (Gen5 software; Biotek, Vermont). Calculated enzyme activity was then normalized to protein content determined by BCA protein assay.

Tissue Processing and Transmission electron microscopy (TEM).
Tissues were fixed in 10% neutral buffered formalin for 24-48 h at 4 °C and processed for paraffin embedding and hematoxylin and eosin (H&E) staining at McMaster University Medical Center (MUMC) Anatomical Pathology core.

Transmission electron microscopy (TEM). Brown adipose tissue (interscapular) was fixed in 2% glutaraldehyde (2% v/v) in 0.1 M sodium cacodylate buffer (pH 7.4) for at least 24 hours. Thin sections were cut on a Leica UCT ultramicrotome and picked up onto Cu grids. Sections were post-stained with uranyl acetate and lead citrate. The preparation, fixation, and sectioning was performed by the electron microscopy group at McMaster University Medical Center.
Electron micrographs shown in the two top panels in Figure 3A and additional images necessary for the quantitative analysis in Figure 3B and 3C were obtained in an AMT 4-megapixel CCD camera (Advanced Microscopy Techniques, Woburn, MA) mounted in a JEOL JEM 1200 EX TEMSCAN transmission electron microscope (JEOL, Peabody, MA, USA) operating at an accelerating voltage of 80 kV.
Higher magnification electron micrographs for publication in the two bottom panels in Figure 3A were obtained in a FEI Tecnai F20 electron microscope operated at 200kV. Images were collected in a Gatan K2 Summit direct detector device camera at a nominal magnification 3,500X, which produced images with a calibrated pixel size of 10.35Å. This detector was used in counting movie mode with ten electrons per pixel per second for 45 seconds exposures and 0.5 seconds per frame. This method produced movies containing 90 frames with an exposure rate of

two electron per square angstrom. Movies were collected using a defocus of 5 μm. Frames were aligned using the program alignframesleastsquares_list.exe and averaged into one single micrograph with the shiftframes_list.exe program from the Rubinstein group (https://sites.google.com/site/rubinsteingroup/home). These programs perform whole frame alignment of the movies using previously published motion correction algorithms (Li et al., 2013). A de-noising filter using Photoshop was applied to the entire images shown in Figure 3A, bottom panel.
To perform the quantification in Figure 3B and 3C, 25 images per sample were acquired by random sampling. Mitochondria with disrupted cristae and total mitochondria were counted from each image, averaged per animal, and then expressed as % cristae disruption (mitochondria with disrupted cristae over total mitochondria). Criteria for disrupted cristae included any observable disorganization, vacuolization, or dissolution of cristae within mitochondria (Poole et al., 2008). The experimenter was blinded when capturing images as well as during the quantification process.

Cell Culture

Isolation, differentiation and treatment of human brown adipocyte strains. Human brown preadipocytes were isolated from the stromal vascular fraction of fat biopsies obtained from the supraclavicular region of adult humans during surgery and sub-cultured as previously described (Jespersen et al. 2013). Cells were grown into confluence and differentiation was initiated two days post confluence by adding a cocktail consisting of 0.1 μM dexamethanzone, 100 nM insulin, 2 nM T3, 10 μg/ml transferrin, 200 nM rosiglitazone and 540 μM IBMX in F12/DMEM (Gibco), 1% penicillin/streptomycin. IBMX was included only the first 3 days of differentiation and rosiglitazone was included only the first 6 days of differentiation. Media was refreshed every third day and the differentiation was progressing during 12 days were lipid droplets were visual. Differentiated brown adipocytes were stimulated with 1μM norepinephrine for 45 minutes and protein was harvested for western blot analysis.

Generation of immortalized brown adipocyte cell line and deletion of AMPK in culture. Isolation of pre-adipocytes from brown adipose tissue was performed as previously described (Klein et al., 1999). Interscapular BAT, isolated from 3-4 day old pups was minced with scissors and digested with collagenase. The primary brown pre-adipocytes were allowed to adhere until the next day. Retrovirus containing the SV40 large T-antigen was produced by transfecting Phoenix ecotropic cells (Swift et al., 2001) with pBABE-puro SV40 LT plasmid (Addgene #13970) and lipofectamine 2000 (Invitrogen) and collecting the subsequent medium containing virus particles through a 0.45 μM filter. Adherent pre-adipocytes were immortalized by retroviral-mediated expression of SV40 large T-antigen and selected with puromycin (2 μg/ml) for one week, followed by a two week treatment with Ciprofloxacin to ensure that the culture was cured of any potential mycoplasma contamination. Once cultures were established, cells were differentiated as previously described (Klein et al., 1999). Briefly, preadipocytes were grown to confluence in culture medium supplemented with 20 nM insulin and 1 nM T3 (differentiation medium). Confluent cells were incubated for 48 h in differentiation medium further supplemented with 0.5 mM isobutylmethylxanthine, 0.5 μM dexamethasone, and 0.125 mM indomethacin (induction medium). Subsequently, the cells were maintained in differentiation medium for two more days and the deletion of AMPK was induced by treating cells with 100 nM 4-hydroxytamoxifen (4-OHT) for two days in differentiation medium. Cells were then maintained for 2-4 days post 4-OHT treatment in differentiation medium. All chemicals were from Sigma and experiments were performed within 20 passages following immortalization.

Isolation of BAT mitochondria and respirometry.

BAT from the interscapular and axillary regions was combined from individual mice and rinsed in ice-cold buffer B1 (250 mM sucrose, 100 mM KCl, 20 mM K-TES pH. 7.2, 0.3% fatty acid free BSA). BAT mitochondria were isolated by differential centrifugation, essentially as described (Cannon and Nedergaard, 2008; Shabalina et al., 2013). All isolation steps were performed on ice or at 4 °C. BAT was finely minced with scissors in buffer B1 and homogenized in a tight fitting Potter homogenizer with Teflon pestle. The homogenized tissue was filtered through gauze sponges and centrifuged at 8,500 g for 10 min. The resulting pellet was resuspended in B1 and centrifuged at 800 g for 10 min, and the resulting supernatant was centrifuged at 8,500 g for 10 min to give a crude mitochondrial pellet. The mitochondrial pellet was resuspended in buffer B2 (100 mM KCl, 20 mM K-TES pH. 7.2, 1 mM EDTA, 0.6% fatty acid free BSA) and centrifuged at 8,500 g for 10 min. The final pellet was resuspended in 60-70 μl of Respiration buffer (125 mM sucrose, 20 mM K-TES pH. 7.2, 2 mM $MgCl_2$, 1 mM EDTA, 4 mM KH_2PO_4 0.1%

fatty acid free BSA) and assessed for respiratory activity. An aliquot of mitochondria was frozen at – 80 °C for later quantification of protein content and western blot analysis.
Mitochondrial respiration was performed in an Oroboros Oxygraph-2k with 2 ml of Respiration buffer. The isolated mitochondria were added to Respiration buffer containing 2 mM malate and 2.5 mM L-carnitine, followed by sequential additions of 30 μM palmitoyl-CoA, 2 mM GDP, 450 μM ADP, 2 mg/ml oligomyocin and FCCP to determine the rate of respiration. The concentration of FCCP was titrated in to a final concentration of 1 μM, which gave maximal respiration rates. All chemical were from Sigma Aldrich (Toronto, Canada).

List of primers used.

Gene	TaqMan Assay ID N°
Cidea	Mm00432554_m1
Cox8b	Mm00432648_m1
Cs	Mm00466043_m1
3-HAD	Mm00492535_m1
MT-CO2	Mm03294838_g1
Pdk4	Mm01166879_m1
Ppara	Mm00440939_m1
Ppia	Mm02342430_g1
Ucp1	Mm01244861_m1
IL-6	Mm00446190_m1
CD68	Mm00839636_g1
TNFα	Mm00443258_m1
Ccl2	Mm00441242_m1
Emr1	Mm00802529_m1

Supplemental References

Cannon, B., and Nedergaard, J. (2008). Studies of thermogenesis and mitochondrial function in adipose tissues. Adipose Tissue Protoc. 109–121.
Ducommun, S., Deak, M., Sumpton, D., Ford, R.J., Núñez Galindo, A., Kussmann, M., Viollet, B., Steinberg, G.R., Foretz, M., Dayon, L., et al. (2015). Motif affinity and mass spectrometry proteomic approach for the discovery of cellular AMPK targets: Identification of mitochondrial fission factor as a new AMPK substrate. Cell. Signal. *27*, 978–988.
Folch, J., Lees, M., and Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. *226*, 497–509.
Galic, S., Fullerton, M.D., Schertzer, J.D., Sikkema, S., Marcinko, K., Walkley, C.R., Izon, D., Honeyman, J., Chen, Z.-P., van Denderen, B.J., et al. (2011). Hematopoietic AMPK β1 reduces mouse adipose tissue macrophage inflammation and insulin resistance in obesity. J. Clin. Invest. *121*, 4903–4915.
Henriksbo, B.D., Lau, T.C., Cavallari, J.F., Denou, E., Chi, W., Lally, J.S., Crane, J.D., Duggan, B.M., Foley, K.P., Fullerton, M.D., et al. (2014). Fluvastatin causes NLRP3 inflammasome-mediated adipose insulin resistance. Diabetes *63*, 3742–3747.
Klein, J., Fasshauer, M., Ito, M., Lowell, B.B., Benito, M., and Kahn, C.R. (1999). β3-Adrenergic stimulation differentially inhibits insulin signaling and decreases insulin-induced glucose uptake in brown adipocytes. J. Biol. Chem. *274*, 34795–34802.
Li, X., Mooney, P., Zheng, S., Booth, C.R., Braunfeld, M.B., Gubbens, S., Agard, D.A., and Cheng, Y. (2013). Electron counting and beam-induced motion correction enable near-atomic-resolution single-particle cryo-EM. Nat. Methods *10*, 584–590.

Mottillo, E.P., Balasubramanian, P., Lee, Y.-H., Weng, C., Kershaw, E.E., and Granneman, J.G. (2014). Coupling of lipolysis and de novo lipogenesis in brown, beige, and white adipose tissues during chronic 3-adrenergic receptor activation. J. Lipid Res. *55*, 2276–2286.
O'Neill, H.M., Maarbjerg, S.J., Crane, J.D., Jeppesen, J., Jorgensen, S.B., Schertzer, J.D., Shyroka, O., Kiens, B., van Denderen, B.J., Tarnopolsky, M.A., et al. (2011). AMP-activated protein kinase (AMPK) 1 2 muscle null mice reveal an essential role for AMPK in maintaining mitochondrial content and glucose uptake during exercise. Proc. Natl. Acad. Sci. *108*, 16092–16097.
Poole, A.C., Thomas, R.E., Andrews, L.A., McBride, H.M., Whitworth, A.J., and Pallanck, L.J. (2008). The PINK1/Parkin pathway regulates mitochondrial morphology. Proc. Natl. Acad. Sci. *105*, 1638–1643.
Rodbell, M. (1964). Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J. Biol. Chem. *239*, 375–380.
Shabalina, I.G., Petrovic, N., de Jong, J.M.A., Kalinovich, A.V., Cannon, B., and Nedergaard, J. (2013). UCP1 in Brite/Beige Adipose Tissue Mitochondria Is Functionally Thermogenic. Cell Rep. *5*, 1196–1203.
Steinberg, G.R., O'Neill, H.M., Dzamko, N.L., Galic, S., Naim, T., Koopman, R., Jorgensen, S.B., Honeyman, J., Hewitt, K., Chen, Z.-P., et al. (2010). Whole Body Deletion of AMP-activated Protein Kinase 2 Reduces Muscle AMPK Activity and Exercise Capacity. J. Biol. Chem. *285*, 37198–37209.
Swift, S., Lorens, J., Achacoso, P., and Nolan, G.P. (2001). Rapid production of retroviruses for efficient gene delivery to mammalian cells using 293T cell–based systems. Curr. Protoc. Immunol. 10–17.