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Supplemental Information

ERRγ Preserves Brown Fat Innate Thermogenic Activity

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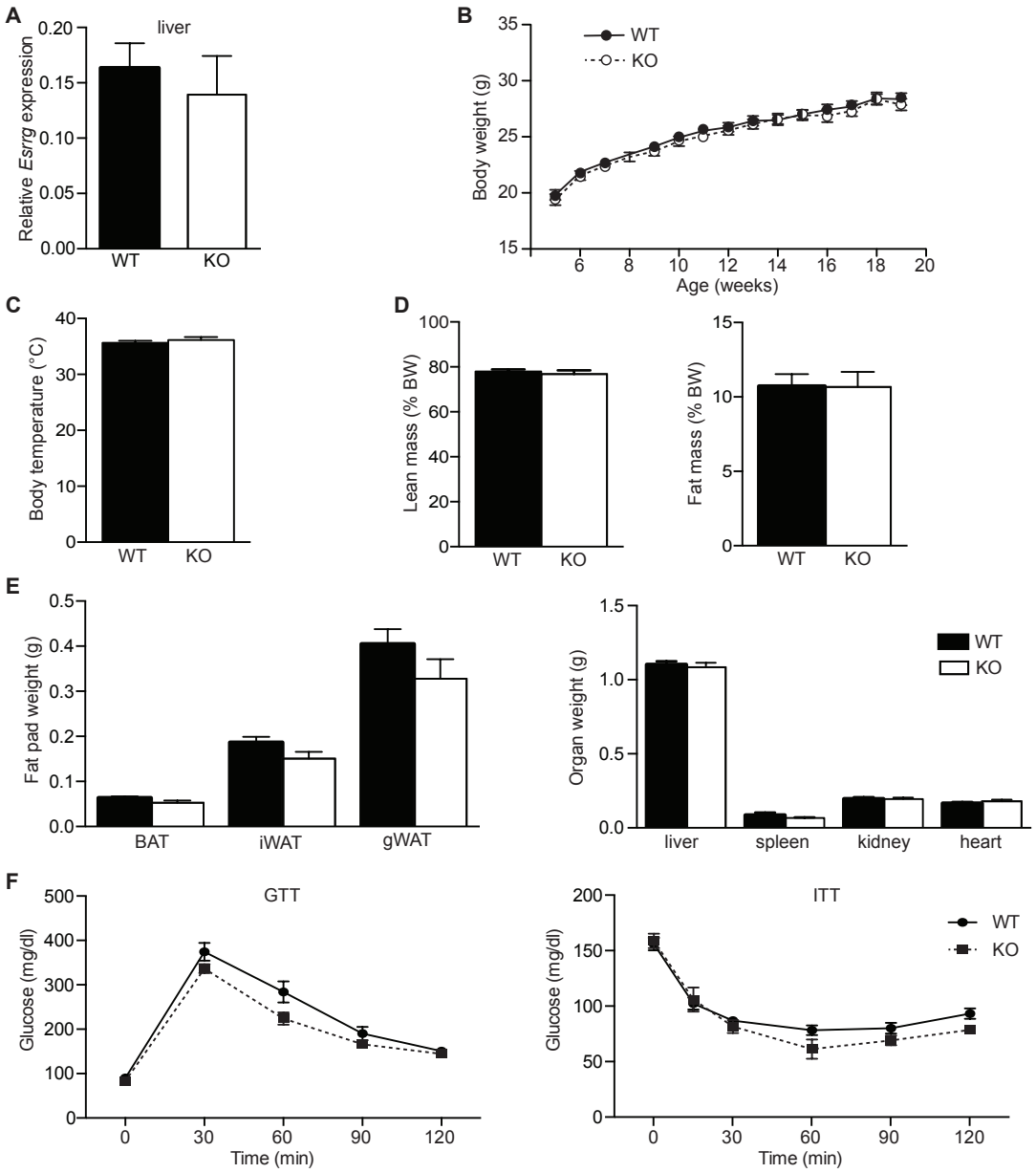
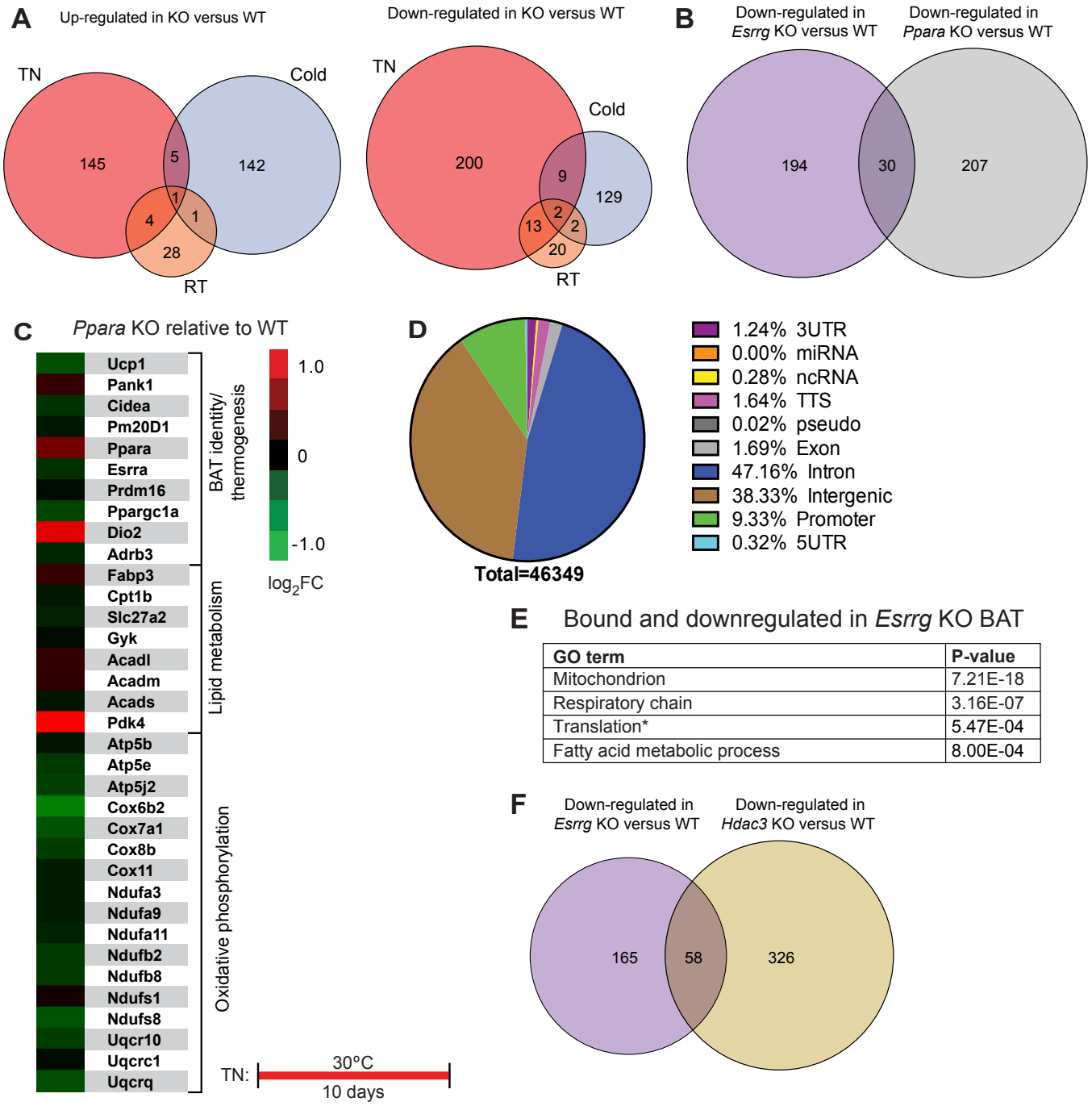


Figure S1. ERRγKO mice exhibit no change in body weight or insulin sensitivity on chow diet at room temperature (related to Figure 1).

A) Relative *Esrrg* mRNA levels from the liver of control flox/flox (WT) and ERRγKO (KO) mice (n=4-7).
B) Body weights of WT and KO mice on a chow diet housed at room temperature (22°C) (RT) (n=8-13).
C) Body temperature of WT and KO mice on a chow diet housed at RT (n=7-10).
D) Lean mass (left) and fat mass (right) of WT and KO mice on a chow diet housed at RT (22°C) (n=7-10).
E) Fat pad weight (left) and organ weight (right) of WT and KO mice on a chow diet housed at RT (n=7).
F) Glucose Tolerance Test (GTT, left) and Insulin Tolerance Test (ITT, right) of WT and KO mice on a chow diet housed at RT (n=6-11). Data represent mean ± SEM.



GO term	P-value
Mitochondrion	7.21E-18
Respiratory chain	3.16E-07
Translation*	5.47E-04
Fatty acid metabolic process	8.00E-04

Figure S2. ERRγ directly regulates BAT genes at thermoneutrality (related to Figure 2).
A) Venn Diagram showing overlap of up (left) and down-regulated genes from RNA-Seq at thermoneutrality (TN), room temperature (RT) and cold in BAT of control flox/flox (WT) and ERRγ KO (KO) mice.
B) Venn diagram showing unique and overlapping genes that are downregulated in ERRγKO BAT and PPARα KO BAT from RNA-Seq.
C) Heatmap of selected BAT signature genes in BAT from PPARα KO mice relative to BAT from WT mice of mice housed at TN. Data are represented as $\log_2$ fold change, n=3 per group.
D) Distribution of ERRγ occupancy from ERRγ ChIP-Seq from BAT of WT mice acclimated to TN.
E) KEGG pathway analysis of bound and downregulated genes from ChIP-Seq for ERRγ and RNA-Seq from ERRγKO BAT, respectively.
F) Venn diagram showing unique and overlapping genes that are downregulated in ERRγKO BAT and HDAC3KO BAT from RNA-Seq.

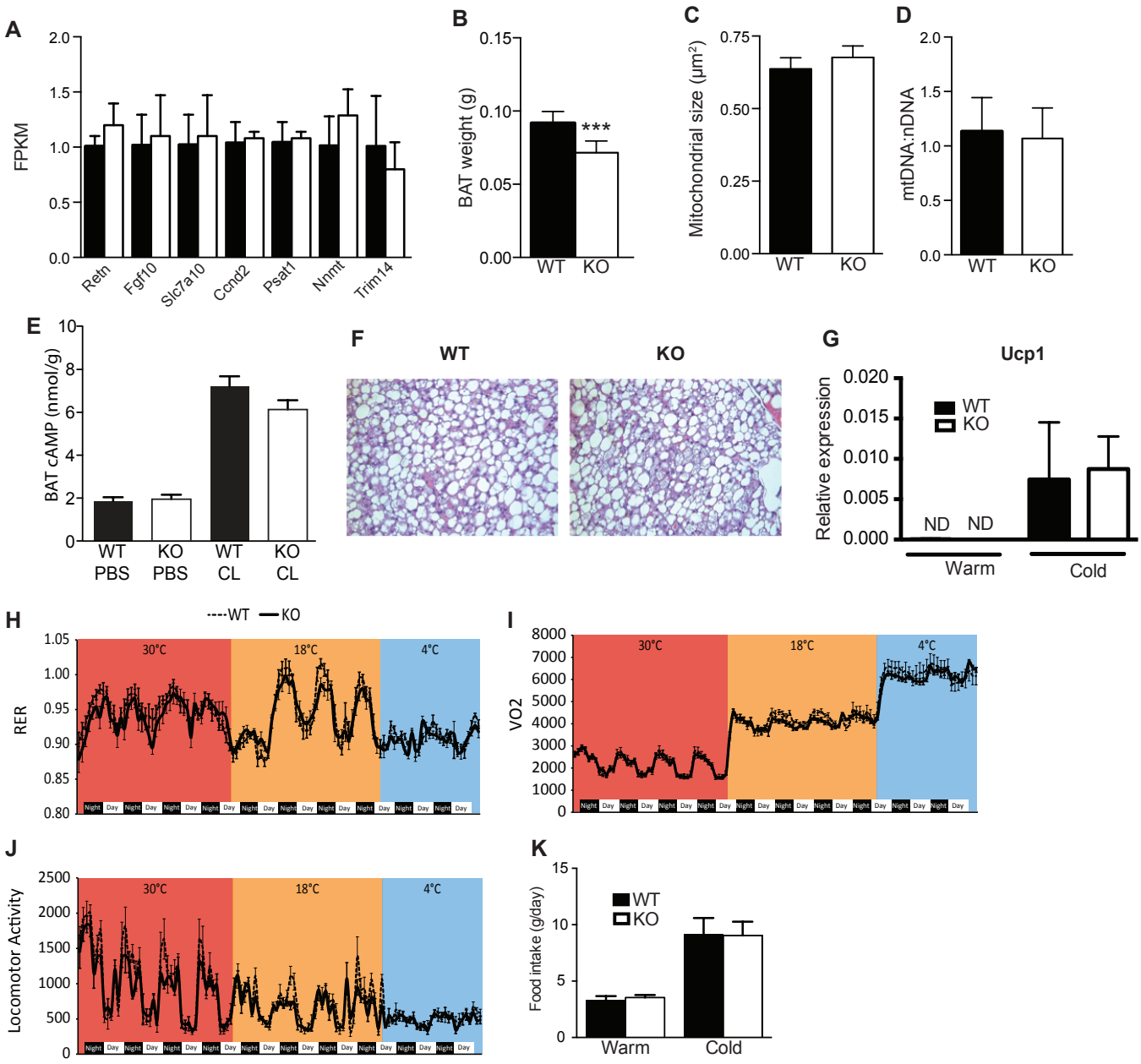


Figure S3. ERRγKO mice exhibit BAT atrophy but no change in expression of WAT-selective genes or in mitochondrial number or size (related to Figure 3).

A) FPKM values from RNA-Seq of selected WAT signature genes in BAT from ERRγKO (KO) mice relative to control flox/flox (WT) mice housed at thermoneutrality (TN) (n=3).

B) BAT weight of WT and KO mice housed at TN on a chow diet (n=5).

C) Mitochondrial size of WT and KO BAT of mice housed at TN on a chow diet.

D) Mitochondrial number of WT and KO BAT of mice housed at TN on a chow diet (n=7-13).

E) cAMP levels in BAT of WT and KO mice 20 minutes after injection of CL316,243 or PBS in mice housed at TN (n=3-6).

F) Representative H&E stain of inguinal WAT from WT and KO mice during gradual cold acclimation.

G) Relative Ucp1 mRNA levels from inguinal WAT of WT and KO mice during gradual cold acclimation (n=4-6).

H) Respiratory Exchange Ratio (RER) of WT and KO mice during gradual cold acclimation (n=6).

I) VO_2 of WT and KO mice during gradual cold acclimation (n=6).

J) Locomotor activity of WT and KO mice during gradual cold acclimation (n=6).

K) Food intake in WT and KO mice at 30°C or after gradual acclimation to cold (1 week 18°C, 1 week 4°C) (n=6) Data represent mean ± SEM. *p<0.05, ****p<0.0001 Student's unpaired t test.

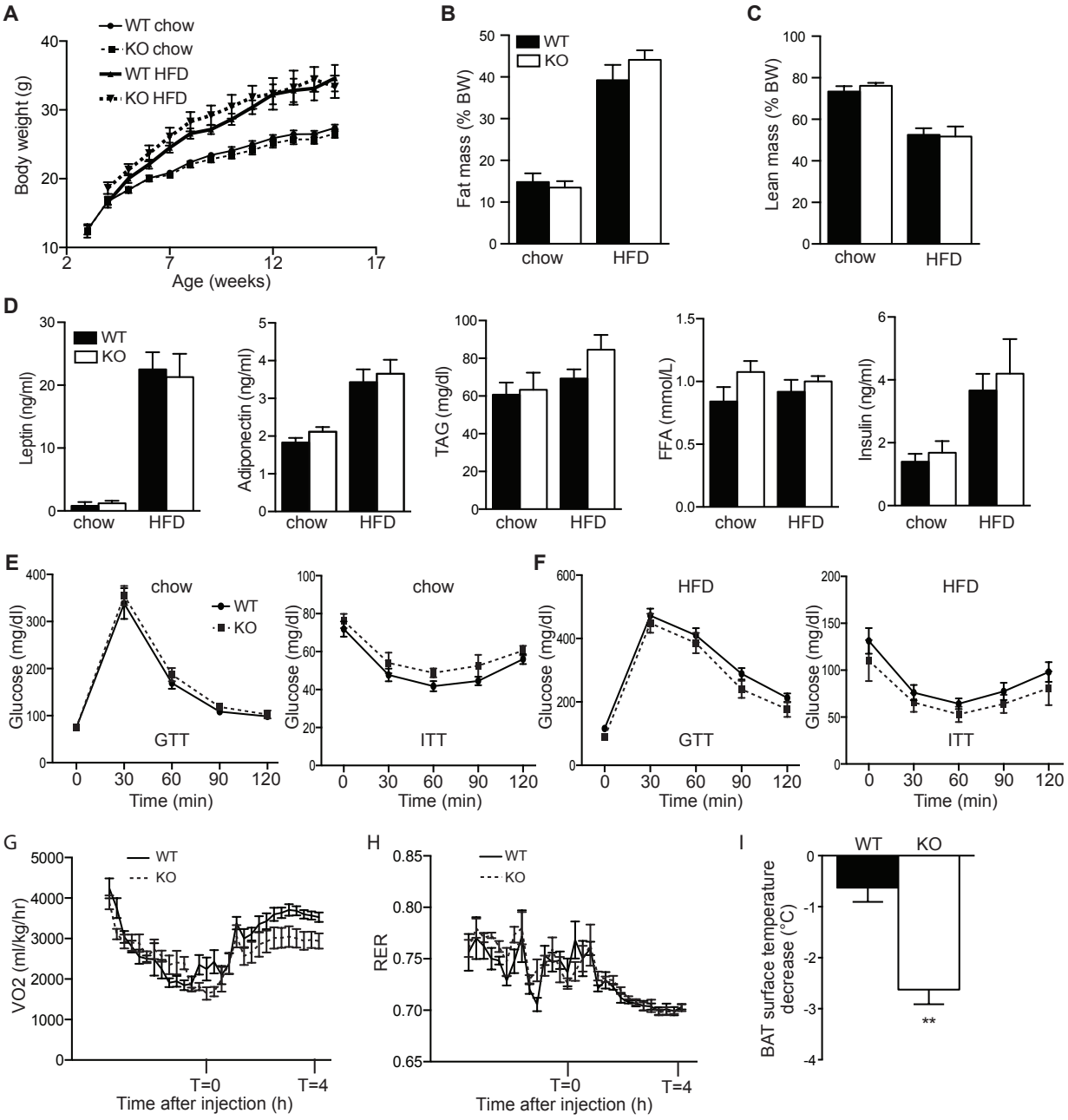


Figure S4. ERRγKO mice housed at thermoneutrality do not gain more weight on high fat diet despite impaired BAT function (related to Figure 4).

A) Body weights on a chow diet and high fat diet (HFD) of ERRγKO (KO) mice and control flox/flox (WT) mice housed at thermoneutrality (TN) (n=6-12).

B) Fat mass in WT and KO mice housed at TN (n=3-9).

C) Lean mass in WT and KO mice housed at TN (n=3-9).

D) Serum levels of Leptin, Adiponectin, Triacylglycerol (TAG), Free Fatty Acids (FFA) and Insulin in mice on a chow diet and HFD, housed at TN (n=6-11).

E) Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT) of mice on a chow diet, housed at TN (n=6).

F) GTT and ITT of mice on a HFD, housed at TN (n=6-11).

G) VO_2 after intraperitoneal injection of CL316243 (1mg/kg BW) from control flox/flox (WT) and ERRγKO (KO) female mice housed at thermoneutrality (n=4).

H) Respiratory Exchange Ration (RER) after intraperitoneal injection of CL316243 (1mg/kg BW) from control flox/flox (WT) and ERRγKO (KO) female mice housed at thermoneutrality (n=4).

I) BAT surface temperature measured with an infrared camera in WT and KO mice housed at thermoneutrality and then acutely exposed to the cold (4°C) (n=3-4). Data represent mean ± SEM. **p<0.01 Student's unpaired t test.

Supplementary Methods

Animal studies

PPARα null mice were purchased from Jackson laboratory. 10 week old male PPARα null mice and WT controls were acclimated to thermoneutrality (TN) for 10 days and brown adipose tissue (BAT) was harvested and prepared for RNA-Sequencing as described in main Methods Section.

Tissue cAMP measurement

Tissue cAMP levels were measured using a cAMP Enzyme Immunoassay Kit (Sigma CA200). Fresh tissue samples were homogenized in 10 volumes of 0.1M HCl, and then spun down and the aqueous faction collected and used in the kit per the manufacturer's instructions.

Histology

Inguinal WAT was fixed in 10% paraformaldehyde, embedded in paraffin, sectioned at 10μm, and stained with hematoxylin/eosin (Pacific Pathology).

Mitochondria number and DNA content

Quantification of mitochondria size was performed using ImageJ software. Mitochondria DNA was determined using the Gentra Puregene Tissue Kit (Qiagen), nuclear and mitochondrial DNA contents were collected using a slightly modified version of manufacturer's recommendations. Ten milligrams of BAT was homogenized in 300μL of Cell Lysis solution followed by addition of 1.5μL of Puregene Proteinase K. The mixture was incubated for 1 hour at 55°C. Then, 1.5μL of RNase A solution was added before mixing by inversion 25 times. After 1hour of incubation at 37°C, the sample was cooled on ice for 5min and 100μL of Protein Precipitation Solution were added. The mixture was vortexed for 20sec and centrifuged for 3min at 15,000g. The upper, aqueous, layer containing the extracted DNA was collected and transferred to a new tube. An equal volume of chloroform was added to the tube followed by a quick vortex and 3min of centrifugation at 13,000g. The upper layer was collected and moved to a new tube containing 300μL of cold isopropanol and 1μL of GlycoBlue (LifeTechnologies). After mixing by inversion, the tube was centrifuged in a 4°C refrigerated centrifuge for 3min at 15,000g. The supernatant was discarded and 300μL of 70% ethanol were added to the pellet followed by a 1min centrifugation at 15,000g, 4°C. The supernatant was discarded and the pellet air dried for 10 min before being resuspended in 50μL of purified water for 1 hour at 65°C. The DNA concentration and purity were measured on a Nanodrop spectrophotometer. DNA was diluted at 0.25ng/μL with SYBR Green (Life Technologies) and primers attaching either intron 9 of the hexokinase 2 gene for nuclear DNA quantitation, or cytochrome B gene for mitochondrial DNA quantitation. For both genes, we used the standard curve method and expressed the mitochondrial DNA content as a ratio of the genomic DNA content.

Gradual cold acclimation

20 week old WT and ERRγKO mice, that had been acclimated to TN, were transferred to metabolic cages housed in temperature controlled cabinets. The temperature was maintained at 30°C for 4 days then switched to 18°C for 4 days then to 4°C for 3 days and RER, VO_2, activity and food intake recorded during this time.

Metabolic Studies

Blood was collected from 20 week old male chow and HFD-fed mice by retro-orbital bleed after an overnight fast and free fatty acids (Wako) and triglycerides (Thermo) were measured using enzymatic colorimetric methods. Serum insulin and leptin levels were measured using an insulin enzyme-linked immunosorbent assay (ELISA) kit (Millipore). Serum adiponectin levels were measured using an ELISA assay (Life Tech). GTTs were conducted after an overnight fast in 20 week male old mice that were intraperitoneally injected with 1gram glucose per kilogram body weight and tail blood glucose was

monitored using OneTouch Ultra glucometer (Lifescan Inc.). ITTs were conducted after a 5hour fast in 20week old male mice. Mice were injected intraperitoneally with insulin (humulin, Eli Lily), which was administered at .75U (chow-fed mice) or 2U (HFD-fed mice) per kilogram body weight. Tail blood glucose was monitored using OneTouch Ultra glucometer (Lifescan Inc.). Analysis of total body composition was performed with EchoMRI-100 (Echo Medical Systems, LLC). BAT surface temperature was measured with an infrared camera (FLIR).