SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Generation of BATI4OE and Ucp1-Cre mice. ***A,*** Schematic of the strategy used to generate BATI4OE mice. ***B***, Schematic of the Ucp1-Cre BAC transgene. ***C,*** QPCR was performed using mRNA from different tissues of Ucp1-Cre X Rosa26-td tomato mice (n=5). ***D,*** Cre expression was measured by QPCR in different fat depots of mice exposed to cold for the indicated time (n=4). ***E, F,*** O_2 consumption and CO_2 production rates of male Ucp1-Cre and WT littermate mice were measured by indirect calorimetry using CLAMS after 16 weeks on HFD (n=8). Related to Fig. 2.

Figure S2. Characteristics of BATI4OE mice on chow and high fat diet. ***A,*** Body weight of BATI4OE mice on chow (n=9-16, **p*<0.05). ***B***, Body composition of the mice from ***A*** (**p*<0.05). ***C,*** Weight of fat pads in high-fat fed control and BATI4OE mice. ***D,*** Locomotor activity of BATI4OE and WT littermates after 4 weeks on HFD (n=8). ***E, F*****,** GTT and ITT of BATI4OE mice after 4-5 wks HFD (n= 8-10, **p*<0.05). ***G,*** Oxidation of ^{14}C-palmitate in isolated mitochondria from BAT (n= 10-12). ***H,*** Western blot analysis of isolated BAT mitochondria from main Fig. 3E. ***I,*** Rate of lipolysis in isolated adipocytes from BAT of male control and BATI4OE (OE) mice (n = 5). ***J,*** Lipolysis in control and BATI4OE (OE) mice on chow diet. Glycerol in plasma was measured in the absence and presence of 10 mg/kg of isoproterenol (n = 5). ***K,*** Lipogenesis in isolated brown adipocytes from BATI4OE and control mice. Results are expressed as mean ± SEM (**p* < 0.05; n = 5). Related to Fig. 2.

Figure S3. Browning of white fat is affected in BATI4OE mice. ***A,*** RNA was harvested from the inguinal WAT of BATI4OE or control littermates housed at 23°C or 4°C for 6 days. Gene expression was measured using QPCR. Data are normalized to *36B4* and expressed as mean ± SEM (n=3-5, **p*<0.05). ***B,*** Immunohistochemical detection of UCP1 in inguinal WAT of mice shown in ***A*** (higher magnification is shown in insets). ***C,*** RNA was harvested from epididymal WAT of BATI4OE or control littermates housed at 23°C or 4°C for 6 days. Gene expression was measured using QPCR. Data are normalized to *36B4* and expressed as mean ± SEM (n=3-5, **p*<0.05). ***D,*** Immunohistochemical detection of UCP1 in epididymal WAT of mice shown in ***C*** (higher magnification is shown in insets). Related to Fig. 3.

Figure S4. Characteristics of BATI4KO mice on chow and HFD. ***A,*** Body weight of BATI4KO mice on chow (n=9-16, **p*<0.05). ***B***, Body composition of the mice from ***A*** (**p*<0.05). ***C,*** Weight of fat pads in high-fat fed control and BATI4KO mice. ***D,*** Locomotor activity of BATI4KO and control littermates after 22 weeks on HFD (n=8). ***E***, Glucose tolerance test in 24-week-old BATI4KO mice on HFD after intraperitoneal injection of glucose (n=8-10, *p<0.05). ***F,*** Insulin tolerance test in 25-week-old BATI4KO mice on HFD after intraperitoneal injection of insulin (n= 8-10, **p*<0.05, ***p*<0.01). ***G*, *H,*** GTT and ITT of BATI4KO mice at 2 and 4 weeks after the initiation of HFD, before divergence of body weight. Related to Fig. 4.

Figure S5. IRF4 affects brown adipose lipid handling and mitochondrial function. ***A,*** Oxidation of ^{14}C-palmitate in isolated mitochondria from BAT (n= 10-12). ***B,*** Rate of lipolysis in isolated adipocytes from BAT of male Flox and BATI4KO mice. Results are expressed as mean ± SEM (**p* < 0.05; n = 5). ***B,*** Rate of lipolysis in isolated adipocytes isolated from BAT of male control and BATI4KO mice (n = 5). ***C,*** Lipolysis in control and BATI4KO mice on chow diet. Glycerol in plasma was measured in the absence and presence of 10 mg/kg of isoproterenol (n = 5). ***D,*** Lipogenesis in isolated adipocytes from BATI4KO and control mice. Results are expressed as mean ± SEM (**p* < 0.05; n = 5). ***E,*** Western blot analysis of isolated BAT mitochondria from main Fig. 5G. ***F,*** Transmission electron microscopy of interscapular BAT in BATI4KO and control mice at 23°C. ***G, H,*** Mitochondrial size and cristae density in sections from mice shown in ***F,*** determined by counting >100 mitochondria from at least three sections/mouse and three mice/genotype. Related to Fig. 5.

Figure S6. Browning of white fat is affected in BATI4KO mice. ***A, D,*** RNA was harvested from inguinal WAT of BATI4KO or control littermates housed at 23°C or 4°C for 6 days. Gene expression was measured using QPCR. Data are normalized to *36B4* and expressed as mean ± SEM (n=3-5, **p*<0.05). ***B,*** Immunohistochemical detection of UCP1 of inguinal WAT of mice shown in ***A*** (higher magnification is shown in insets). ***C,*** RNA was harvested from epididymal WAT of BATI4KO or control littermates housed at 23°C or 4°C for 6 days. Gene expression was measured using QPCR. Data are normalized to *36B4* and expressed as mean ± SEM (n=3-5, **p*<0.05). ***D,***

Immunohistochemical detection of UCP1 of epididymal WAT of mice shown in ***C*** (higher magnification is shown in insets). Related to Fig. 5.

Figure S7. Genetic, physical, and functional interactions between IRF4 and PGC-1α in brown fat. ***A,*** *Irf4* mRNA expression in interscapular BAT from control vs. adipocyte-specific *Ppargc1a* null mice (Adipoq-cre x *Ppargc1a*^{flox}) (n=4, **p*<0.05). ***B,*** SVF from BAT of control vs. adipocyte-specific *Ppargc1a* null mice was differentiated into adipocytes and then treated with Forskolin or vehicle for 4 hours. Gene expression was measured using QPCR. Data are normalized to *36B4* and expressed as mean ± SEM (n=3-4, **p*<0.05). ***C,*** Schematic representation of mouse PGC-1α. Red lines indicate the location of the three LXXLL motifs. ***D,*** Co-IP of IRF4 with the N terminus of PGC-1α, but not a C-terminal fragment. ***E,*** IRF4 does not interact with a mutant allele of PGC-1α lacking the second and third LXXLL motifs of PGC-1α (PGC-1α-LALA). ***F,*** Schematic representation of mouse IRF4, showing the DNA binding domain (DBD) and protein interaction associated domain (IAD). ***G,*** Co-IP of PGC-1α with the C-terminal fragment of IRF4, but not an N-terminal fragment. ***H,*** Fragments of the *Ucp1* promoter fused to a luciferase reporter gene were co-transfected into primary brown adipocytes together with pCDH-GFP (control) or IRF4 in the presence or absence of a PGC-1α expression plasmid. Luciferase activity was corrected for Renilla luciferase activity and normalized to control activity (n=3, *p<0.05). Related to Fig. 7.

SUPPLEMENTAL METHODS

Generation of Ucp1-cre transgenic mice

A C57Bl/6 mouse bacterial artificial chromosome (148M1) containing the Ucp1 gene was transformed into the recombinogenic EL250 bacteria cells, and homologous recombination was performed as described elsewhere (Lee et al., 2001). The Cre-FRT-Kan-FRT cassette was transformed into the Ucp1 BAC host EL250 cells and recombined to insert the Cre ATG into the Ucp1 ATG. Ucp1-Cre-FRT-Kan-FRT BAC host EL250 clones were identified by PCR screening. The FRT-Kan-FRT cassette was removed, and an Ucp1-Cre BAC host EL250 clone without mutation in the Cre coding sequence was obtained. The loxP site present in the vector sequence of the Ucp1-Cre BAC was removed. The transgenic construct was microinjected into pronuclei of fertilized one-cell stage embryos of FVB mice (Jackson Laboratories) via standard

methods. The Ucp1-Cre line was back-crossed more than eight times onto C57BL/6J prior to use in these experiments; these mice are maintained as hemizygotes. The Ucp1-Cre mice are now available through Jackson Labs (JAX Stock No. 024670; B6.129-Tg(Ucp1-Cre)1Evdr/J).

Generation of Rosa26-LSL-IRF4 transgenic mice

The murine IRF4 cDNA was knocked into a locus immediately downstream of the endogenous Rosa26 gene promoter and a loxP-STOP-loxP sequence in the Ai9 plasmid (Madisen et al., 2010). The IRF4 cDNA was fused in frame to an N-terminus biotin ligase recognition peptide, and was designed to be expressed as a single peptide that includes the bacterial biotin ligase BirA, separated from IRF4 by an autocleavable viral 2A sequence. The construct was linearized with KpnI and microinjected into 129/Sv embryonic stem cells. Successful integration of a single copy of the transgene was validated using Southern blotting and long-range PCR (5' primers: 5'-GCCAAGTGGGCAGTTTACCG-3' and 5'-TAGGTAGGGGATCGGGACTCT-3'; 3' primers: 5'-GCCAGCTCATTCCTCCCACTC-3' and 5'-GGCATGGCAATGTTCAAGCAG-3'). Intact microinjected eggs were transferred into pseudopregnant recipients by the BIDMC transgenic core.

Generation of BATI4KO and BATI4OE Mice

Irf4$^{flox/flox}$ mice (Klein et al., 2006) were first crossed to Ucp1-Cre mice, and BATI4KO cohorts were established by mating F1 *Irf4*$^{flox/+}$*;Cre*$^{+}$ mice to littermate *Irf4*$^{flox/+}$*;Cre*$^{-}$ mice. The BATI4OE cohorts were established by directly mating the Rosa26-LSL-IRF4 mice to Ucp1-Cre mice. Mice were maintained under a 12 hr light/12 hr dark cycle at constant temperature (23°C) with free access to food and water and were fed either a standard chow diet (8664, Harlan Teklad) or a high-fat diet (D12331; Research Diets Inc.). Body weight was measured weekly. Mice were subjected to magnetic resonance imaging (MRI) (Echo Medical Systems) to examine body composition.

Primary preadipocytes and adipocytes

The interscapular brown fat pad (total of 6-8 fat pads) was dissected from newborn mice (postnatal day 2-4), minced, and then digested for 45 min at 37°C in isolation buffer [123mM NaCl, 5mM KCl, 1.3mM $CaCl_2$, 5mM glucose, 100mM Hepes, 4% BSA, 1.5mg/ml Collagenase B (Roche)]. Digested tissue was filtered through a 100 um cell strainer to remove large pieces, and the flow-through was then centrifuged for 10 minutes at 600xg to pellet the stromal-vascular fraction (SVF) cells. SVF cells were resuspended in complete culture medium (DMEM/F12 (1:1; Invitrogen) plus Glutamax,

Pen/Strep, and 10% FBS (Gemini Bio-products), and then plated onto 10cm tissue culture dishes. For preadipocyte differentiation, cells grown to 100% confluence (Day 0) were exposed to induction DMEM/F12 GlutaMAX™ containing dexamethasone (5μM; Sigma), insulin (0.5μg/ml; Sigma), isobutylmethylxanthine (0.5mM; Sigma), rosiglitazone (1μM; Cayman), T3 (1nM; Sigma) and 10% FBS. Four days after induction (from Day 4), cells were maintained in media containing insulin (0.5μg/ml), T3 (1nM) and 10% FBS until ready for harvest (generally day 6-7 post-differentiation).

Lipolysis assay

For *ex vivo* studies, adipocytes were isolated from brown fat pads by collagenase digestion. Cells were resuspended in Krebs-Ringer HEPES (KRH) buffer at $2X10^5$ cells/ml. Cells were incubated in the presence or absence of 1 mM isoproterenol for 0–2 hr at 37°C with gentle shaking. Glycerol content of conditioned medium was determined with the Free Glycerol Determination Kit (Sigma). For *in vivo* studies, mice were fasted for 4hr and injected with isoproterenol (10mg/kg body weight). Blood was collected from the tail vein before and 15 min after injection, and glycerol content of serum was measured as above.

Lipogenesis assay

For *ex vivo* studies, adipocytes were isolated from brown fat pads as in the lipolysis assay. Cells were incubated in the presence of 100nM insulin for 15 min and incubated with 1μCi ^{14}C-glucose per well for 1hr at 37°C. Cells were then washed three times with ice-cold PBS. Radiolabeled glucose incorporation into lipid was assessed by placing cells into 0.5ml PBS and shaking vigorously with 3 ml Betaflour Liquid Scintillation Fluid (National Diagnostics). After samples settled overnight, radioactivity partitioning into the organic phase was determined by scintillation counting.

Fatty acid oxidation assay

BAT mitochondria were extracted as mentioned above. 350 μg mitochondria were incubated for 1 hr with reaction buffer (100mM sucrose, 5mM KH_2PO4, 10mM Tris-HCl, 0.2 mM EDTA, 0.2% fatty acid-free BSA, 50mM KCl, 1mM $MgCl_2$, 2mM L-carnitine, 0.1mM malate, 0.05mM coenzyme A, 1mM DTT, 2mM ATP and 0.1mM palmitic acid) supplemented with [1-^{14}C] palmitic acid. The oxidation reaction was terminated and CO_2 was released from the media by the addition of 6N HCl. Filter paper saturated with NaOH was placed in the tube cap to capture CO_2. Following 2hr incubation, $^{14}CO_2$ resulting from oxidized fatty acid was quantified by scintillation counting of the filter paper. The results were normalized to total protein.