SUPPLEMENTAL FIGURES AND LEGENDS

Fig. S1, related to Fig. 1. Additional characterization of UCP1-TRAP mice. (A) Immunohistochemistry staining for UCP1 and GFP from the classical BAT (A) or immunofluorescence of UCP1, GFP, and DAPI in iWAT (B) of 6-week old female UCP1-TRAP mice after two weeks cold exposure at 4°C. (C, D) Relative mRNA expression of indicated genes from UCP1-TRAP (C) or whole tissue (D) samples. For (C) and (D), data are presented as means ± standard error; n = 3-5/group. *, ** $p < 0.05$ or < 0.01, respectively, in BAT versus pgWAT samples; #, ## $p < 0.05$ or < 0.01, respectively, in BAT versus iWAT samples.

Fig. S2, related to Fig. 2. Additional analysis of the common and differentially expressed UCP1-TRAP genes. (A) DAVID gene ontology analysis of the 1000 most abundant "equivalent" genes from the UCP1-TRAP dataset. (B) Relative expression of equivalently (*Fabp4*, *Ucp1*) or differentially (*Fasn*, *Scd1*) expressed genes. These data are presented as means ± standard error and are taken directly from the UCP1-TRAP-Seq data; n = 2 for iWAT and n = 3 for pgWAT and BAT. (C) Venn diagram showing the overlap of core thermogenesis genes and general adipocyte genes. For this analysis (also see Supplemental Experimental Procedures), core thermogenesis genes (blue circle) were identified using the UCP1-TRAP-Seq dataset by FPKM > 1 in all depots and ratio between high and low depot ≤ 3; general adipocyte genes (red circle) were identified using whole adipose tissues by Affymetrix probe signal > 200 and ratio between high and low depot ≤ 3.

Fig. S3, related to Fig. 3. Validation of depot-selective UCP1-TRAP gene expression. UCP1-TRAP-Seq genes that were identified to be selective for each depot (Groups 1, 2, and 3) from Fig. 3A were validated by qPCR in an independent cohort of UCP1-TRAP mice. Data are presented as means; n = 3-4/group. Color coding was performed for each table where the relative high and low values are indicated by red and blue, respectively.

Fig. S4, related to Fig. 4. Characterization of *Adiponectin*-TRAP mice. (A) Western blotting for GFP and β-actin loading control from various fat depots in Adipoq-TRAP or control (Cre-negative, TRAP-positive) mice. (B) Relative expression of *Ucp1* from immunopurified RNA (TRAP) samples of Adipoq-TRAP mice. Data are presented as means ± standard error; n = 3-5/group.

Fig. S5, related to Fig. 5. Additional characterization of the SMC-like signature in UCP1-TRAP samples. (A) Relative expression in UCP1-TRAP samples of the indicated genes from the iWAT, axWAT (axillary), or BAT depots. Data are presented as means ± standard error; n = 3-5/group. (B) Relative expression of the indicated genes from the UCP1-TRAP-Seq dataset. Units are in FPKM. Individual replicates for each of the depots are shown; n = 2 for pgWAT and n = 3 for each iWAT and BAT. Red and blue indicate relative high and low expression, respectively, for the row.

Fig. S6, related to Fig. 6. Gene expression of *Myh11*-labeled and unlabeled cells in the stromal vascular fraction. Relative expression of the indicated genes from RFP-positive and RFP-negative cells in the iWAT stromal vascular fraction (SVF) of *Myh11*-

tdTomato reporter mice. After harvesting SVF and passaging once, cells were sorted by RFP fluorescence and replated, and total mRNA was collected one day later. Data are presented as means ± standard error; n = 3-5/group. *, ** $p < 0.05$ or < 0.01, respectively, for RFP-positive versus RFP-negative comparisons. Ref. 1-5 are indicated below:

Ref. 1: Rodeheffer, M.S., Birsoy, K., and Friedman, J.M. (2008). Identification of white adipocyte progenitor cells in vivo. Cell *135*, 240-249.

Ref. 2: Lee, Y.H., Petkova, A.P., Mottillo, E.P., and Granneman, J.G. (2012). In vivo identification of bipotential adipocyte progenitors recruited by beta3-adrenoceptor activation and high-fat feeding. Cell Metabolism *15*, 480-491.

Ref. 3: Gupta, R.K., Mepani, R.J., Kleiner, S., Lo, J.C., Khandekar, M.J., Cohen, P., Frontini, A., Bhowmick, D.C., Ye, L., Cinti, S.*, et al.* (2012). Zfp423 expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. Cell Metabolism *15*, 230-239.

Ref. 4: Tang, W., Zeve, D., Suh, J.M., Bosnakovski, D., Kyba, M., Hammer, R.E., Tallquist, M.D., and Graff, J.M. (2008). White fat progenitor cells reside in the adipose vasculature. Science (New York, NY) *322*, 583-586.

Ref. 5: Wu, J., Bostrom, P., Sparks, L.M., Ye, L., Choi, J.H., Giang, A.H., Khandekar, M., Virtanen, K.A., Nuutila, P., Schaart, G.*, et al.* (2012). Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell *150*, 366-376.

Table S1, related to Fig. 1. The UCP1-TRAP-Seq dataset. This Excel sheet provides the raw UCP1-TRAP-Seq dataset, organized by gene name and the relative expression (in FPKM) of the genes across UCP1-TRAP samples from the iWAT (n = 3), pgWAT (n =

2), or BAT (n = 3). Tissues were harvested from 6-week old UCP1-TRAP females after two weeks cold exposure at 4°C.

Table S2, related to Fig. 2. Analysis of equivalent or differentially expressed genes in the UCP1-TRAP-Seq dataset. This Excel sheet provides an initial equivalent/different filter to the raw UCP1-TRAP-Seq dataset. First, genes were identified as detected if they were present (FPKM > 1) in at least two samples from the UCP1-TRAP-Seq. Next, these 9015 detected genes were classified as either equivalently or differentially expressed between the UCP1-TRAP samples as described in the Experimental Procedures. This gene list is ordered from most to least abundant genes, as determined by the sum of the signals (total FPKM) across all UCP1-TRAP samples.

Table S3, related to Fig. 2. Identification of the UCP1-TRAP core thermogenesis gene set. This Excel sheet provides the individual gene names for the common thermogenesis and general adipocyte gene sets (see Experimental Procedures). This gene list is ordered from most to least abundant genes in UCP1-TRAP samples from each depot.

Table S4, related to Fig. 3. Identification of differentially expressed genes from the UCP1-TRAP dataset. The initial identification of depot-specific genes (Groups 1, 2, and 3 in Fig. 3A) from the UCP1-TRAP dataset is shown as three individual tabs in this Excel sheet. The criteria for generating these gene lists are provided in the Experimental Procedures.

Table S5, related to Fig. 4. Genes analyzed for the identification of anatomy-independent markers of brown and beige cells. This Excel sheet provides the fold change and p-values of all UCP1-TRAP depot-specific genes evaluated between interscapular BAT versus interscapular WAT, or between UCP1-TRAP versus Adipoq-TRAP mice. Data are presented as means; n = 4-6/group.

Table S6, related to Experimental Procedures. qPCR primers for all genes used in this study.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Generation of TRAP mice

The EGFP-L10a portion was PCR amplified from a construct provided by David Olson (Heiman et al., 2008) and subcloned between the *Bsu36I* and *SexAI* sites of a pCAG-HS-mG (hH2B-mCherry-2A-EGFP-GPI) plasmid kindly provided by Dr. Richard Behringer (Stewart et al., 2009). This transgene was further subcloned into a modified *Rosa26*-pCAG-LSL-WPRE-bGHpA targeting vector generously provided by Hongkui Zeng at the *FseI* site between the LSL and WPRE sequences (Madisen et al., 2010). LSL sequence contains specifically *lox*P- Stop codons - 3x SV40 polyA – *lox*P. The targeting vectors were linearized (*KpnI*) and transfected into the 129/B6 F1 ES cell line G4. G418-resistant ES clones were first screened by PCR using primers spanning the 1.1 kb 5′ genomic arm (forward primer: 5′-GCCAAGTGGGCAGTTTACCG-3′, reverse primer:

5′-TAGGTAGGGGATCGGGACTCT-3′), and then confirmed using primers spanning the 4.4 kb 3' genomic arm (forward primer: 5′-GCCAGCTCATTCCTCCCACTC-3′, reverse primer: 5′-GGCATGGCAATGTTCAAGCAG-3′). Positive ES clones were injected into C57BL/6J blastocysts to obtain chimeric mice following standard procedures. Chimeric mice were bred with C57BL/6J mice to obtain germline transmitted F1 mice. The TRAP mice were backcrossed at least six generations prior to use in the expeirments.

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. 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 a *Ucp1*-Cre BAC host EL250 clone without mutation in the Cre coding sequence was obtained. The *lox*P 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 founder was backcrossed at least eight generations onto C57BL/6J prior to use in the experiments.

UCP1-TRAP RNA Sequencing

RNA-Seq was performed by the Dana-Farber Cancer Institute Center for Cancer Computational Biology Sequencing Facility. Total RNA was first put through quality control using the Qubit (Life Tech) and the Bioanalyzer (Agilent). RNA quantity was determined on the Qubit using the Qubit RNA Assay Kit (Life Tech) and RNA quality was determined on the Bioanalyzer using the RNA Pico Kit (Agilent). Using the NEBNext Ultra RNA Library Prep Kit for Illumina (NEB), 100 ng of total RNA was converted into a DNA library following the manufacturer's protocol, with no modifications. Following library construction, DNA libraries were then put through quality control. Library quantity was determined using the Qubit High Sensitivity DNA Kit (Life Tech) and library size was determined using the Bioanalyzer High Sensitivity Chip Kit (Agilent). Finally, libraries were put through qPCR using the Universal Library Quantification Kit for Illumina (Kapa Biosystems) and run on the 7900HT Fast qPCR machine (ABI). Libraries passing quality control were diluted to 2 nM using sterile water and then sequenced on the HiSeq 2000 (Illumina) at a final concentration of 12 pM on a single read flowcell with 50 sequencing cycles, following all manufacturer protocols.

TRAP RNA isolation

For the preparation of anti-GFP-conjugated dynabeads, for each sample 50 µl protein G dynabeads (Life Technologies) was first washed with PBS-T and then incubated with 2 µl anti-GFP antibody (Abcam, ab290) in a total volume of 200 ul PBS-T. After incubating for at least 20 min at room temperature, the PBS-T was removed and the tissue lysates were immediately added to the beads, as described below. Each tissue was harvested, briefly minced with a razor, and then manually dounce homogenized in 3 ml

(iWAT, pgWAT) or 1 ml (BAT) of IP buffer [50 mM Tris, pH 7.5; 12 mM $MgCl_2$; 1% NP-40; 100 µg/ml cycloheximide (Sigma); 0.5 mM DTT; 100 mM KCl; 1x HALT protease inhibitor EDTA-free (Thermo); 1 mg/ml sodium heparin (Sigma); 0.2 units/µl RNasin (Promega)]. Following vortexing and centrifugation (12,000 x g for 10 min), the lipid layer was removed and the remaining supernatant was incubated with the anti-GFP-conjugated dynabeads. After 1 h at 4°C, dynabeads were separated and washed thrice with 1 ml high salt buffer [50 mM Tris, pH 7.5; 12 mM $MgCl_2$; 1% NP-40; 100 µg/ml cycloheximide (Sigma); 0.5 mM DTT; 300 mM KCl]. Following the last wash, TRIzol (500 µl, Life Technologies) was immediately added to the beads and RNA was purified using RNeasy Micro Kit (Qiagen) according to the manufacturer's instructions.

Bioinformatic analyses

For analysis of the "core" thermogenesis set (Fig. S2C and Table S3), the approach was to first define a common gene set to the UCP1-TRAP dataset and then to remove genes that are common to adipocytes generally (e.g., *Gapdh* or *Fabp4*). The UCP1-TRAP common gene set was defined using the UCP1-TRAP-Seq dataset by FPKM > 1 in all depots and ratio ≤ 3. The adipocyte common gene set was defined using Affymetrix data GSE53307 (Cohen et al., 2014) from whole adipose tissues by Affymetrix probe signal > 200 and ratio ≤ 3. Genes that were not detected due to technical limitations (e.g., missing probes) were excluded from this analysis.

Immunofluorescence and immunohistochemistry

For immunohistochemical stainings, adipose tissues excised, fixed in 10% formalin

overnight at 4°C, washed in PBS, embedded in paraffin, and cut into 5 μm of sections. Haematoxylin and eosin staining was performed according to standard protocols. Slide sections were deparaffinized and hydrated, antigen retrieval was performed using sodium citrate, and endogenous peroxidase activity was blocked with hydrogen peroxide. Then sections were incubated with 5% normal donkey serum (JacksonImmunoResearch Lab Inc, West Grove PA) for an hour at room temperature. Slides were then incubated with anti-GFP antibody (1:500) (Novus NB-100-1678) or anti-UCP-1 antibody (1:1000) (Abcam ab10983) overnight at 4°C. The slides were then washed and incubated with biotinylated donkey anti-goat secondary antibody (1:500) or biotinylated donkey anti-rabbit secondary antibody (1:500) for an hour at room temperature. Samples were enhanced with Elite Vectashield ABC kit (Vector Lab), developed in DAB (Diaminobenzidine) metal enhanced kit (Vector lab) and counter stained with hematoxylin.

For immunofluorescence stainings, tissues were fixed in 4% PFA for 24 h and 20% sucrose for 24 h before embedding and cryosectioning at 50 μm thickness. Slides were blocked in PBS-T supplemented with 10% fetal bovine serum (FBS) for 30 min before incubation with either rabbit polyclonal UCP1 antibody (abcam, ab10983) at 2 μg/ml or goat polyclonal perilipin (Abcam, ab61682) over night at 4°C in PBS-T/ 1% FBS. Slides were washed three times in PBS-T and incubated with Alexa Flour conjugated antibodies and nuclei was stained with Hoechst 33342 (Invitrogen). Immunofluorescence stainings were observed with a Nikon Ti w/ A1R confocal laser scanning microscope using 10× and 20× objective lenses and Nikon Elements acquisition software.

Isolation of primary aortic smooth muscle cells

Primary murine aortic SMCs were obtained as follows: aortas from six female mice were obtained sterilely and placed into a 100 mm dish containing media. The adventia was cleaned off and the aorta was cut into 1-2 mm pieces (horizontally). After placing into a collagen Biocoat plate (Fisher, catalog #08-772-69), the pieces were allowed to briefly dessicate and then cell culture media was added carefully. The next day, additional culture media was added if necessary. Explants were grown out in 3-7 days and removed. The next day, wells were washed (PBS), trypsinized, and passaged until P5, at which point they were used for experiments.