SUPPLEMENTAL METHODS

Mice

C57BL/6J wild-type mice and whole-body *Ucp1* knock out mice (*Ucp1*$^{-/-}$) were obtained from Jackson Laboratory (Bar Harbor, ME). *Bmp3b*$^{-/-}$ whole-body knockout mice were obtained courtesy of Dr. Se-Jin Lee (Johns Hopkins University) and have been described previously (41). All animals were fed a standard diet with food and water given *ad libitum* and maintained on a standard 12:12-h light/dark cycle. All animal procedures were approved by the University of Pennsylvania's Institutional Animal Care and Use Committee. All animals received humane and ethical care in compliance with the NIH *Guide for the Care and Use of Laboratory Animals* (National Academies Press, 2011). In the model of cardiac I/R, we only used male mice as the function of brown adipose tissue has been shown to be different between male and female sex (for a Review, see (42)).

Myocardial ischemia-reperfusion

Mice at 8-week-old age were anesthetized with a mixture of intraperitoneal ketamine (100 mg/kg) and xylazine (5 mg/kg) and placed supine on a heated operating table for the ischemia/reperfusion (I/R) surgery. Briefly, a small left parasternal incision was made between intercostal spaces VI and V. After retraction of the chest wall, we rotated the mouse into the right lateral position to expose the left ventricle. The left anterior descending (LAD) artery was visualized and an 8–0 suture was passed around this coronary artery just under the tip of the left auricle. PE-10 tubing was placed under the suture on top of the vessel to occlude the coronary artery. Body temperature was maintained at 37°C using a heating pad, and temperature was monitored using a rectal thermometer. After 45 min of ischemia, the slipknot was released, and the heart was reperfused for 3 or 24 hours based upon the assays. Mice were maintained at

thermoneutrality on the day of the procedure. Sham-operated control mice underwent the same surgical procedure, except the suture placed under the left coronary artery was not tied.

In mice treated with BMP3b (10 ng/g body weight or 50 ng/g body weight, according to the experiment), the jugular vein was exposed, and a silicone catheter was introduced for the administration of BMP3b at the time-point indicate in each experiment. K02288, a selective type I BMP receptor inhibitor, was dissolved with β-cyclodextrin and administrated intraperitoneally 1 hour before I/R surgery at a dose of 3.5 mg/kg body weight when indicated.

Myocardial injury evaluation

After 24 hours of reperfusion, mice were killed with an overdose of anesthesia and MI size was determined by 2,3,5-triphenyltetrazolium chloride (TTC) and microspheres (FluoSpheres™ Polystyrene Microspheres, #F8836; Life Technologies Corp). Briefly, the LAD was retied in the same position of the previous ligation and the microspheres were injected through the apex directly into the left ventricle. The heart was removed, cut into 1 mm slices, and soaked in 5% TTC PBS buffer at 37ºC for 5 min. The area devoid of microspheres (area at risk), and the TTC-negative area (MI) were measured on each slice using ImageJ software. Volumes were obtained for each slice and the total area at risk (AAR) and MI size calculated. To blind the analysis, each sample was given a number, and the relevant characteristics of this sample (genotype of the mouse, procedure, treatment, timing of the sample) were entered in a master file. The investigator analyzing the variables of interest was only aware of the number and blinded to the characteristics of the sample that was analyzed.

BAT transplantation

Interscapular BAT was transplanted from 6-week-old mice to 7-8 week-old mice. After cervical dislocation of donor mice, BAT was dissected, incubated in sterile saline for 5 min during

which the peripheral white adipose tissue was excluded. Epididymal WAT was harvested from the same donor. Recipient mice were anesthetized and transplanted with the BAT, epididymal WAT fat pad or a glass bead (as a sham transplant) into the subcutaneous dorsal region, adjacent to the endogenous interscapular fat pad of the recipient mice.

RNA extraction, transcriptome sequencing, and RNA-seq

Alzet osmotic minipumps (no. 1002; Alza Durect Corp.) containing isoproterenol (60 mg/kg/d) or saline were subcutaneously and dorsally inserted in mice under isoflurane anesthesia. After 3 days, interscapular BAT of WT and *Ucp1*$^{-/-}$ mice was harvested, and total RNA extracted by using an RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. Total RNA was assayed for quantity and quality with an Agilent 2100 Bioanalyzer (Agilent Technologies). Libraries of coding and small non-coding RNAs were generated using TruSeq Stranded mRNA HT Sample Prep Kit (Illumina) as per standard protocol in the kit's guide. A MiSeq micro test lane was run to check pool balance followed by 75 bp single-read sequencing on an Illumina HiSeq 2000 sequencer.

Fastq files were aligned against mouse reference (mm39/mGRC39) using the STAR aligner (v2.7.9a) (43). Duplicate reads were removed using MarkDuplicates from Picard tools, and per gene read counts for Ensembl (v104) gene annotations were computed. Expression levels in counts per million (CPM) were normalized and transformed using VOOM in the limma R package (44). Surrogate variables to account for sources of latent variation such as batch were calculated using the svaseq function from the R SVA package (45). Differential gene expression analysis was conducted using the limma package. Gene Ontology and pathway analysis was performed using the clusterProfiler (v4.4.4) (46). All plots were constructed in R using ggplot2 or

Complexheatmap. All RNA-seq data generated during this study have been deposited at the Gene Expression Omnibus database (accession number GSE210613).

Glucose tolerance and insulin tolerance tests

Mice were fasted for 6 h for the glucose tolerance test, and 2 h for the insulin tolerance test. Blood glucose was measured in awake mice by tail bleeding at 0, 15, 30, 60, 90, and 120 minutes after injection. For glucose tolerance tests, mice received an intraperitoneal injection of glucose (2 mg/kg body weight). For the insulin tolerance tests, mice received an intraperitoneal injection of insulin (1 U/kg body weight) (17).

Histology

Mice tissues were fixed in 4% PFA overnight, dehydrated in 100% ethanol, and embedded in paraffin for sectioning (5 µm). BAT was stained with hematoxylin and eosin and stained for immunohistochemistry against PECAM1 (DIA-310; Dianova), TH (AB152; Millipore) antibodies. Negative controls where the primary antibody was omitted were included to exclude non-specific binding.

Cell culture

Neonatal Rat Cardiomyocytes (NRCMs) were isolated from 1- to 2-day-old heart pups of Sprague-Dawley rats using a cardiomyocyte isolation kit (ThermoFisher Scientific). Cardiomyocytes were cultured for 24 h before adding the cardiomyocyte cell culture growth supplement. Cells were then serum starved for 2 hours and treated with BMP3b for 30 minutes. To inhibit BMP signaling, NRCMs were pre-treated with K02288 (1µM) for 30 minutes.

Determination of apoptosis

Heart samples were collected 24 hours after reperfusion, fixed with 4% paraformaldehyde, and embedded in paraffin. DNA fragmentation was detected with the use of TUNEL assay (In Situ

Cell Death Detection Kit, Roche) according to the manufacture's instructions. DAPI-staining was used for nuclear staining. Nuclear density and TUNEL-positive nuclei were counted by two blinded investigators (JT and LG) examining the same fields. Three fields from seven sections were examined for each heart.

In vitro, the apoptosis was evaluated by measuring the Caspase 3/7 activity on NRCMs cells with the Caspase-Glo® 3/7 assay kit (Promega), according to manufacturer's guidelines.

Gene expression analysis

The interscapular brown adipose tissue (BAT), white adipose tissue (WAT), and left ventricle were excised immediately after euthanasia, and stored at -80°C for subsequent procedures. Total RNA was isolated from tissues using TRIzol® reagent (Life Technologies) and complementary DNA was synthesized using High Capacity cDNA Archive kit (Thermo Fisher Scientific). Quantification of mRNA levels was performed by real-time PCR. PrimeTime® qPCR probe assays (Integrated DNA Technologies) were used for *Bmp3b* (Mm.PT.58.31156962) and *Ucp1* (Mm.PT.58.7088262). TATA-binding protein (*Tbp*) (Mm.PT.39a.22214839) was used as endogenous controls.

Immunoblotting analysis

Total proteins were extracted in RIPA buffer containing protease inhibitors and their concentrations were determined using the BCA Protein Assay kit. Protein extracts were resolved by SDS-PAGE and transferred to Immobilon PVDF membranes. Blots were incubated with antibodies against phospho-Smad2/3 (#8828), total-Smad2/3 (#5678), phospho-Smad1/5 (#9516), total-Smad1 (#6944) β-actin (#3700) and Gapdh (#2118) all of them purchased from CST, and Ucp1 (#NB100-2828; Novus), Bmp3b (PA5-70041; Thermo Fisher). Detection was performed using Supersignal detection system.

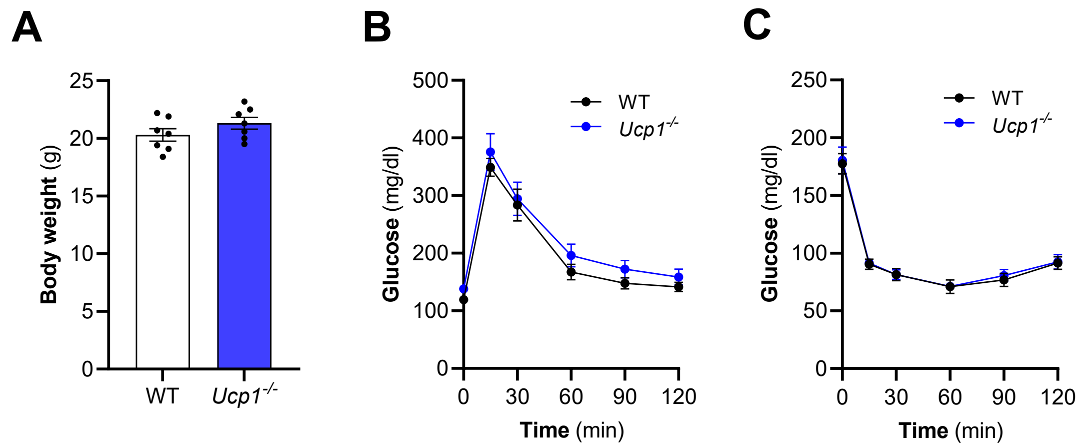
ELISA

Mice plasma was collected 24 hours after reperfusion for cardiac troponin I (cTnI) (CTNI-1-HSP; Life Diagnostic, Inc) and Bmp3b (#IT5716; GBiosciences) measurement following manufacturer's protocols.

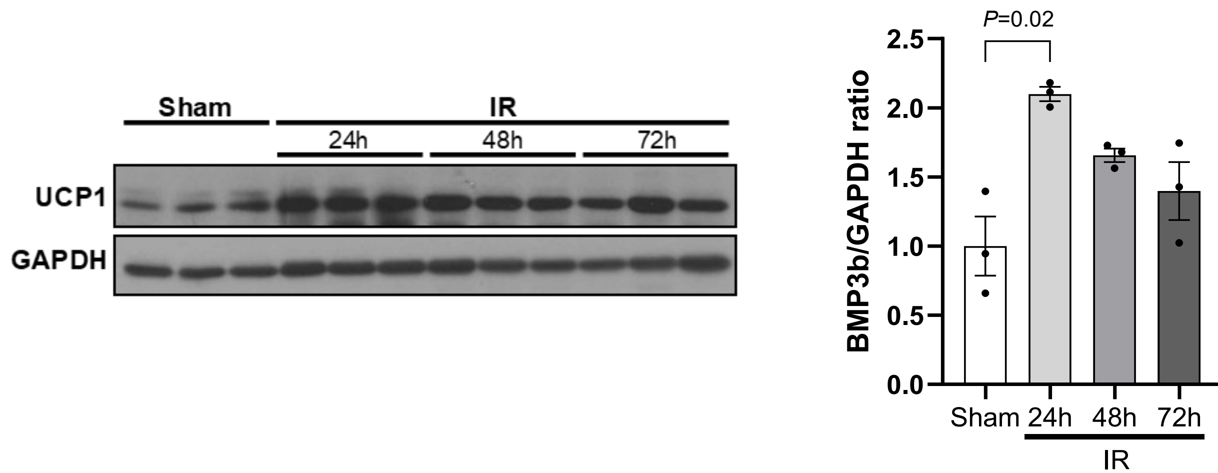
Levels of BMP3b were measured in plasma from patients undergoing alcohol septal ablation procedure according to the manufacturer's instructions (LS-F26940-1; LifeSpan Biosciences, Inc.). Troponin T was measured as recommended by the manufacturer. The plasma samples were obtained 1 hour before alcohol ablation and 1 hour after alcohol ablation procedure.

Statistics

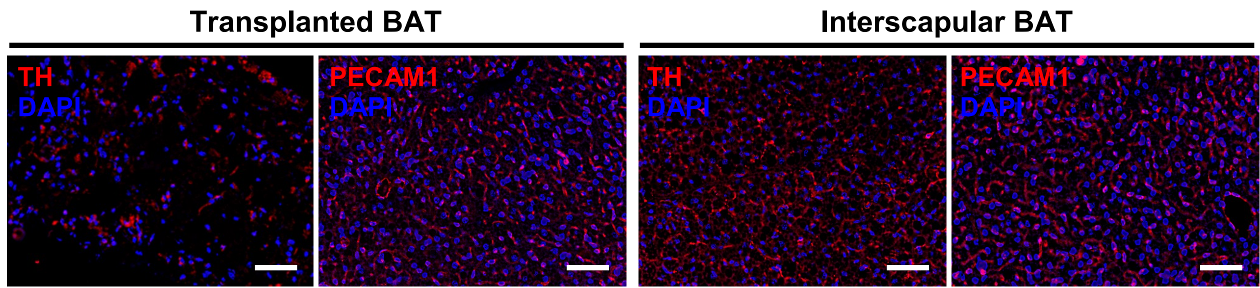
Statistical data analysis was performed using GraphPad Prism. For the comparison between two genotypes, significant differences were established by unpaired Student's t-test. For the comparison between three conditions or more, we used one-way ANOVA followed by Tukey correction for multiple comparisons. For the analysis of changes over time, repeated measures two-was ANOVA followed by Tukey correction was applied. When two independent variables were studied, a two-way ANOVA followed by Tukey correction for multiple comparisons was applied. To evaluate plasma human samples, we used paired t-test and Spearman correlation analysis. Data are expressed as means ± SEM, unless otherwise stated. In all experiments, differences were considered significant at $p<0.05$. Representative images on western blot and immunohistochemistry were selected based on the closest similarity with the mean value of each experimental group from all experiments performed for each experiment.

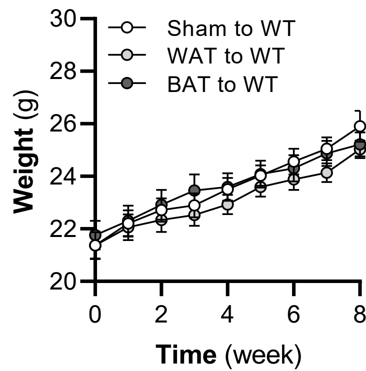


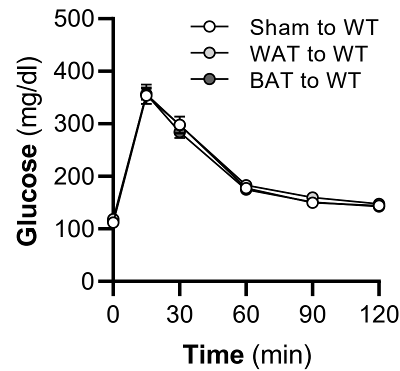
Supplemental Figure 1. **Body weight, glucose tolerance (GTT) and insulin tolerance (ITT) tests in 2-month-old wild-type (WT) and *Ucp1*-knockout (*Ucp1*$^{-/-}$) male mice.** *A*) Body weight of male WT and *Ucp1*$^{-/-}$ mice was measured (*n* = 7 mice in each group). Glucose tolerance test (*B*) and ITT (*C*) were performed on WT and *Ucp1*$^{-/-}$ mice. Mice were fasted for 6 h and injected with 2 g/kg body weight glucose (GTT) or fasted for 2 h and injected with 1 U/kg body weight insulin (ITT) (*n* = 7 mice in each group). Values expressed as means ± SE. *P* values were determined by unpaired Student *t* test (*A*) and repeated measurements two-way ANOVA with Tukey correction (*B* and *C*) .

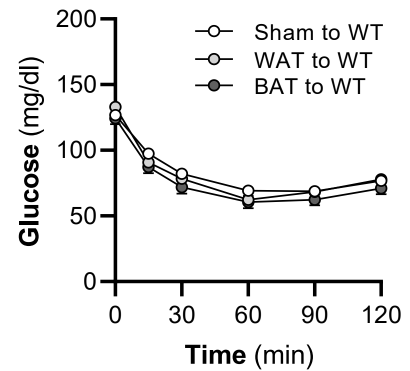


Supplemental Figure 2. Ucp1 protein expression was analyzed by Western blot in brown adipose tissues from witd-type mice that underwent sham or myocardial ischemia-reperfusion (IR) surgery with different times of reperfusion (24, 48 or 72h). Gapdh is shown as a loading control. Values expressed as means ± SE. *P* values were determined by Kruskal-Wallis test with Dunn correction.

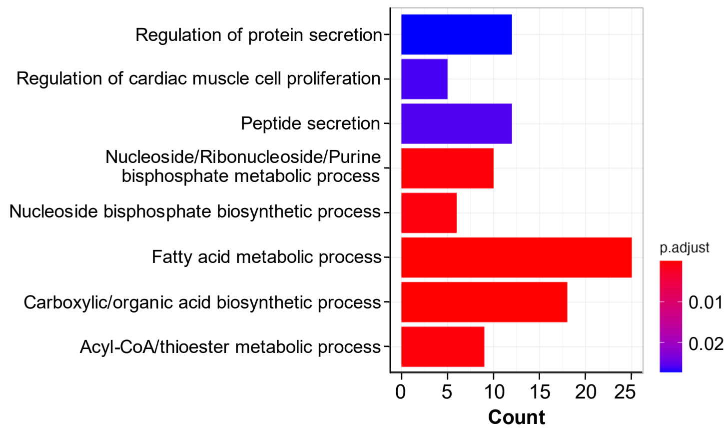


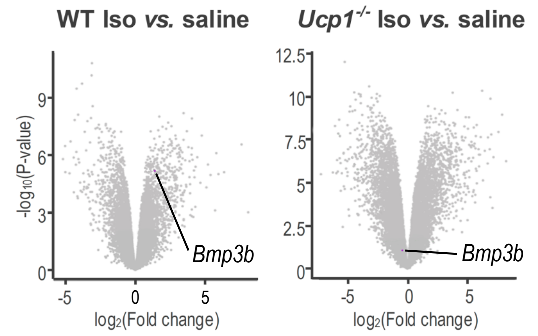




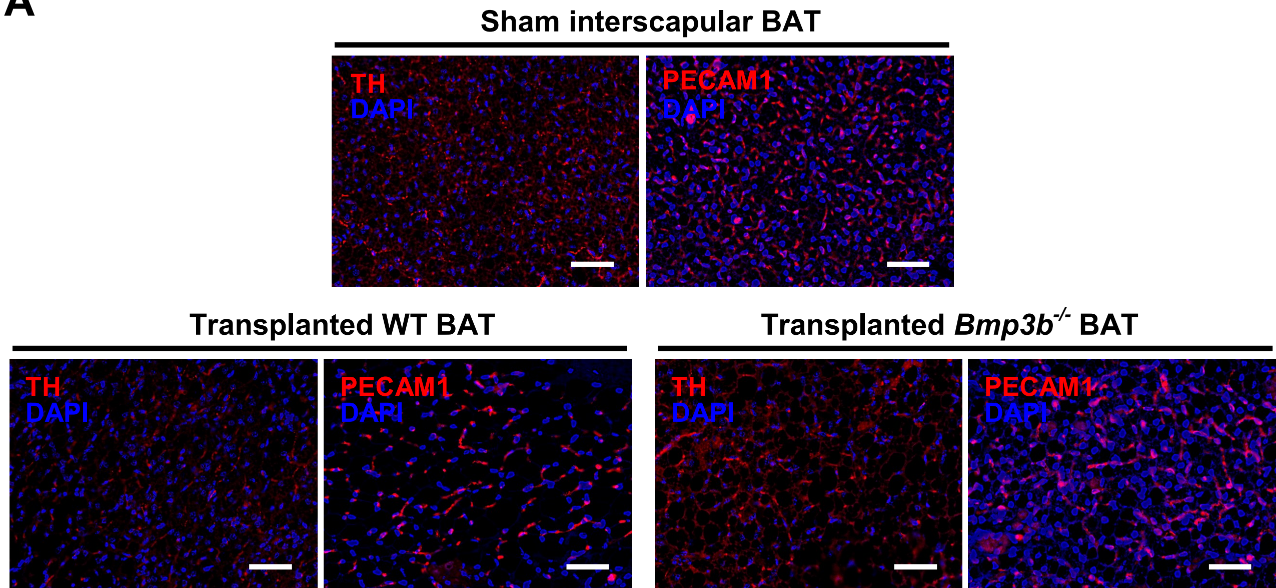


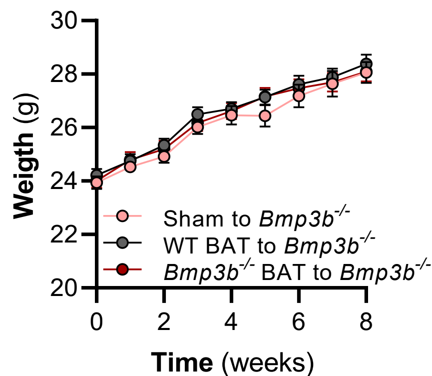
Supplemental Figure 3. **Analysis of the innervation, vascularization of transplanted brown adipose tissue (BAT), and of the metabolic effect of BAT transplantation.** *A*) Representative images of the immunohistochemistry analysis of tyrosine hydroxylase (TH to assess innervation) and platelet endothelial cell adhesion molecule (PECAM1 to assess vascularization) of transplanted BAT eight weeks after transplantation and interscapular endogenous BAT. Scale bars: 50µm. *B*) Body weight of wild-type (WT) mice that underwent a sham operation, transplantation of white adipose tissue (WAT) or of BAT from WT donors. Body weight was measured weekly (Sham to WT, *n* = 13; WAT to WT, *n* = 15; BAT to WT, *n* = 15). Glucose tolerance test (GTT) (*C*) and insulin tolerance test (ITT) (*D*) were performed 7 weeks after fat transplant surgery in male WT. Mice were fasted for 6 h and injected with 2 g/kg body weight glucose (GTT) or fasted for 2 h and injected with 1 U/kg body weight insulin (ITT). (Sham to WT, *n* = 13; WAT to WT, *n* = 15; BAT to WT, *n* = 15). Values expressed as means ± SE. *P* values were determined by repeated measurements two-way ANOVA with Tukey correction (B-D).

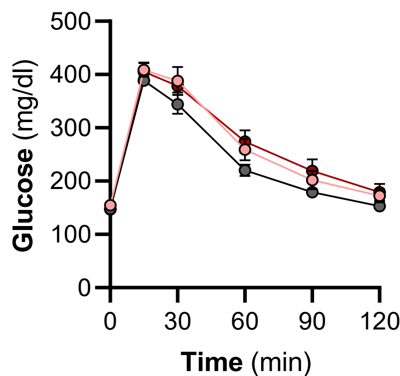


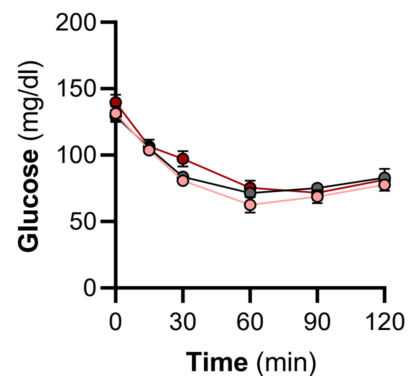


Supplemental Figure 4. *A*) Gene Ontology (GO) enrichment analysis for differentially regulated genes between isoproterenol-treated WT mice and isoproterenol-treated *Bmp3b*$^{-/-}$ mice and (*B*) the volcano plot representation. Benjamini-Hochberg Procedure for multiple test correction was applied.









Supplemental Figure 5. Analysis of the innervation, vascularization of transplanted brown adipose tissue (BAT) and of the metabolic effect of BAT transplantation, from wild-type (WT) and BMP3b deficient (*BMP3b*$^{-/-}$) donors into *BMP3b*$^{-/-}$ recipients. *Bmp3b*$^{-/-}$ mice received BAT transplant from WT or *Bmp3b*$^{-/-}$ donors, or underwent a sham procedure. *A*) Representative images of the immunohistochemistry analysis of tyrosine hydroxylase (TH to assess innervation) and platelet endothelial cell adhesion molecule (PECAM1 to assess vascularization) of transplanted BAT eight weeks after transplantation and interscapular endogenous BAT. Scale bars: 50µm. *B*) Body weight of *Bmp3b*$^{-/-}$ mice that underwent a sham operation, transplantation of BAT from *Bmp3b*$^{-/-}$ or WT donors. Body weight was measured weekly (Sham to *Bmp3b*$^{-/-}$, *n* = 15; WT BAT to *Bmp3b*$^{-/-}$, *n* = 15; *Bmp3b*$^{-/-}$ BAT to *Bmp3b*$^{-/-}$, *n* = 16). Glucose tolerance test (GTT) (*C*) and insulin tolerance test (ITT) (*D*) were performed 7 weeks after BAT transplant surgery in male *Bmp3b*$^{-/-}$. Mice were fasted for 6 h and injected with 2 g/kg body weight glucose (GTT) or fasted for 2 h and injected with 1 U/kg body weight insulin (ITT). (GTT: *n* = 13 in each group. ITT: Sham to *Bmp3b*$^{-/-}$, *n* = 9; WT BAT to *Bmp3b*$^{-/-}$, *n* = 13; *Bmp3b*$^{-/-}$ BAT to *Bmp3b*$^{-/-}$, *n* = 9). Values expressed as means ± SE. *P* values were determined by repeated measurements two-way ANOVA with Tukey correction (B-D).

Supplemental Table 1. List of genes significantly up-regulated in WT mice compared to *Ucp1*$^{-/-}$ mice after isoproterenol treatment (60 mg/kg/day during 3 days).

SYMBOL	ENTREZ ID	logFC	FC	adj.P.Val
Bmp3b	14560	1.39	2.62	3.49E-04
Sorl1	20660	2.72	6.60	3.86E-04
Crispld1	83691	1.33	2.51	4.80E-04
Pon1	18979	2.27	4.81	0.001
Postn	50706	1.03	2.04	0.001
Mfap4	76293	2.88	7.35	0.001
Cilp	214425	1.61	3.05	0.001
Atp6v0a2	21871	1.01	2.01	0.002
Orm3	18407	1.64	3.12	0.002
Muc2	17831	1.86	3.62	0.002
Fndc5	384061	2.79	6.91	0.002
Itih4	16427	2.15	4.43	0.003
Bcap31	27061	1.03	2.04	0.005
Ncan	13004	4.31	19.81	0.006
Fuom	69064	1.18	2.26	0.007
Rbp4	19662	1.05	2.06	0.007
Fgf9	14180	1.54	2.90	0.008
Col12a1	12816	1.19	2.28	0.008
Fcna	14133	2.11	4.33	0.008
Hapln4	330790	1.65	3.14	0.010
Adamts12	239337	1.08	2.11	0.010
Lpl	16956	1.35	2.54	0.010
Otop1	21906	1.23	2.35	0.017
Zpbp2	69376	1.43	2.70	0.019
Fbn1	14118	1.02	2.03	0.020
Fmod	14264	1.62	3.07	0.020
C3	12266	1.11	2.16	0.036
Slit2	20563	1.15	2.21	0.036
Mmp9	17395	1.28	2.43	0.048
Tpsb2	17229	1.22	2.33	0.048