Supplemental Methods

Animals

Male animals on a C57BL/6 background were used in this study. Mice were maintained on a 12hr light/dark cycle in a temperature-controlled environment and given free access to water and food. *Pdgfrb*^{rtTA} transgenic mice (C57BL/6-Tg(Pdgfrb-rtTA)58Gpta/J; JAX028570; RRID: IMSR_JAX:028570), TRE-Cre (B6.Cg-Tg(tetO-cre)1Jaw/J; JAX006234; RRID:IMSR_JAX:006234) and *Il33*$^{flox/flox}$-eGFP (C57BL/6(Il33)tm1.1Bryc; JAX030619; RRID:IMSR_JAX:030619) were obtained from Jackson Laboratories and previously described (Vishvanath et al. 2016; Mahlakoiv et al. 2019; Spallanzani et al. 2019).

Rodent diets and drug treatments

Mice were maintained on a standard rodent chow diet or chow diet containing 600 mg/kg doxycycline (DOX) (Bio-Serv, #S4107). For cold exposure, mice were housed at room temperature until 8 weeks of age and then switched to cold housing chambers (6ºC) for the indicated times. For thermoneutrality experiments, mice were housed for 2 months in thermoneutral housing chambers (30ºC) beginning at the age of 6 weeks. For isoproterenol administration, mice were injected i.p. with vehicle (PBS) or isoproterenol (10 mg per kg body weight; Sigma, #I6504) and sacrificed after 1.5 hours. For methionine-enkephalin peptide (MetENK, Sigma, #82362172) administration, mice were subcutaneously injected with vehicle (PBS) or MetENK (10 mg per kg body weight) daily during the 7-day cold exposure.

Indirect Immunofluorescence assays

For IL-33 staining, indirect immunofluorescence assay of CD31 and IL-33 expression was performed for determining the localization of IL-33-expressing cells. Antigen retrieval of the rehydrated sections was performed using Antigen Retriever 2100 (Electron Microscopy Sciences) and R-Buffer A pH 6.0 solution (Electron Microscopy Sciences, #62706). Antibodies and concentrations used for immunofluorescence assays include: goat anti-IL-33 1:500 (R&D, #AF3626); rabbit anti-CD31 1:500 (Abcam, #ab28364); donkey anti-rabbit Alexa flour 488 1:200 (Invitrogen, #A21206); donkey anti-goat Alexa flour 647 1:200 (Invitrogen, #A21447). Confocal images were captured using Zeiss LSM880 Airyscan system at the Live Cell Imaging Core at UTSW. Bright-field images were acquired using a Keyence BZ-X710 microscope.

Reverse transcription and quantitative PCR

cDNA was synthesized using random hexamer primers (ThermoFisher Scientific, #N8080127) and M-MLV reverse transcriptase (ThermoFisher Scientific, #28025013) according to the manufacturer's instructions. Relative levels of mRNAs were determined by quantitative real-time PCR using SYBR Green PCR system (Applied Biosystems) and *Rps18* were used as an internal control for calculation using the $\Delta\Delta$-Ct method. All primers sequences used in this study are listed in Supplementary Table 5.

RNA-seq analysis

Reads with phred quality scores less than 20 and less than 35 bp after trimming were removed from further analysis using trimgalore version 0.4.1. Quality-filtered reads were then aligned to the mouse reference genome GRCm38 (mm10) using the HISAT (v 2.0.1) (Kim et al. 2015) aligner using default settings and marked duplicates using

Sambamba version 0.6.6 (Tarasov et al. 2015). Aligned reads were quantified using 'featurecount' (v1.4.6) (Liao et al. 2014) per gene ID against mouse Gencode version 20 (Frankish et al. 2019). Differential gene expression analysis was done using the R package DEseq2 (v 1.6.3) (Love et al. 2014). Cutoff values of absolute fold change greater than 2 (LogFC>1or <-1) and FDR <= 0.01 were then used to select for differentially expressed genes between sample group comparisons. Unadjusted and adjusted p values of all comparisons are provided in Supplementary tables 1-4. All RNA-seq data have been deposited to GEO.

Isolation of adipose stromal vascular fraction and flow cytometric assays

The stromal vascular fraction of iWAT was prepared as previously described (Peics et al. 2020). Briefly, minced inguinal fat depots were incubated for 1.5 hours in digestion buffer (1×Hank's Balanced Salted Solution, 1.5% bovine serum albumin, and 1 mg/ml Collagenase D [Roche, #11088882001]) at 37°C within a shaking water bath. The digested mixture was sequentially filtered through a 100 μm cell strainer and a 40 μm cell strainer. The filtered SVFs were shortly incubated in 1 ml 1×RBC lysis buffer (eBioscience, #00-4300-54) and then resuspended in blocking buffer (2% FBS/PBS containing anti-mouse CD16/CD32 Fc Block 1:200). For FACS isolation of DPP4+ and DPP4- APCs, primary antibodies were added to the cells in blocking buffer for 20 min while incubating at 4°C. The cells were then washed once and resuspended in 2% FBS/PBS before sorting. FACS was performed using a BD Biosciences FACSAria cytometer at the Flow Cytometry Core Facility at UT Southwestern. The primary antibodies and the working concentrations are as following: CD45-PerCP/Cyanine5.5 1:400 (BioLegend, clone 30-F11, #103132), CD31-PerCP/Cyanine 5.5 1:400 (BioLegend, clone 390, #102420), PDGFRβ-PE 1:75

(BioLegend, clone APB5, #136006), DPP4-APC 1:200 (BioLegend, clone H194-112, #137807).

For the analysis of adipose tissue ILC2 cells, isolated SVF cells were incubated with flow antibodies for 40 minutes at 4°C. The primary antibodies and the working concentrations are as following: CD45-PerCP/Cyanine5.5 1:400 (BioLegend, clone 30-F11, #103132), CD11b-BV421 1:300 (BioLegend, clone M1/70, #101236), CD3e-BV421 1:300 (BioLegend, clone 145-2C11, #100335); ST2-PE-Cyanine7 1:100 (Invitrogen, clone RMST2-2, #25-9335-80), KLRG1-PE-eFluor 610 1:200 (eBiosciences, clone 2F1, #61-5893-80). After two washes with 2% FBS/PBS, cells were fixed by incubation with BD Cytofix (BD Biosciences, #554655) for 20 min.

For the analysis of intracellular IL-33 levels, isolated SVFs cells were first incubated with flow antibodies as described above to label DPP4+ and DPP4- APCs. After two washes with 2% FBS/PBS, cells were fixed with the incubation of 1X Fix/Perm buffer (eBiosciences, #00-5123-43, #00-5223-56) at room temperature for 30 min. After two washes with 1X Permeabilization Buffer (eBiosciences, #00-8333-56), cells were suspended in 1X Permeabilization Buffer containing IL-33-AF594 1:200 (R&D, clone 396118, #IC3626T) and incubated in 4°C for 1 hour. After twice wash with 1X Permeabilization Buffer, cells were resuspended with 2% FBS/PBS. Flow cytometry analysis was performed using a BD Biosciences LSR Fortessa. All the flow cytometry datasets were analyzed and graphed using FlowJo Version 10.5.3.

Culture of PDGFRβ+ subpopulations

Freshly isolated DPP4+ and DPP4- APCs were cultured in DMEM/F12 media containing 10% FBS and 1% Pen/Strep and gentamicin (growth media). DPP4+ or DPP4- APCs

maintained in growth media were treated with isoproterenol (ISO, 10μM; Sigma, #I6504), or CL316243 (10μM; Sigma, #C5976) for the indicated times. For the study β-adrenergic signaling inhibitors, cells were pretreated with the indicated inhibitors for 1 hour and then in combination with ISO (10μM) for an additional 4 hours. Chemical inhibitors utilized are: PKA, H-89 (10μM; Cayman Chemical Company, #10010556); β1- and β2-AR, Propranolol (10μM; Cayman Chemical Company, #23349); β1-AR, CGP-20712A (1μM; Sigma-Aldrich, #C231); β2-AR, ICI-118551 (1μM; Sigma-Aldrich, #I127-5MG).

For adipogenesis assays, freshly isolated primary cells were plated in 48-well plates at a density of $4x10^4$ cells/well and maintained in growth media at 37°C until confluency. Confluent cultures were stimulated with adipogenic induction media (growth media supplemented with 5 μg/ml insulin, 1 μM dexamethasone, and 0.5 mM isobutylmethyxanthine) for 48 hours. Then the cells were maintained in growth media supplemented with 5 μg/ml insulin (maintenance media) until harvest.

CRISPR/Cas9 gene targeting in cells

Lentiviral CRISPR plasmids targeting *Creb1* were constructed by cloning gRNAs (*Creb1* #1: 5'-TGTACTGCCCACTGCTAGTT-3'; *Creb1* #2: 5'-AATGGTAGTACCCGGCTGAG-3') into the LentiCRISPR V2 (Addgene #52961) plasmid backbone. For lentivirus production, 10 μg lentiviral CRISPR plasmids were transfected using Lipofectamine LTX (Invitrogen) into Phoenix packaging cells along with 5 μg psPAX2 (Addgene #12260) and 5 μg pMD2.G (Addgene # 12259). Viral supernatants were harvested 48 hours after transfection. For viral transduction, viral supernatants were added to primary DPP4+ APCs for 24 hours in the presence of 8 μg/ml polybrene (Sigma). After switching to virus-

free media, cells were cultured for another 24 hours before being used for the experiments described.

Immunoblotting and antibodies

Cultured cells and iWAT were lysed by homogenization in RIPA lysis buffer (ThermoFisher Scientific, #89900). Protein extracts were separated by SDS-PAGE and then transferred onto PVDF membrane (Millipore, #IPVH00010). Following overnight incubation with the indicated primary antibodies at 4ºC, membranes were incubated with IR dye-coupled secondary antibodies (LI-COR) and then visualized by the using of the LI-COR Odyssey infrared imaging system (LI-COR). The primary antibodies utilized included: anti-phospho-CREB 1:1000 (Ser133; Cell Signaling Technology, #9198); anti-CREB 1:1000 (Cell Signaling Technology, #9197T); anti-IL-33 1:1000 (R&D, #AF3626); anti-UCP-1 1:1000 (Abcam, #Ab10983); anti-β-actin 1:5000 (Sigma, #A1978); anti-α-Tubulin 1:5000 (Cell Signaling Technology, #2128T). Full-lengths scan of western blots presented in this study are in Supplemental Dataset 6.

Chromatin Immunoprecipitation

Chromatin Immunoprecipitation (ChIP) was performed as described (Shan et al. 2020). DPP4+ APCs were treated with isoproterenol (10μM; Sigma, #I6504) for 4 hours and then cross-linked with 1% formaldehyde in PBS for 10 minutes at 37 °C. Crosslinking was quenched by the addition of 125 mM glycine in PBS for 5 minutes at 4 °C. DPP4+ cells were then lysed in Farnham lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0.5% NP-40, 1 mM DTT, and Protease inhibitor cocktail [Sigma, #P8340]) to obtain nuclear material. Crude nuclear pellets were collected by centrifugation and then lysed by incubation in

lysis buffer containing 5 mM Tris-HCl pH 7.9, 1% SDS, 10 mM EDTA, 1mM DTT, and Protease inhibitor cocktail [Sigma, #P8340]. Chromatin fragmentation (200-500 bp length) was performed at 4 °C by Bioruptor 300 using the setting of 10 cycles of 30 seconds on and 60 seconds off. Soluble chromatin was diluted 1:10 with dilution buffer (20 mM Tris-HCl pH 7.9, 0.5% Triton X-100, 2 mM EDTA, 150 mM NaCl, 1 mM DTT, and Protease inhibitor cocktail [Sigma, #P8340]) and pre-cleared using Protein G Sepharose 4 Fast Flow (GE Healthcare Bio-sciences, #17-0618-01) for 1 hour at 4 °C. Pre-cleared samples were incubated overnight with the indicated antibodies at 4 °C. Antibody-protein-DNA complexes were captured by incubation with Protein G Sepharose 4 Fast Flow (GE Healthcare Bio-sciences, #17-0618-01) at 4 °C for 2 hours. Immunoprecipitated material was consecutively washed with low salt wash buffer (20 mM Tris-HCl pH 7.9, 2 mM EDTA, 125 mM NaCl, 0.05% SDS, 1% Triton X-100, and Protease inhibitor cocktail [Sigma, #P8340]), high salt wash buffer (20 mM Tris-HCl pH 7.9, 2mM EDTA, 500 mM NaCl, 0.05% SDS, 1% Triton X-100, and Protease inhibitor cocktail [Sigma, #P8340]), LiCl wash buffer (10 mM Tris-HCl pH 7.9, 1 mM EDTA, 250 mM LiCl, 1% NP-40, 1% sodium deoxycholate, and Protease inhibitor cocktail [Sigma, #P8340]), and 1x Tris-EDTA (TE). After elution (100 mM $NaHCO_3$, 1% SDS), the immunoprecipitated material was digested with RNase (Roche, #11119915001) and proteinase K (ThermoFisher Scientific, #EO0491) prior to the purification and concentration of the immunoprecipitated genomic DNA by ChIP DNA Clean & Concentrator kit (Zymo Research, #D5201). ChIP-isolated DNA was subjected to qPCR (ChIP-qPCR) or library production (ChIP-seq) using Nebnext NGS DNA Library Preparation for Illumina kit (New England BioLabs, #E7645). Sequencing was performed

with Illumina NextSeq 500 Mid Output (130M) by the UT Southwestern McDermott Center Next Generation Sequencing Core.

ChIP-seq analysis

Trimgalore version 0.4.1 (Martin, 2011) was used to remove adapter sequences and to remove reads shorter than 35bp or with phred quality scores less than 20. Trimmed reads were then aligned to the mouse reference genome (GRCm38/mm10) using default parameters in BWA version 0.7.12 (Li and Durbin 2009). The aligned reads were subsequently filtered for quality and uniquely mappable reads were retained for further analysis using Samtools version 1.3 (Li et al. 2009) and Sambamba version 0.6.6 (Tarasov et al. 2015). Library complexity was measured using BEDTools version 2.26.0 (Quinlan and Hall 2010) and meets ENCODE data quality standards (Landt et al. 2012). Relaxed peaks were called using MACS version 2.1.0 (Feng et al. 2012) with a p-value of $1x10^{-2}$.

Cellular respiration measurements

Cellular respiration was measured using a Seahorse XFe24 Extracellular Flux Analyzer (Agilent, Santa Clara, CA, USA). In brief, mitochondrial stress tests were performed according to a manufacturer-recommended BOFA (basal-oligomycin-FCCP-antimycin A/rotenone) protocol. *Ex vivo* mitochondrial function was assessed using whole adipose tissue (5-10 mg). For tissues, a standard Seahorse basal medium (Agilent Seahorse XF Base Medium, # 102353-100) supplemented with glucose (7 mM), pyruvate (1 mM), and glutamine (2 mM) as substrates was utilized, and oligomycin (2 μM), FCCP (8 μM), and antimycin A (10 μM)/rotenone (3 μM) were added. Oxygen consumption rates (OCRs) and extracellular acidification rates (ECARs) were recorded.

Statistics and Reproducibility

No statistical method was used to predict sample sizes. We determined samples sizes based on prior experience and reported experimental designs. No randomization or blinding was performed to allocate the samples. No criteria of inclusion or exclusion of data were used. Statistical analysis was carried out as indicated in figure legends and Supplemental Table 4. All data are presented as the mean + SEM unless otherwise indicated in figure legends. Data variance was examined by *F*-test or Bartlett's test. The data meet the assumptions of the indicated statistical analysis. All tests were performed as two sided. *P* value less than 0.05 was considered statistically significant. All statistical analyses were performed using Microsoft Excel or GraphPad Prism 8.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Data Availability

All animal models, reagents, and datasets are freely available to investigators upon reasonable request. Bulk RNA-seq and ChIP-seq data have been deposited to Gene Expression Omnibus under the accession numbers GSE165974, GSE169669, GSE169672.