Supplementary Figures:

**Fig. S1**

(A) Gating strategy of the flow cytometric analysis of the eosinophils isolated from iWAT or bone marrow (BM).

(B) Gating strategy of the flow cytometric analysis of the eosinophils isolated from iWAT or bone marrow (BM).

**Fig. S1.** The eosinophils are analyzed by flow cytometry.

(A-B) Gating strategy of the flow cytometric analysis of the eosinophils isolated from iWAT or bone marrow (BM).
Fig. S2. The strategies to generate cell-specific knockout of Ngf^fl/fl and functional characterization.

(A) Schematic graph showing the strategy for generating Ngf^fl/fl conditional knockout mice. E, exon.

(B) Deletion of Ngf exon 4 in immune cells in Vav^Cre;Ngf^fl/fl mice, determined by qPCR of genomic DNA isolated from inguinal lymph node (iLN). n=6 for Ngf^fl/fl and n=5 for Vav^Cre;Ngf^fl/fl group.

(C) The mice housed at thermoneutral condition were subjected to cold challenge for 6 days. Salivary glands were collected for RNA extraction.
The wildtype mice were housed at thermoneutral or cold for 36 hours. The CD45<sup>−</sup>CD31<sup>+</sup> endothelial cells, CD45 CD31<sup>−</sup> stromal cells, and adipocytes were isolated and analyzed for Ngf expression. n=3 mice.

Wildtype bone marrow cells were transferred to lethally irradiated Vav<sup>Cre</sup>;Ngf<sup>fl/fl</sup> mice and their littermate Ngf<sup>fl/fl</sup> controls. Eight weeks post transplantation, the recipient mice were subjected to thermoneutral or cold conditions for six days. The expression of beiging-associated genes in iWAT was shown. n=6 for TN Ngf<sup>fl/fl</sup> and n=5 for each of the rest group.

The mice housed at thermoneutral condition were subjected to cold challenge for 6 days. The iWAT were processed for the whole-mount immunostaining and volume fluorescence imaging at 12.6× magnification on the lightsheet microscope. The 3D-projection images of CD31 (F) stained with the anti-CD31 antibody were shown from a depth of 500 µm. The vasculature was quantified and shown (G). n=3 mice for each group.

Schematic graph showing the strategy for generating Epx<sup>CreERT2</sup>conditional knockout mice. E, exon.

The mice with indicated genotypes were subjected to tamoxifen treatment for 5 days, Ai32 reporter expression was determined by flow cytometric analysis in bone marrow eosinophils (CD11b<sup>+</sup>Siglec-F<sup>+</sup>) 11 days post treatment. Data are presented as mean ± s.e.m. P values were calculated by: two-tailed unpaired t-test (B, D); two-tailed unpaired Welch’s t-test (C); two-way ANOVA and Tukey’s post hoc test (E, G). ns (non-significant) p> 0.05, *p≤ 0.05, **p≤ 0.01, ***p≤ 0.001, ****p≤ 0.0001.
Fig. S3. Sympathetic nerves innervate human adipose tissues.

(A) Human adipose tissues were dissected from various locations (subcutaneous and pelvic wall), and subjected to whole-mount immunostaining and volume-fluorescence imaging. The 3D-projection images TH staining were shown with a depth of 1000 µm.

(B) Human adipose tissues were dissected from various locations (epiploic appendices, omental, pelvic, and abdominal wall) and subjected to whole-mount immunostaining and volume fluorescence imaging. The 3D-projection images of co-staining of Tuj1 and TH were shown with a depth of 500-600 µm.
Fig. S4. Sympathetic nerves are the predominant nerve subtype innervating human adipose tissues.

(A) Human adipose tissues were dissected from various locations (epiploic appendices, omental, pelvic, and subcutaneous), and subjected to whole-mount immunostaining and volume-fluorescence imaging. The 3D-projection images of Tuj1 and VACHT stained with the respective antibodies were shown with a depth of 500 µm.
Fig. S5. IL-33 promotes intra-adipose axonal outgrowth.

(A) Schematic graph showing the strategy for generating $\text{Il33}^{/-}$ mice. E, exon.

(B) Verification of IL-33 deficiency in $\text{Il33}^{/-}$ mice determined by qPCR of mRNA in iWAT. n=3 mice.
(C) Expression levels of the beiging-related genes in iWAT of the indicated genotypes post cold stimulation were determined by the qPCR analysis. n=4 for WT TN group, n=8 for WT cold group, n=3 for Il33−/− TN group, and n=9 for Il33−/− cold group.

(D and E) Appearance of multilocular beige cells in iWAT with indicated genotypes post cold stimulation was examined by HE staining. The percentage of beige cells among adipocytes was shown (E). n=4-6 lobules.

(F-J) The wildtype mice housed at thermoneutral conditions were administered with recombinant IL-33 through intraperitoneal injection.

(F and G) The immune cell frequency was assessed by flow cytometric analysis 3 days after IL-33 treatment. The proportion of CD45+Ter119− immune cells in live cells was quantified (F). The eosinophils were identified as CD11b+Siglec-F+ and the proportion was quantified in CD45+Ter119− immune cells (G). n=6 mice for each group.

(H and I) The iWAT from the indicated treatments for 6 days were processed for the whole-mount immunostaining and volume fluorescence imaging. The 3D-projection images of Siglec-F (H) were shown with a depth of 500 µm. The cell numbers were quantified (I). n=5 mice for each group.

(J) The iWAT from the indicated treatments were homogenized and the levels of NGF protein were determined by ELISA. n=5 mice for PBS group, n=6 for IL-33 group.

(K and L) The iWAT from the indicated treatments were processed for the whole-mount immunostaining and volume fluorescence imaging. The 3D-projection images stained with anti-TH and STMN2 (K) antibodies were shown with a depth of 500 µm. The nerve fiber length was quantified for TH and STMN2 (L). For TH staining, n=5 mice for PBS and IL-33 groups. For STMN2 staining, n=6 mice for PBS group, n=2 for IL-33 group.

Data are presented as mean ± s.e.m. P values were calculated by: two-tailed unpaired Welch’s t-test (B, I); two-way ANOVA and Tukey's post hoc test (C); two-tailed unpaired t-test (E, F, G, J, L). ns (non-significant) p> 0.05, *p≤ 0.05, **p≤ 0.01, ***p≤ 0.001, ****p≤ 0.0001.
Fig. S6. The Irl1l−/− knockout mouse line is generated by CRISPR/Cas9 strategy.

(A) Schematic graph showing the strategy for generating Irl1l−/− mice. E, exon.

(B) Verification of Irl1l−/− deficiency determined by qPCR of mRNA in iWAT. n=4 for wildtype group, n=6 for knockout group. Data are presented as mean ± s.e.m. P values were calculated by two-tailed unpaired Welch’s t-test (B). ns (non-significant) p> 0.05, *p≤ 0.05, **p≤ 0.01, ***p≤ 0.001, ****p≤ 0.0001.

Supplementary Video 1. Whole-mount immunostaining and volume fluorescence imaging of sympathetic nerves and immune cells in iWAT.
The iWAT of the wildtype mice were processed for anti-TH (green) and anti-CD45 (red) co-immunostaining, and imaged at 12.6× magnification on the lightsheet microscope. Image stacks were used to reconstruct the video.

Supplementary Video 2. Whole-mount immunostaining and volume fluorescence imaging of sympathetic nerves and eosinophils in iWAT.
The iWAT of the wildtype mice were processed for anti-TH (green) and anti-Siglec-F (red) co-immunostaining, and imaged at 12.6× magnification on the lightsheet microscope. Image stacks were used to reconstruct the video.

Supplementary Video 3. Whole-mount immunostaining and volume fluorescence imaging of sympathetic nerves in human adipose tissue.
The human pelvic wall adipose tissue was processed for anti-TH (green) immunostaining, and imaged at 12.6× magnification on the lightsheet microscope. Image stacks were used to reconstruct the video.
Supplementary Video 4. Whole-mount immunostaining and volume fluorescence imaging of sympathetic nerves and IL-33 in iWAT.
The iWAT of the wildtype mice were processed for anti-TH (green) and anti-IL-33 (red) co-immunostaining, and imaged at 12.6× magnification on the lightsheet microscope. Image stacks were used to reconstruct the video.

Supplementary Video 5. The time-lapse imaging of intracellular calcium in adipose stromal cells.
Time lapse fluorescence images of adipose stromal cells loaded with Rhod-2 without stimulation. Scale bar, 20 µm.

Supplementary Video 6. The time-lapse imaging of intracellular calcium in adipose stromal cells treated with ionomycin.
Time lapse fluorescence images of adipose stromal cells loaded with Rhod-2 and treated with ionomycin. Scale bar, 20 µm.

Supplementary Video 7. The time-lapse imaging of intracellular calcium in adipose stromal cells treated with NE.
Time lapse fluorescence images of adipose stromal cells loaded with Rhod-2 and treated with NE. Scale bar, 20 µm.

Supplementary Video 8. The time-lapse imaging of intracellular calcium in adipose stromal cells treated with PE.
Time lapse fluorescence images of adipose stromal cells loaded with Rhod-2 and treated with PE. Scale bar, 20 µm.

Supplementary Methods
Bone Marrow Transplantation
For transplantation, 2 x 10^6 bone marrow cells from WT mice were intravenously injected to lethally irradiated Vav^Cre;NGF^{fl/fl} mice and their littermate NGF^{fl/fl} controls. Eight weeks postinjection, the mice were subjected to TN or cold condition for 6 d.

Flow Cytometry and Cell Sorting
Mice were euthanized using CO2. iWAT were dissected out and placed into Dulbecco’s modified Eagle’s medium (DMEM) containing 2% fetal bovine serum (FBS). The tissues were minced and mechanically dissociated. The tissues were further incubated with collagenase type I (4 mg/mL) (Sigma-Aldrich) and CaCl$_2$ (10 mM) at 37°C for 30 min with constant shaking. Single-cell suspensions were washed with DMEM + 2% FBS, filtered through a 70-μm cell strainer, and further washed.

For the analysis of bone marrow cells, the cells were flushed with 1-mL syringes for single-cell preparation. For sorting of peritoneal cells, the mice were intraperitoneally injected with 5 mL 1xPBS and the cells in the peritoneal cavity were collected with 5-mL syringes.

For flow cytometry of surface staining, cell preparations were subjected to red blood cell lysis by incubating with 0.5 to 1 mL ammonium-chloride-potassium lysing (ACK) buffer (Lonza) for 1 min at 4°C, washed, and resuspended in PBS + 2% FBS. The cells were blocked with Fc blocking antibody (BioLegend Cat no. 101302, RRID: AB_312801) and then stained with antibodies to surface markers for 30 min at 4°C. 7-aminoactinomycin D (7-AAD) (ThermoFisher Scientific) incubation was performed for 10 min at room temperature for dead cell exclusion. Antibodies included Ter119-PE/Cy7 (BD Biosciences Cat no. 557853, RRID: AB_396898), CD45-BV510 (BioLegend Cat no 103138, RRID: AB_2563061), Siglec-F-PE (BD Biosciences Cat no 552126, RRID: AB_394341), CD11b-APC (BioLegend Cat no 101212, RRID: AB_312795), Siglec-F-BV421 (BD Biosciences Cat no 562681, RRID: AB_2722581), CCR3-PE (BioLegend Cat no 144506, RRID: AB_2561534), CD45-APC/Cy7 (BioLegend Cat no 103116, RRID: AB_312981), CD90.2-BV510 (BioLegend Cat no 105335, RRID: AB_2566587), Sca1-APC (BioLegend Cat no 122512, RRID: AB_756197), KLRG1-PE/Cy7 (BioLegend Cat no 368614, RRID: AB_2728371), CD3-FITC (BioLegend Cat no 100306, RRID: AB_312671), CD4-FITC (BioLegend Cat no 100406, RRID: AB_312691), CD5-FITC (BioLegend Cat no 100606, RRID: AB_312735), CD8-FITC (BioLegend Cat no 100706, RRID: AB_312745), B220-FITC (Thermo Fisher Scientific Cat no 11-0452-82, RRID: AB_465054), CD11c-FITC (BioLegend Cat no 117306, RRID: AB_313775), CD11b-FITC (Thermo Fisher Scientific Cat no 11-0112-85, RRID: AB_464936), Gr-1-FITC (BioLegend Cat no 108406, RRID: AB_313371), TER119-FITC (BioLegend Cat no 116206, RRID: AB_313707), NK1.1-FITC (BioLegend Cat no 108706, RRID: AB_313393), CD31-APC (BioLegend Cat no 102509, RRID: AB_312916). Samples were analyzed on an LSR II (BD Biosciences) with five lasers or sorted using FACS Aria with five lasers (BD Biosciences).
**Tissue Section Immunohistochemistry**

Human adipose tissues were fixed in PBS/1% PFA/10% sucrose and then treated with PBS/30% sucrose for an additional 24 h. Cryosections were obtained and blocked in PBS/0.5% TritonX-100/5% donkey serum (blocking buffer) for 1 h and then stained with primary antibodies (1:500) for 24 h at room temperature. After being washed three times each for 5 min in PBS, the sections were incubated with indicated secondary antibodies (1:500) for 10 h. Finally, the slides were washed and mounted with mounting medium (ThermoFisher Scientific). Images were acquired on a Nikon A1RMP confocal microscope. “Advanced Denoising” was applied onto the raw data of human IL-33 staining for display purpose.

Mouse iWAT from $Epx^{\text{CreERT}}, \text{Ngf}^{+/\text{fl}}$ mice and their littermate $Epx^{\text{CreERT}}$ controls were fixed in PBS/1% PFA overnight and processed for paraffin sectioning. The sections were then deparaffinized and hydrated for UCP1 staining. After being permeabilized with 0.5% Tween-20/PBS for 30 min at room temperature, the sections were blocked, immunolabeled, and imaged following the same protocol mentioned above. The UCP1 intensity from three or more independent lobules for each iWAT was quantified by ImageJ.

**Tissue Processing and qPCR Analysis**

To determine expression levels of the genes, iWAT or salivary glands were acutely dissected from the mice at the indicated time after treatment and homogenized in 4 mL TRIzol. The total RNAs were extracted for reverse transcription and SYBR Green qPCR analysis. To determine the levels of Ngf mRNA, the WT mice were housed at TN (32°C) or cold (4°C) condition for 36 h. The iWAT from two mice were pooled and digested with type 1 collagenase. After centrifugation the floating adipocytes were collected in TRIzol for RNA extraction. The stromal-vascular fractions were stained with CD45-BV510 and CD31-APC to sort CD45$^-$CD31$^+$ endothelial cells and CD45$^+$CD31$^-$ stromal cells. After sorting, the cells were collected by centrifugation and lysed in 1 mL TRIzol for further analysis.

To confirm the knockout efficiency of $Vav^{\text{Cre}}, \text{NGF}^{+/\text{fl}}$ mice, the inguinal lymph nodes (iLNs) from the conditional knockout mice and their littermate controls were lysed in lysis buffer [50 mM Tris-HCl, 2.5 mM EDTA, 5 mM KCl, 0.45% Nonidet P-40, 0.4% Tween-20 (pH=8.0)]. The DNA was purified by phenol/chloroform and then used for qPCR analysis.
To examine the appearance of the cold-induced beige adipocytes, iWAT were fixed in PBS/1% PFA at 4°C overnight, and processed for paraffin sectioning and HE staining. The percentage of beige cells was manually counted. For each iWAT three or more independent lobules were randomly selected for the quantification.

**Primers List**

| Primer   | Forward Sequence                  | Reverse Sequence                  |
|----------|-----------------------------------|-----------------------------------|
| Ppib-F   | TGGAGAGCACCAAGACAGACA             |                                    |
| Ppib-R   | TGCCGGAGTCCGACAATG               |                                    |
| Ucp1-F   | GGAGAGAAACACCTGCCTCT             |                                    |
| Ucp1-R   | ATTGTAGGTCCCCGTGTAGC             |                                    |
| Dio2-F   | CGATTTGATGTGGCTCCCTAAA           |                                    |
| Dio2-R   | TCTGACTTTCTGCTTCGCTATC           |                                    |
| Cidea-F  | ATCACAACTGGCCTGGTTACG            |                                    |
| Cidea-R  | TACTACCCGGTGCCATTTTCT            |                                    |
| Pgc1a-F  | AGCGTGACCACACTGACAACGAG          |                                    |
| Pgc1a-R  | GCTGCATGGTTCTGAGTGCTAAG          |                                    |
| Ngf-F    | TCTATAGTGCCGACCTGAGG             |                                    |
| Ngf-R    | GGACATTTGCTATCTGTGTCG            |                                    |
| Il33-F   | ACCCAGAAAAGATATTCACTAAAA         |                                    |
| Il33-R   | CAAGCAAGGATCTCTTCTAGAAT          |                                    |
| Il5-F    | ACATTCGGCAAAAAAAGAG             |                                    |
| Il5-R    | ATCCAGGAACTGCGCTC               |                                    |
| Ngf-exon4-F | TGTTGTCTACACTCTGATCACTGCG      |                                    |
| Ngf-exon4-R | GGGCAGCTATTGGGTCAGTAG            |                                    |
| Acta-F   | GGACATCTGTTGGATTCTG             |                                    |
| Acta-R   | CACGAAAGGATAGCCACGC             |                                    |
| Il1rl1-F | TGTCTCAAGAGATCGTCTGAAGTT        |                                    |
| Il1rl1-R | TGGTGACATTCAAGATCCAGTCT         |                                    |