Supporting Information

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SI Materials and Methods

Mice aP2-nSREBP-1c transgenic males (strain 003393, C57BL/6J x SJL) were purchased from Jackson Laboratories and crossed to P8, using WT C57BL/6J females to avoid potential consequences of maternal diabetes; offspring were genotyped by PCR following established protocols. Study mice had free access to water and chow (Purina 5008). In selected experiments 10-wk-old C57BL/6J mice were fed for 16 weeks with either standard chow (Research Diets D12450B, 10% kcal fat) or HFD (Research Diets D12492, 60% kcal fat); 11-wk-old ob/ob and control littermates (Jackson Laboratories) were maintained on standard chow for 2 weeks in our facility to acclimatize prior the experiments. Sodium salicylate (4 g/kg) was incorporated into Purina 5008 chow by Harlan Teklad. Lipopolysaccharide (40 pg/g body weight, *E. coli* 0111:B4, SIGMA L3012) was injected intraperitoneally and mice were killed 2 h later. *LyzM-Cre* and *Ikk*$\beta^{Flox/Flox}$ mice on C57BL/6J backgrounds were crossed to generate *Ikk*$\beta^{\Delta mye}$ mice with myeloid-specific deletion of *Ikk*β (1). aP2-nSREBP-1c transgenic males were crossed with *Ikk*$\beta^{\Delta mye}$ females to generate Tg/*Ikk*$\beta^{\Delta mye}$ mice and control Tg/*Ikk*$\beta^{Flox/Flox}$ and WT/*Ikk*$\beta^{Flox/Flox}$ littermates. All mice were housed under alternating 12 h light and dark cycles, and experiments were conducted in accordance with the National Institutes of Health guidelines under protocols approved by the Joslin Institutional Animal Care and Use Committee.

Circulating Metabolites

Blood samples were obtained from the tails of unanesthetized mice after an overnight 16 h fast. Blood glucose concentrations were measured using a Glucometer Elite (Bayer). Serum insulin (Crystal Chem), TNFα and IL-6 (Invitrogen, Biosource), and IL-10 (R&D) were measured by ELISA in the Joslin DERC Assay Core. Serum MCP-1, leptin, and resistin were measured by Luminex (Linco).

Quantitative Real-Time RT-PCR

Total RNA was extracted from frozen, pulverized mouse tissue using RNeasy (QIAGEN); cDNA was synthesized using hexamer primers with the Advantage RT-for-PCR kit (BD Biosciences). PCR amplifications using SYBR Green or TaqMan Universal PCR Master Mix (Applied Biosystems) were normalized against 18S and TATA box binding protein (Tbp). Primers from Applied Biosystems include 18S (4310893E), TNFα (Mm00443258-m1), IL-6 (Mm00446190-m1), CXCL10 (Mm00445235-m1), IL-12α (Mm00434165-m1), IL-1Rα (Mm00434237-m1), IL-1Rβ (Mm00439622-m1), TGFβ (Mm00441724-m1), and IL-10 (Mm00439616-m1). Forward/reverse primers for other genes:

Adiponectin: 5′-CCCTCCACCCAAGGGAACT-3′/5′-TGTCATTCCAACATCTCCTGTCTC-3′
Arginase 1: 5′-CTCCAAGCCAAAGTCCTTAGAG-3′/5′-AGGAGCTGTCATTAGGGACATC-3′
F4/80(Emr1): 5′-TTTCCTCGCCTGCTTCTTC-3′/5′-CCCCGTCTCTGTATTCAACC-3′
IκBα: 5′-GGGATGGCCTCAAGAAGGA-3′/5′- TGCCAAGTGCAGGAACGA-3′
IL-1β: 5′-GCCCATCCTCTGTGACTCAT-3′/5′-AGGCCACAGGTATTTTGTCG-3′
Leptin: 5′-TCAAGCAGTGCCTATCCAGA-3′/5′-AAGCCCAGGAATGAAGTCCA-3′
MCP1: 5′-TCCCAATGAGTAGGCTGGAG-3′/5′-AAGTGCTTGAGGTGGTTGTG-3′
Mgl1: 5′-TGAGAAAGGCTTTAAGAACTGGG-3′/5′-GACCACCTGTAGTGATGTGGG-3′
Mgl2: 5′-TTAGCCAATGTGCTTAGCTGG-3′/5′-GGCCTCCAATTCTTGAAACCT-3′
Mrc2: 5′-TACAGCTCCACGCTATGGATT-3′/5′-CACTCTCCCAGTTGAGGTACT-3′
Nos2: 5′-CAGCTGGGCTGTACAAACCTT-3′/5′-CATTGGAAGTGAAGCGTTTCG-3′
PU.1: 5′-CGGATGTGCTTCCCTTATCAAAC-3′/5′-TGACTTTCTTCACCTCGCCTGTC-3′
Resistin: 5′-CCCTCCTTTTCCTTTTCTTCCTTG-3′/5′-AGACTGCTGTGCCTTCTGGG-3′
Tbp: 5′-ACCCTTCACCAATGACTCCTATG-3′/5′-TGACTGCAGCAAATCGCTTGG-3′
UCP-1: 5′-CACTCAGGATTGGCCTCTACG-3′/5′-GGGGTTTGATCCCATGCAGA-3′
Ym1/Chi3l3: 5′-AGAAGGGAGTTTCAAACCTGGT-3′/5′-GTCTTGCTCATGTGTGTAAGTGA-3′

Immunohistochemistry

Immunohistochemistry was performed using 4 μm thick formalin-fixed, paraffin-embedded tissue sections done at the Histology Core from the Joslin Diabetes Center. Immunostaining was performed at the Longwood Specialized Histopathology Core at the Brigham and Women's Hospital. Briefly, slides soaked in xylene were passed through graded alcohols and into distilled water. Slides were then pretreated with 1 mM EDTA, pH 8.0 for CD3 or with 10 mM citrate, pH 6.0 for Mac2, B220 and Caspase3 in a steam pressure cooker (Decloaking Chamber, BioCare Medical) followed by a distilled water wash as instructed by the supplier (Zymed). Subsequent steps were performed at room temperature in a hydrated chamber. Slides were pretreated with Peroxidase Block (DAKO) for 5 min to quench endogenous peroxidase activity. Each of the following steps was conducted in DAKO diluent for 1 h. For macrophage staining: 1° monoclonal rat anti-murine Mac2 (clone M3/38, Cedarlane) at 1:20,000; 2° rabbit anti-rat Ig at 1:750. For T cells staining: 1° polyclonal rabbit anti-murine CD3 antibody (CMC363, Cell Marque) at 1:1,500. For B cells staining: 1° monoclonal rat anti-murine B220 (clone RA3-6B2, BD Pharmingen) at 1:200; 2° rabbit anti-rat Ig at 1:750. For apoptosis: monoclonal rabbit anti-murine Caspase3 antibody (Cell Signaling #9664) at 1:100. For perilipin, guinea pig anti-perilipin (RDI Division of Fitzgerald, Concord, MA #RDI-PROGP29) at 1:100. Slides were washed in 50 mM Tris-Cl, pH 7.4, and detected with anti-rabbit Envision+ (DAKO). After further washing, brown immunoperoxidase staining was developed using a DAB chromogen (DAKO) and counterstained with hematoxylin (blue). For mast cells: 0.1% toluidine blue (Electron Microscopy Sciences, #22050) was applied for 20 seconds followed by washing steps with water and 95% ethanol.

Flow Cytometry

Epididymal WAT, inguinal s.c. WAT, and interscapular BAT were excised and minced into 10 mL of KRB solution containing 12.5 mM hepes pH 7.4, 120 mM NaCl, 6 mM KCl, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, 2% BSA, and 2.5 mM glucose. Collagenase II (Sigma C6885; 1 mg/mL) and DNase I (Sigma DN25; 0.2 mg/mL) were added and incubated at 37 °C for 20 min with shaking. 0.01 M EDTA was added 5 min before the end of the incubation. Larger particles were removed using 250 μm nylon

sieves and the filtrates were centrifuged at 300 *g* for 5 min to separate floating adipocytes from the stromal-vascular fraction (SVF) pellets. Adipocyte fractions resuspended in 5 mL of KRB were centrifuged a second time (300 *g* for 5 min at room temperature). The two SVF pellets were combined, washed (300 *g* for 5 min at 4 °C), resuspended in 300 μL of staining buffer (PBS containing 2% FCS) containing FcBlock (BD Biosciences), and cells were stained with the following conjugated antibodies (15 min at 4 °C in the dark): CD45-PE-Cy7, F4/80-biotin, CD40-PE, Ter119-APC, CD3e-APC, B220-APC (all from E-Bioscience), and CD11b-APC-Cy7, CD11c-APC, and NK1.1-APC (BD-Pharmingen). Biotinylated antibodies were visualized following a second incubation with SA-Alexa PE-610 (inVitrogen). Spleens were mashed in 1 mL staining buffer and centrifuged (800 *g* for 3 min at 4 °C); cells were treated with 100 μL of ACK lysis buffer (Biowhitaked; 10 min at room temperature), washed, and stained as described above. Cells from adipose tissue SVF or spleen were resuspended in 200 μL of staining buffer containing 0.2 μg/mL propidium iodide (Sigma), filtered through a 100 μm mesh, and analyzed by FACS (LSRII, BD Biosciences). Sorted macrophages (CD11b+F4/80+) analyzed by FACS (FACSAria, BD Biosciences) were collected in 0.5 mL of staining buffer. RNA was extracted with 0.5 mL TRIzol (Invitrogen) and precipitated with isopropanol.

Fat Transplantation

WAT transplantation experiments were performed as previously described (2), using 5-wk-old Tg female recipients and 5-wk-old WT female littermate donors. Briefly, recipients were anesthetized with ketamine/xylazine (100 mg/kg; Henry Schein). Donor parametrial fat pads (4 × 100–150 mg each, approximately 500 mg total) were transplanted s.c. through small incisions in the shaved back skin, 1 piece per incision. Mice were housed individually for 1 week after the surgery. To measure triglyceride content in transplanted mouse liver, pulverized frozen tissue (100-200 mg) was homogenized in 500 μL PBS. Chloroform-extracted lipid was dried under a N_2 stream, redissolved in isopropanol, and triglyceride was quantified using the L-Type TG H kit (Wako). Adipocyte area was measured using the ImageJ software.

NF-κB Assay

Nuclear proteins were isolated from liver and muscle. Electrophoretic mobility shift assays (EMSA, Promega) and ELISAs of NF-*k*B binding activity were performed as previously described (3). Oct1 activity was measured as a control transcriptional factor for all samples.

TG Content

Liver and muscle triglycerides were extracted with chloroform and quantified (L-Type TG H kit, Wako).

Statistics

Data are presented as mean ± SEM. Student *t* tests were used for statistical analysis. A probability level of $P < 0.05$ was considered to be statistically significant.

1. Arkan MC, et al. (2005) IKK-β links inflammation to obesity-induced insulin resistance. *Nat Med* 11:191–198.
2. Gavrilova O, et al. (2000) Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest* 105:271–278.
3. Cai D, et al. (2004) IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. *Cell* 119:285–298.

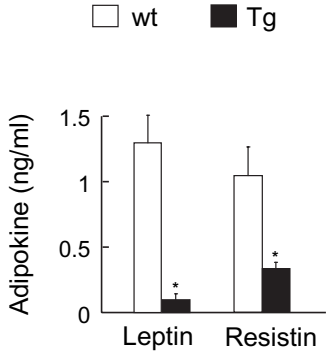

Fig. S1. Circulating adipokines. Circulating leptin and resistin concentrations in 36-wk-old male WT control (open bars) vs. Tg (filled bars) littermates ($n = 6$). *$P < 0.05$ WT vs. Tg.

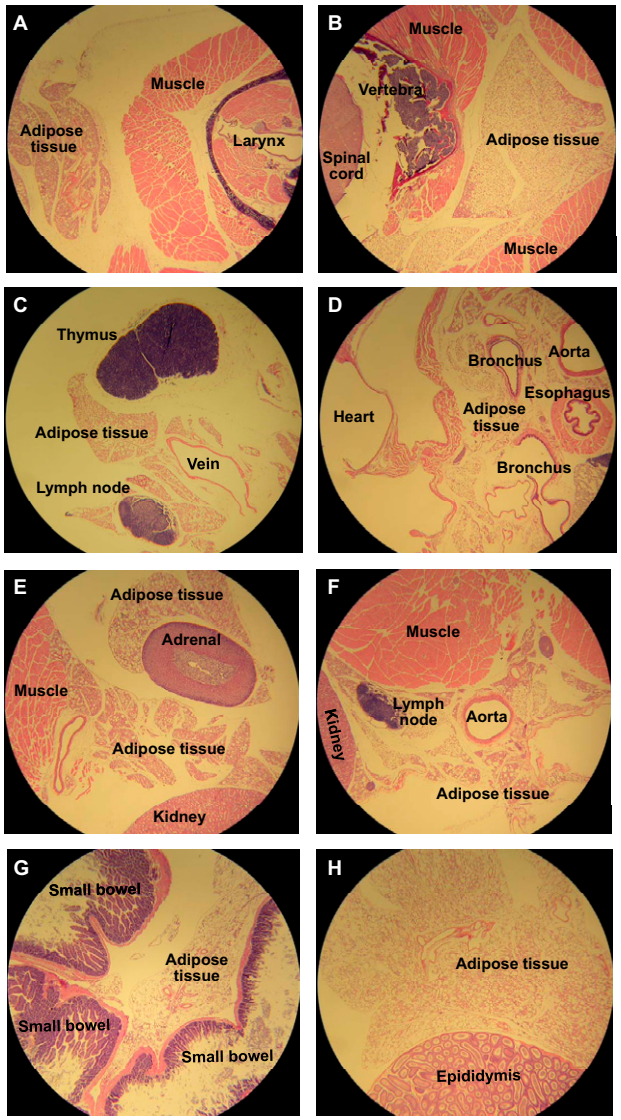

Fig. S2. Nose-to-tail histological examination. An intracardiac perfusion fixation with formalin was performed in 16-wk-old male WT control and Tg littermates. (*A–H*) Histological sections (H&E) from Tg mice from the cervical (*A, B*), mediastinum (*C, D*), retroperitoneum (*E, F*), mesentery (*G*) and pelvic (*H*) areas. The identity of the tissues in each case is specified.

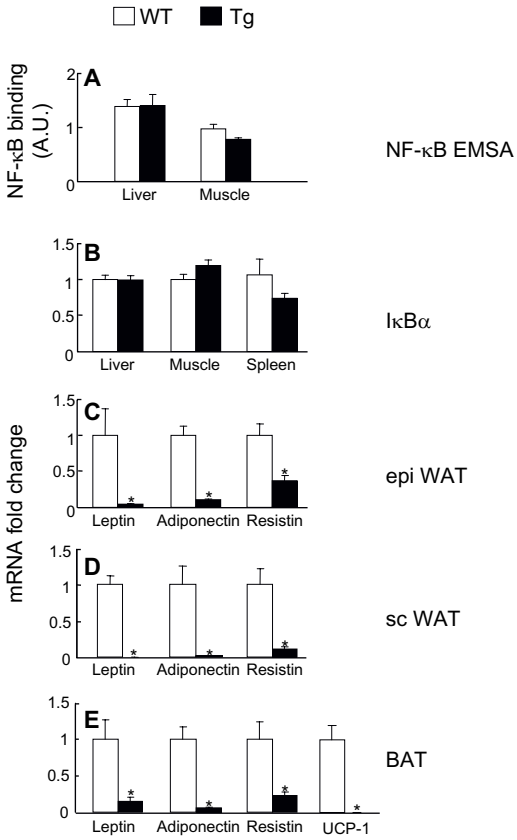

Fig. S3. NF-κB binding activity and adipose tissue gene expression. (*A*) NF-κB binding activity in liver and muscle from 18-wk-old male WT control (open bars) vs. Tg (filled bars) littermates ($n = 4$). (*B–E*) mRNA expression in liver, muscle, spleen, epididymal (epi) WAT, s.c. (sc) WAT and interscapular BAT of 18-wk-old male WT control (open bars) vs. Tg (filled bars) littermates ($n = 6$). *$P < 0.05$ WT vs. Tg.

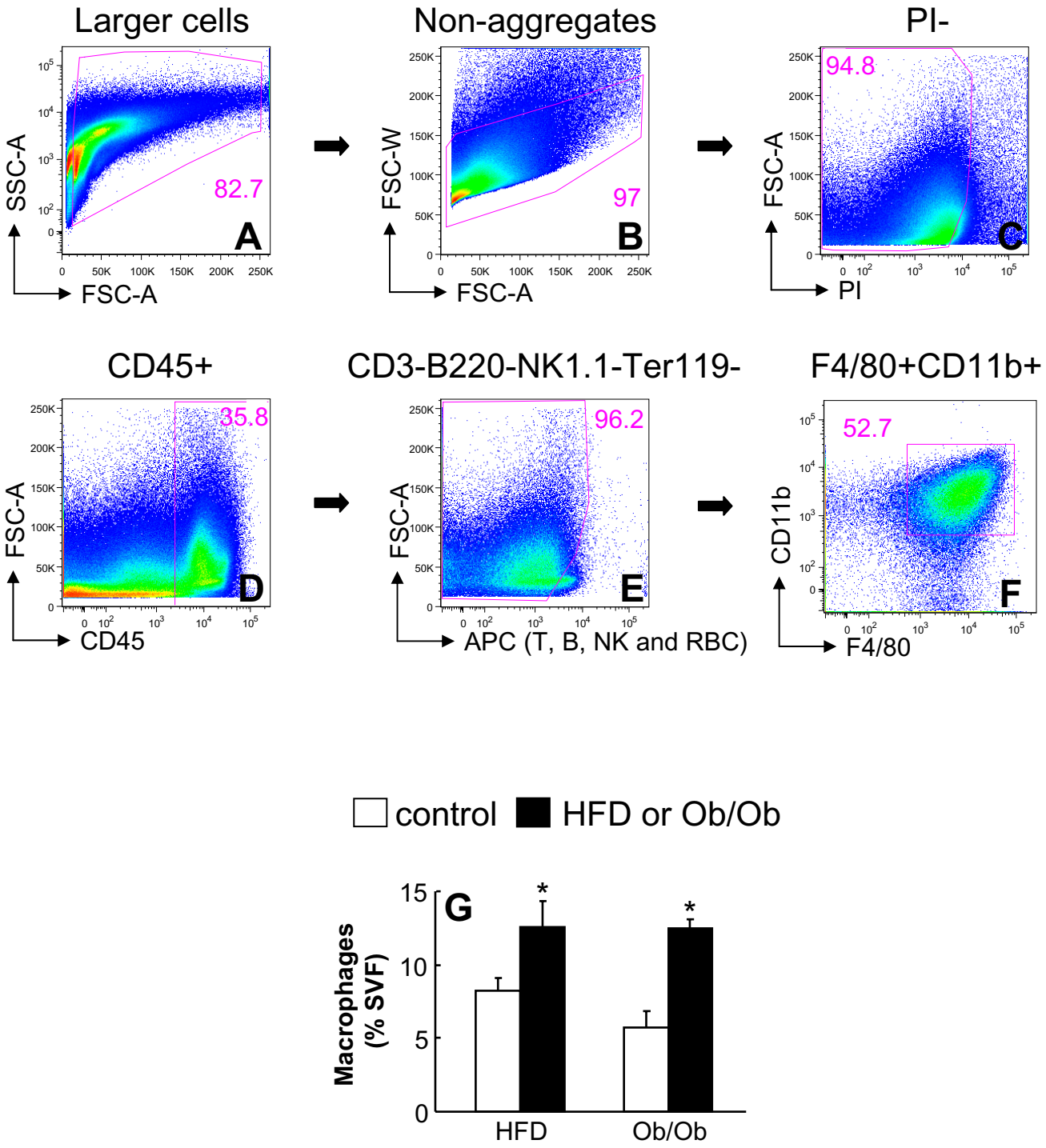

Fig. S4. Flow cytometry analyses. Stromal-vascular cells from epididymal WAT were stained with fluorescent conjugated antibodies and analyzed by flow cytometry. (*A*–*F*) Cells were gated according to forward and side scatter (to eliminate debris, cell fragments, and aggregates), propidium iodide- (PI-, viability), CD45+ (leukocytes), CD3-, B220-, NK1.1-, and Ter119- (to eliminate T, B, NK, and red blood cells, respectively) and F4/80+CD11b+ (macrophages). (*G*) Quantitation of epididymal WAT macrophage numbers from HFD-induced and ob/ob models of obesity. Six-wk-old C57BL/6J males were fed chow (control) vs. HFD for 16 weeks and killed at 22 weeks old. Controls and ob/ob male littermates were 14 weeks old ($n = 5$, $^*P < 0.05$).

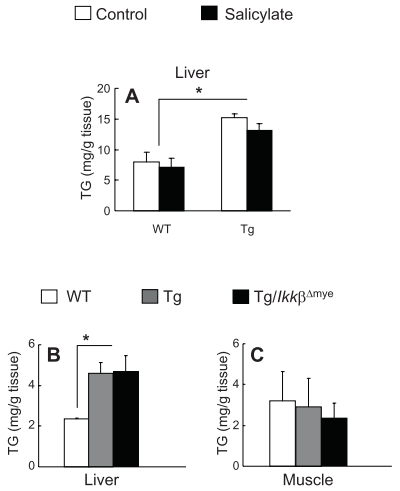

Fig. S5. Liver and muscle TG content. (*A*) Liver triglyceride content of salicylate-treated mice. WT control (open bars) and Tg (filled bars) littermates were fed normal chow (control) or chow containing 4 g/kg sodium salicylate for 5 weeks. (*B*, *C*) Liver and muscle triglyceride content in male mice with myeloid-selective deletion of IKKβ. 24-wk-old WT control (open bars), Tg (gray bars), and Tg/Ikkβ$^{\Delta mye}$ (black bars) littermates were fed normal chow. n = 4–7, *P < 0.05.

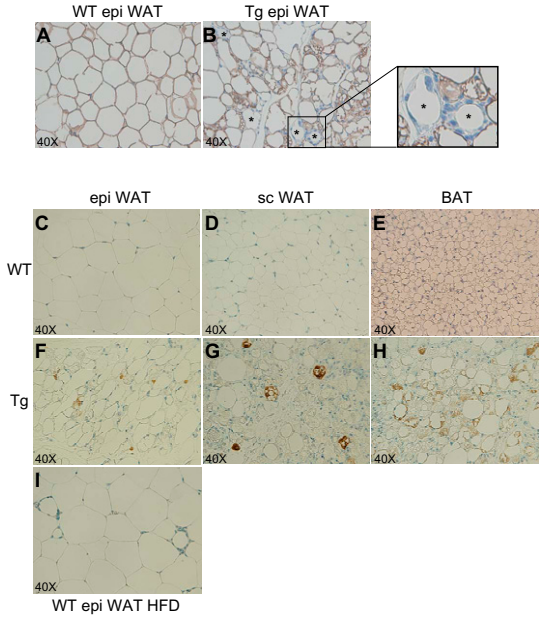

Fig. S6. Adipose tissue apoptosis. (*A*, *B*) Histological sections of epididymal WAT from 12-wk-old male WT control and Tg littermate. Sections were stained with anti-perilipin antibody followed by colorimetric detection (brown) and counterstaining with hematoxylin (blue). (*) indicates dying adipocytes with perilipin-negative lipid droplets. (*C*–*I*) Histological sections of epididymal WAT (*C*, *F*), inguinal s.c. WAT (*D*, *G*) and interscapular BAT (*E*, *H*) from a representative WT control and Tg littermates. Sections were stained with anti-caspase 3 antibody followed by colorimetric detection (brown) and counterstaining with hematoxylin (blue). Caspase-3 staining is also shown for a 22-wk-old C57BL/6J male mouse fed HFD for 12 weeks (*I*).

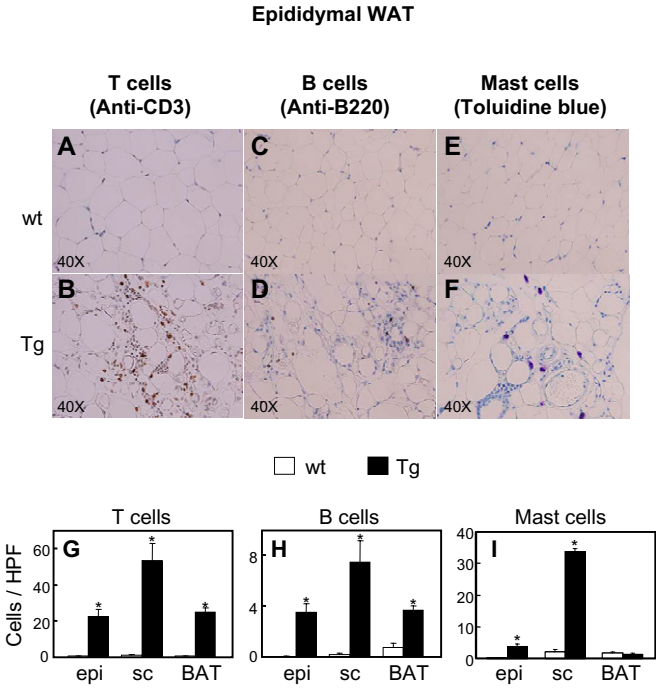

Fig. S7. Adipose tissue histology. (*A–F*) Histological sections from epididymal WAT from a representative 28-wk-old male WT control and Tg littermates stained with anti-CD3 (T cells), anti-B220 (B cells), and toluidine blue (mast cells) followed by colorimetric detection (brown) and counterstaining with hematoxylin (blue) for the case of T and B cells. (*G–I*) Counting of cells per high power field (HPF) from the above male WT control (open bars) and Tg (filled bars) littermates (10 HPF counted per mouse, $n = 5$ mice per genotype). *$P < 0.05$ WT vs. Tg.