SUPPORTING ONLINE MATERIAL

MATERIALS AND METHODS

Mice and Diet

4get (*S1*), 4get/KN2 (*S2*), YARG (*S3*), IL-5tg (*S4*), IL-4/IL-13-deficient (*S5*) and ΔdblGATA male mice (*S6*), all on the BALB/c background, or wild-type (WT) C57BL/6 or C57BL/6 *ob/ob* male mice (Jackson Labs) were maintained in the UCSF specific pathogen-free barrier according to institutional guidelines and were fed normal chow diet (Picolab Mouse diet 20 #5058) and used between 8 and 15 wk of age, unless otherwise noted. Where indicated, mice were fed high-fat diet D12451 (45% kcal fat, Research Diets, Inc.) for 15-24 wk as noted. Experiments with WT C57BL/6 mice utilized high-fat diet D12492 (60% kcal fat, Research Diets, Inc.) as noted. Unless noted, results are shown from male mice on the BALB/c background.

Cells and flow cytometry

For 'walkout' after cell dispersion (*S7*), perigonadal adipose tissues were minced in media (RPMI, 10% fetal calf serum, L-glutamine, penicillin-streptomycin) and incubated overnight at 37^{o} C in 5% CO_2. Cells were passed through 100 μm filters to generate single-cell suspensions and analyzed by flow cytometry. For isolation of an adipose stromal vascular fraction (SVF), we first confirmed that the conditions of tissue digestion did not alter the expression of surface markers used for cell identification. Perigonadal adipose was finely minced with a razor blade, dispersed by shaking in medium (low-glucose DMEM, 0.2 M HEPES, 10 mg/ml fatty acid-poor BSA (USB Corp.)) containing 1.2 U Liberase Blendzyme 3 (Roche) and 25 μg/ml DNase I (Roche) at 37^{o} C for 20 min,

and passed through 100 μm filters to generate single-cell suspensions. After retaining the cellular pellet, the adipose fraction was digested for a further 20 min, and the cellular pellets were pooled for flow cytometric analysis. Red blood cells were lysed using PharmLyse (BD Pharmingen). Cells were washed with FACS buffer (PBS, 3% FCS, 0.05% NaN_3), incubated with FcBlock and stained with the indicated antibodies. Monoclonal antibodies used for flow cytometry were APC-eFluor 780-anti-CD4 (RM4-5; eBioscience), PE-anti-Siglec-F (E50-2440; BD Biosciences), APC-anti-CD11b (M1/70; BioLegend), PE-Cy7-anti-F4/80 (BM8; eBioscience); biotin-anti-pan-NK (CD49b) (DX5; eBioscience) and biotin-anti-FcεRIα (MAR-1; eBioscience). Secondary fluorophore for biotin-conjugated antibodies was PE-Cy7-streptavidin (BD Pharmingen). Total adipose CD4+ T-cells were identified as SSC/FSC-lo, CD4+, CD3+, and negative for other lineage markers. Adipose tissue macrophages were identified as CD11b+ and F4/80+, a population that included a subset (5-10%) of contaminating eosinophils, as described (fig. S1). Eosinophils were identified as shown (fig. S1B) and were constitutively GFP+ in 4get mice. Other IL-4 competent (4get+) cells in adipose tissues (Fig 1A) from 4get mice were identified using GFP positivity, SSC/FSC profiles, and the following markers: CD4 T cells: CD4+, CD3+; basophils: DX5+, FcεRI+; innate helper type 2 cells: T/B/NK lineage-negative, c-Kit+ (*S8*); Undefined: conjugates or other positive cells without the clear combination of markers noted.

***In vivo* metabolic analyses**

DEXA scanning was performed using a PIXImus instrument according to the manufacturer's instructions (GE Healthcare). Glucose tolerance testing was performed after fasting mice overnight for 14 hr and challenging with 2 g per kg glucose by

intraperitoneal injection. Fasting blood glucose was measured after a 4 hr morning fast. Insulin tolerance tests were performed after a 4-5 hr morning fast, injecting insulin IP (0.75 mU per g human insulin (Eli Lilly)), and measuring blood glucose at the times indicated. For biochemical analysis of insulin signaling via measurement of tissue phosphorylated AKT, mice were fasted for 4 hr in the morning, anesthetized and injected in the portal vein with 5 mU per g human insulin (Eli Lilly). Liver, quadriceps muscle and perigonadal adipose tissue were isolated after 2, 5 and 5 min, respectively. Homogenized proteins were immunoblotted for total Akt and phospho-Akt (S473, Cell Signaling). Data were normalized by a ratio of pAkt/Akt total net pixels within each band. Blood glucose was measured at indicated times using a glucose meter (Bayer).

Bone marrow reconstitution and adoptive transfers

Bone marrow cells were collected from femurs and tibias of 4get x IL-5tg mice or IL-5tg x IL-4/IL-13 DKO mice and, after lysis of red blood cells, were transferred intravenously (10-15 x 10^6 cells/recipient) into sublethally irradiated (400 rad) ΔdblGATA x YARG male recipient mice. Doses were selected to enable reconstitution by transferred hematopoietic cells while preserving the viability of tissue macrophages capable of expressing the arginase-1 YFP allele. Bone marrow reconstitution was monitored by blood collections at 3 wk post-transfer and mice were euthanized for analysis of perigonadal fat 4-6 wk after bone marrow transfer.

For adoptive transfer, spleens from 4get x IL-5tg mice were homogenized, passed through a 70 μm filter, red blood cells were lysed, and single-cell suspensions were generated. To normalize numbers of transferred eosinophils, aliquots of the samples were incubated with FcBlock, stained with indicated antibodies, and Siglec-F positive

eosinophils were quantified by flow cytometry using CountBright Absolute Counting Beads (Invitrogen). Total 4get x IL-5tg spleen cells, predominantly eosinophils (50 x 10^6), were transferred to recipient mice by intravenous tail vein injection. For integrin blockade, recipient mice were pre-treated with intravenous injection of 100 μg each of anti-α_4 (PS/2, kindly provided by D. Erle, UCSF) and anti-α_L antibodies (M17/4, Bio X Cell), or isotype-matched control monoclonal antibodies, followed by cell transfer 2 hr later as described above.

Immunohistochemistry

Perigonadal white adipose tissue samples were fixed in formalin and embedded in paraffin prior to sectioning. Tissue sections were stained with hematoxylin and eosin or with anti-Siglec-F (BD Pharmingen) and biotin-goat anti-rat secondary antibody (BD Pharmingen) followed by tyramide amplification (PerkinElmer). Nuclei were counterstained with DAPI.

Separation of stromal vascular and adipocyte fractions for qRT-PCR

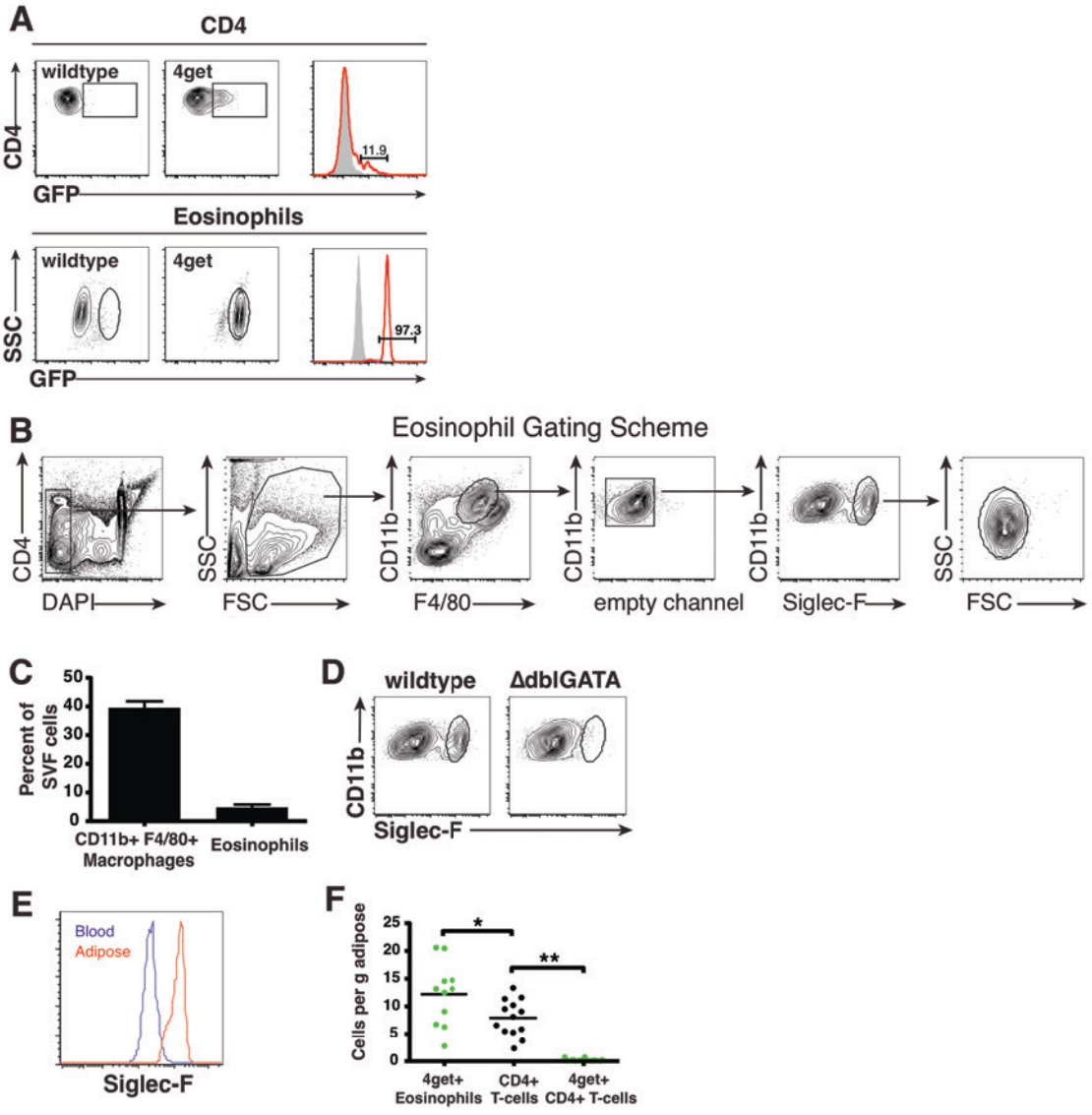
Perigonadal white adipose tissue was collected, minced, dispersed by shaking in medium (low-glucose DMEM, 0.2 M HEPES, 10 mg/ml fatty acid-poor BSA (USB Corp.)) containing 1.2 U Liberase Blendzyme 3 (Roche) and 25 μg/ml DNase I (Roche), incubated 30 min at 37 ^{o} C, and passed through 100 μm filters to generate single-cell suspensions. Samples were centrifuged at 500 g x 5 min to separate SVF (pellet) and adipocyte fractions (floating cells). Each fraction was washed and re-centrifuged at 500 g x 5 min to purify SVF and adipocyte fractions.

Quantitative RT-PCR

RNA was isolated with Trizol (Invitrogen) followed by further purification with RNeasy Plus Mini Kit (Qiagen), according to provided protocol. cDNA was reverse transcribed using Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen). Quantitative PCR was run on a Step-One Plus Real-Time PCR system (Applied Biosystems) using SYBR Green (Invitrogen). Samples were run in triplicate and were normalized to GAPDH or β-actin, as noted. Mouse IL-4 and IL-13 primers are proprietary Qiagen Quantitect qPCR primers. GAPDH primer sequences are 5'-tgaagcaggcatctgaggg-3' and 5'-cgaaggtggaagagtgggag-3'. Arg1 primer sequences are 5'-gtatgacgtgagagaccacg-3' and 5'-ctcgcaagccaatgtacacg-3'. Fizz1 primer sequences are 5'-ccatagagattatcgtggag-3' and 5'-tggtccagtcaacgagtaag-3'.

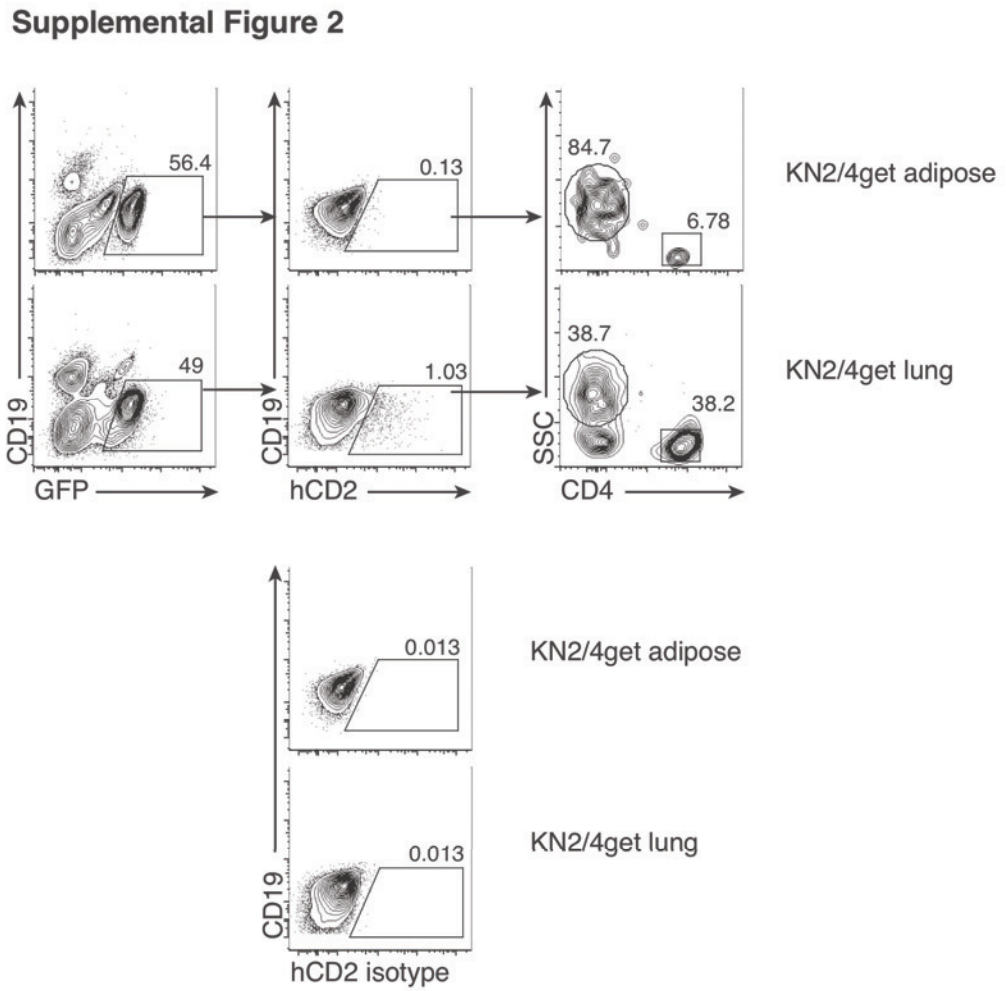
SUPPLEMENTAL FIGURES

Supplemental Figure 1



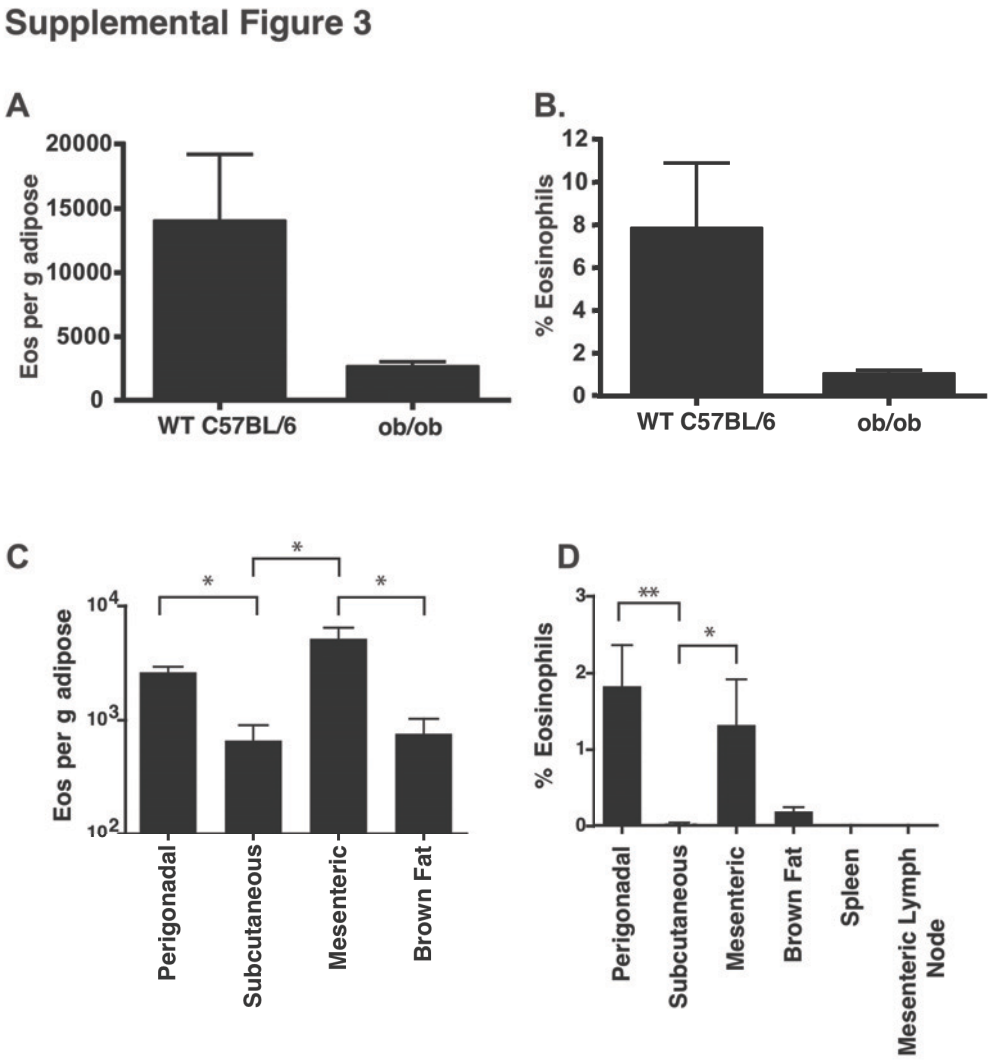
Supplemental Figure 1. Flow cytometric gating and quantitation of IL-4 expressing cells in adipose tissue. (**A**) Minced perigonadal adipose tissues from wildtype (WT) and IL-4 reporter (4get) mice were incubated overnight. 'Walkout' cells were collected and live cells were analyzed for expression of the GFP-marked IL-4 reporter. Top panels, CD4 T cells; bottom panels, side-scatter (SSC) high eosinophils. Outlines indicate GFP gates as set using cells from WT mice (left). Histograms show WT (gray) and 4get (red) cells analyzed for GFP (percentages indicated above bar for positive gate) among total recovered CD4 T cells (top) and eosinophils (bottom). **(B)** Stromal vascular fraction (SVF) cells were isolated and gated for live, DAPI-negative and side- and forward-scatter (SSC/FSC) before analysis using CD11b and F4/80 antibodies. Eosinophils are $CD11b^{int}F4/80^{int}$ and are additionally selected with an empty channel to gate out autofluorescent cells. Eosinophils are Siglec-F+ within the $CD11b^{int}$ population and constitutively express GFP in 4get mice (not shown). Backgating on Siglec-F+ cells confirmed that the cells are eosinophils with a characteristic SSC/FSC profile. **(C)** Adipose eosinophils or CD11b+ F4/80+ macrophages were gated as described above and in the supplemental methods, and results are shown as a percent of viable SVF cells. Note CD11b+ F4/80+ eosinophils account for approximately 5-10% of the "macrophage" population as gated. *$p<0.05$, **$p<0.01$ as determined using Student's t-test, error bars are SEM. (**D)** Analysis of perigonadal adipose from WT and eosinophil-deficient (ΔdblGATA) mice. Eosinophil gate outlined by Siglec-F and CD11b expression. CD11b+Siglec-F-negative cells are adipose tissue macrophages. (**E)** Siglec-F expression levels on adipose (red) and blood eosinophils (blue) from the same mouse. **(F)** Adipose 4get+ eosinophils, total CD4+ T-cells, or 4get+ CD4+ T-cells were gated as described

above and in supplemental methods, and results are shown per g adipose tissue. *p<0.05, **p<0.01 as determined using ANOVA with Bonferroni's post-test correction for multiple comparisons. Results for **(C)** and **(F)** are pooled data from three or more independent experiments.



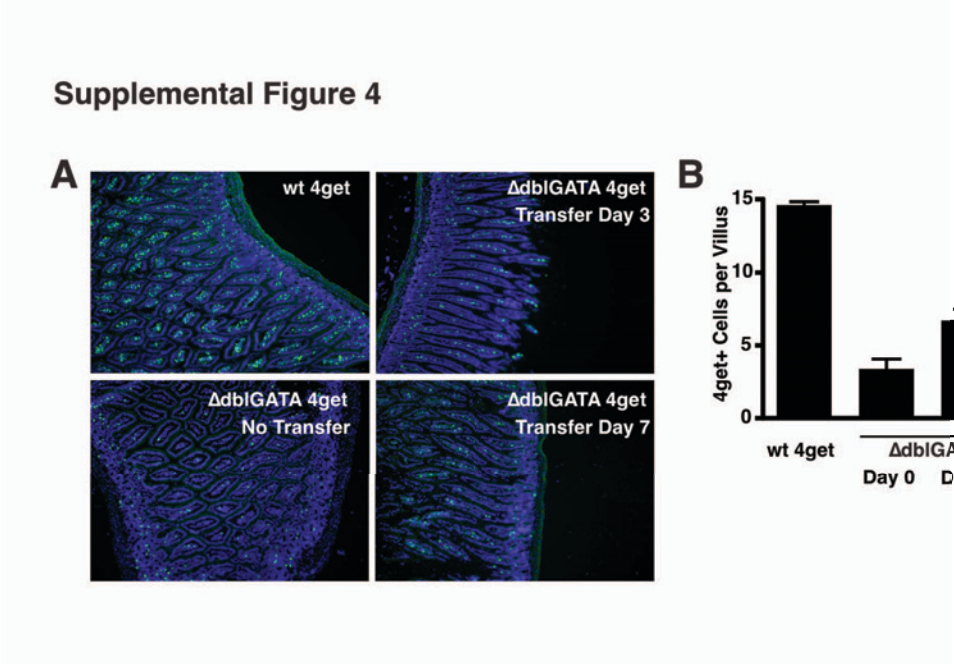
Supplemental Figure 2. Eosinophils are major IL-4-secreting cells in perigonadal adipose tissue. 4get/KN2 double heterozygous mice contain a 4get allele that marks IL-4 competency by GFP expression and a KN2 allele that contains a knock-in human *CD2*

replacement gene at the *il4* start site to mark cells that have recently secreted IL-4 protein. These mice were infected with the migratory helminth *Nippostrongylus brasiliensis* in order to induce IL-4-producing cells in the lung tissues for a positive control as previously described (*S9*). Ten days after infection, single-cell suspensions of lung and perigonadal adipose were prepared and analyzed for GFP fluorescence to assess IL-4 competence (4get+) and for human CD2 expression to assess recent IL-4 secretion as described (*S2*). Live cells were gated using DAPI exclusion and side- and forward-scatter (not shown). After gating for IL-4-competent cells based on expression of GFP (left panels; percentages of live cells shown), the positive cells were further gated based on expression of human CD2 (hCD2) as a marker for recent IL-4 secretion *in situ* (middle panels; percentages of live GFP+ cells shown). Isotype control antibody for hCD2 shown in lower panels. Gated values in right panels represent hCD2-positive cells as a percent of total hCD2-positive cells within the eosinophil and CD4 T cells gates circled. In adipose tissues, approximately 85% of IL-4-secreting cells are eosinophils with the remainder CD4 T cells, whereas in lung, eosinophils, CD4 T cells and SSC^{lo} CD4-negative cells comprise approximately equal portions of IL-4-secreting cells. Results shown are representative of two independent experiments with 2-4 animals per cohort.



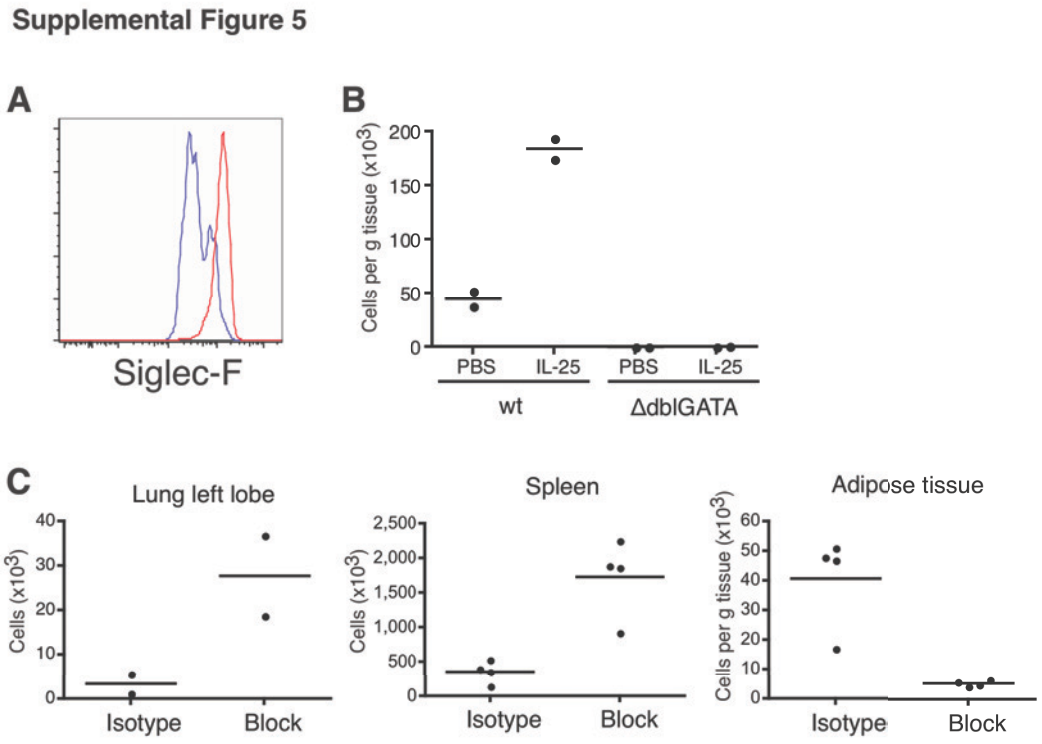
Supplemental Figure 3. Flow cytometric analysis to identify eosinophils in *ob/ob* C57BL/6 mice and in multiple adipose tissue types in BALB/c mice. (A,B) Male 8-12 wk old C57BL/6 *ob/ob* genetically obese mice on normal chow or WT C57BL/6 controls were quantitated for perigonadal adipose eosinophils per g adipose **(A)** or as a percent of SVF **(B)**. Results depict one experiment with 5 animals per cohort, and are representative of 3 independent experiments. **(C,D)** Male 4-6 month old 4get BALB/c mice on normal chow were euthanized and the indicated adipose tissues were dissected. SVF were

isolated as described for perigonadal adipose tissue and the adipose tissue eosinophil content was assayed. Results are pooled data from 2 independent experiments with 2-4 animals per cohort. *p<0.05, **p<0.01 as determined using the Kruskal-Wallis non-parametric test with Dunn's post-test correction for multiple comparisons.



Supplemental Figure 4. 4get+ eosinophils traffic to and are sustained in the small intestines. Spleen cells from 4get+ IL-5tg hypereosinophilic mice, containing 50 x 10^6 eosinophils, were transferred into ΔdblGATA mice lacking eosinophils. Intestines were collected from non-transplanted controls or collected at days 3 and 7 post-transfer, and analyzed by immunofluorescent microscopy for GFP+ cells as a surrogate marker for eosinophils. The majority of 4get+ cells within intestinal villi are Siglec F+ (data not shown), indicative of eosinophils. Slides were scored by identifying intact longitudinal sections of villi and scoring numbers of intravillar GFP+ cells. Animals were scored on duplicate slides of at least 40 villi per slide. Total percent GFP+ cells were also scored,

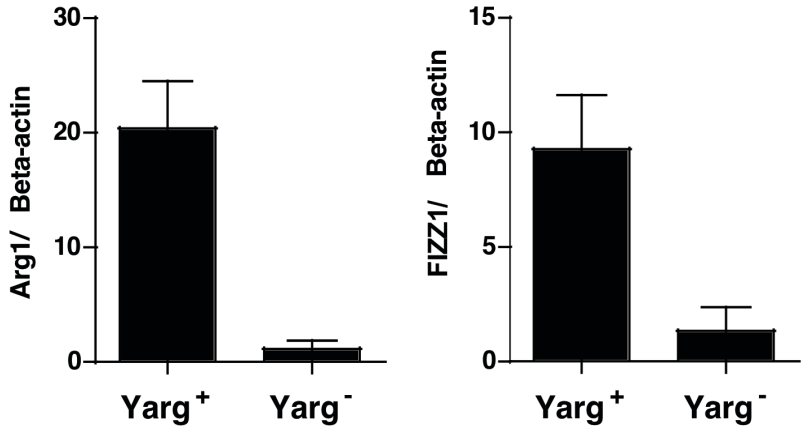
with comparable results (data not shown). Data are means +/-SEM from 2-4 animals from each group.



Supplemental Figure 5. IL-25-induced eosinophil migration to adipose tissue is integrin-mediated. (**A**) Siglec-F expression levels on adoptively transferred eosinophils isolated from adipose (red) or spleen (blue) in the same animal. (**B**) 500 ng recombinant mIL-25 was administered intraperitoneally to 8 wk-old 4get or eosinophil-deficient (ΔdblGATA) x 4get mice for 4 consecutive days (day 0-3) and adipose tissue was analyzed for eosinophils (4get GFP+, CD11b+, Siglec-F+, SSC-hi) on day 8. (**C**) 500 ng of recombinant mIL-25 was administered intraperitoneally to 4get mice on 4 consecutive days. Mice received 100 μg each of antibodies against α4 and αL integrins or isotype control IgG2a and IgG2b antibodies on day 3 and 6. Tissues were analyzed for

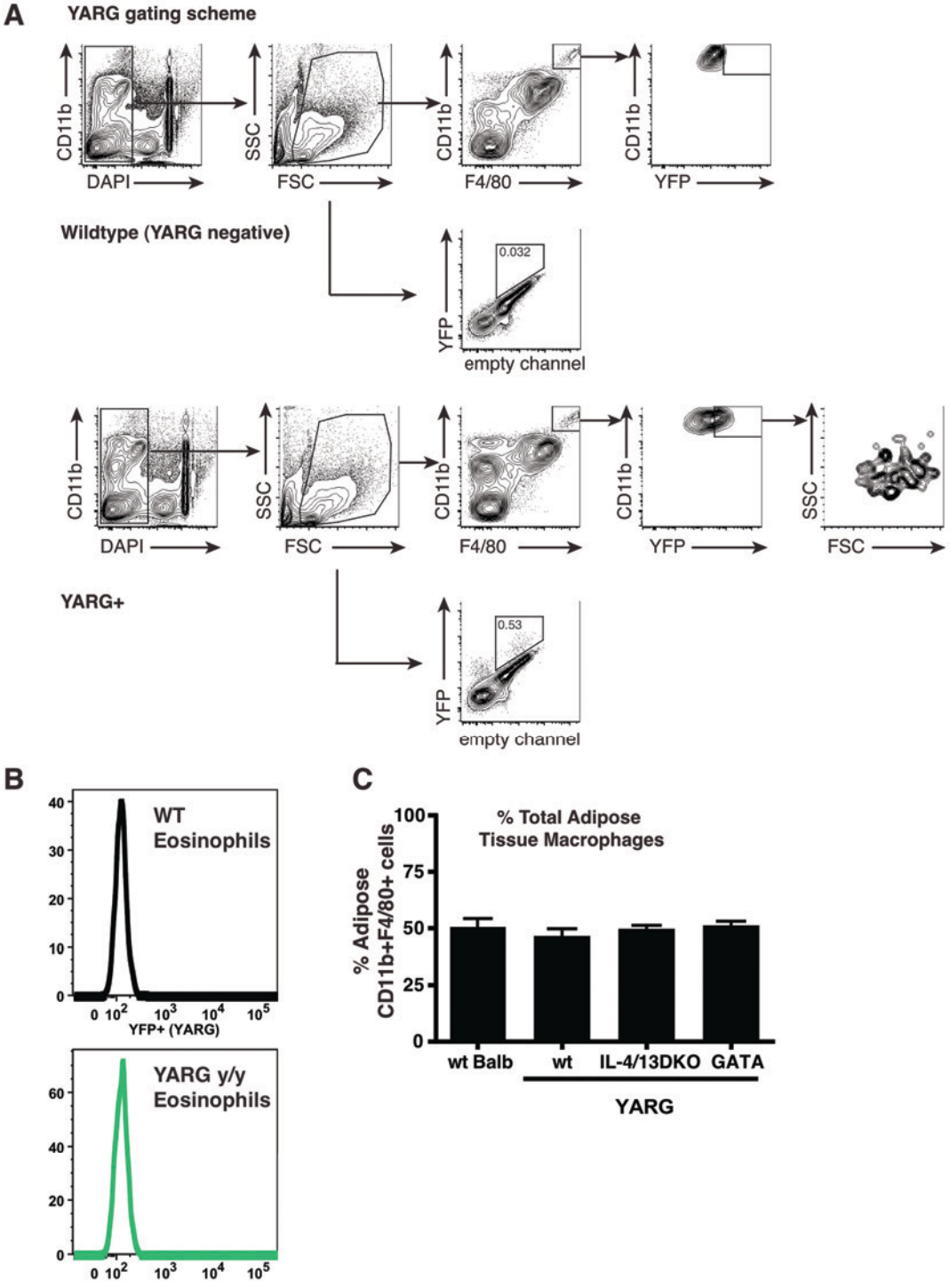
eosinophils by flow cytometry on day 8. Data is compiled from 2 independent experiments.

Supplemental Figure 6



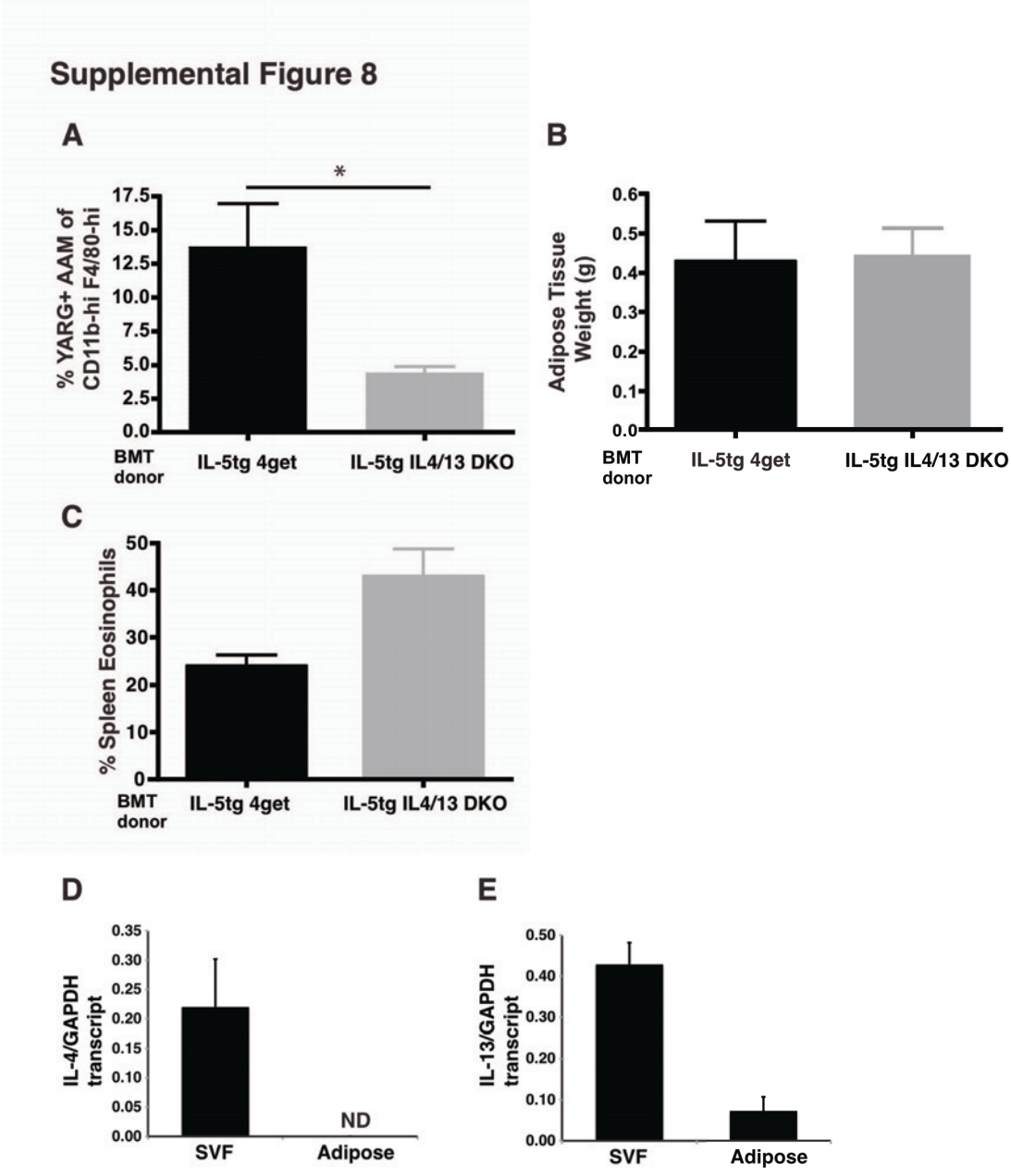
Supplemental Figure 6. Transcripts of alternative activation are increased in arginase1+ YFP (YARG) macrophages. Gene expression was assessed by quantitative RT-PCR in YARG+ F4/80^{+}CD11b^{+} and YARG- F4/80^{+}CD11b^{+} macrophages sorted from *Nippostrongylus brasiliensis*-infected lung at 7 days after infection. Arginase 1 and FIZZ1 are analyzed as representative transcripts of AAM. Data are shown as the mean ± SEM for 4 individual mice. Data is representative of 2 independent experiments.

Supplemental Figure 7



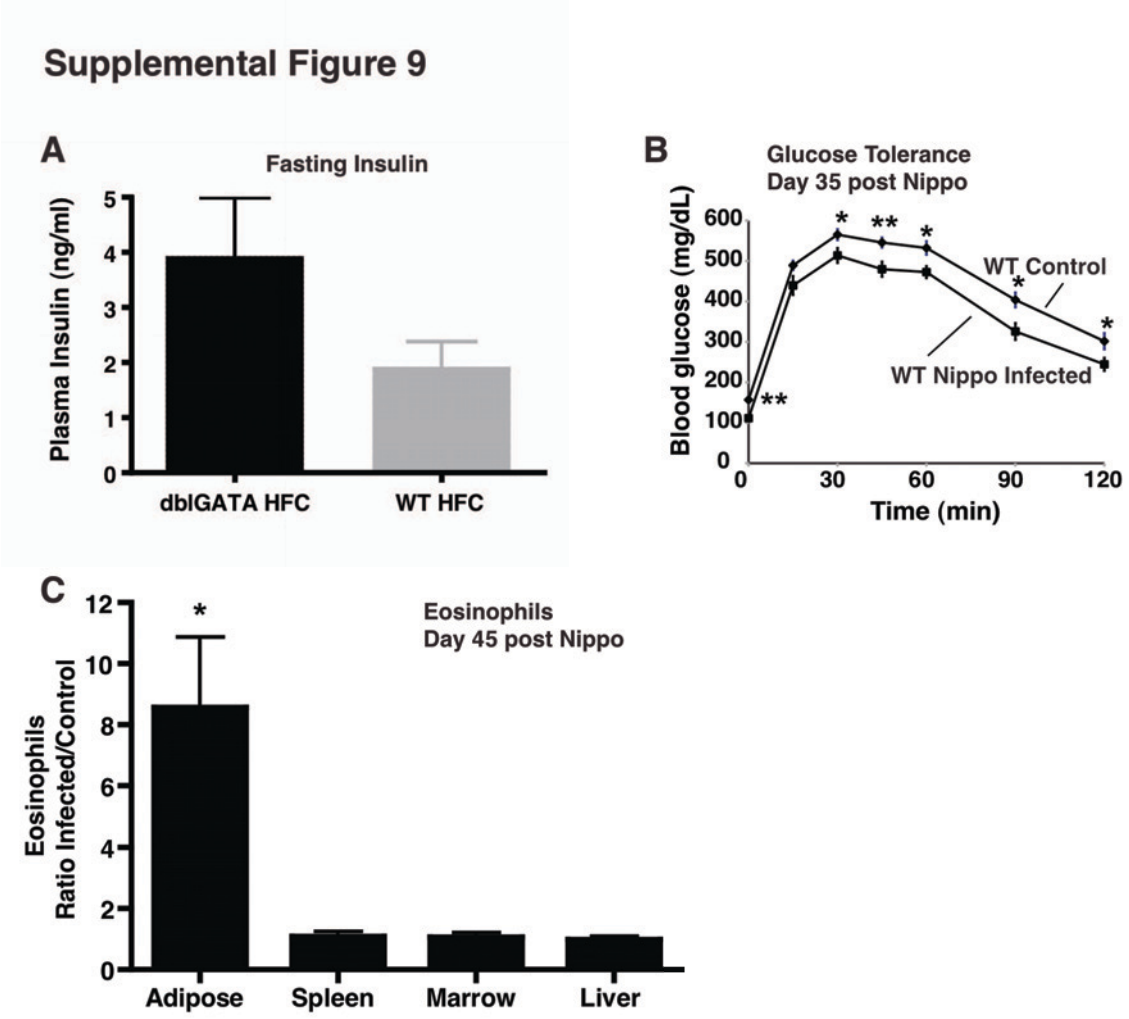
Supplemental Figure 7. Flow cytometric gating for YFP expression in adipose tissue macrophages from YARG mice and quantitation of total adipose macrophages. (A)

Live cells were gated by DAPI exclusion and by side-/forward-scatter (SSC/FSC). $CD11b^{hi}F4/80^{hi}$ cells were analyzed further to identify YFP+ macrophages in YARG mice (lower panels) that are absent in WT mice (upper panels). Backgating confirmed that the YFP+ cells are macrophages based on large SSC/FSC profile. Live YFP+ cells could also be identified by gating with an empty channel to exclude autofluorescent cells falling along the horizontal axis, confirming that YARG+ macrophages are lacking in adipose tissues in wildtype mice (upper panels) but YFP+ in YARG mice (lower panels). **(B)** In contrast, eosinophils were gated as shown in fig S1B and do not express YFP in YARG homozygous mice (lower panel, green) as compared with WT non-reporter controls (upper panel, black). **(C)** Total percent adipose CD11b+ F4/80+ cells were quantitated from the strains indicated. This population predominantly includes adipose tissue macrophages, but also includes a subset (5-10%) of eosinophils. Results are pooled data from 3-5 independent experiments with 2 or more animals per experiment.



Supplemental Figure 8. IL-5tg bone marrow transplant comparisons and IL-4 and IL-13 transcripts analysis in adipose tissue. Perigonadal fat pads and spleens were harvested from YARG x dblGATA animals that had received sublethal irradiation and subsequent bone marrow transplants 4-6 wk prior from either IL-5tg or IL-5tg IL4/13 double-knockout mice, as described in Figure 3C, and analyzed for percent YARG+

macrophages **(A)**, perigonadal fat pad weight **(B)**, or percent spleen eosinophils **(C)**. Data is pooled from 2 or more independent experiments with 2-5 animals per experiment. **(D,E)** Wild-type C57BL/6 ten-week old male mice were euthanized and perigonadal adipose tissue was pooled into two cohorts of 5 mice each. RNA was isolated from the SVF and adipocyte fractions, and cDNA was analyzed for IL-4 **(D)** and IL-13 transcripts **(E)**, with data normalized to GAPDH levels and expressed relative to levels of transcript in the mesenteric lymph node. ND = not detected up to 40 cycles.



Supplemental Figure 9. Fasting insulin levels and metabolic values 35-45 days post-*Nippostrongylus* infection. (**A**) Either WT or eosinophil-deficient BALB/c mice were

kept on high-fat (HF) diet for 20-22 wk and fasting plasma insulin levels were determined after a 4-hr morning fast. **(B)** Standard glucose tolerance test was performed on C57BL/6 wild-type male mice maintained on HF diet for 6 weeks pre-infection, either infected (Nippo) or not (Control), and maintained on HF diet for 35 days before glucose tolerance testing. Data are representative of 2 independent experiments with 20-30 total mice per experiment. **(C)** Perigonadal adipose, spleen, liver and bone marrow were harvested and eosinophils were quantitated from WT C57BL/6 mice maintained on HF diet for 6 weeks pre-infection, infected (Nippo) or not (Control), and maintained on HF diet for 45 additional days. Results are shown as a ratio of total tissue eosinophils in *Nippostrongylus*-infected animals relative to uninfected controls. Data are representative of 2 independent experiments with 5 mice per cohort. *$p<0.05$, **$p<0.01$ as determined using Student's t-test; error bars = SEM; n.s. = not significant.

Supporting Online Material References

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Competing interests statement. The authors declare that they have no competing financial interests.