Supplemental Materials and Methods section.

Dyes, recombinant proteins, and antibodies. The following antibodies were used: TCRab-FITC (H57-597), Ly6C-FITC (AL-21), Ly6G (Gr1)-APC (RB6-8C5, Ly6G-specific antibody), CD68-PE (FA11), IL-17A-PE or –eFluor 660 (TC11-1810), CD11b-eFluor 450 (M1/70.15), IFNγ-eFluor 450 (XMG1.2), CD3-APC eFluor 780 (17A2), CD4-PerCP eFluor 710 (RM4-5), CD29-PE (eBioHMB1-1), CD31-eFluor 450 (390), (all from eBioscience, Inc), CD45-PO (30-F11, Molecular Probes, Life Technologies, Carlsbad, CA), α-Smooth Muscle Actin-FITC (1A4, Sigma-Aldrich, St. Louis, MO), and CD16/CD32 antibodies (The Lymphocyte Culture Center, University of Virginia).

For IL-17C cytokine flow cytometry experiments, unlabeled Rat anti-mouse IL-17C (IgG2a, 311522) and Rat IgG2a (MAB006, both from R&D Systems, Bio-Techne, Minneapolis, MN) antibodies were labeled with Alexa Fluor 647 dye with Alexa Fluor 647 Antibody Labeling Kits (Molecular Probes, A-20186), following the manufacturer's protocol. Labeled antibody preparations were dialyzed (Slide-A-Lyzer Dialysis Cassettes, Pierce, Thermo Fisher Scientific) after the labeling procedure and concentrated to 0.2 mg/ml. The degree of labeling for each preparation was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA) to be 4.5-5 moles of Alexa Fluor 647 dye/mole of antibody. 0.2µg/1x10^6 million cells of IL-17C-Alexa Fluor 647 or IgG2a-Alexa Fluor 647 antibody was used for each flow cytometry test. Recombinant murine IL-17C (2306-ML-025/CF R&D Systems) was used in the *ex vivo* explant studies described.

En Face preparations. 12 week WD-fed *Apoe*$^{-/-}$ and *Il17c*$^{-/-}$*Apoe*$^{-/-}$ aortas were excised and stained for atherosclerotic lesions using Oil Red O as previously described[1]. Photomicrographs were taken and the percent area occupied by lesions was determined using Image-J (v1.44). Three independent observers quantified the Oil Red O staining and the aggregate scores are presented (Figure 3).

Histology. Hearts from the 12 week WD-fed *Apoe*$^{-/-}$ and *Il17c*$^{-/-}$*Apoe*$^{-/-}$ En Face cohorts were perfused with 4% Para-formaldehyde in phosphate buffered saline by cardiac puncture and collected for histology. Sequential 5µm aortic root sections (totaling >300µm of coverage) from the point of the appearance of the aortic valve leaflets were collected. Sections at depths 50, 150, and 250µm were deparafinized, rehydrated, and stained with antibodies against α-Smooth muscle actin (1A4, Sigma-Aldrich, St. Louis, MO) following antigen retrieval (Vector Laboratories, Burlingame, CA). α-SMA staining was detected using the following biotin-streptavadin reagents: Rabbit-Anti-Mouse-Biotin IgG (B8520, Sigma-Aldrich, St. Louis, MO) and Vectastain ABC kit (Vector Labs, Burlingame, CA). Sections at depths 55, 155, and 255µm were stained for collagen fibers using a pico sirius red staining kit (Polysciences, Inc., Warrington, PA) following the manufacturer's instructions. Images of the stained sections were acquired by white light microscopy and polarized light microscopy (Picosirius Red stained sections) and quantified by two independent observers using Image J (v1.44). The results are presented as a percentage area of marker positive staining within the total area of the atherosclerotic plaques.

Quantative real time PCR. Total RNA was isolated from atherosclerotic aortas using Trizol reagent (Invitrogen, Life Technologies). To identify major producers of pro-collagen, we sorted primary *Apoe*$^{-/-}$ and *Il17c*$^{-/-}$*Apoe*$^{-/-}$ CD45^{+} aortic leukocytes, CD45^{-}CD31^{+} endothelial cells, CD45^{-}CD31^{-}CD29^{-} SMCs, and CD45^{-}CD31^{-}CD29^{+} fibroblasts by FACS. The primary cells were then assessed for *Col1a1* expression by RT-PCR. To examine cell-specific expression of IL-17C aortas from 12 week CD C57Bl6, *Apoe*$^{-/-}$, and 12 week WD-fed *Apoe*$^{-/-}$ mice were collected and expression of *Il17c* in whole *Apoe*$^{-/-}$ aortas, magnetically sorted *Apoe*$^{-/-}$ aortic leukocytes (CD45^{+}, Stemcell Technologies), *Apoe*$^{-/-}$ vascular cells (CD45- cells), and FACS-sorted *Apoe*$^{-/-}$ CD45^{-}CD31^{-}CD29^{-} SMCs was determined. Co-isolated genomic DNA was removed from the preparations by DNase I treatment using RNeasy kits (Qiagen, Valencia, CA) following the manufacturer's instructions. 1µg of Total RNA was reverse transcribed as described[2] using Moloney murine leukemia virus reverse transcriptase, 10µM dNTPs, and random hexamers (Invitrogen, Life Technologies). RT-PCR was conducted using mouse Th17 PCR profiler arrays (SA Bioscience, Qiagen, Frederick, MD) and gene-specific mouse Taqman probes (*Actb* (Mm00607939_s1), *Gapdh* (Mm99999915_g1), *Il17c* (Mm00521397_m1), *Il17a* (Mm00439618_m1), *Ccl2* (Mm00441242_m1), *Ccl7* (Mm00443113_m1), *Cx3cl1* (Mm00436454_m1), *Cxcl1* (Mm04207460_m1), *Cxcl2* (Mm00436450_m1), *Cxcl5* (Mm00436451_g1), and *Il6* (Mm00446190_m1), Applied Biosystems, Carlsbad, CA). Ct values were determined using an iCycler iQ Real-time detection system (Bio-Rad laboratories, Hercules, CA). All PCR results were normalized to *Actb*, *Gapdh*, and *Hsp90ab1* as housekeeping gene controls and the data is presented as a fold change compared to *Apoe*$^{-/-}$ or vehicle controls ($2^{-\Delta\Delta CT}$ method).

Flow Cytometry. Aortic, splenic, peripheral lymph node, and peri-aortic lymph node single cell suspensions were prepared as previous described[1, 3, 4]. In brief, mice were anesthetized and their vasculature was perfused by cardiac puncture with 20U/ml sodium heparin in phosphate buffered saline. The aortas with surrounding aortic adventitia were sterilely microdissected, collected, and subsequently digested with the following cocktail of enzymes in PBS for 1 hour at 37C, as described:[1, 3, 4] 125 U/ml Collagenase type XI, 60 U/ml hyaluronidase type I-s, 60 U/ml DNAse1, and 450 U/ml Collagenase type 1 (all from Sigma-Aldrich, St. Louis, MO). For studies with peripheral primary and secondary lymphatic organs, the following organs were collected from each mouse: The spleen, peripheral lymph nodes (superficial and deep cervical, auxiliary, brachial, and inguinal lymph nodes), peri-aortic lymph nodes (sacral, lumbral, renal, and mediastinal lymph nodes), and blood.

For intracellular cytokine staining experiments, aortic, splenic, peripheral lymph node, and peri-aortic lymph node single cell suspensions were cultured for 5 hours in RPMI 1640 supplemented with 10% FBS, 1% Penicillin/Streptomycin, 10ng/ml Phorbol 12-myristate 13-acetate (PMA, Sigma Aldrich), 500ng/ml Ionomycin (Sigma Aldrich), and GolgiStop (BD Biosciences). Intracellular staining for IFNγ, IL-17A, and Foxp3 or appropriate isotype controls was performed using eBioscience Foxp3/Transcription Factor Staining Buffer (eBioscience). In the T cell phenotyping experiments, aortic IL-17A^{+} CD3^{+}TCRαβ^{-} T cells were considered to be IL-17A^{+} γδ^{+} T cells as we demonstrated previously that TCRαβ^{+} Th17 cells and IL-17A^{+} TCRγδ^{+} T cells are the major cellular producers of aortic IL-17A *in vivo*. Intracellular staining for CD68, IL-17C, and α-Smooth muscle actin were conducted using Fix&Perm cell permeabilization reagents (BD Biosciences). For some experiments aortas from 2-3 mice were combined in order to have sufficient number of analyzed cells. For all flow cytometry experiments involving aortas, data from the entire sample was acquired on the flow cytometer. A CytekDXP 8 color

488/637/407 (Cytek Development Inc.) upgraded FACSCalibur (BD Biosciences) was used to acquire samples, and FlowJo (Tree Star Inc., Ashalnd, OR) was used to analyze the data. For all flow cytometry experiments, the gates were placed based on isotype and fluorescent minus one controls.

Adoptive Transfer Experiments.

For adoptive splenocyte transfer experiments, 3-4x 12 week WD *Apoe*$^{-/-}$ spleens were collected sterilely and single cell suspensions were prepared. Erythrocytes were lysed using ACK lysis buffer (8.29mg/ml NH_4Cl, 1mg/ml $KHCO_3$, 0.372 mg/ml EDTA, pH 7.2, all from Sigma Aldrich). Leukocytes were subsequently labeled with 5μM CellTrace Violet (CTV) dye (Molecular Probes, Life Technologies) in PBS at 37C for 10 minutes and washed twice with PBS containing 1% FBS. 30x10^6 CTV$^+$ splenocytes in 0.25ml of PBS were injected into 12 week WD *Apoe*$^{-/-}$ and *Il17c*$^{-/-}$*Apoe*$^{-/-}$ recipient mice via tail vein injection (i.v.). As a negative control for the injected CTV$^+$ splenocytes, 12 week *Apoe*$^{-/-}$ mice were injected with 0.25ml of PBS. 72 hours post injection, the recipient and sham control aortas, spleens, and peri-aortic lymph nodes were collected and processed for intracellular cytokine flow cytometry experiments. The gates for CTV$^+$ donor cells and cytokine$^+$CTV$^+$ donor cells were set based on sham negative controls and isotype controls, respectively.

Transwell migration assays.

12 week WD-fed *Apoe*$^{-/-}$ and *Il17c*$^{-/-}$*Apoe*$^{-/-}$ aortic smooth muscle cells (CD45$^-$CD31$^-$CD29$^-$ aortic cells) were sterilely FACS sorted and cultured for an hour in 10 % of FBS complete RPMI in vitro to collect cell supernatants. CD4$^+$ T cells were isolated in parallel from 40 week CD *Il17a*$^{icre/icre}$ *R26R*$^{tdTomato/tdTomato}$ *Apoe*$^{-/-}$ mice. 0.3x10^6 *Il17a*$^{icre/icre}$*R26R*$^{tdTomato/tdTomato}$*Apoe*$^{-/-}$ CD4$^+$ T cells were cultured in 200μl of migration media in the top wells of transwell plates and allowed to migrate towards 600μl of: a migration media vehicle control, 1000 ng/ml recombinant mouse CCL20, *Apoe*$^{-/-}$ or *Il17c*$^{-/-}$*Apoe*$^{-/-}$ aortic smooth muscle cell supernatants for 2 hours. The transmigrated cells were collected and assessed for IL-17A$^{tdTomato+}$ Th17 cells by flow cytometry and normalized to the percentage of Th17 cells in the starting population. All assays were performed in triplicate.

Statistical Analysis.

Statistical comparisons between two groups were conducted using unpaired Student's T Tests and the data reported as mean±SEM. For statistical comparisons between three or more groups, a one way ANOVA with Tukey HSD post-hoc tests were used. Statistical significance was set at $p<0.05$.

Reference List

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