Online supplemental materials.

Recombinant Proteins and Antibodies. Abs used were as follows (all from BD-Pharmingen, San Diego, CA): Per-CP or PE-Texas Red-CD45, PE-IL-17A, Pacific blue-CD3ε, APC-Cy7-CD4, FITC or APC-TCR β chain, biotinylated or FITC-γδ TCR, LIVE/DEAD® Aqua Dead Cell Stain Kit (Invitrogen) and CD16/CD32 (The Lymphocyte Culture Centre**,** UVA).

Flow cytometry. Single cell suspensions from the aorta and surrounding adventitia were prepared as previously described.[1] Briefly, mice were anesthetized, and their vasculature was perfused by cardiac puncture with phosphate-buffer saline (PBS) containing 20 units/ml of heparin. Harvested aortas were microdissected and digested with 125 U/ml collagenase type XI, 60 U/ml hyaluronidase type I-s, 60 U/ml DNAse1 and 450 U/ml collagenase type I (all enzymes Sigma, St. Louis, MO) in PBS at 37^{o}C for 1 hour.[1] Single cell suspensions from spleen, mesenteric lymph nodes (MLN), peripheral LN (PLN), lamina propria (LP)[2] and aortas were treated with 10 ng/ml PMA, 500 ng/ml calcium ionophore and GolgiStop for 5 h. Intracellular staining for IL-17A and CD4 was performed using Fix&Perm® cell permeabilization reagents. In some experiments, 1-2 aortas were pooled for the intracellular staining. To determine the location of IL-17-expressing cells, harvested aortas with surrounding adventitia were digested with 312.5 U/ml collagenaseII and 5.625 U/ml elastase (Worthington Biochemical Corp., Lakewood, NJ) for 1 hr at 37°C, and adventitia was carefully removed. Aortas and the adventitia were further digested separately for 30 min at 37°C. Cell suspensions from pooled 3-4 aortas were treated as described above. Flow cytometry analysis was performed on a FACSCalibur^{TM} or CyanADP^{TM}, data analyzed using FlowJO (Tree Star Inc., Ashland, OR) software. Gates were set by isotype controls or fluorescent minus one control.

Preparation of Mouse Aortas and histochemistry. Aortas were excised as previously described[1] and stained with Oil Red O. Images were scanned, and the percent of surface areas occupied by lesions were determined with Image-ProPlus (Media Cybernetics, Inc). For immunohistochemistry, mice were perfused by cardiac puncture with 4% PFA and hearts were collected. Sequential 5 μm thick sections of the aortic root were cut from the point of the appearance of aortic valve leaflets. Tissues were stained with Abs against Mac-2 (Cedarlane Lab) and CXCL1 (R&D Systems) with avidin-biotin technology. Images were scanned and analyzed by Image-ProPlus. The percent of the surface area occupied by the staining was determined as a ratio of positively stained area to the total cross-sectional area of the aortic root. Alternatively, the surface area of the plaque and the total number of Mac-2^+ macrophages within the plaques was determined. The total numbers of macrophages were then normalized to the total area of the plaques to determine the number of macrophages per $10^4 \mu m^2$.

mRNA quantification. To analyze IL-17A expression, RNA was extracted from the aorta, small intestines, spleen, MLN and PLN following homogenization in Trizol® (Invitrogen), according to manufacturer's instructions. Reverse transcription and PCR steps were performed using QuantiTect SYBR Green RT-PCR Kit (Qiagen, Valencia, CA). 1 μg of total RNA was used for all tissues. Values were determined using iCycler iQ Real-Time Detection System Software (Qiagen, Valencia, CA). The corresponding values were normalized to Ribosomal RNA control reagent and then normalized to individual *C57BL/6* mouse organs as the calibrator control (always equal to 1), thereby expressing the values as relative quantification (RQ) values. Alternatively, to determine the expression of the different members of IL-17 family, mouse Th17 PCR profiler array (SABioscience) was used according to the manufacturer's instructions.

Adenovirus transfer and western blotting. To test the role for IL-17A in atherosclerosis, *Apoe*$^{-/-}$ mice were injected retro-orbitally with $0.5x10^9$ PFU of Ad-IL-17RA:Fc[3] at 4 day old, then with $1x10^9$ PFU of Ad-IL-17RA:Fc at 52 and 104 day (Supplemental Fig.2). As a control, Ad-Lu[3] was injected into the control group of *Apoe*$^{-/-}$ mice at the same time points. *Apoe*$^{-/-}$ mice were fed chow diet for 6 weeks and then WD for 15 weeks. Immunoprecipitation was carried out by pre-absorbing rat monoclonal anti-mouse IL-17RA Ab (R &D systems) to protein A/G agarose beads (PIERCE). Plasma soluble IL17-RA was detected by western blot analysis using rabbit anti mouse IL-17RA Ab (Santa Cruz Biotechnology, Inc, CA) followed by incubation with HRP conjugated anti-rabbit IgG (Zymed, Invitrogen). After 5 minute incubation with super signal west pico chemiluminescent substrate (PIERCE, IL) membranes were exposed to film for detection of the soluble IL-17RA protein.

Measurements of cytokines and chemokines. Mouse IL-17A was measured using mouse multi-cytokine detection kit (Millipore, MA). Plasma IFN-γ and IL-6 were measured by BD Cytometric Bead Array (BD Pharmingen, CA). G-CSF and IL-17F were quantified by ELISA (R&D Systems and eBioscience, respectively).

Measurements of plasma lipids. Plasma triglyceride and total cholesterol levels were determined via an automated enzymatic technique (Boehringer Mannheim GmbH, CA).

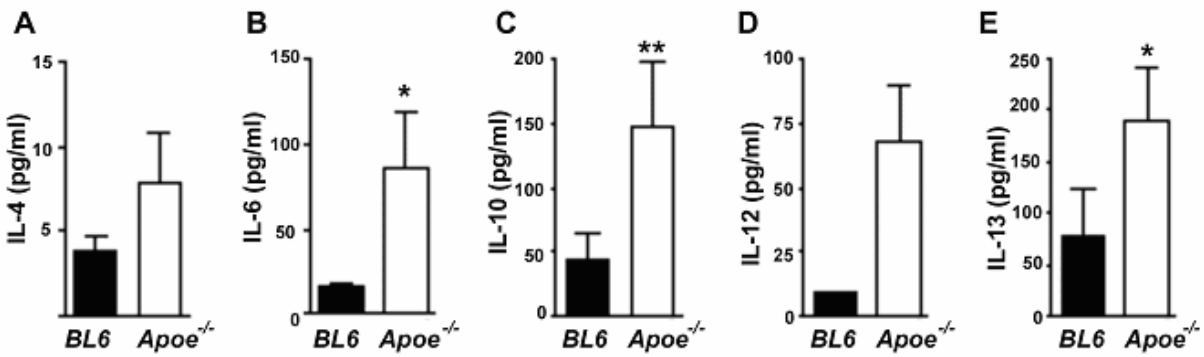
Ex vivo monocyte adhesion assay. Assay was performed as described previously[4]. Briefly, aortas were isolated from 27-30 week old *Apoe*$^{-/-}$ mice, and incubated overnight in RPMI-1640

media containing 10% FBS with or without 10ng/ml of rIL-17A (R&D Systems). Next, aortas were washed either with media or kept with media containing IL-17A, opened longitudinally and pinned to sterile agar. Monocytes were isolated from peripheral blood by positive selection with anti-CD115-bio Abs and streptavidin-specific microbeads (Miltenyi Biotec) and labeled with 2 μM carboxyfluorescein diacetate-succinimidyl ester (CFSE) as previously described.[1] CFSE-labeled monocytes were incubated with the pinned aortas, and 1 hour later, unbound monocytes were washed away with PBS. The number of monocytes adherent to the aorta was counted using fluorescent microscopy. To determine the expression of CXCL-1 in IL-17A-treated aortas, we isolated total RNA and performed real time PCR using CXCL1 and Beta-Actin Taqman primers (Applied Biosystems, CA) as described above.

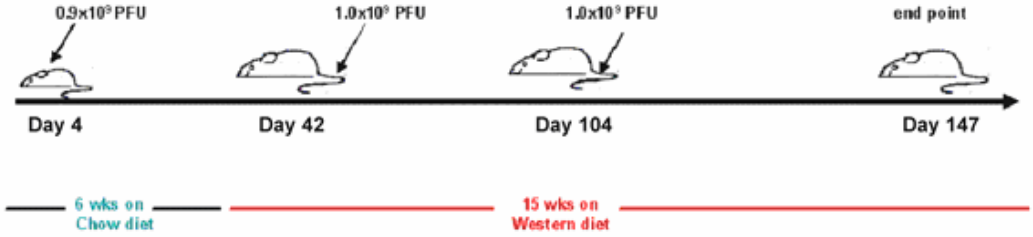
Supplemental Figure S1: Cytokine levels are elevated in *Apoe*$^{-/-}$ mice. Serum levels of (A) IL-4, (B) IL-5, (C) IL-10, (D) IL-12 and (E) IL-15 were analyzed in *C57BL/6* and *Apoe*$^{-/-}$ mice. *P<0.05, **P<0.01 by unpaired Students t test.

Supplemental Figure S2: Scheme of the injection of Ad-IL-17RA:Fc or control Ad-Lu into *Apoe*$^{-/-}$ recipient mice. *Apoe*$^{-/-}$ mice were injected retro-orbitally with $0.5x10^9$ PFU of Ad-IL-17RA:Fc at 4 days-old, then with $1x10^9$ PFU of Ad-IL-17RA:Fc at 52 and 104 days. As a control, Ad-Luc was injected into the control group of *Apoe*$^{-/-}$ mice at the same time points. *Apoe*$^{-/-}$ mice were fed chow diet for 6 weeks and then WD for 15 weeks.

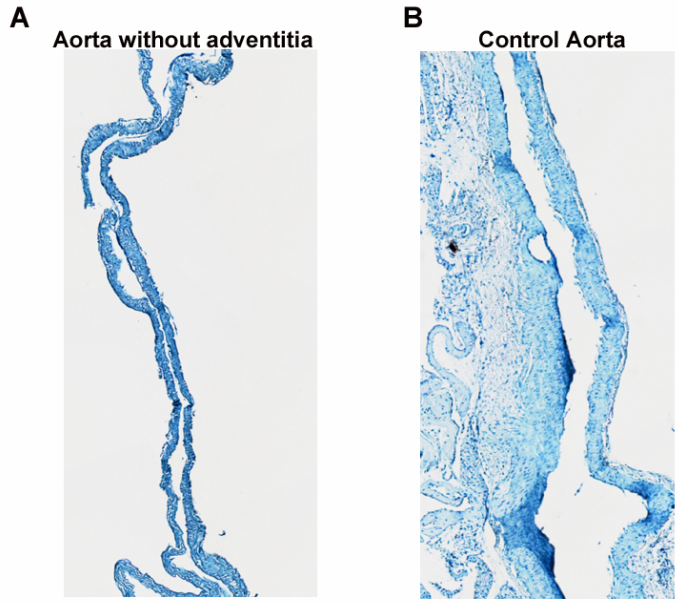
Supplemental Fig.S3: Isolation murine aorta and adventitia. (A) Harvested *Apoe*$^{-/-}$ aortas with surrounding adventitia were digested with 312.5 U/ml collagenaseII and 5.625 U/ml elastase for 1 hr at 37°C, and adventitia was carefully removed. (B) Control aortas with surrounding adventitia were harvested from *Apoe*$^{-/-}$ mice. Longitudinal 5 μm thick sections of the aortas were cut and tissues were stained with hematoxylin.



Supplemental Fig.1S



Supplemental Fig.2S



Supplemental Fig.3S

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