**Online Data Supplement**

## Methods

### Construction of recombinant helper-dependent adenovirus (HDAd)

HDAd expressing human apoE3 (HDAd-gE3) was constructed using pΔ21.[1] In brief, a 10.7-kb Hind III fragment containing the human apoE3 gene and a 1.7-kb Pst I fragment containing the human apoE liver-control region at its 3'-end were subcloned into pΔ21. Human IL-7 cDNA was first cloned into an apoAI expression cassette and then subcloned into pΔ21.[2] Rescue and amplification of the HDAd were performed as described.[3] A control empty vector (HDAd-0) contained no expression cassette. Helper virus contamination and vector DNA were analyzed by Southern blot analysis and by real-time PCR. Helper virus contamination was less than 0.1%. The infectious titre of HDAd was determined by real-time PCR to quantify competition with an Adenovirus Type 5 Reference Material (VR-1516, ATCC) in infection of 293 cells.[4] The infectivity of HDAd-gE3, HDAd-0 and HDAd-IL-7 were 64 ± 7, 55 ± 34 and 60 ± 0 (mean ± S.D, n=3), respectively.

### Animals

Female $Apoe^{-/-}$ mice (6-8 weeks of age, Stock No. 002052) on a C57BL/6 background were purchased from The Jackson Laboratory and were fed a diet containing 0.2% (w/w) cholesterol and 10% (v/w) coconut oil to induce atherosclerosis.[3] After 30 weeks of the diet, 10 mice were sacrificed to obtain the initial extent of atherosclerosis (baseline). The remaining mice were divided into two groups and treated with a tail vein injection of HDAd-gE3 ($5 \times 10^{12}$ VP/kg, n=11) or HDAd-0 ($5 \times 10^{12}$ VP/kg, n=11). Having confirmed that plasma cholesterol was normalized in the HDAd-gE3 group, the diet was changed to normal chow at 5 weeks and maintained throughout the remainder of the experiments. Mice were euthanized at 41 weeks. The experimental design is shown in Figure 1. Blood was collected from the saphenous vein into EDTA-containing tubes after a 5-hour fast. For FPLC analysis, mice were anesthetized with isoflurane before collecting blood from the retro-orbital plexus. For microarray analysis, mice were fed a high cholesterol diet for 30 weeks, divided into 3 groups (n=15/group), treated with HDAd-0, HDAd-gE3 or phosphate buffered saline (PBS) and sacrificed 10 days after confirming the reduced plasma cholesterol levels in the HDAd-gE3 group. Three aortas extending from sinus to arch were pooled for RNA extraction with an RNeasy kit (Qiagen). For induction of human IL-7 in the blood circulation, 9 week old female $Apoe^{-/-}$ mice were treated by HDAd-IL-7 ($5 \times 10^{12}$ VP/kg, n=8) or HDAd-0 ($5 \times 10^{12}$ VP/kg, n=7) and sacrificed after 1 week. For bone marrow transplantation (BMT), female $Ldlr^{-/-}$ mice (Stock No. 002207, The Jackson Laboratory) were fed a high cholesterol diet for 6 weeks to induce atherosclerosis.[5] One group of mice (n=10/group) was sacrificed to obtain baseline levels. BM cells were then isolated from either male $Il\text{-}7^{-/-}$ mice[6] or $Il\text{-}7^{+/+}$ litter mate. In brief, BM cells were prepared by flushing the femur and the tibia with fresh complete media (FCM) composed of RPMI-1640 with 2% heat inactivated foetal bovine serum (FBS) and 10 mM HEPES into a 50 mL conical tube. BM cells were filtered through a 70 μm nitrex mesh filter (BD Falcon #352350) into a 15 mL tube and centrifuged for 5 min at 1,500 rpm at room temperature and rinsed with 5 mL FCM. BM cells were gently resuspended in 9 mL of sterile water over 17 sec and 1 mL of 10 x PBS was immediately added and rapidly dispersed by swirling, inverting and pipetting up and down. BM cell suspension was filtered through a 70 μm nitrex mesh filter, centrifuged for 5 min at 1,500 rpm to remove lysed red blood cell debris and resuspended in 1 mL of PBS without $Ca^{2+}$ and

$Mg^{2+}$ for cell counts. Recipient mice were irradiated at 4.5 Gy twice over 2-3 hours and then received 4 x $10^6$ BM cells in 0.2 ml PBS intravenously 5 hours after the first irradiation.[7] Mice were sacrificed 24 weeks after transplantation for analysis. All experimental protocols were performed according to the guidelines of the Institutional Animal Care and Usage Committee at Baylor College of Medicine.

**Immunohistochemistry, histology, and quantitation of atherosclerotic lesions**

Aortic *en face* lesions and cross sections were evaluated by quantitative morphometry.[3] For immunohistochemistry, sequential sections (5 μm thick) of fresh-frozen OCT-embedded proximal aorta were stained using the following primary antibodies: Mac3 (Santa Cruz Biotechnology, 1:100); rabbit anti-mouse vascular cell adhesion molecule-1 (VCAM-1, Santa Cruz Biotechnology, 1:100); rabbit anti-α-actin (Abcam, 1:100); goat anti-mouse IL-7 (Santa Cruz, 1:100); rabbit anti-γ-sarcoglycan (Abcam, 1:100); rabbit anti-mouse CD3 (Abcm, 1:100); mouse anit-mouse TNF-α (Abcam, 1:100). Goat anti-mouse or anti-rabbit antibodies (1:200, Vector laboratories) were used as secondary antibodies. In brief, sections were incubated with a primary antibody at 4°C overnight and then with the secondary antibody for 1 hour at room temperature. Immunoreactive proteins were detected by avidin-biotin horseradish peroxidase using Vextastain Elite ABC reagent (Vector laboratories) or a NovaRed substrate kit for peroxidase (Vector Laboratories). Histology was performed using standard techniques for Oil-Red O, H&E, trichrome, and Van Kossa. 6 sections for each mouse were quantified by SigmaScanPro.[5]

**Expression microarray analysis of aortas**

The following procedures were performed in the Baylor College of Medicine Microarray Core Facility (http://www.bcm.edu/mcfweb/). The quality and quantity of isolated RNA were measured by Agilent 2100 Bioanalyzer and Nanodrop ND-1000 Spectrophotometer. RNA was amplified using a NuGen's Ovation V2 Amplification kit and labelled by a FL-Ovation kit (NuGen Technologies, Inc., CA). The probes were then hybridized to a Mouse 430 2.0 GeneChip® array that represents 39,000 transcripts. The array was scanned on the Affymetrix GeneChip® Scanner 3000 and was analyzed using Affymetrix GCOS software version 1.1.2. The GeneSifter® microarray data analysis system (VizX Labs LLC, http://www.genesifter.net) was used to analyze data generated from a pairwise comparison between the HDAd-0 and the HDAd-gE3 groups or between PBS and vector treated groups. The parameters used to identify differentially expressed genes were: global median normalization; Student's *t*-test (two-tailed, unpaired); quality = 1; threshold = 2.0 and with log transformation. The filtering criteria was at least 1.5 fold change and $p<0.05$. We used Pathway Express and Ontology Express for functional profiling (http://vortex.cs.wayne.edu/projects.htm). Ontology Express was used to establish biological significance of differentially expressed genes based on a classification scheme devised by the Gene Ontology Consortium (http://www.geneontology.org/GO.doc.html).[8] Pathway Express was used to analyze lists of differentially expressed genes for functional content, as defined in the KEGG database (http://www.genome.ad.jp/kegg/). The two approaches used in Pathway Expresses were statistical analysis for overrepresentation[9] and a novel impact analysis.[10] A Fisher's exact test was performed to evaluate the probabilities using *R* (http://www.r-project.org/). Correction for multiple testing was performed using the method of Benjamini and Hochberg[11] to estimate a false discovery rate from the raw p-values.

**Cell culture**
RAW 264.7 cells and rat thoracic aorta SMCs[12] were maintained in Dulbecco's modified Eagle's medium (DMEM)/10% FBS. THP-1 cells were maintained in RPMI-1640/10% FBS. HAECs were purchased from Lonza and maintained in EGM-2 BulletKits (Lonza Walkersville, Inc.). Peritoneal macrophages (PMs) were harvested from the peritoneal fluid of $Apoe^{-/-}$ mice 3 days after i.p. injection of 1 mL of thioglycolate (30g/L) in saline. PMs were washed, seeded into a 6-well plate and incubated in DMEM/10% FBS for 24 hrs. Cells were incubated with 50 μg/mL oxidized LDL (oxLDL, Kalen Biomedical, LLC, MD) in the presence of lipoprotein-deficient FBS for 4 hours. Endotoxin-free FBS (Hyclone) was used for incubation with human IL-7 (R&D). For adhesion assay, HAECs were plated in a 96-well plate at the density of $5 \times 10^4$ cells/well one day before assay. THP-1 cells were labelled with 5 mM calcein-acetoxymethyl ester (calcAM, Molecular Probes). Monolayers of HAECs were stimulated by IL-7 (100 ng/mL) for 6 hours, washed three times with PBS and then $1 \times 10^5$ cells of calcAM labelled THP-1 were added to HAECs. After 1 hour incubation, unbound THP-1 cells were washed and quantified. The PI3K inhibitor (LY294002) and the JAK inhibitor I were purchased from Calbiochem. The NF-κB inhibitors, pyrrolidine dithiocarbamate (PDTC) and N-acetyl-L-cycteine (NAC) were obtained from Sigma-Aldrich.

**Construction of lentiviral reporters**
The human ICAM-1 (−485 to +45) and VCAM-1(−213 to +119) promoter regions containing the NF-κB binding site[13] were cloned by PCR using genomic DNA isolated from HAECs as a template and subcloned into pGL3-basic luciferase reporter plasmid vector (Promega). After verifying by DNA sequence analysis, the fragments containing ICAM-1 or VCAM-1 promoter plus firefly luciferase gene were excised by Kpn I and XbaI digestion. The XbaI site was blunt-ended and cloned into the KpnI/blunt-ended NotI sites of pLentiLox4.0 (Invitrogen). The resulting plasmids were cotransfected with psPAX2 packaging plasmid and pMD2.G enveloping plasmid (both are gifts of D. Trono, Global Health Institute, School of Life Sciences, Ecole Polytechnique Federale de Lausanne, Switzerland) into 293T cells by calcium phosphate precipitation method. The medium was harvested 48 hours after transfection and the vector was concentrated by ultracentrifugation.[14] The infectious titre was estimated by infection of 293T cells followed by quantitative PCR for vector DNA converted from the RNA genome.

**Luciferase assay**
HAECs in 48-well plate were infected by lentiviral reporter constructs for 48 hours and then incubated for 6 hours in the presence of IL-7 (100 ng/mL). The cells were harvested and the promoter activities were analyzed by luciferase assay using a kit (Promega). pLentiLox4.0-TK-renilla-luciferase was used as an internal control. The luciferase activities were normalized by renilla luciferase.

**Other procedures**
Lipid and FPLC (GE Healthcare) analyses were performed as previously described.[3] Human plasma apoE3 levels were quantified by an ELISA using polyclonal goat anti-apoE antibody (Chemicon, 1:1000). Human apoE3 (Calbiochem) was used to generate a standard curve in the assay. For immunoblot analysis, 1 μL of pooled plasma was electrophoresed on a 12% denaturing SDS-polyacrylamide gel, transferred to a PVDF membrane and incubated with goat anti-apoE antibody (1:2000) or goat anti-albumin (Abcam, 1:12000). For detection of

intercellular adhesion molecule-1 (ICAM-1) and VCAM-1 in HAECs, mouse anti-ICAM-1 (R&D system, 1.5 μg/mL), rabbit anti-VCAM-1 (Santa Cruz, 1:500) and mouse anti-GAPDH (Chemicon, 1:1000) were used. RNA abundance was determined by real time RT-PCR using SYBR Green. All data were normalized to β-actin and expressed as variables of fold change over the controls. The primer sequences are shown in Online Supplementary Table S1.

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**Table S1. Sequences of primers used for real-time RT-PCR**

| Gene name | Forward primer | Reverse primer |
|---|---|---|
| **Mouse** | | |
| IL-7 | 5'-CCTCCACTGATCCTTGTTCTG-3' | 5'-TGCGAGCAGCACGATTTAG-3' |
| α-Actin | 5'-ATCAGATGAGGAAGCCAAGG-3' | 5'-ACACAAAAACCCAACCAACC-3' |
| γ-Sarcoglycan | 5'-GCTGACCCATTTCAAGACC-3' | 5'-TGACTTCACCAGCCATAGAC-3' |
| CCR7 | 5'-ACGCAACTTTGAGCGGAAC-3' | 5'-TGACGCCGATGAAGGCATAC-3' |
| β-Actin | 5'-ATTGTGATGGACTCCGGAGA-3' | 5'-ATGTCACGCACGATTTCCCT-3' |
| IL-7Rα | 5'-AAAGCATGATGTGGCCTACC-3' | 5'-TTGATTCTTGGGTTCTGGAG -3' |
| MCP-1/CCL2 | 5'-AGCACCAGCCAACTCTCACT-3' | 5'-TCATTGGGATCATCTTGCTG-3' |
| ICAM-1 | 5'-CGAAGGTGGTTCTTCTGAGC-3' | 5'-GTCTGCTGAGACCCCTCTTG-3' |
| VCAM-1 | 5'-TGGAGGAAATGGGCATAAAG-3' | 5'-TGAGCAGGTCAGGTTCACAG-3' |
| E-selectin | 5'-AGCTACCCATGGAACACGAC-3' | 5'-ACGCAAGTTCTCCAGCTGTT-3' |
| TNF-α | 5'-ACGTGGAACTGGCAGAAGAG-3' | 5'-AGGGTCTGGGCCATAGAACT-3' |
| **Rat** | | |
| IL-7 | 5'-CCTCCACTGATCCTTGTTCTG-3' | 5'-TGCGAGCAGCACGATTTAG-3' |
| IL-7Rα | 5'-AAAGCATGATGTGGCCTACC-3' | 5'-TTGATTCTTGGGTTCTGGAG -3' |
| **Human** | | |
| IL-7 | 5'-ATGTTCCATGTTTCTTTTAGGTATATC-3' | 5'-TCAGTGTTCTTTAGTGCCCATC-3' |
| IL-7Rα | 5'-AGCCAGTTGGAAGTGAATGG -3' | 5'-CAGGTCAAAAGGAGCCTCAG -3' |
| β-Actin | 5'-GCCATGTACGTTGCTATCCA -3' | 5'-CCTCGTAGATGGGCACAGT -3' |
| MCP-1/CCL2 | 5'-CCCCAGTCACCTGCTGTTAT -3' | 5'-TGGAATCCTGAACCCACTTC -3' |
| IL-6 | 5'-TACCCCCAGGAGAAGATTCC -3' | 5'-TTTTCTGCCAGTGCCTCTTT -3' |
| ICAM-1 | 5'-AGCTTCTCCTGCTCTGCAAC -3' | 5'-CATTGGAGTCTGCTGGGAAT-3' |
| VCAM-1 | 5'-CGCTGACAATGAATCCTGTT -3' | 5'-AGGGCCACTCAAATGAATCT-3' |
| E-selectin | 5'-TGAACCCAACAATAGGCAAA -3' | 5'-CCACTGCAGGATGTATTGGT -3' |

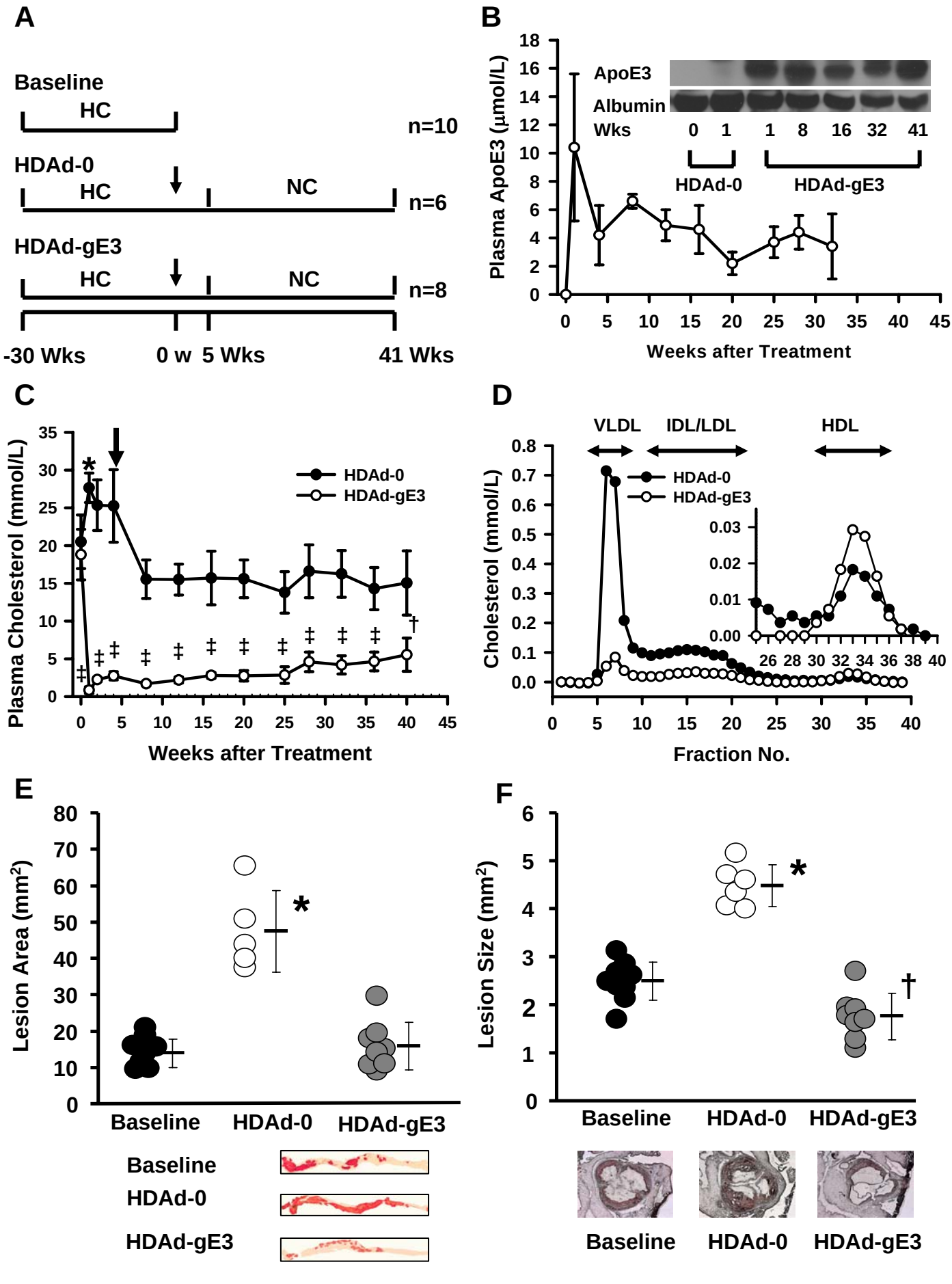
A
Baseline
HC
n=10
HDAd-0
HC
NC
n=6
HDAd-gE3
HC
NC
n=8
-30 Wks
0 w
5 Wks
41 Wks
B
Plasma ApoE3 (μmol/L)
ApoE3
Albumin
Wks
0 1 1 8 16 32 41
HDAd-0
HDAd-gE3
Weeks after Treatment
C
Plasma Cholesterol (mmol/L)
HDAd-0
HDAd-gE3
Weeks after Treatment
D
VLDL
IDL/LDL
HDL
Cholesterol (mmol/L)
HDAd-0
HDAd-gE3
Fraction No.
E
Lesion Area (mm²)
Baseline
HDAd-0
HDAd-gE3
F
Lesion Size (mm²)
Baseline
HDAd-0
HDAd-gE3

**Figure S1** A single intravenous injection of HDAd-gE3 induces regression of advanced atherosclerosis in Apoe$^{-/-}$ mice. A, Experimental design. Eleven mice for each group were treated with HDAd. The n indicates number of mice which survived and were used for analysis at the end of experiment. Arrows indicate the injection of HDAd. HC: high cholesterol diet; NC: normal chow. B, Human apoE3 level. ApoE3 was measured by ELISA. N=8. Inset: Immunoblot analysis of plasma apoE3. 1 µl of pooled plasma collected at indicated times was separated on 4-15% SDS-PAGE and transferred to a PVDF membrane. Duplicate membranes were incubated with anti-apoE or anti-albumin antibody. C, Plasma cholesterol levels. Arrow indicates the point of change from a high cholesterol diet to a normal chow at 5 weeks. *$p<0.05$ vs. baseline, †$p<0.01$ vs. HDAd-0, ‡$p<0.001$ vs. HDAd-0. N=6 (HDAd-0) and 8 (HDAd-gE3). D, FPLC analysis. Plasma was collected 41 weeks after vector treatment. 0.2 mL of pooled plasma was separated with FPLC and cholesterol was determined. Magnified HDL profiles are shown in the inset panel. E, *En face* quantification of aortic lesions. *$p<0.01$ vs. baseline. F, Quantification of lesion areas on cross-sections. *$p<0.005$ vs. baseline, †$p=0.012$ vs. baseline. Lower panel: representative aortas and cross-sections. Data are presented as mean ± standard deviation (SD).

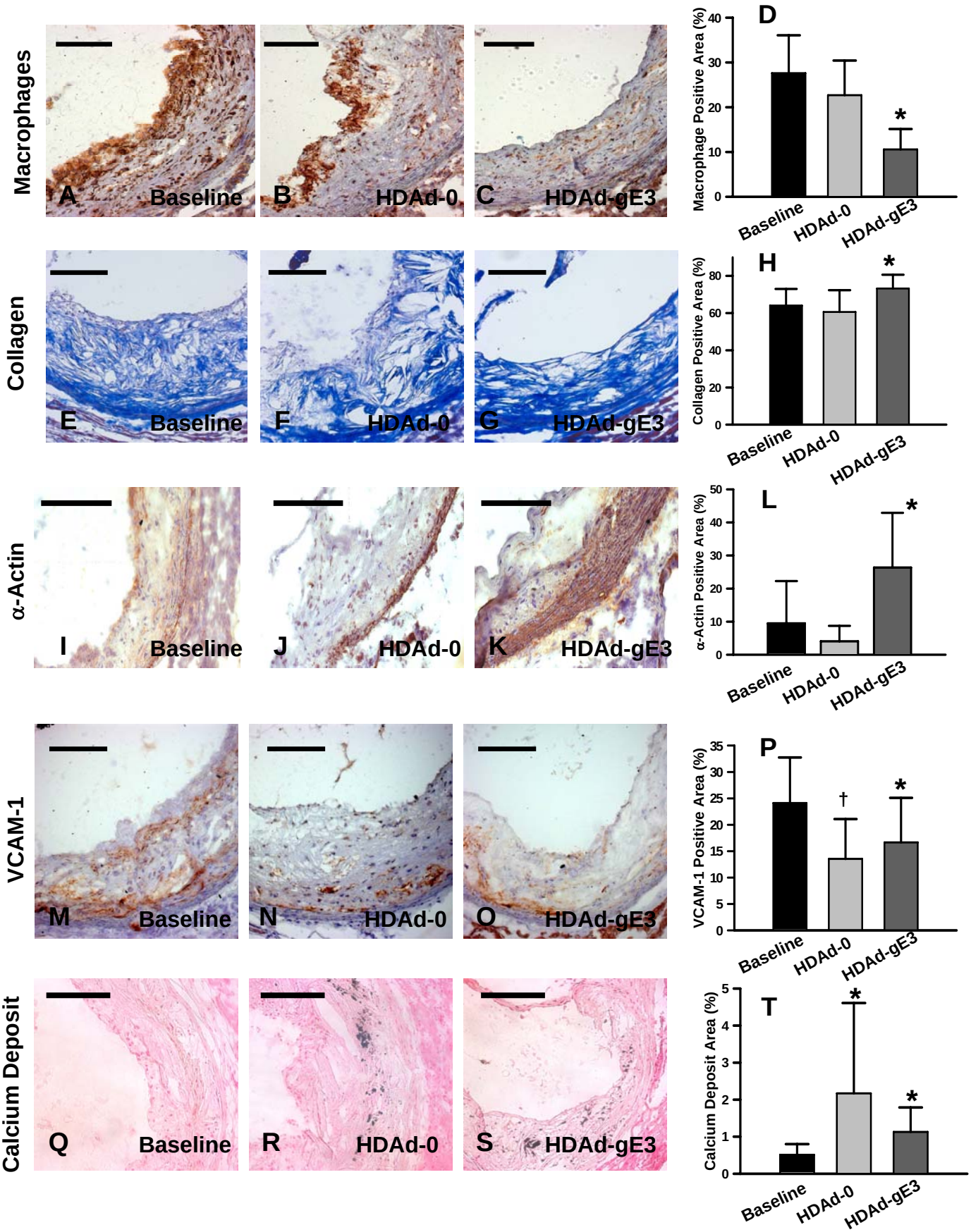
Macrophages
A
Baseline
B
HDAd-0
C
HDAd-gE3
D
Macrophage Positive Area (%)
40
30
20
10
0
*
Baseline
HDAd-0
HDAd-gE3
Collagen
E
Baseline
F
HDAd-0
G
HDAd-gE3
H
Collagen Positive Area (%)
80
60
40
20
0
*
Baseline
HDAd-0
HDAd-gE3
α-Actin
I
Baseline
J
HDAd-0
K
HDAd-gE3
L
α-Actin Positive Area (%)
50
40
30
20
10
0
*
Baseline
HDAd-0
HDAd-gE3
VCAM-1
M
Baseline
N
HDAd-0
O
HDAd-gE3
P
VCAM-1 Positive Area (%)
35
30
25
20
15
10
5
0
†
*
Baseline
HDAd-0
HDAd-gE3
Calcium Deposit
Q
Baseline
R
HDAd-0
S
HDAd-gE3
T
Calcium Deposit Area (%)
5
4
3
2
1
0
*
*
Baseline
HDAd-0
HDAd-gE3

**Figure S2.** Immunochemical and histological features in atherosclerotic lesion regression. A-C, Mac3 (macrophage) staining. D, Macrophage-positive areas were quantified and expressed as mean ± SD % areas. *p<0.0001 vs. baseline and HDAd-0. N=10 (baseline), 6 (HDAd-0) and 8 (HDAd-gE3). E-G, Trichrome staining. H, Collagen positive areas. *p=0.02 vs. baseline and HDAd-0. I-K, Smooth muscle α-actin. L, α-Actin positive areas. *p=0.01 vs. baseline, and 0.0003 vs. HDAd-0 group. M-O, VCAM-1 staining. P, VCAM-1 positive areas. *p<0.05, †p<0.01 vs. baseline. Although relative VCAM-1 positive areas were reduced in both treatment groups, actual VCAM-1 positive area was increased in the HDAd-0 group due to increased lesion area. Q-S, Van Kossa staining. T, Calcium deposit positive areas. *p<0.005 vs. baseline. Bar: 50 μm.

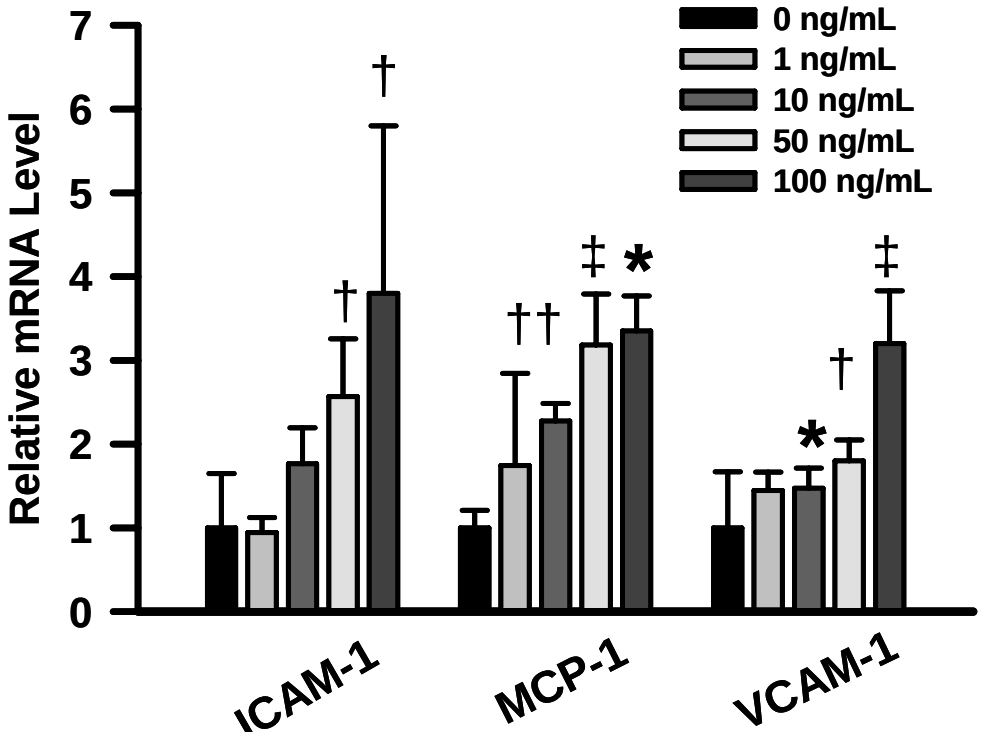


**Figure S3.** Relative mRNA expression of ICAM-1, MCP-1 and VCAM-1 in HAECs with increasing concentrations of IL-7. Human aortic endothelial cells (HAECs) were incubated with increasing concentration of IL-7 for 6 hours and mRNAs were quantified by real time RT-PCR. The mRNAs were normalized to β-actin mRNA and the results are expressed as mean ± SD. N=4/group. *$p<0.05$ (vs. 0 ng/mL), †$p<0.01$, ‡$p<0.001$. The experiment was repeated three times and the representative results are shown.

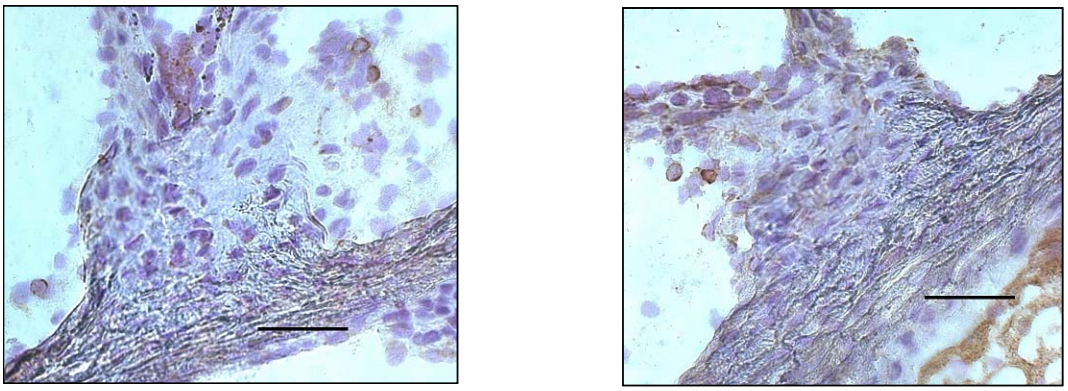


**Figure S4.** High IL-7 plasma levels increase CD3 positive immunoreactivity in aortas. 9 week old female Apoe$^{-/-}$ mice on normal chow diet were treated by HDAd expressing human IL-7 (HDAd-IL-7, 5 x $10^{12}$ VP/kg) or HDAd-0 (empty vector, 5 x $10^{12}$ VP/kg). Mice were euthanized 7 days after treatment and CD3 immunoreactive protein was detected by immunohistochemistry. Representative CD3 immunostaining in HDAd-0 treated mice (left panel) and in HDAd-IL-7 treated mice (right panel). x400, Bar, 50 µm.

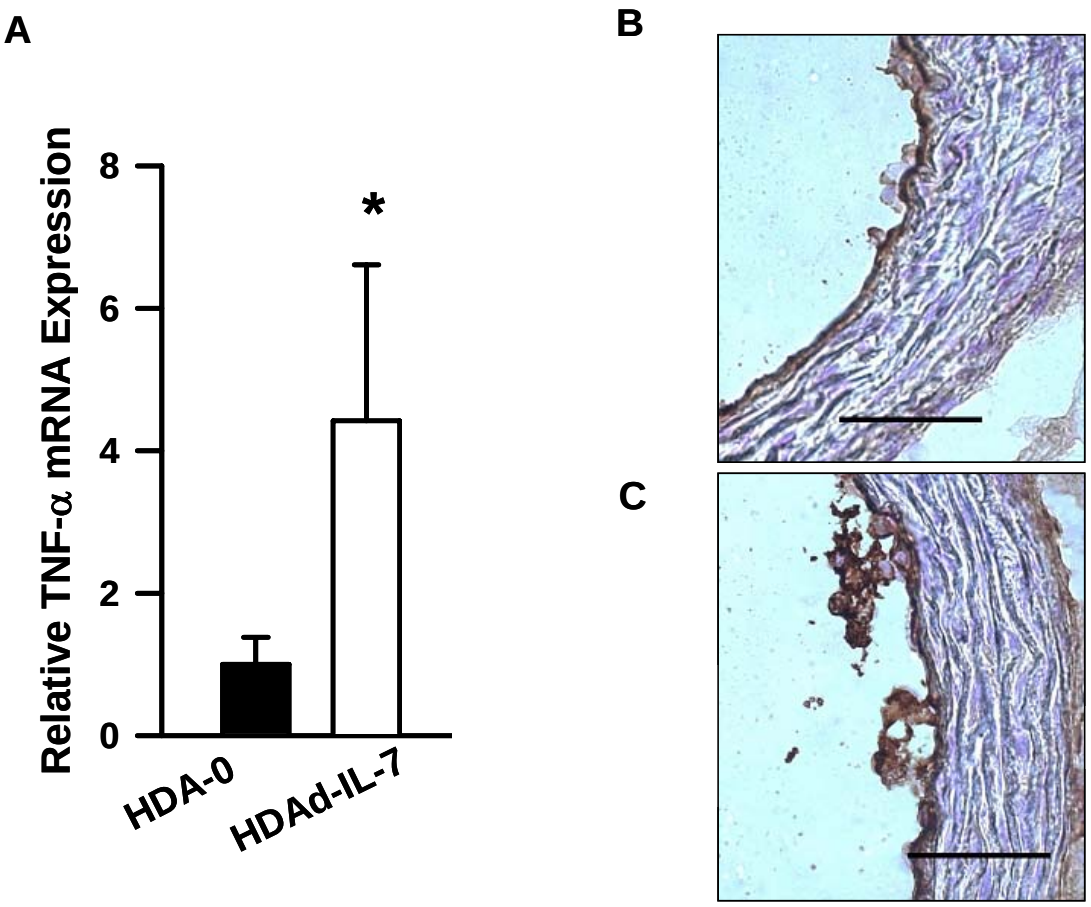


**Figure S5.** High IL-7 plasma levels increase TNF-α mRNA expression and TNF-α immunoreactivity in aortas. 9 week old female Apoe$^{-/-}$ mice on normal chow diet were treated by HDAd expressing human IL-7 (HDAd-IL-7, 5 x $10^{12}$ VP/kg) or HDAd-0 (empty vector, 5 x $10^{12}$ VP/kg). Mice were euthanized 7 days after treatment and TNF-α mRNA expression was determined by qRT-PCR and protein expression was characterized by immunohistochemistry. A, Relative TNF-α mRNA expression. TNF-α mRNA was normalized to β-actin mRNA and the results are expressed as mean ± S.D. *p=0.001 (vs. HDAd-0, n=6/group). Representative TNF-α immunostaining in HDAd-0- (B) or HDAd-IL-7-treated mice (C). x400, Bar, 50 μm.

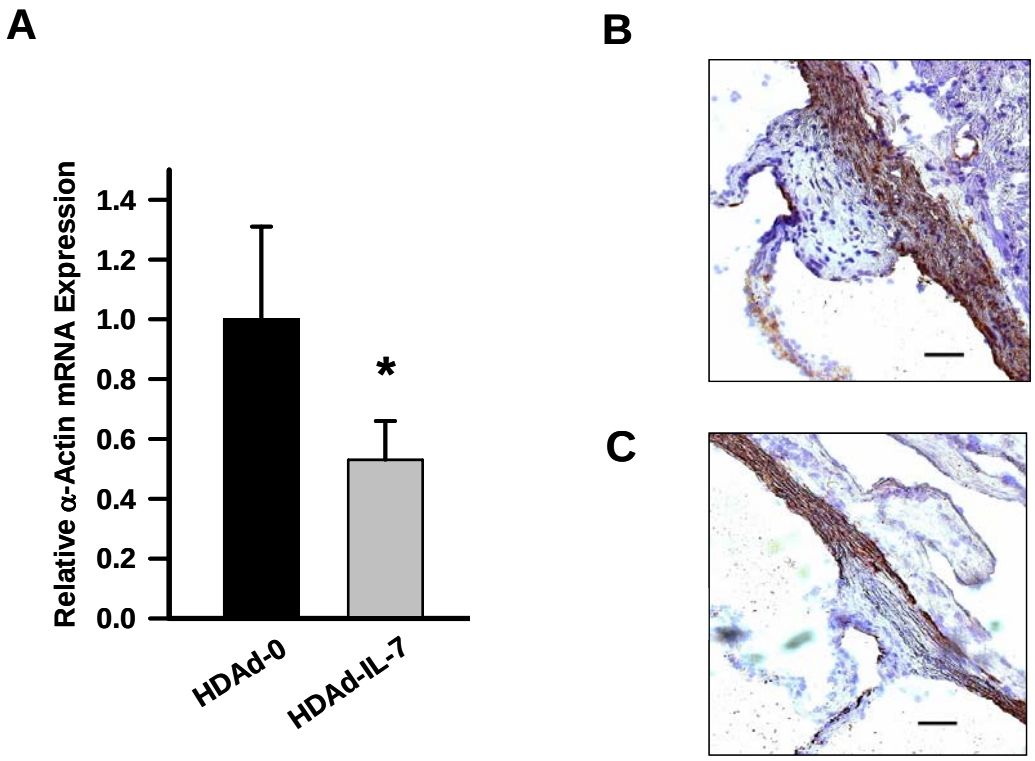


**Figure S6.** High IL-7 plasma levels reduces α-actin mRNA expression in aortas. 9 week old female Apoe$^{-/-}$ mice on normal chow diet were treated by HDAd expressing human IL-7 (HDAd-IL-7, 5 x $10^{12}$ VP/kg) or HDAd-0 (empty vector). Mice were euthanized 7 days after treatment and α-actin mRNA expression was determined by qRT-PCR or protein expression was characterized by immunohistochemistry. A, Relative α-actin mRNA expression. α-Actin mRNA was normalized to β-actin mRNA and the results are expressed as mean ± S.D. N=5/group. *p=0.01 (vs. HDAd-0). Representative α-actin immunostaining in HDAd-0 treated mice (B) and in HDAd-IL-7 treated mice (C). x200, Bar, 50 μm. **Note**: due to small lesion size, quantitation of α-actin positive area/lesion area was not performed.

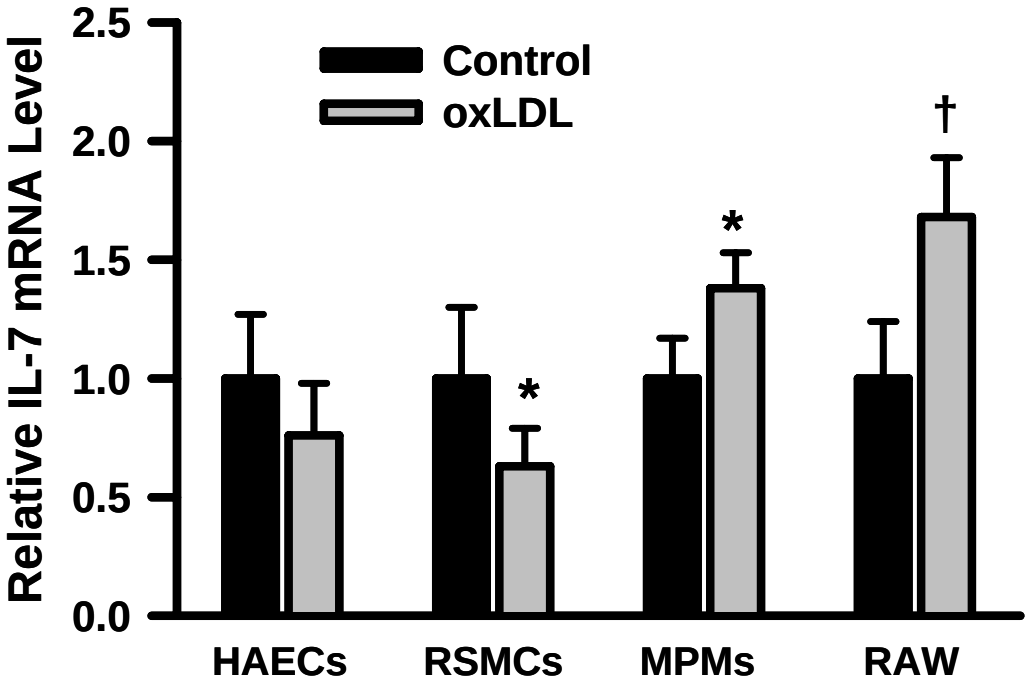


**Figure S7** Regulation of IL-7 expression by oxidized LDL (oxLDL). Cells were incubated in the presence or absence of oxLDL (50 μg/mL) for 4 hours and IL-7 mRNA was quantified by qRT-PCR. The results are normalized to β-actin mRNA and expressed as mean ± S.D. HAECs: human aortic endothelial cells; RSMCs: rat aortic smooth muscle cells; MPMs: peritoneal macrophages isolated from $Apoe^{-/-}$ mice; RAW: RAW 264.7 cells. N=3/group. *$p<0.05$ (vs. control), †$p<0.01$. The experiment was repeated three times and the representative results are shown.

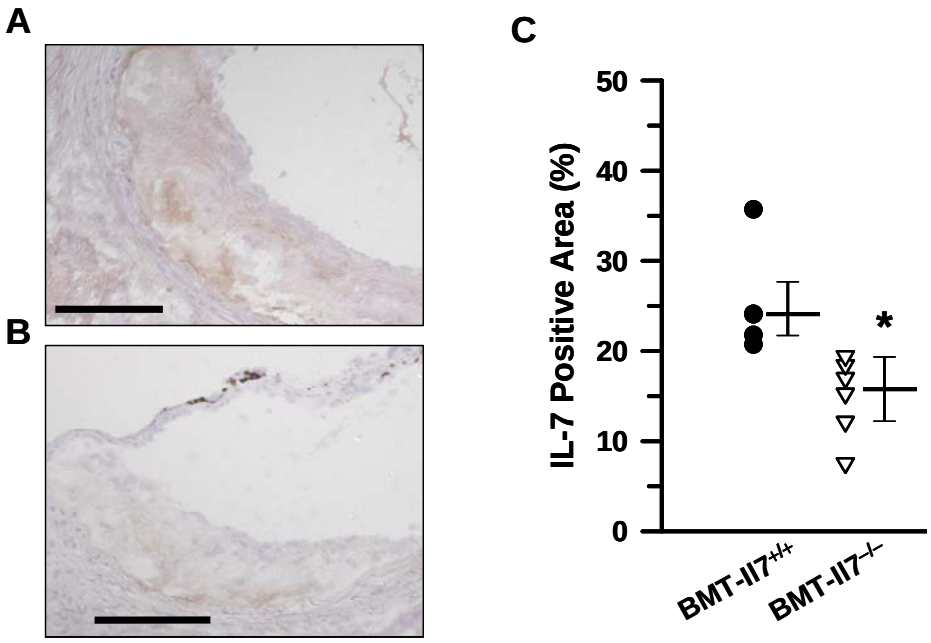


**Figure S8.** Lack of IL-7 in bone marrow-derived cells decreases IL-7 positive areas in atherosclerotic lesions. Representative IL-7 immunohistochemical staining of aortas in mice which received bone marrow cells isolated from $Il7^{+/+}$ mice (A) or $Il7^{-/-}$ mice (B). C, IL-7 positive area. IL-7 positive areas were quantified by morphometric analysis and analyzed by Mann-Whitney Rank Sum Test. Median, 25$^{th}$ percentile and 75$^{th}$ percentile values are indicated. *$p<0.05$ (n=5 for the $Il7^{+/+}$ group and n=6 for the $Il7^{-/-}$ group).

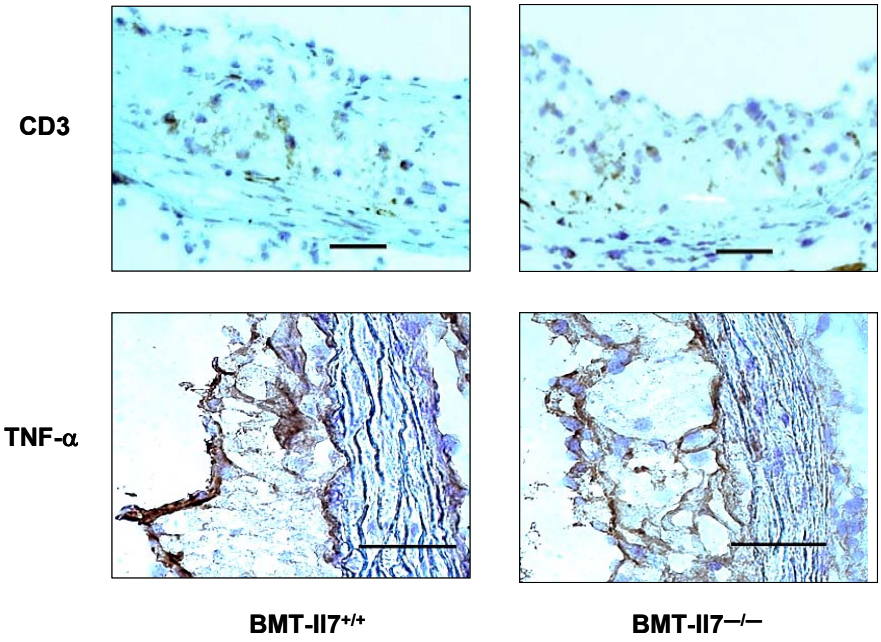


**Figure S9.** Lack of IL-7 in bone marrow-derived cells does not affect either CD3 positive cells or TNF-α immunoreactivity in atherosclerotic lesions. Representative CD3 (top panels) or TNF-α (bottom panels) immunohistochemical staining of aortas in mice which received bone marrow cells isolated from $Il7^{+/+}$ mice (left panels) or $Il7^{-/-}$ mice (right panels) is shown. Top panels, x200 and Bottom panels, x400. Bar: 50 μm.