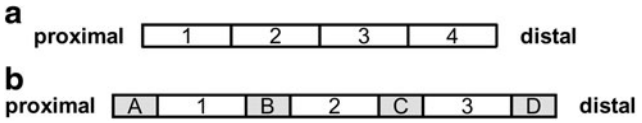
Supplementary Data



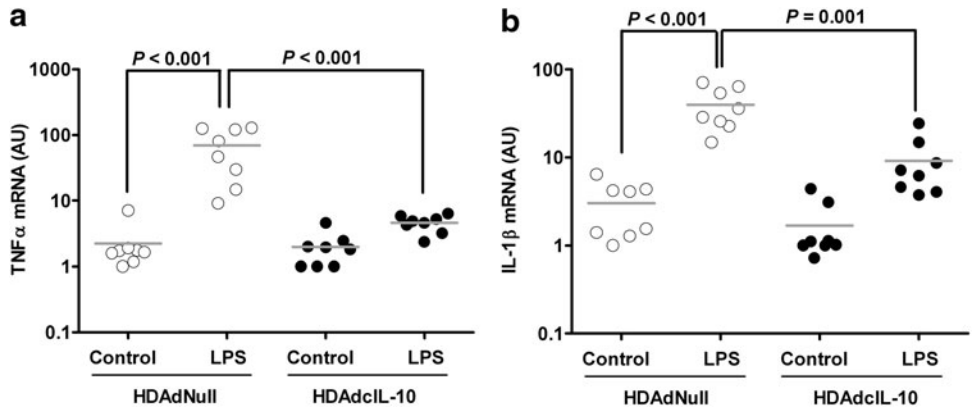
SUPPLEMENTARY FIG. S1. Division of arteries for endpoint analyses. (**a**) Some arteries were cut into 4 segments (1 – 4, proximal to distal). (**b**) Other arteries were cut into 7 segments, of the relative sizes illustrated. Segments A, B, C, and D were embedded in OCT medium and cryosectioned for histological and immunohistochemical studies. Segment 1 was snap-frozen for RNA extraction. Segments 2 and 3 were placed in explant culture overnight for conditioned medium collection, then frozen for DNA extraction.

Supplementary Table 1. Primers and Probes

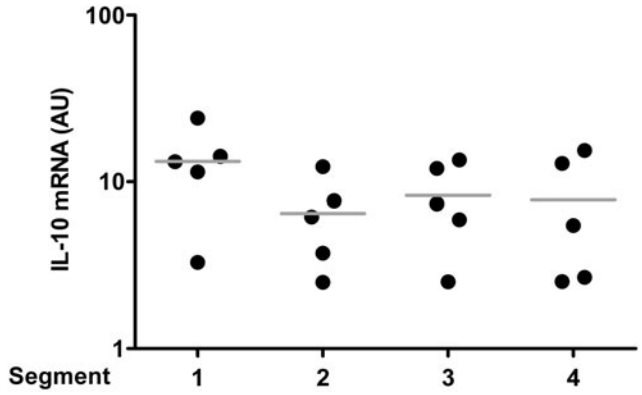
Primer/Probe[a] *Name*	*Sequence (5′ – 3′)*
IL-10 Forward	AGAACAGCTGCATTCACTTTCCA
IL-10 Reverse	CCTTCGATTGAAAGAAAGTCTTCAC
IL-10 Probe	CTCCGCGAGCTCCGTGCTGC
GAPDH Forward	TCATTGACCTCCACTACATGGTCTA
GAPDH Reverse	CGCTCCTGGAAGATGGTGAT
GAPDH Probe	TCCAGTATGATTCCACCCACGGCAA
CMV Forward	CATCTACGTATTAGTCATCGCTATTACCA
CMV Reverse	TGGAAATCCCCGTGAGTCA
CMV Probe	ACCGCTATCCACGCCCATTGGTGT
E1A Forward	AATGGCCGCCAGTCTTTTG
E1A Reverse	AAATGGCTAGGAGGTGGAAGATT
E1A Probe	TCAGCCAGTACCTCTTCGATCAGCTGGT
Helper Virus Forward	TCTGAGTTGGCACCCCTATTC
Helper Virus Reverse	GTTGCTGTGGTCGTTCTGGTA
Helper Virus Probe	TTCAGGGATGCCACATCCGTTGA
TNFα Forward	TGCTGCACTTCAGGGTGATC
TNFα Reverse	ATCTGGGCCACAGGGTTGA
TNFα Probe	CCCTCAGGAGGAAGAGTCCCCAAACA
IL-1β Forward	TCCAGACGAGGGCATCCA
IL-1β Reverse	CTGCCGGAAGCTCTTGTTG
IL-1β Probe	CTGCGCATCTCCTGCCAACCCT
IFN-γ Forward	TTTGCTTTCAAATATGCCTTTAGGT
IFN-γ Reverse	TCTGCCTCATCTTGGGTTCTTAC
IFN-γ Probe	TGCCAGGACACACTAACCAGAGAAACAGAA
MCP-1 Forward	GTGAAGAGGCTAATGAGCTATAGAAGAA
MCP-1 Reverse	GCCAGTTTGGTCATGAAGATCA
MCP-1 Probe	CAACAGCACCAAGTGTCCCAAAGAAGCT
IL-6 Forward	GTCCTTGCTTGCGGAATTTC
IL-6 Reverse	CAATGGACAGGATGGTGTGTTC
IL-6 Probe	TGGGCTCTGCCTCCCACGGTC

IL-10, interleukin-10; GAPDH, glyceraldehyde phosphate dehydrogenase; CMV, cytomegalovirus; TNFα, tumor necrosis factor alpha; IL-1β, interleukin 1-beta; IFN- γ, interferon-gamma; MCP-1, monocyte chemotactic protein-1; IL-6, interleukin-6.

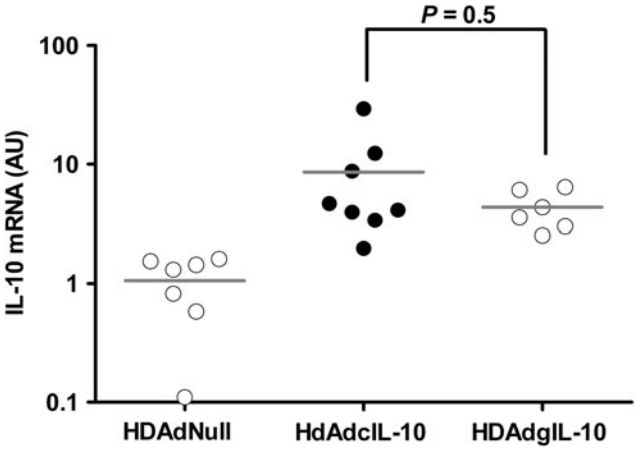
[a]All probes are labeled with 5′-FAM and 3′-TAMRA as the reporter and the quencher dyes.



SUPPLEMENTARY FIG. S2. Activity of vector-expressed IL-10. Rabbit peripheral blood mononuclear cells were treated with conditioned medium generated by transduction of 293 Cre4 cells with either HDAdNull or HDAdcIL-10. Aliquots of cells in both groups were treated with LPS. After 5 hr, RNA was extracted and the expression of (**a**) TNFα and (**b**) IL-1β were measured by qRT-PCR. Data points are individual wells of cells; bars represent group means.



SUPPLEMENTARY FIG. S3. Expression of IL-10 mRNA along transduced arteries. IL-10 mRNA was measured in each of 4 segments of 5 separate arteries infused with HDAdgIL-10 (5×10^{10} part/ml), with segment 1 most proximal and segment 4 most distal (see Supplementary Fig. 1). Bars are group means. The mean IL-10 mRNA value for an untransduced artery segment assayed in parallel was 1. $P = 0.4$ among the groups by one-way ANOVA.



SUPPLEMENTARY FIG. S4. In vivo IL-10 expression from HDAd expressing cDNA or genomic IL-10 clones. The two HDAdIL-10 or HDAdNull were infused in rabbit carotid arteries 4 wk after initiation of a high-fat diet. Arteries were removed 3 d later, RNA extracted, and IL-10 mRNA quantified by qRT-PCR. Data points are individual arteries; bars represent group means.

Supplementary Table 2. Plasma Cholesterol

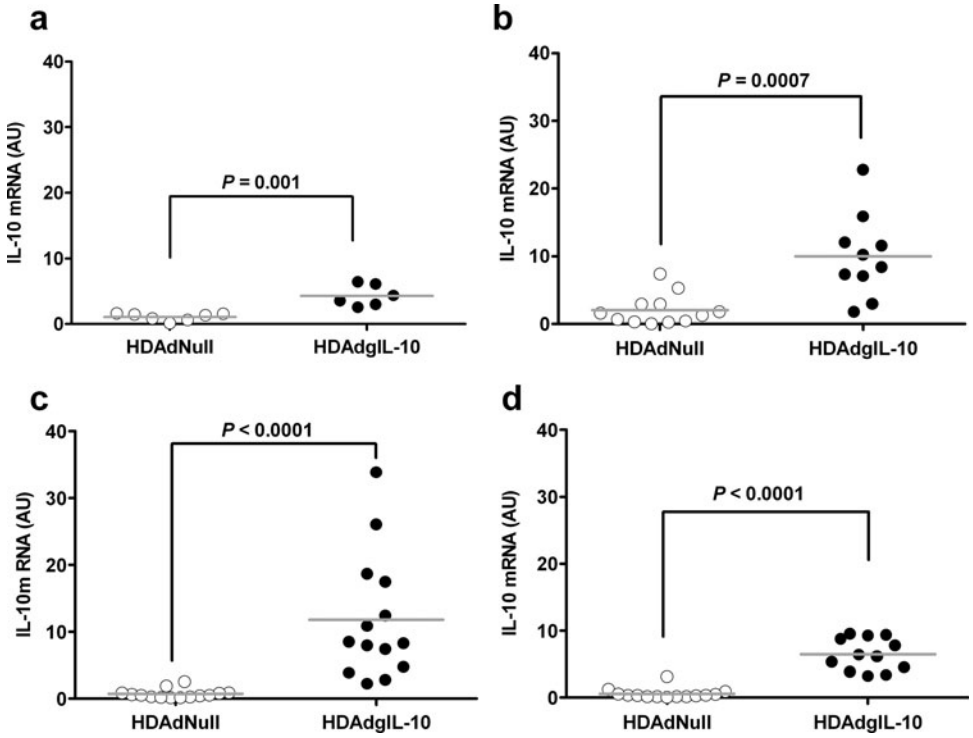
Before surgery		*At harvest*		
HDAdNull	*HDAdgIL-10*	*HDAdNull*	*HDAdgIL-10*	*Time of Harvest (days post surgery)*
374 ± 91	352 ± 96	489 ± 31	475 ± 45	3
390 ± 69	388 ± 70	676 ± 96	537 ± 87	14
463 ± 116	462 ± 138	485 ± 80	472 ± 125	28
364 ± 74	330 ± 83	490 ± 83	460 ± 71	56

Values are in mg/dL and are mean ± SEM of n = 8 – 14 rabbits per group.

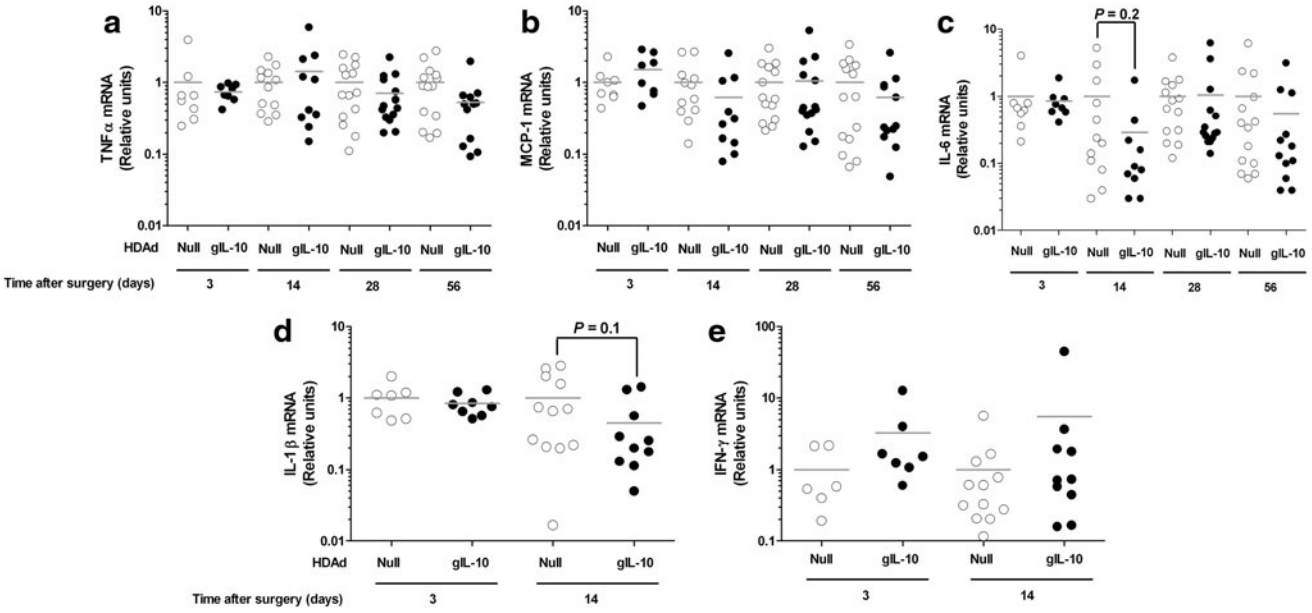
Supplementary Table 3. Blood Cell Counts and Serum Chemistries

	14 d		*56 d*	
	HDAdNull	*HDAdgIL-10*	*HDAdNull*	*HDAdgIL-10*
White blood cells ($10^3/mm^3$)	5.22 ± 0.52	6.67 ± 0.85	5.32 ± 0.48	4.45 ± 0.59
Red blood cells ($10^6/mm^3$)	6.10 ± 0.11	5.84 ± 0.10	5.85 ± 0.22	5.70 ± 0.15
Hemoglobin (g/dL)	12.6 ± 0.3	11.7 ± 0.3	12.6 ± 0.4	12.0 ± 0.4
Hematocrit (%)	35.3 ± 0.8	34.2 ± 0.4	34.8 ± 1.0	33.8 ± 1.0
Platelets ($10^3/mm^3$)	248 ± 26	397 ± 74	313 ± 21	344 ± 33
Polymorphonuclear Leukocytes (cells/mm^3)	1590 ± 420	2060 ± 391	1120 ± 301	856 ± 220
Lymphocytes (cells/ mm^3)	3230 ± 357	4080 ± 677	3830 ± 221	3400 ± 507
Monocytes (cells/ mm^3)	211 ± 49	151 ± 37	106 ± 38	39 ± 14
Eosinophils (cells/ mm^3)	56 ± 37	120 ± 39	76 ± 22	50 ± 20
Basophils (cells/ mm^3)	138 ± 27	263 ± 97	195 ± 50	100 ± 21
Glucose (mg/dl)	153 ± 23	127 ± 12	ND	ND
BUN (mg/dl)	21.8 ± 1.7	22.4 ± 0.8	ND	ND
Creatinine (mg/dl)	1.5 ± 0.1	1.7 ± 0.1	ND	ND
Total protein (g/dl)	5.5 ± 0.1	5.5 ± 0.1	ND	ND
Alkaline phosphatase (U/L)	82 ± 10	81 ± 7	ND	ND
Alanine aminotransferase (U/L)	40 ± 4	39 ± 8	ND	ND

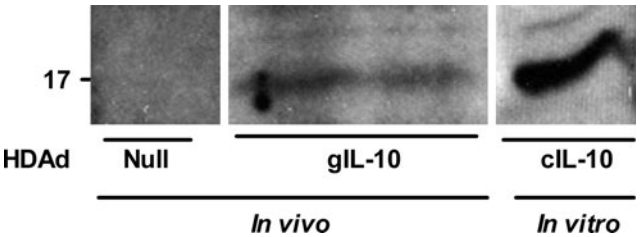
Data are mean ± SEM with n = 5 –7 in each group. $P > 0.2$ for all comparisons between HDAdNull and HDAdgIL-10 groups. ND = Not done.



SUPPLEMENTARY FIG. S5. Increased IL-10 mRNA in HDAdgIL-10 arteries. Carotid arteries were transduced with either HDAdNull or HDAdgIL-10 and harvested 3, 14, 28, and 56 days later (**a – d**, respectively). IL-10 mRNA was measured by qRT-PCR, normalized to GAPDH mRNA in the same extracts, and expressed as arbitrary units (AU). Data points are individual arteries; bars are group means. mRNA from the HDAdNull and HDAdgIL-10 arteries harvested at each of the time points was measured simultaneously.



SUPPLEMENTARY FIG. S6. HDAdgIL-10 does not alter expression of cytokines in rabbit carotid arteries. Carotid arteries were transduced with HDAdNull or HDAdgIL-10 and harvested 3 – 56 d later. (**a – c**) mRNA encoding TNFα, MCP-1, and IL-6 were measured in extracts of arteries harvested at 3, 14, 28, or 56 d. (**d – e**) mRNA encoding IL-1β and IFN-γ was measured only in the arteries harvested at 3 and 14 d. Cytokine mRNA was measured by qRT-PCR. Data points are individual arteries; bars represent group means.



SUPPLEMENTARY FIG. S7. IL-10 protein secreted by *in vivo* transduced arteries. Carotid arteries transduced with HDAdNull or HDAdgIL-10 were harvested after 14 d and placed in explant culture. Conditioned medium from the arteries was pooled, concentrated, and analyzed by Western blot. Unconcentrated conditioned medium from HEK 293 cells transduced with HDAdcIL-10 was used as a positive control (right lane). Size marker is in kDa.