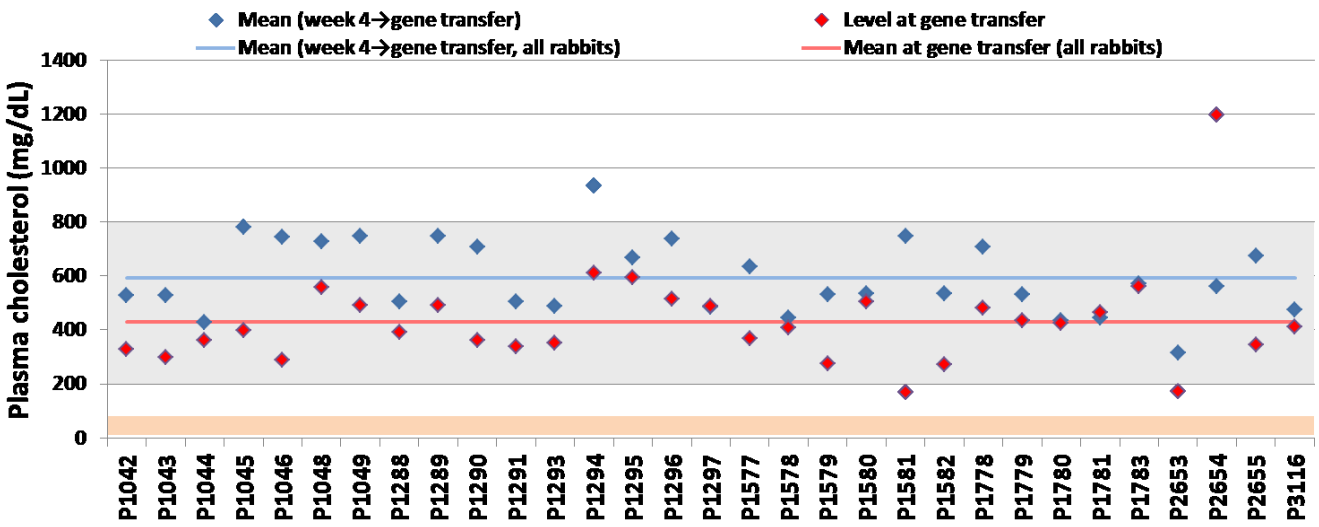
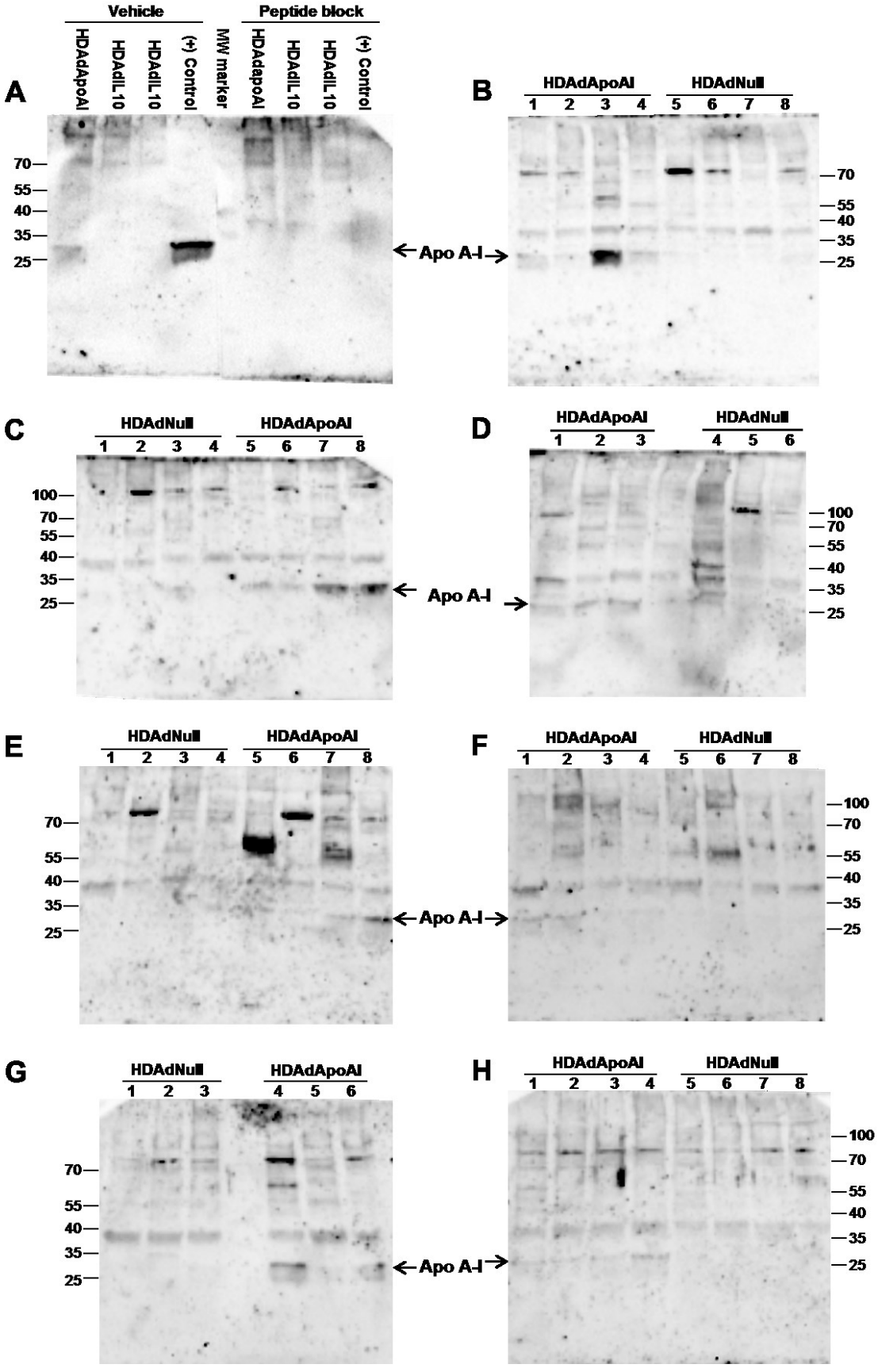
Supplemental Table I. Complete Blood Count and Serum Chemistries

Parameter	Units	Mean ± SD		Reference Range
WBC	10^3/µL	5.6 ± 1.4		3.7-11.0
RBC	10^6/µL	6.1 ± 0.5		4.90-7.00
HGB	g/dL	12.9 ± 0.8		10.0-15.0
HCT	%	37.1 ± 2.7		31.0-48.0
MCV	fL	61.2 ± 2.9		57.0-67.0
MCH	pg	21.2 ± 0.7		NA
MCHC	%	34.7 ± 1.1		31.0-35.0
PLT	10^3/µL	218 ± 63		200-650
Polys #	/µL	1653 ± 830		500-4000
Lymphs #	/µL	3415 ± 1145		1500-9000
Monos #	/µL	162 ± 114		0-500
Eosin #	/µL	91.1 ± 64.3		0-300
Basos #	/µL	297 ± 139		0-700
% Polys	%	29.2 ± 10.1		NA
% Lymphs	%	60.3 ± 13.3		NA
% Monos	%	3.3 ± 3.0		NA
% Eosin	%	1.6 ± 1.1		NA
% Basos	%	5.6 ± 3.2		NA
Glucose	mg/dL	164 ± 19		110-170
BUN	mg/dL	19.4 ± 2.1		11-21
Creatinine	mg/dL	1.1 ± 0.2		0.8-1.7
Calcium	mg/dL	12.3 ± 0.5		12.0-15.0
Phosphorus	mg/dL	3.5 ± 0.5		2.3-6.6
Ca/Phos Ratio		3.7 ± 0.8		NA
Total Protein	g/dL	5.2 ± 0.3		4.4-7.0
Albumin	g/dL	3.6 ± 0.3		3.2-4.1
Globulin	g/dL	1.5 ± 0.1		1.5-3.0
A/G Ratio	-	2.4 ± 0.3		2.2-3.2
Tot Bilirubin	mg/dL	0.1 ± 0.0		0.0-0.1
ALP	U/L	37.3 ± 11.8*	L	50-350
GGT	U/L	4.6 ± 1.2*	L	5-14
ALT	U/L	37.0 ± 16.2		18-80
AST	U/L	16.3 ± 6.8		11-55
CK	U/L	691 ± 324*	H	100-300
Cholesterol	mg/dL	81.5 ± 56.8		20-90
Amylase	U/L	271 ± 38		NA
Lipase	U/L	862 ± 654		NA
Bile Acid	µmol/L	6.3 ± 2.8		NA

NA = Not available. * Mean values that are outside the normal range for rabbits (according to the outside laboratory) are marked H (higher than range) or L (lower than range). All of these values are within published normal ranges for New Zealand White rabbits.[1, 2] n = 8 rabbits for all tests.



Supplemental Figure I. Plasma cholesterol levels of 31 rabbits that completed the study. Blue data points indicate each rabbit's mean cholesterol level during the period of sliding-scale feeding. Red data points indicate each rabbit's cholesterol level at the time of gene transfer. Horizontal bars are the corresponding mean values for all rabbits.


A
Vehicle
Peptide block
HDAdApoAI
HDAdIL10
HDAdIL10
(+) Control
MW marker
HDAdapoAI
HDAdIL10
HDAdIL10
(+) Control
70
55
40
35
25
Apo A-I
B
HDAdApoAI
HDAdNull
1 2 3 4 5 6 7 8
70
55
40
35
25
C
HDAdNull
HDAdApoAI
1 2 3 4 5 6 7 8
100
70
55
40
35
25
Apo A-I
D
HDAdApoAI
HDAdNull
1 2 3 4 5 6
100
70
55
40
35
25
E
HDAdNull
HDAdApoAI
1 2 3 4 5 6 7 8
70
55
40
35
25
Apo A-I
F
HDAdApoAI
HDAdNull
1 2 3 4 5 6 7 8
100
70
55
40
35
25
G
HDAdNull
HDAdApoAI
1 2 3 4 5 6
70
55
40
35
25
Apo A-I
H
HDAdApoAI
HDAdNull
1 2 3 4 5 6 7 8
100
70
55
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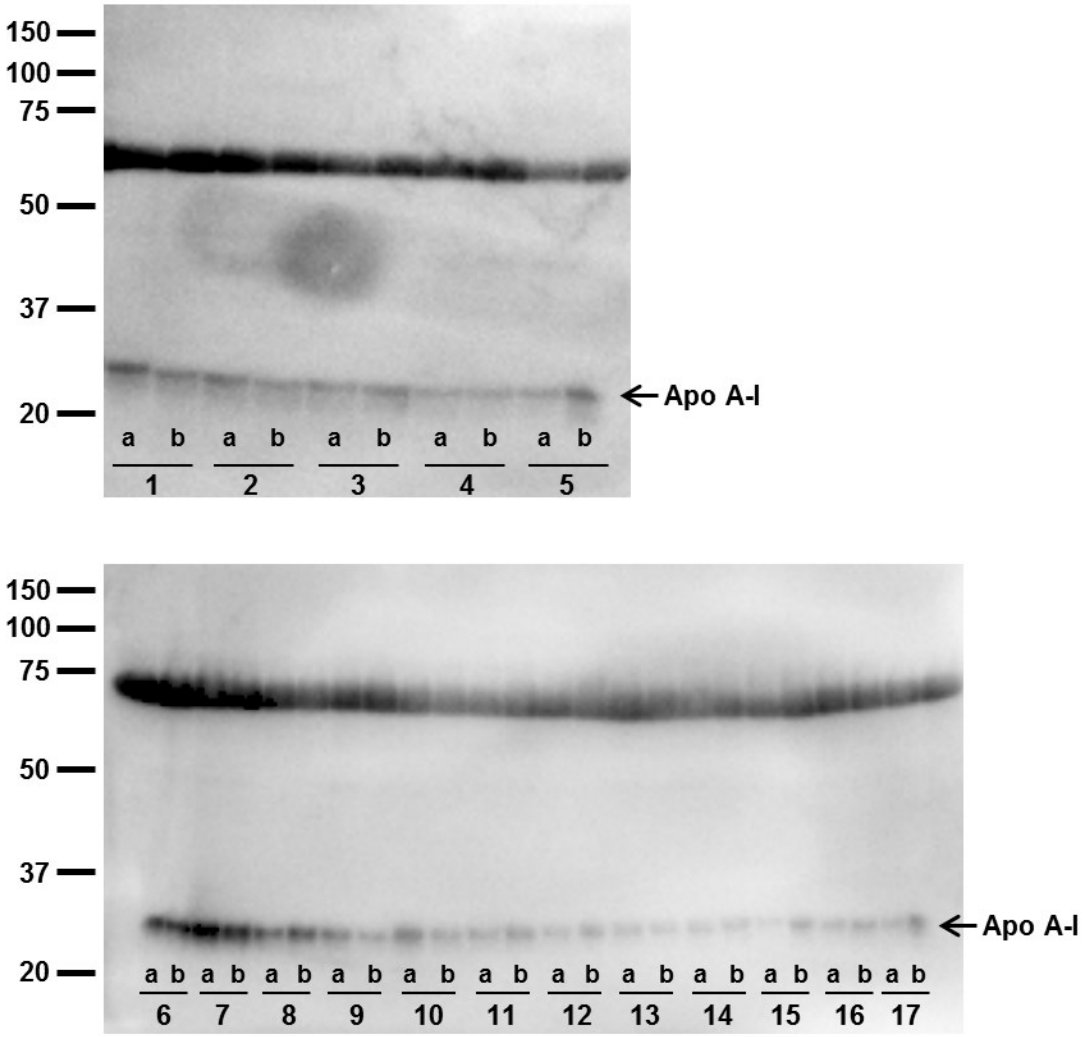
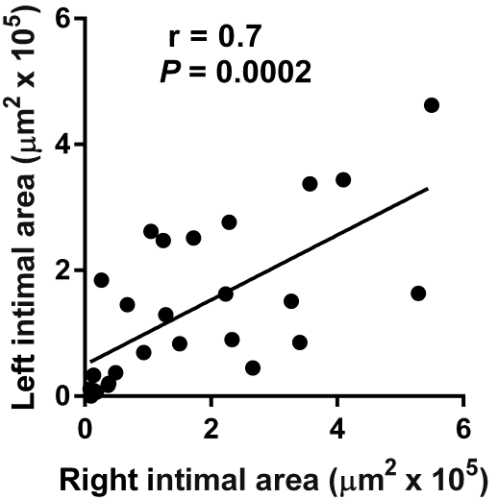
25

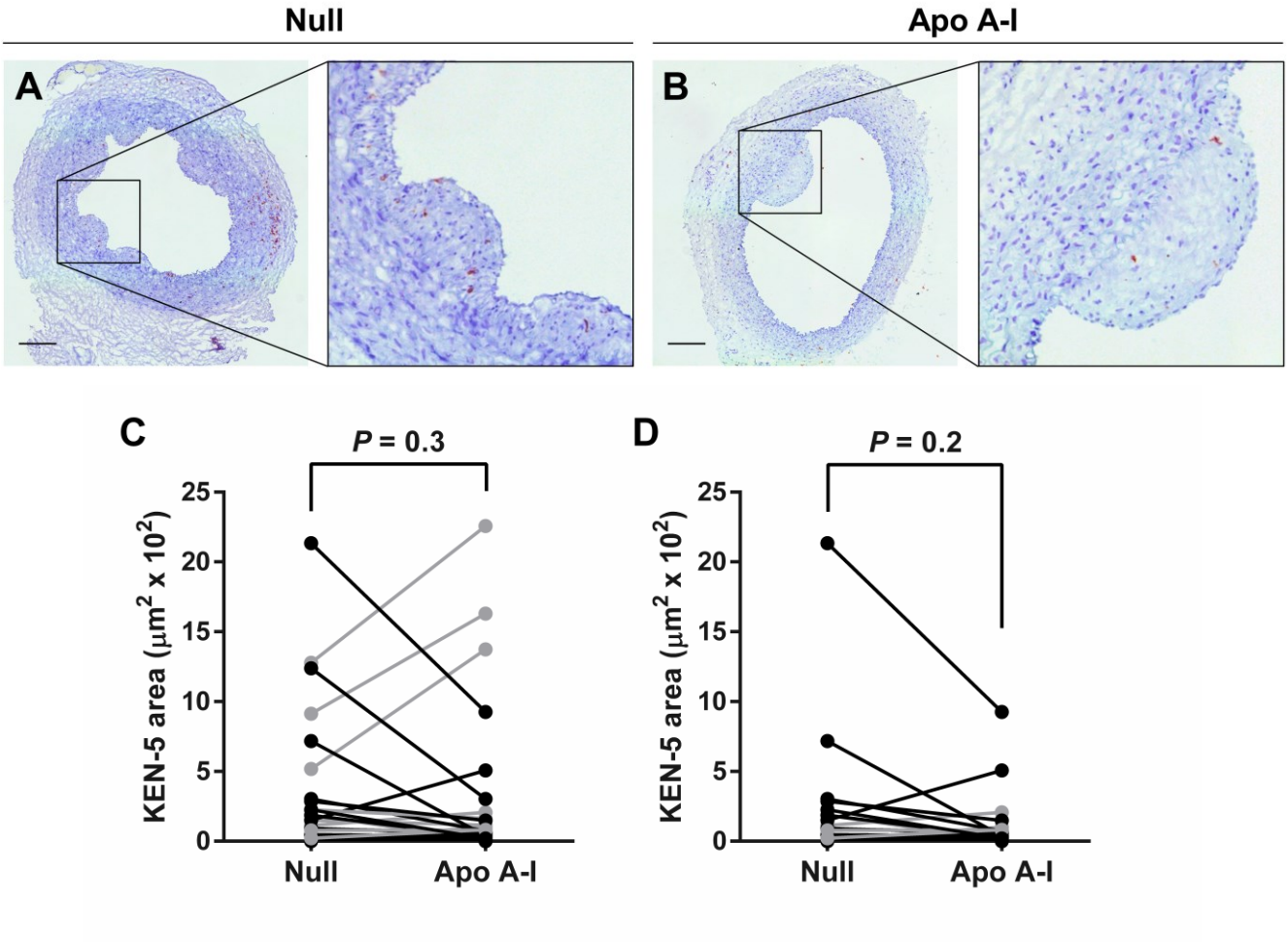
Supplemental Figure II. Expression of apo A-I protein from in vivo transduced carotid artery segments. (**A**) Testing of new anti-rabbit apo A-I antibody. Samples include explant culture medium from rabbit carotid arteries transduced in vivo with either HDAdApoAI or a control vector (HDAdIL10; archival samples), and medium conditioned by bovine aortic endothelial cells transduced in vitro with HDAdApoAI (+ control). Blots were probed with the new antibody either in the presence of vehicle or the peptide immunogen (peptide block). (**B**–**H**) Western analysis of medium conditioned by explanted carotid arteries from all 26 rabbits. In each rabbit, one carotid artery was transduced with HDAdNull, the other with HDAdApoAI. The arteries were harvested 7 weeks later and segments placed in explant culture. (**B**,**C**,**E**,**F,H**) Carotid arteries from the same rabbit are in lanes 1 and 5, 2 and 6, 3 and 7, and 4 and 8. (**D**,**G**) Arteries from the same rabbit are in lanes 1 and 4, 2 and 5; and 3 and 6. Size markers are in kilodaltons.



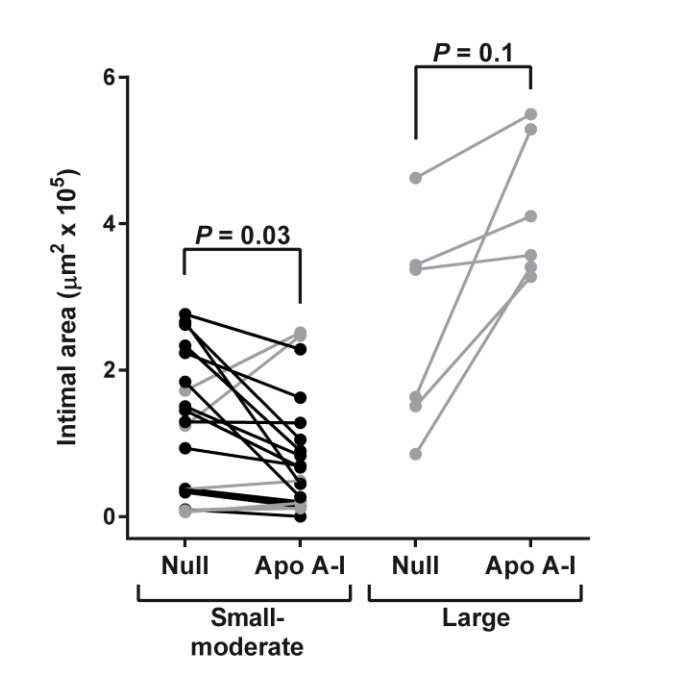
Supplemental Figure III. Western analysis of plasma apo A-I. Plasma is from 17 of the study rabbits and was collected from each rabbit: (a) just before gene transfer; and (b) 7 weeks later. The difference in band density between paired lanes (a) and (b) was compared with the Wilcoxon signed rank test ($P > 0.7$). Size markers are in kilodaltons.



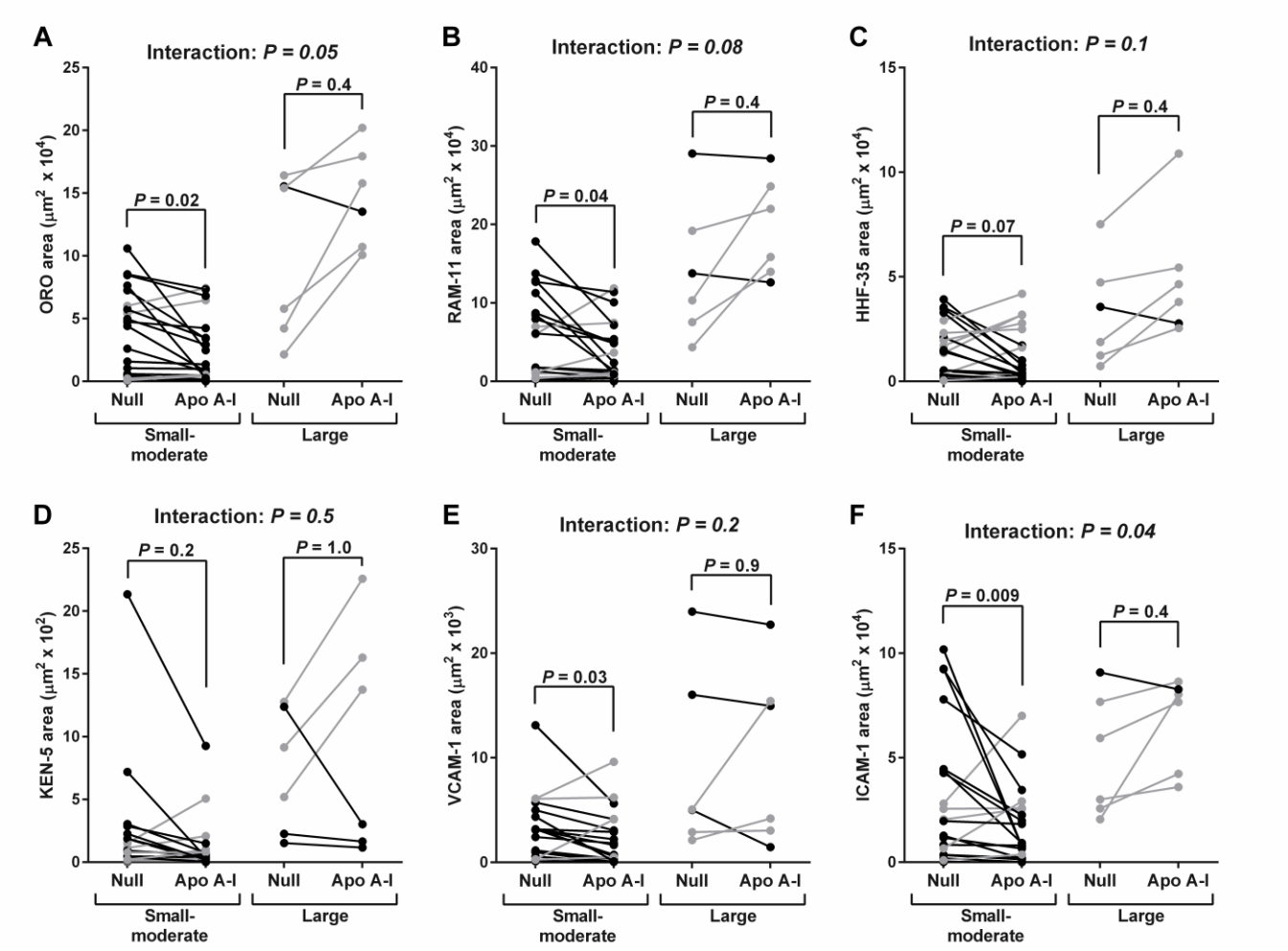
Supplemental Figure IV. Relationship of intimal areas in left and right carotid arteries of same rabbits. Each data point represents one rabbit. The intimal area of the rabbit's right carotid artery is indicated with reference to the x-axis. The intimal area of the rabbit's left carotid artery is indicated with reference to the y-axis. Pearson correlation coefficient (r) is indicated.



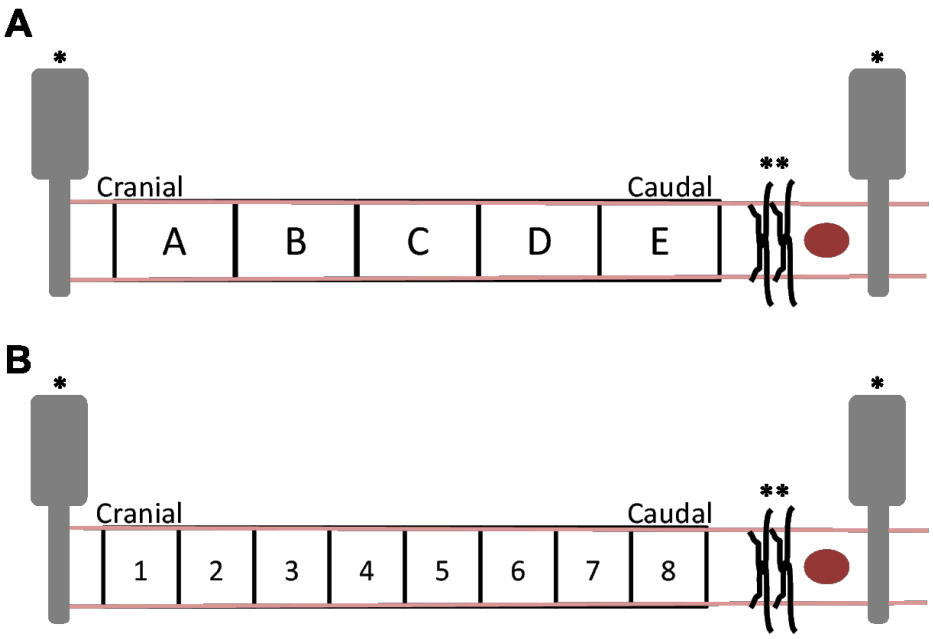
Supplemental Figure V. Intimal T cell content. Carotid arteries were removed 7 weeks after infusion of HDAdNull or HDAdApoAI, embedded in OCT, sectioned, and stained with the KEN-5 antibody to detect T cells. (**A**, **B**) Representative sections of arteries treated either with HDAdNull (Null) or HDAdApoAI (Apo A-I). (**C**) Intimal areas staining with the KEN-5 antibody in arteries infused with HDAdNull or HDAdApoAI; arteries from all 26 rabbits are included. (**D**) Intimal areas staining with the KEN-5 antibody; arteries are from a subgroup of 20 rabbits, none of which had an intimal lesion area more than one standard deviation above the mean of all lesions. Data points are means for each artery; points from arteries in the same rabbit are connected by bars. Rabbits in which HDAdApoAI-treated arteries had lower values than HDAdNull-treated arteries are indicated in black; rabbits in which HDAdNull-treated arteries had lower values than HDAdApoAI-treated arteries are indicated in grey. (**A**, **B**) Hematoxylin counterstain; scale bars = 200 μm.



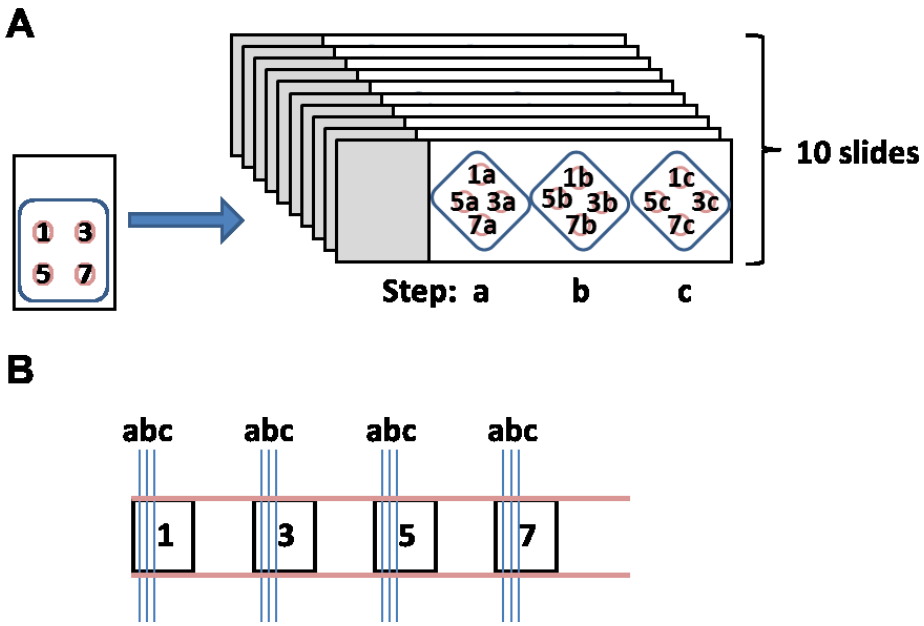
Supplemental Figure VI. Interaction of lesion size and response to treatment. Data are from two groups of lesion size, determined post-hoc: "Small-moderate" includes 20 rabbits (40 arteries) with intimal lesion areas that are not greater than one standard deviation above the mean of all lesions. "Large" includes contains 6 rabbits (12 arteries) in which either the left or right carotid artery had an intimal area greater than one standard deviation above the mean of all arteries. Data are the same as in Figure 4, but are split here into the 2 groups to enable analysis of interaction of lesion size and response to treatment. *P* values are for the effect of treatment within each group of lesion size. *P*=0.02 for interaction of lesion size group and treatment. Arteries were removed 7 weeks after treatment with either HDAdNull (Null) or HDAdApoAI (Apo A-I), sectioned, stained, and analyzed with computer-assisted planimetry.



Supplemental Figure VII. Interaction between intimal lesion size and response to treatment as measured by: (**A**) intimal Oil Red O area; (**B**) intimal RAM-11 area; (**C**) intimal HHF-35 area; (**D**) intimal KEN-5 area; (**E**) intimal VCAM-1 area; and (**F**) intimal ICAM-1 area. Data are from two groups of lesion size, determined post-hoc: "Small-moderate" includes 20 rabbits (40 arteries) with intimal lesion areas that are not greater than one standard deviation above the mean of all lesions. "Large" includes contains 6 rabbits (12 arteries) in which the mean area of either left or right carotid artery intimal lesion was greater than one standard deviation above the mean of all arteries. Data are the same as in Figure 4, Figure 6, and Supplemental Figure V, but are split here into the 2 groups to enable analysis of interactions between lesion size and response to treatment. *P* values are for the effect of HDAdApoAI treatment within each of the 2 lesion size groups and for the interaction of lesion size and response to treatment. Arteries were removed 7 weeks after treatment with either HDAdNull (Null) or HDAdApoAI (Apo A-I), sectioned, stained, and analyzed with computer-assisted planimetry. Data points are means for each artery; points from arteries in the same rabbit are connected by bars. Rabbits in which HDAdApoAI-treated arteries had lower values than HDAdNull-treated arteries are indicated in black; rabbits in which HDAdNull-treated arteries had lower values than HDAdApoAI-treated arteries are indicated in grey.



Supplemental Figure VIII. Protocols for processing rabbit carotid arteries for histologic and molecular analyses. (**A**) Arteries harvested 3 days after vector infusion were cut into 5 segments. Segments A, C, and E were processed for RNA; segments B and D were used for DNA analyses. (**B**) Arteries harvested 7 weeks after vector infusion were cut into 8 segments. Segments 1, 3, 5, and 7 were embedded in OCT for histology. Segments 2 and 6 were snap-frozen in liquid nitrogen for RNA analyses. Segments 4 and 8 were placed into DMEM and later processed for protein and DNA analysis. *Position of vascular clips, placed before vector infusion to isolate arterial segment. Red ellipses are sites of the arteriotomy used to rinse the lumen and infuse either DMEM or an HDAd vector. **Position of sutures used to secure cannula during vector infusion.



Supplemental Figure IX. Protocol for sectioning of arteries. (**A**) Each OCT block contains 4 segments (1, 3, 5, and 7; see Supplemental Figure VIII) from the same artery. Ten serial 6-µm-thick sections are cut from the block and placed on 10 different slides (step a). Ten more serial 6-µm-thick sections are cut at a 150 µm step from step a (step b). These 10 sections are placed on the same 10 slides, adjacent to the step a sections. The process is repeated to yield 10 step c sections. (**B**) Diagram of an artery showing position of the 4 segments (1, 3, 5, 7) and the 3 steps at which each segment is sectioned (vertical lines). The protocol yields sections at 12 steps along the artery. Each of 10 slides per artery contains sections from all 12 steps.

References

1. Hewitt, CD, Innes, DJ, Savory, J,Wills, MR (1989). Normal biochemical and hematological values in New Zealand white rabbits. *Clin Chem* **35**: 1777–1779.
2. Gil, AG, Silvan, G, Villa, A, Millan, P, Martinez-Fernandez, L,Illera, JC (2010). Serum biochemical response to inhalant anesthetics in New Zealand white rabbits. *J Am Assoc Lab Anim Sci* **49**: 52–56.