

Supplemental Figure Legends

Figure I. Relatively persistent transgene expression from HDAd. Arteries of cholesterol-fed rabbits were infused with either FGAduPA or HDAduPA (both at 7.5×10^{11} vp/mL) and harvested 3 d or 4 wk later. uPA mRNA was measured in arterial extracts by quantitative RT-PCR. Results are expressed as arbitrary units (AU), with the mean of the group with the lowest expression (FGAduPA at 4 wk) assigned a value of 1. Data points are from individual arteries; bars are group means.

Figure II. Transgene expression in arteries infused with FGAd or HDAd. FGAdCMVnLacZ (A and B) or HDAdGFP (C and D) were infused in arteries of either chow-fed (A, C) or cholesterol-fed (B, D) rabbits and harvested 3 d later. Sections of HDAdGFP arteries were viewed with fluorescence microscopy. FGAdCMVnLacZ arteries were stained with X-gal, embedded, sectioned, and counterstained with nuclear fast red. Transgene expression is primarily in luminal endothelium. Limited transgene expression in the adventitia (arrows) is likely due to leakage of vector during the surgical procedure.

Figure III. Histochemical and immunohistochemical detection of lipid, macrophages, and adhesion molecule expression in arteries of hyperlipidemic rabbits. Arteries were infused with DMEM, FGAdNull, or HDAdNull (both at 7.5×10^{11} vp/mL) and harvested 14 d later. Lipid content was detected with oil red O staining. Sections were stained for expression of RAM-11 (to identify macrophages), ICAM-1, or VCAM-1

Figure IV. Infusion of high dose (7.5×10^{11} vp/mL), but not low dose (2×10^{11} vp/mL) HDAdNull causes intimal growth, accumulation of lipid and macrophages, and expression of adhesion molecules in carotid arteries of cholesterol-fed rabbits. Arteries were harvested 4 wk after infusion of DMEM or HDAdNull. A, Intimal area; B, Percentage of oil red O-stained area

in the intima; C, Percentage of RAM-11-stained area in the intima; D and E, semiquantitative immunohistochemistry for intimal expression of VCAM-1 and ICAM-1.

Figure V. Co-infusion of FGAd increases HDAd-mediated transgene expression at 3 d and does not expedite loss of HDAd expression. Arteries of chow-fed rabbits were infused with the indicated vectors alone or in combination and were harvested either 3d or 4 wk later. A, uPA mRNA (expressed by HDAduPA) was measured by quantitative RT-PCR of artery RNA and expressed as arbitrary units (AU). B, Plasminogen activator (PA) activity was measured in medium conditioned by arteries explanted 3 d or 4 wk after vector infusion. PA activity (IU) was normalized to wet artery weight.

Figure 1

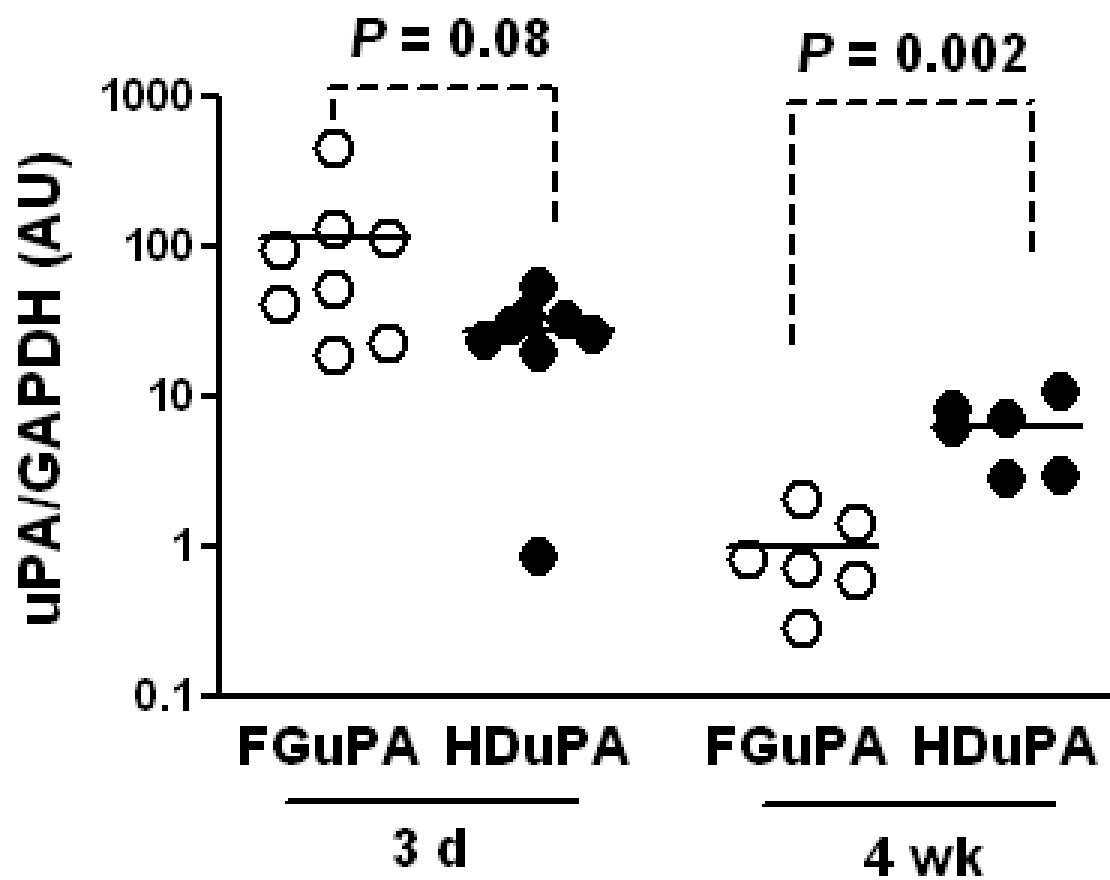


Figure II

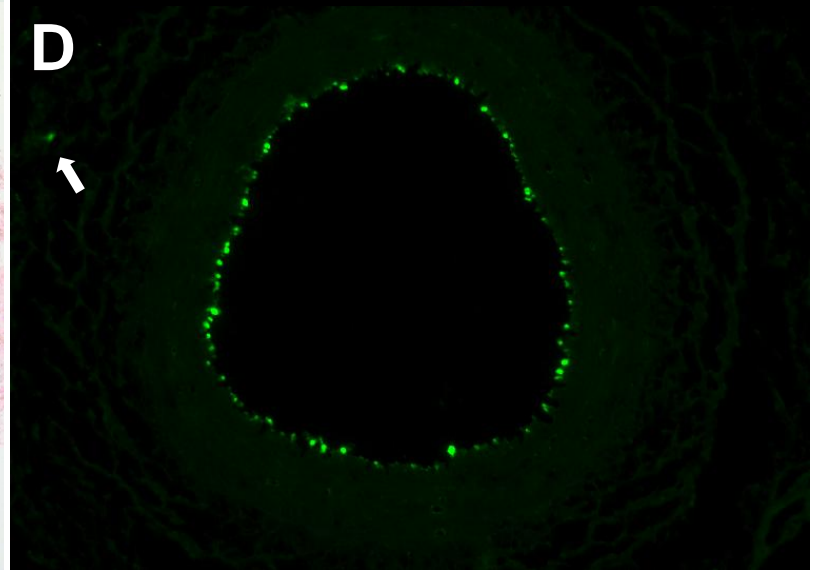
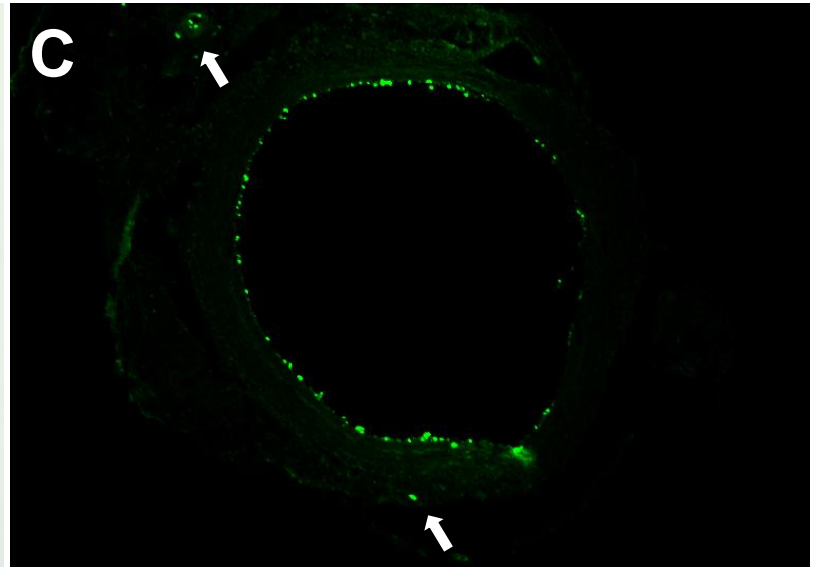
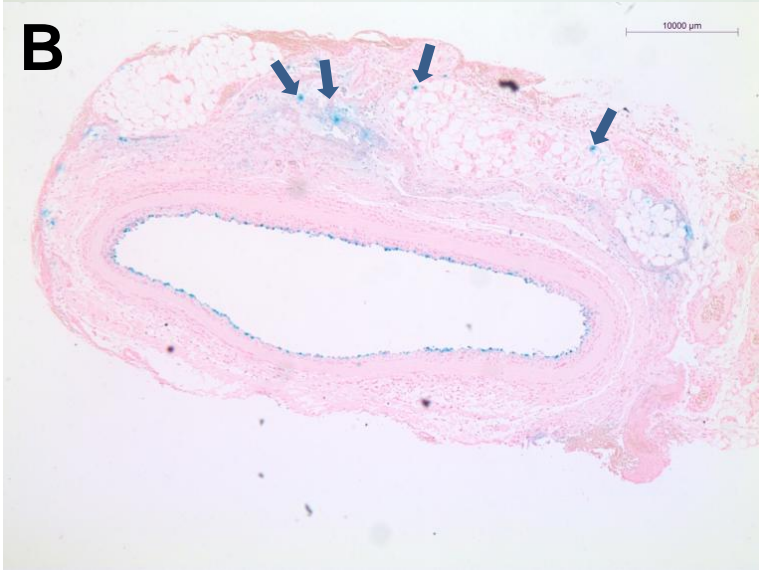
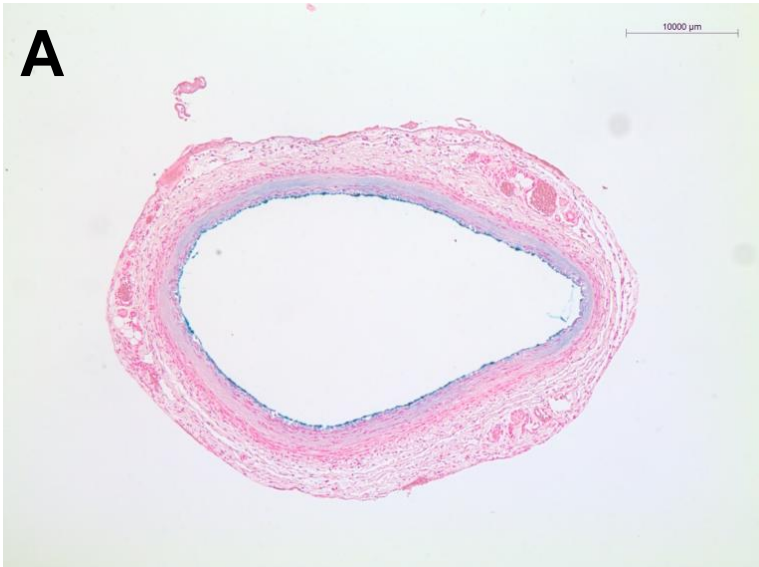


Figure III

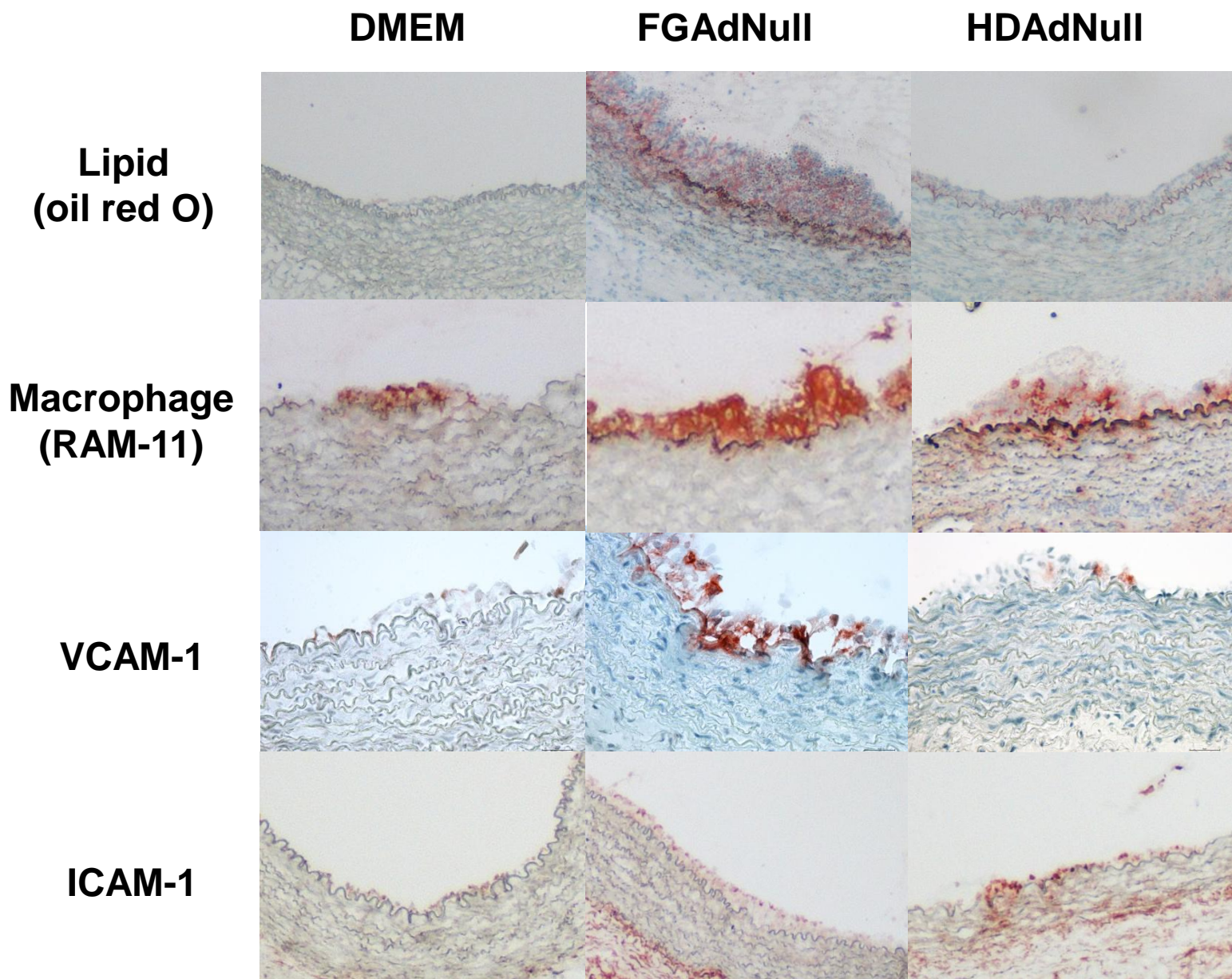


Figure V

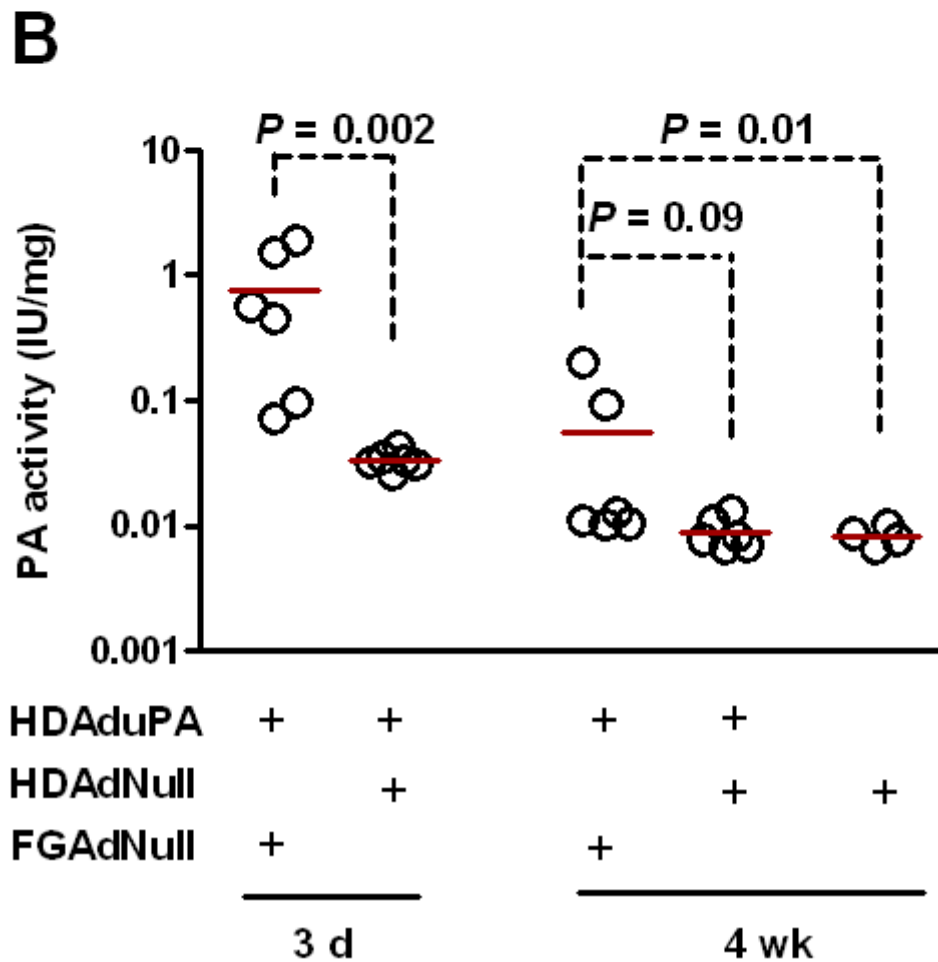
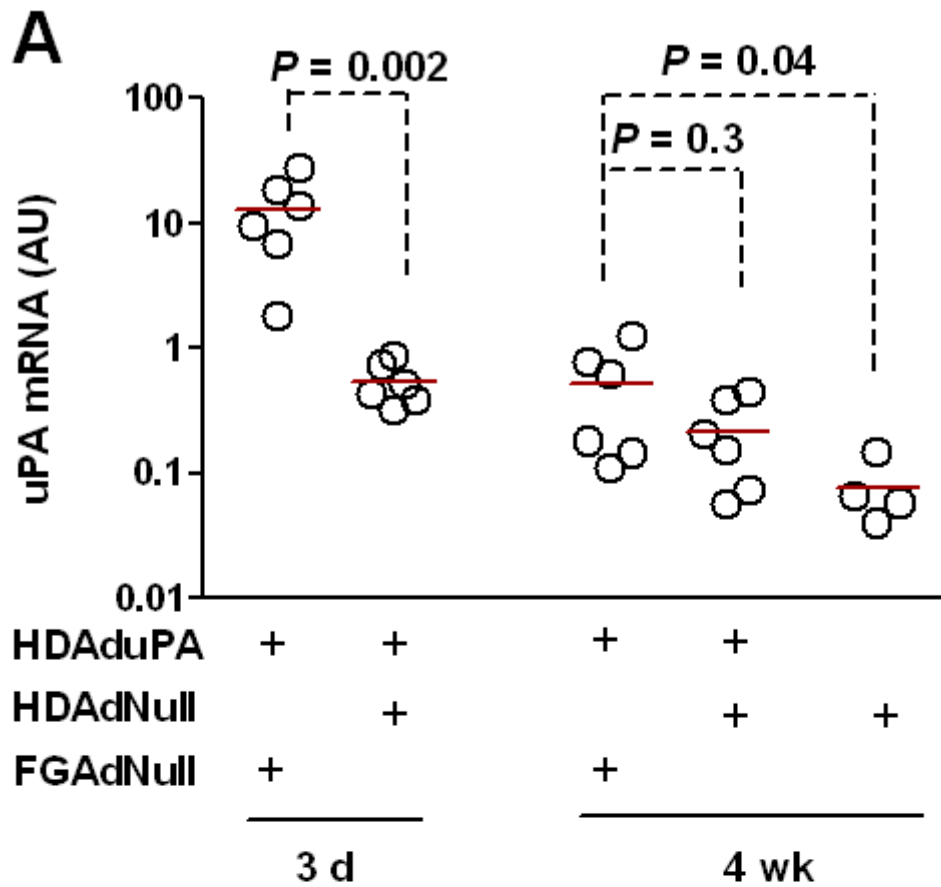


Table I. Primers and probes for qRT-PCR

MCP-1	Primers: GTGAAGAGGCTAATGAGCTATAGAAGAA GCCAGTTTGGTCATGAAGATCA Probe: CAACAGCACCAAGTGTCCCAAAGAAGCT
TNF- α	Primers: TGCTGCACTTCAGGGTGATC ATCTGGGCCACAGGGTTGA Probe: CCCTCAGGAGGAAGAGTCCCCAAACA
IL-6	Primers: GTCCTTGCTTGCGGAATTTC CAATGGACAGGATGGTGTGTTC Probe: TGGGCTCTGCCTCCCACGGTC
uPA	Primers: TACGAAAACATACCATGCCCA TGCACATAGCACCAGGGTATTC Probe: CACAATTACTGCAGGAACCCAGACCACCA
GAPDH	Primers: TCATTGACCTCCACTACATGGTCTA CGCTCCTGGAAGATGGTGAT Probe: TCCAGTATGATTCCACCCACGGCAA

Table II. Plasma cholesterol levels at time of gene-transfer surgery

Experiments examining intimal growth, vascular inflammation, and vessel reactivity 2 wk after gene-transfer surgery (Figures 2, 3 and 5; n = 6 per group)					
Infusate	DMEM	FGAdNull	HDAdNull		
Cholesterol (mg/dL)	298 ± 79	403 ± 160	351 ± 68		
Experiments examining intimal growth at 4 or 8 wk after gene-transfer surgery (Figure 6; n = 5 – 8 per group)*					
Infusate	DMEM	FGAdNull	HDAdNull	HDAdNull	FGAd+HDAd
Time of harvest	4 wk	4 wk	4 wk	8 wk	4 wk
Cholesterol (mg/dL)	266 ± 34	284 ± 36	285 ± 35	333 ± 52	442 ± 104
Experiments examining effects of low dose and high dose of HDAd at 4 wk after gene-transfer surgery (Figure 4; n= 4 – 6 per group)					
Infusate	DMEM	HDAdNull	HDAdNull		
		(2 × 10 ¹¹ vp/mL)	(7.5 × 10 ¹¹ vp/mL)		
Cholesterol (mg/dL)	469 ± 170	500 ± 129	505 ± 126		

*Primary data for the DMEM 8 wk group are not available; however, at the time these rabbits were operated, cholesterol levels of 400 – 700 mg/dl were required for entry to the study (see reference 17).