SUPPLEMENTAL MATERIAL

Supplemental Methods:

Generation of Adamts7 mouse strains: Adamts7$^{-/-}$ mice were generated by request at the Knockout Mouse Project (KOMP, University of California, Irvine). More information about the construction of these mice can be fond on the KOMP website (https://www.komp.org/geneinfo.php?geneid=18338). Vascular injury studies were performed in female mice, and wild-type (WT) littermates were used as controls. All mice were generated on pure C57B/6 backgrounds, and all animal experiments were reviewed and approved by the Institutional Animal Care and Use Committees of the University of Pennsylvania.

The expression of *Adamts7* mRNA was analyzed in tissues of 12 week old WT and *Adamts7*$^{-/-}$ mice by RT-PCR using multiple TaqMan probe-sets (Mm01239070_g1 and Mm01239067_m1, Life Tech, Grand Island, NY) and levels expressed relative to β-Actin expression. For determining *Adamts7* gene expression in mouse tissues, RNA was prepared from the indicated tissue from three female C57BL/6 mice. TaqMan expression analysis was carried out using probe Mm01239067_m1 and normalized to β-Actin. The tissue with the lowest level of expression was set to one (ovaries in this instance), and all tissue expression values are reported as relative fold-difference. Tissue distribution of *Adamts7* was examined by X-gal staining for the β-galactosidase reporter as positioned in the exon-trapping cassette in the *Adamts7* locus of the *Adamts7*$^{-/-}$ mouse.

X-gal Staining: Tissues were dissected and fixed in 2% paraformaldehyde (PFA) for 1 hour. After six 30 minute PBS washes, tissues were incubated at 37°c overnight in X-gal stain (PBS containing 2mM MgCl2, 5mM Potassium Hexacyanoferrate (iii), 5mM

Potassium Hexacyanoferrate (ii) trihydrate, 0.01% NP40, 0.1% Deoxycholate, and 1.0 mg/ml X-Gal in dimethylformamide). Tissues were then washed six more times in PBS, and then imaged.

Vascular Injury Studies: 8-week old *Adamts7*$^{-/-}$ and WT littermates were subjected to femoral artery wire-injury or sham surgery as previously described, with slight modification[1]. Briefly, one side of the femoral artery was exposed by blunt dissection while mice were under anesthesia a flexible angioplasty wire (0.35-mm diameter; Cook Inc, Bloomington, IN) was inserted into the femoral artery toward the iliac artery for 3 minutes to denude and dilate the artery. The femoral artery on the other side was sham-operated as a control. Mice (N=5 and 4, for *Adamts7*$^{-/-}$ and WT respectively) were euthanized 28 days post-surgery and perfused with 4% PFA. Femoral arteries were harvested and paraffin embedded for morphometric and histological analysis. Morphometric analysis was performed on 4 serial cross sections with a customized program (Phase 3 Imaging Systems, Glen Mills, PA) of Image Pro. The area of the lumen, the area inside the internal elastic lamina, and the area inside the external elastic lamina were estimated. Percentage stenosis was calculated as the ratio of the intimal area to the area inside the original internal elastic lamina, and the ratio of intima to media was determined.

Primary Aortic VSMC studies: Primary aortic smooth muscle cells were isolated as previously described [2] from *Adamts7*$^{+/+}$ and *Adamts7*$^{-/-}$ mice aged 8-10 weeks. Migration assays were performed using the Radius™ Cell Migration Assay (Cell Biolabs, Inc, San Diego, CA) as per manufacturers instructions. Cells were treated for 24 hours prior to gel spot removal or X-gal staining with 25ng/ml recombinant TNFα (Life Tech, Grand Island, NY) or water.

Aortic SMC Immunofluorescence: hAoSMCs were obtained commercially (Lonza, Switzerland) and maintained in Smooth Muscle Growth Medium-2 (SmGM-2, Clonetics, Walkersville, MD) per manufacturer protocol. For immunofluorescence, $1.5x10^5$ hAoSMCs were seeded on chamber slides. Twenty-four hours later, cells were fixed in 4% PFA for 15 minutes, permeabilized in 0.2% Triton X-100 for 5 minutes, washed and incubated in 1% BSA/PBS for 30 minutes at room temperature. Cells were treated with antibodies for ADAMTS7 (H00011173-A01, Novus Biologicals, Littleton, CO), Na+/K+ ATPase (sc-28800, Santa Cruz Biotechnology, Santa Cruz, CA), or Cortactin (ab11066, Abcam, Cambridge, MA), and then incubated with Donkey anti-mouse Alexa Fluor 488 and Goat anti-Rabbit Alexa Fluor 647 secondary antibodies (1:1000; Life Tech, Grand Island, NY) in 0.3%BSA/PBS. Purified rabbit and mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA) served as controls (Supplemental Figure 8). Deconvolution microscopy images were acquired using the Deltavision Core Deconvolution Microscope system (Applied Precision Inc Issaquah, WA), using an Olympus IX70 microscope and softWoRx acquisition and analysis software (Applied Precision Inc Issaquah, WA).

Immunohistochemistry: All IHC, including that for sections of mouse aortic root, mouse BCA, mouse femoral artery, and human coronary arteries was performed in the UPenn Cardiovascular Institute Histology Core, and all relevant protocols are available at http://www.pennmedicine.org/heart/research-clinical-trials/core-facilities/histology-gene-expression/. All tissue sections were deparaffinized and pretreated using heat antigen retrieval with Bull's Eye Decloaker (BioCare Medical, Concord, CA) or 1.5% Proteinase K. After washing with 0.1% PBST and blocking with normal serum for 30-60 minutes at 25°C, sections were incubated with primary antibody in 0.1% PBST overnight at 4°C. For all work with mouse tissues, antibodies used included mouse anti-Fibronectin (1:50; Santa Cruz, Santa Cruz, CA), mouse anti-Collagen IV (1:100; Millipore, Billerica, MA),

goat anti-Sm22a (1:100; ab10135, Abcam, Cambridge, MA), mouse anti-SM-α-actin (1:200; A5228, Sigma, St. Louis, MO), mouse anti-SM-MyHC (1:100; Biomedical Technologies Inc., Stoughton, MA), and rat anti-Mac-3 (1:100; #550292, BD BioSciences, San Jose, CA). For staining of human coronary artery sections, we used anti-ADAMTS7 (1:100; ab45044, Abcam, Cambridge, MA), anti-CD68 (1:100; ab955, Abcam, Cambridge, MA) and the same anti-Sm22α and anti-SM-α-actin listed above. After washing with 0.1% PBST, sections were incubated with Alexa Fluor 488 or 568 Goat anti-mouse or Donkey anti-goat IgG (1:250; Life Tech, Grand Island, NY) for 1 hour at 25°C, or were subjected to ImmPRESS TSA detection for 60 minutes at room temperature. Secondary antibodies alone served as controls (Supplemental Figure 8). All slides then washed with 0.1% PBST and mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA).

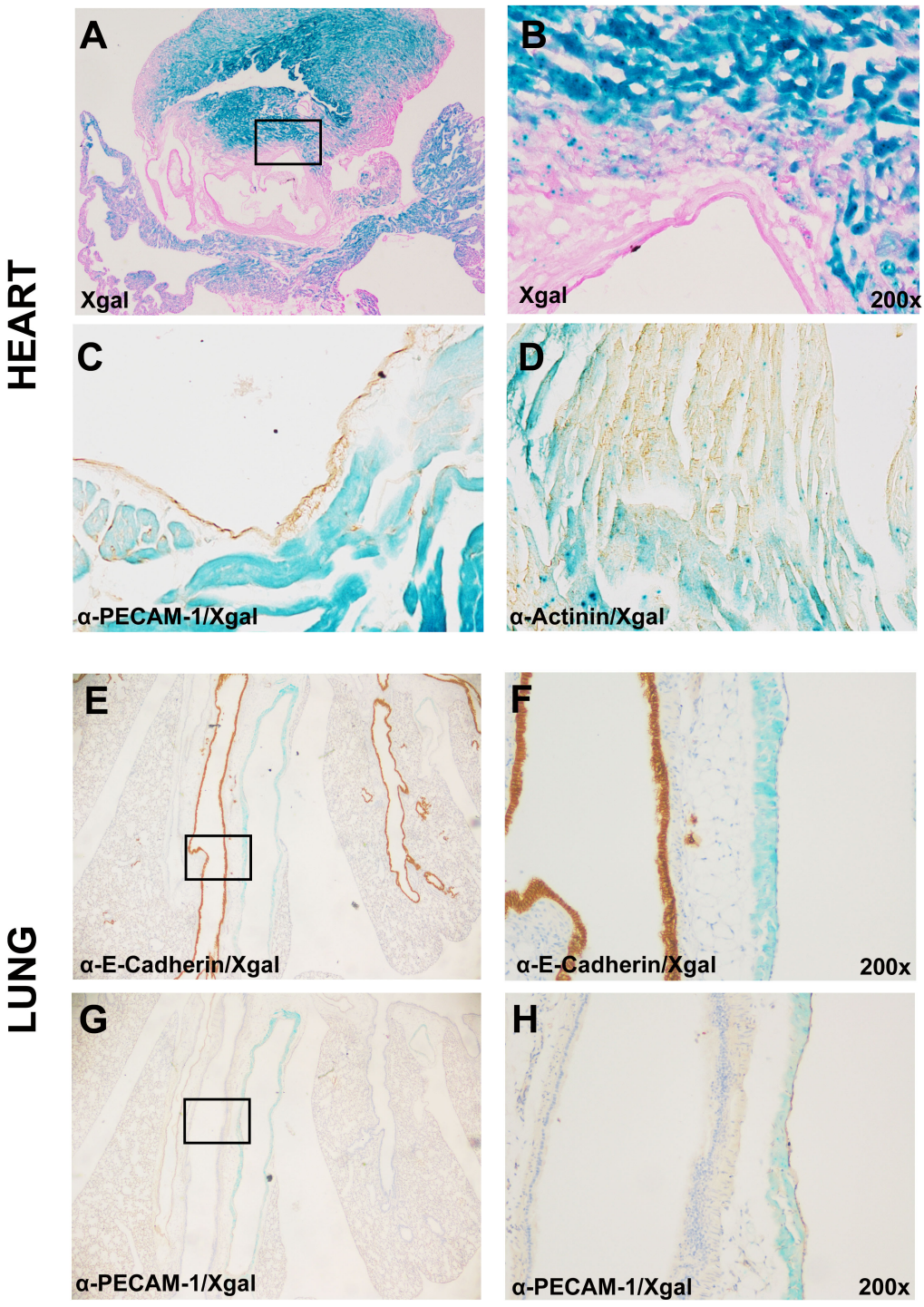
Plasma Lipid Measurements: Whole blood (200 μL) was collected via retro-orbital sinus plexus from mice after 4 hours of fasting for plasma lipid analyses. Plasma was obtained after centrifugation for 7 minutes at 10,000 rpm in a microcentrifuge and stored in aliquots at −80°C.

Supplemental Tables:

		Prebleed				Terminal Bleed			
Sex	Genotype	TC	HDL	Non-HDL	TG	TC	HDL	Non-HDL	TG
M	$Ats7^{+/+};Ldlr^{-/-}$	278.5 (46.4)	116.9 (14.3)	161.6 (33.5)	70.8 (18.6)	1795.7 (488.3)	258.9 (75.6)	1536.9 (429.1)	435.2 (164.0)
M	$Ats7^{-/-};Ldlr^{-/-}$	271.5 (28.1)	109.0 (12.8)	162.5 (28.8)	83.4 (25.6)	1924.2 (504.0)	302.6 (74.6)	1621.6 (441.5)	434.1 (165.8)
F	$Ats7^{+/+};Ldlr^{-/-}$	236.9 (23.9)	88.9 (9.2)	148.1 (18.8)	54.1 (16.1)	1140.6 (254.2)	187.0 (3.4)	953.6 (229.8)	164.3 (56.1)
F	$Ats7^{-/-};Ldlr^{-/-}$	264.1* (22.1)	95.2 (9.6)	168.9* (16.1)	63.7 (24.4)	1176.6 (250.6)	193.1 (40.9)	983.5 (219.5)	149.1 (60.9)
M	$Ats7^{+/+};Apoe^{-/-}$	645.0 (30.5)	56.2 (4.1)	588.8 (28.5)	107.5 (3.4)	1374.9 (116.8)	129.2 (19.4)	1245.7 (122.9)	101.1 (40.8)
M	$Ats7^{-/-};Apoe^{-/-}$	719.3 (82.6)	44.5* (6.8)	674.8 (88.1)	112.3 (9.5)	1413.4 (193.3)	138.9 (25.1)	1274.5 (191.2)	84.1 (34.4)
F	$Ats7^{+/+};Apoe^{-/-}$	(578.3 (33.9)	32.8 (5.1)	545.5 (30.0)	26.3 (9.1)	1078.8 (92.0)	89.8 (28.1)	989.0 (75.4)	48.6 (17.2)
F	$Ats7^{-/-};Apoe^{-/-}$	733.5 (120.9)	29.6 (2.5)	703.9 (123.5)	34.0 (45.3)	1006.7 (98.5)	104.8 (16.4)	901.9 (100.7)	57.8 (31.0)

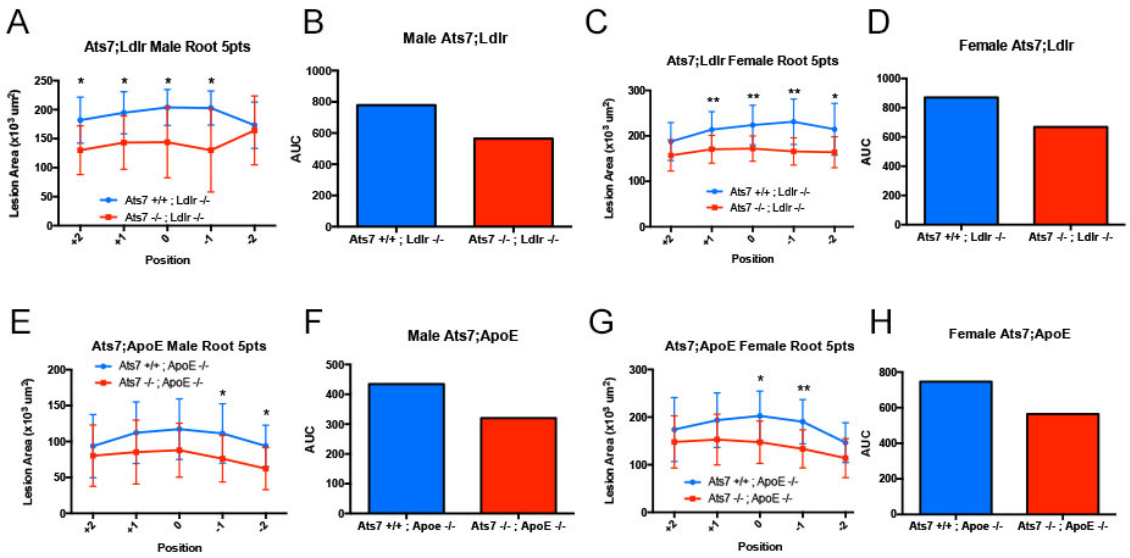
Supplemental Table 1: Plasma lipid levels in both the *Ldlr* and *Apoe* hyperlipidemic KO mouse models are unchanged in the setting of *Adamts7* deficiency. Plasma lipid values for total cholesterol (TC), HDL, Non-HDL, and triglycerides (TG) are shown for all atherosclerosis experimental groups. The units for all measurements are mg/dL. The value in parentheses is the standard deviation of the group. * denotes a p-value < 0.05 as compared to control group by Student's t-test.

Supplemental Figures:

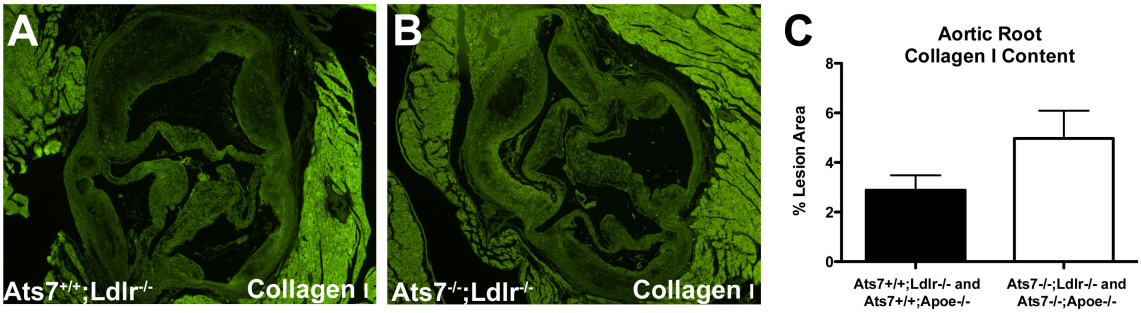


Supplemental Figure 1: *Adamts7* is expressed in myocardium and pulmonary vasculature as evidenced by Xgal staining. A) Xgal staining of whole heart reveals

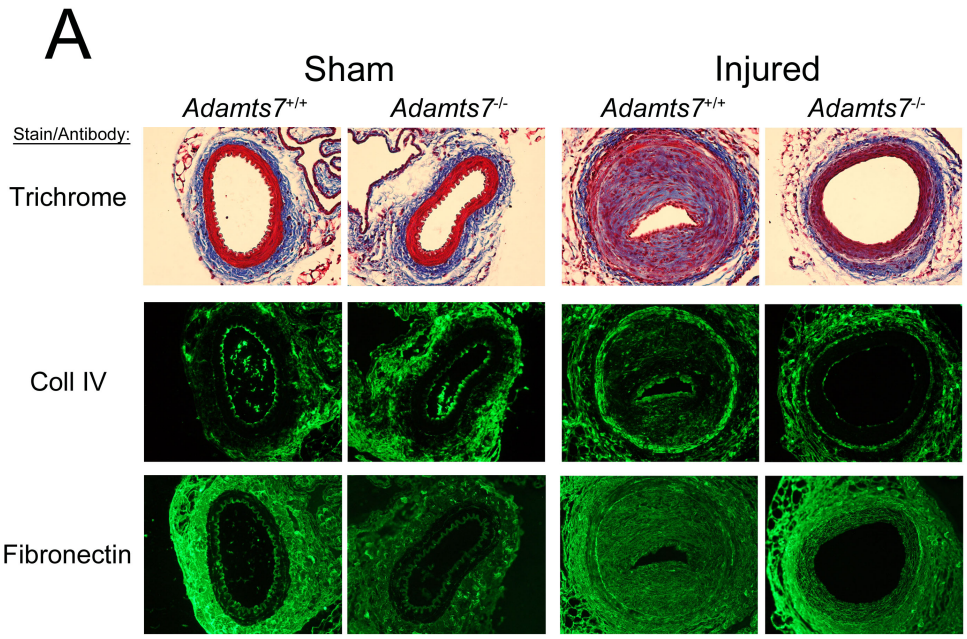
ubiquitous expression of *Adamts7* in cardiomyocytes throughout the heart. B) 200x zoom of the boxed region from panel A showing that while myocardium expression of *Adamts7* is high, in the resting state there is little to no positive staining in the wall of the aortic root. C) X-gal staining in heart does does not colocalize with markers (PECAM-1) for endothelial cells, but D) does localize with markers (α-Actinin) of cardiomyocytes. E-H) Staining of Xgal-stained lung sections with antibodies directed at E-cadherin and PECAM-1 to differentiate between trachea and pulmonary vasculature. *Adamts7* expression is localized to the media of the vasculature, as positive X-gal staining colocalizes with positive PECAM-1 staining for the endothelial layer and not positive E-Cadherin staining for bronchial epithelial cells. Panels F and H provide 200x enlargement of boxed regions in E and G.

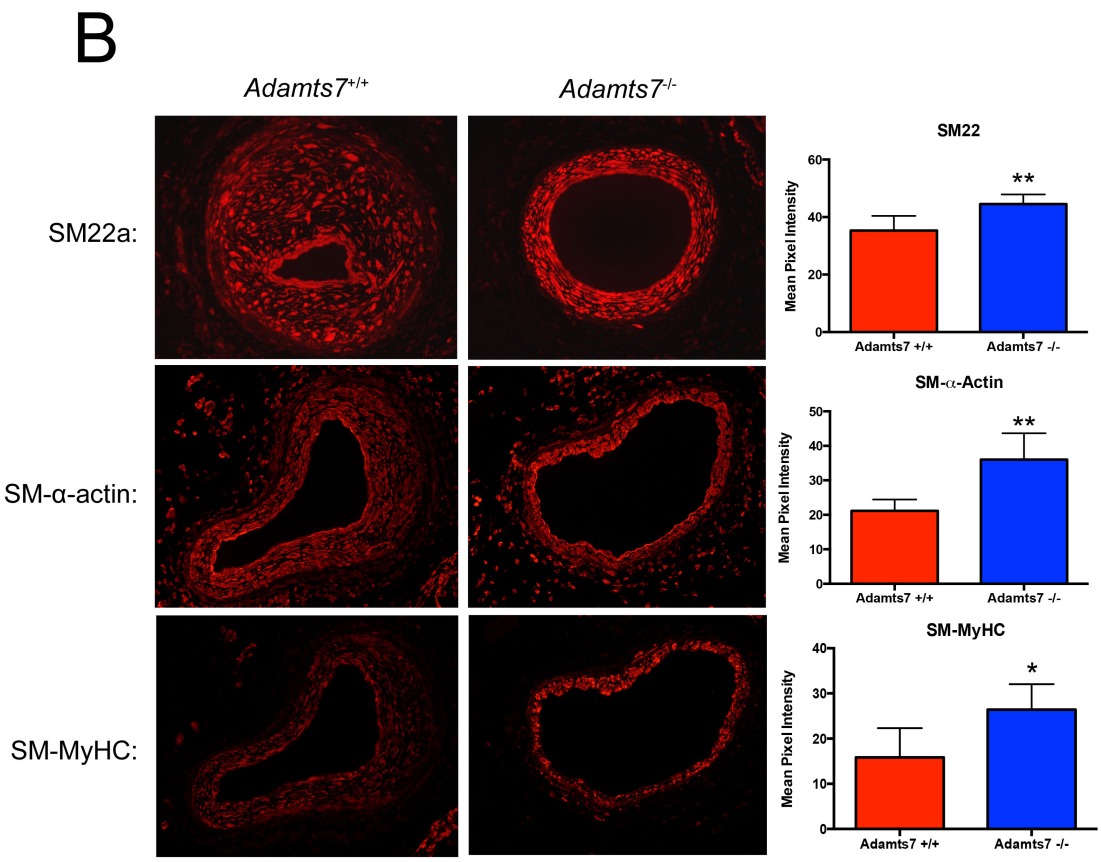


Supplemental Figure 2: Integrated AUC analysis of aortic root lesion area in both *Ats7*/*Ldlr* and *Ats7*/*Apoe* atherosclerotic experimental models reveals decreased lesion area. For each animal, a "Zero Point" was established at the first section where all 3 leaflets of the aortic valve were visible. Lesion area was quantified at this section and the two sections above (+1 and +2) and below (-1 and -2). The average lesion area of these 5 sections is shown (A, C, E, G) for all experimental groups, and the area under the resultant curve is also shown (B, D, F, H). * denotes *p*-value < 0.05, ** denotes *p*-value < 0.01.



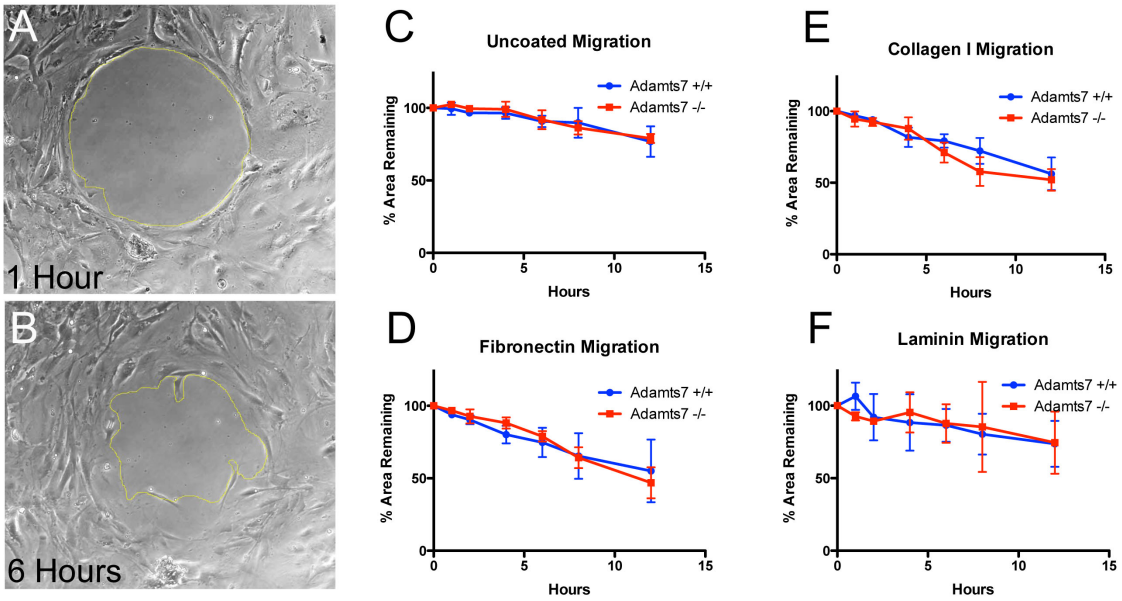
Supplemental Figure 3: Trend towards increased collagen content of aortic root lesions in *Adamts7*-deficient atherosclerotic mice. A-C) Representative images of Collagen I IHC in aortic root sections from hyperlipidemic *Adamts7* WT (N=6) and KO mice (N=10), and quantitation of lesion Collagen I content represented as percent lesion area.



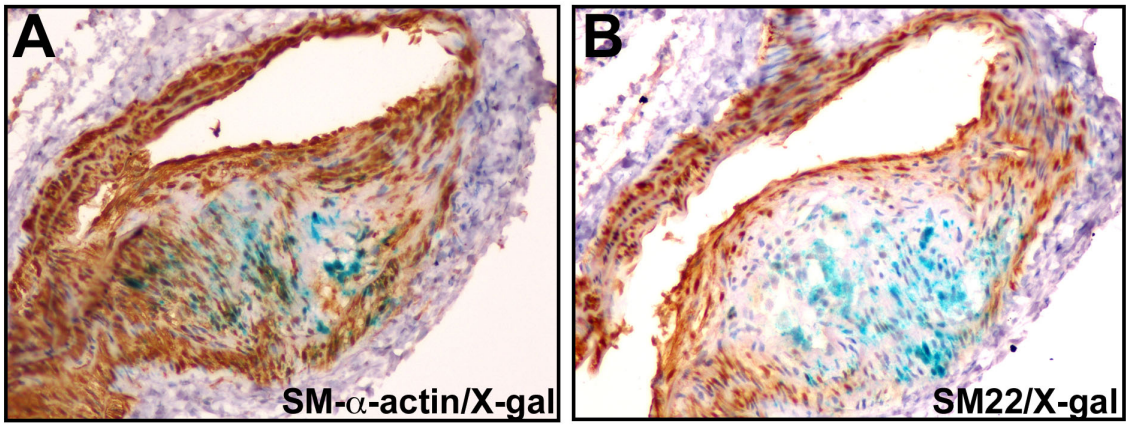


Supplemental Figure 4: Vascular ECM deposition is reduced and SMC phenotype

markers preserved in *Adamts7*$^{-/-}$animals following femoral artery injury. (A) Trichrome staining, as well as collagen-IV and fibronectin immunohistochemistry revealed greatly reduced ECM deposition in injured vessels of *Adamts7*$^{-/-}$ compared to WT. (B) IHC for contractile VSMC markers (SM22α, SM-α-actin, and SM-MyHC) revealed preserved expression in the injured vessels of *Adamts7*$^{-/-}$ mice compared to WT (N=5 and 4, respectively). Far right column is quantification of signal from wire-injury lesions, as measured as mean pixel intensity in neointimal region. * denotes *p*-value < 0.05, ** denotes *p*-value < 0.01.



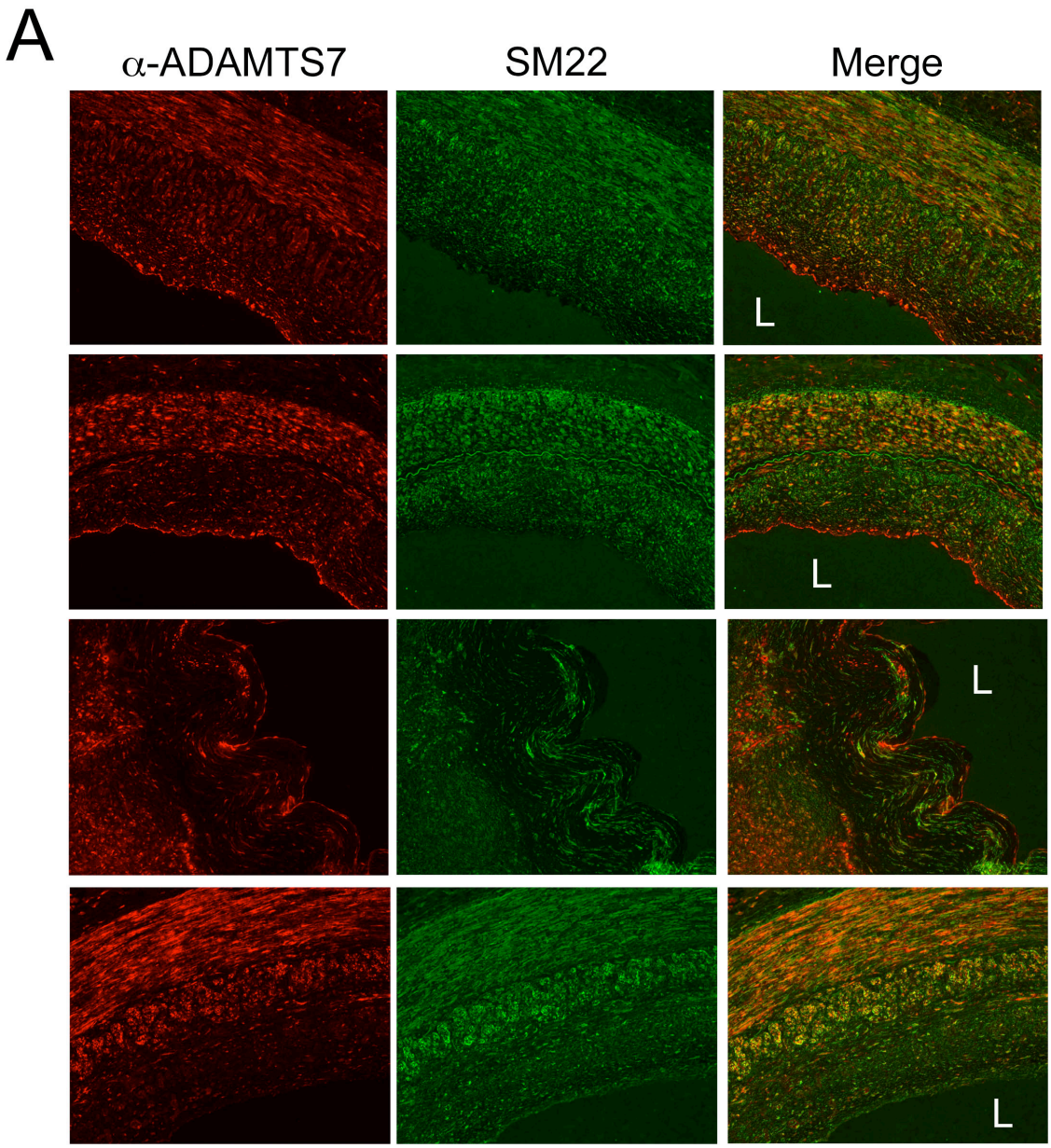
Supplemental Figure 5: Primary *Adamts7*$^{+/+}$ and *Adamts7*$^{-/-}$ VSMCs display no difference in migration on extracellular matrices under resting (non-TNFα) stimulated conditions. A-B) Representative images from Radius™ 24-Well Cell Migration Assay used for quantitation at 1-hour and 6-hours post-gel spot removal. C-F) Migration of *Adamts7* WT and KO primary aortic VSMCs (N=3/timepoint) for 12 hours on uncoated tissue culture plates (C), plates coated with fibronectin (D), collagen I (E), and laminin (F).

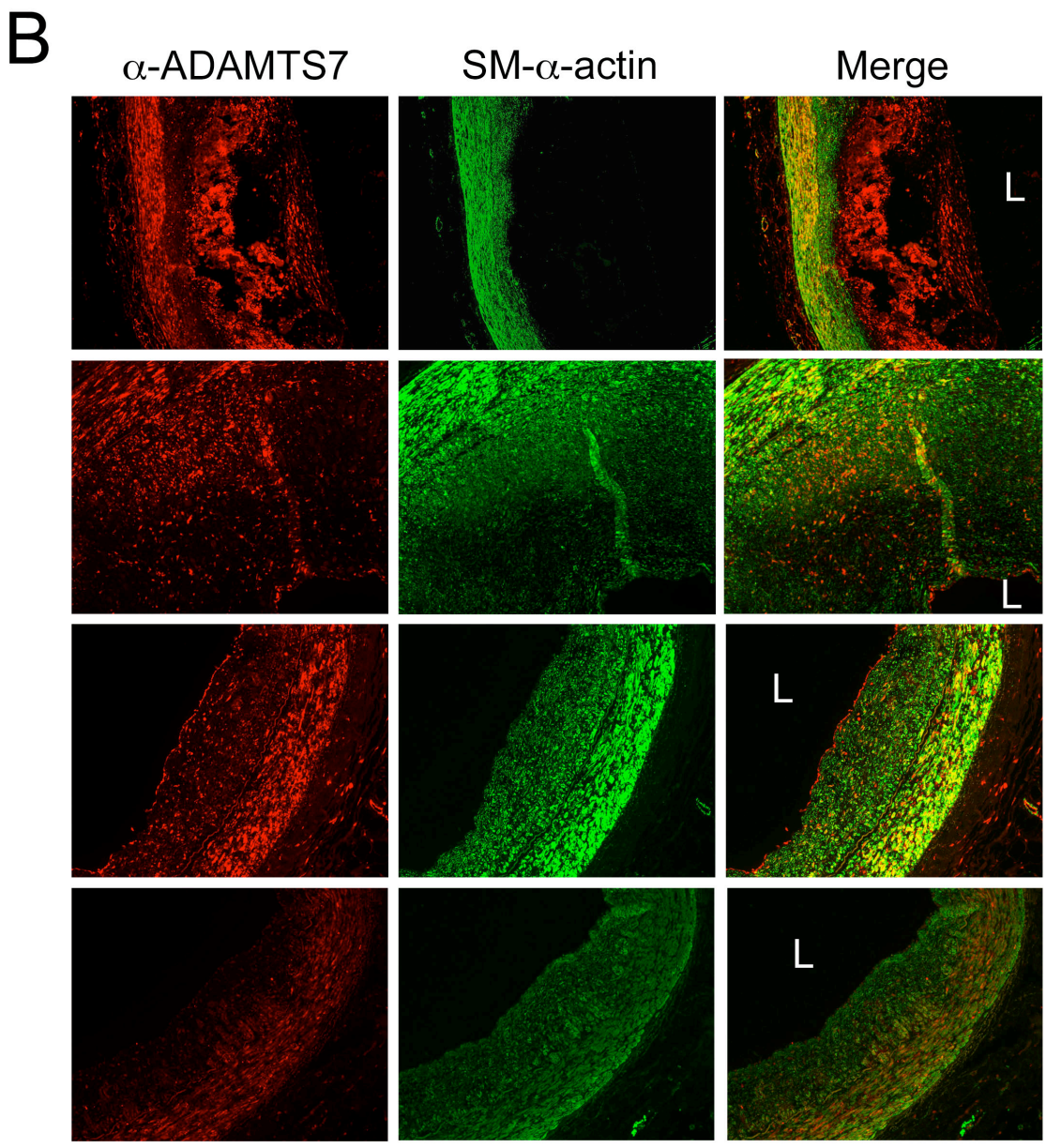


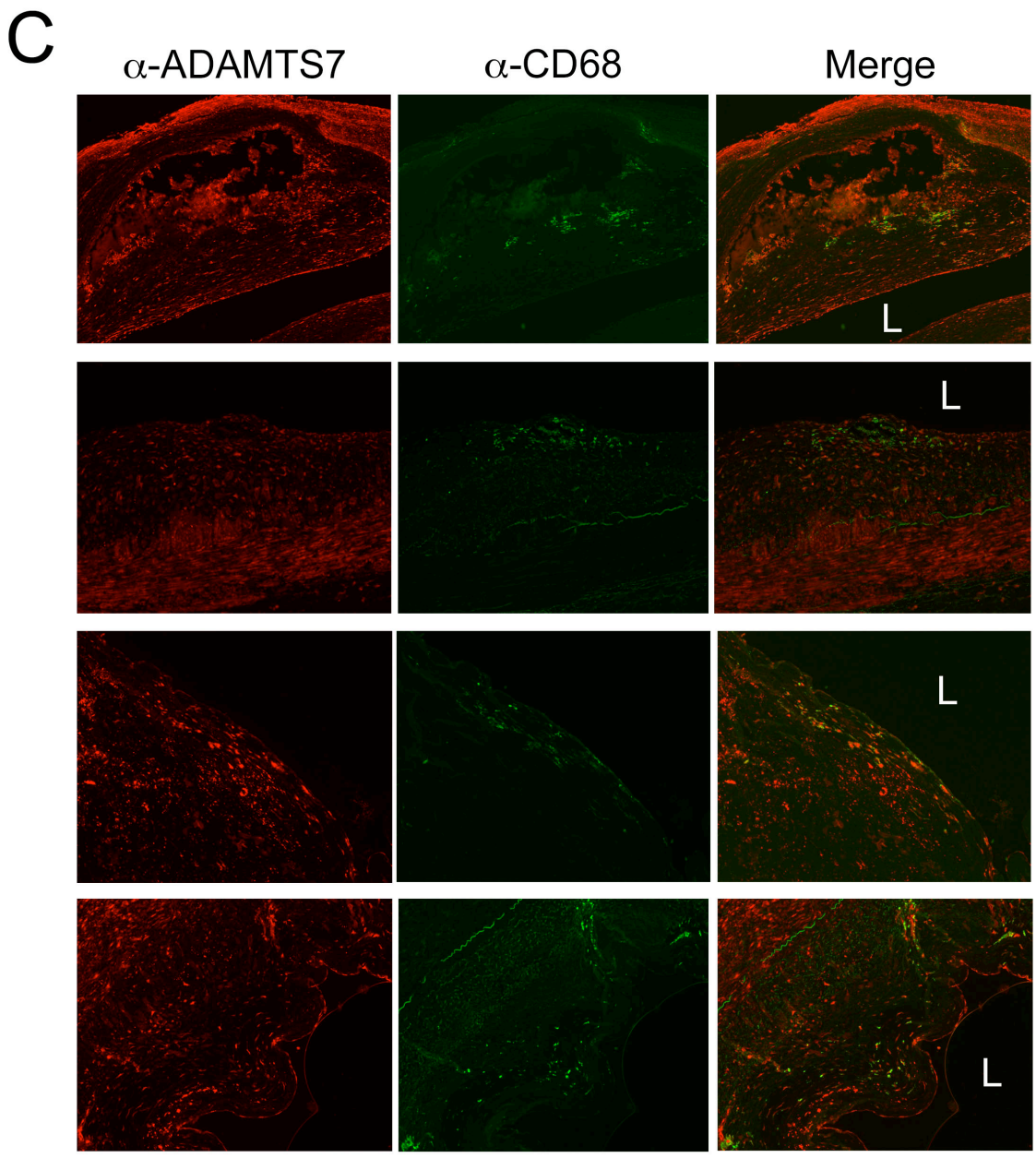
Supplemental Figure 6: Positive X-gal staining in BCA lesions of *Ats7*/*Apoe* dKO mice after 4-weeks of western diet feeding co-localize with SM-α-actin positive cells. IHC analysis of X-gal stained BCA atherosclerotic lesions from *Ats7*/*Apoe* dKO mice after 4-weeks of western diet feeding. Positive X-gal staining overlaps with SM-α-actin staining (A) but not that of SM22 (B).

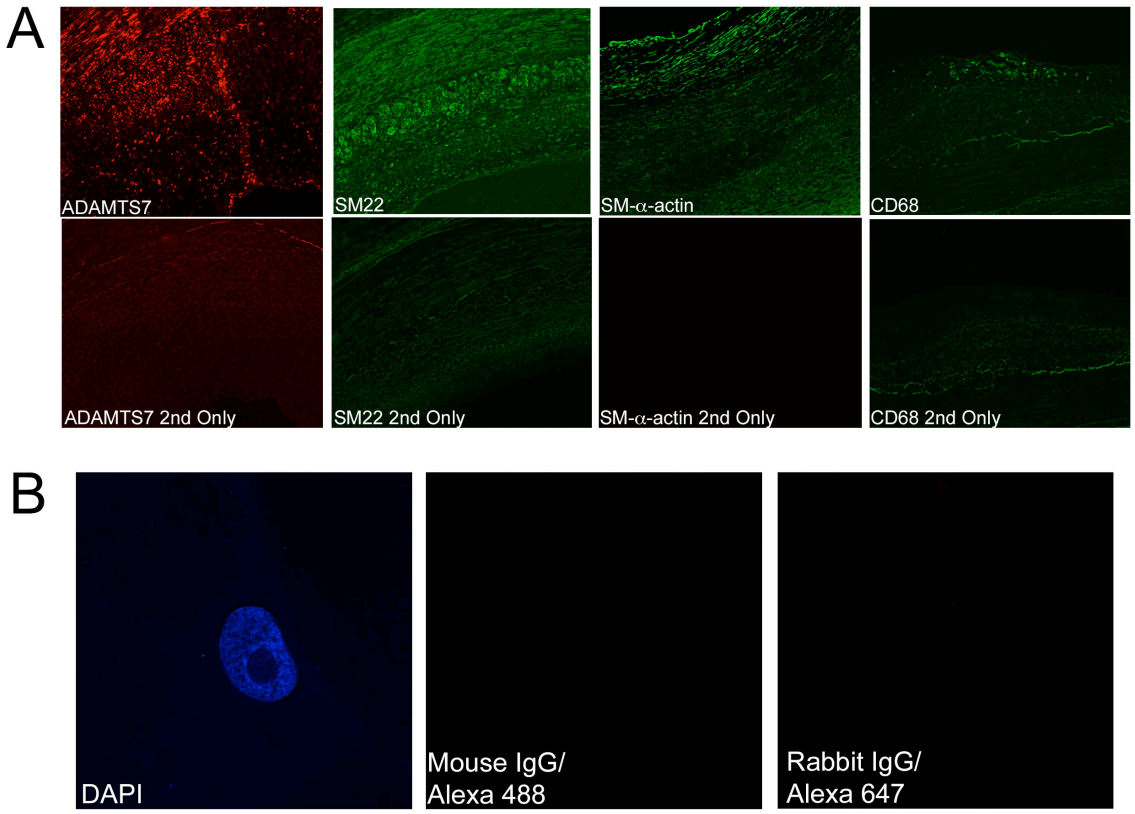
Supplemental Figure 7: Additional IHC staining of atherosclerotic human coronary arteries (HCAs) for ADAMST7 and markers of SMCs and macrophage.

Representative IHC staining from 4 HCA samples is shown for ADAMTS7 and A) SM22, B) SM-a-actin, and C) CD68, with merge of ADAMTS7 and cell marker staining in right column with the lumen demarcated by “L”.




B
α-ADAMTS7
SM-α-actin
Merge
L
L
L
L


C
α-ADAMTS7
α-CD68
Merge
L
L
L
L



Supplemental Figure 8: Negative controls for immunostaining. A) Negative controls shown for each type of staining done in human coronary arteries. Top row is example of positive IHC staining with indicated antibody, and bottom row is 2nd antibody only in the same region of sample. B) Immunofluorescence of hAoSMCs incubated with mouse or rabbit IgG and Alexa Fluor 488 or 647. All panels are of the same view indicated in the DAPI panel.

Supplemental Material References

1. Wang M, Ihida-Stansbury K, Kothapalli D, Tamby MC, Yu Z, Chen L, Grant G, Cheng Y, Lawson JA, Assoian RK, Jones PL, FitzGerald GA. Microsomal Prostaglandin E2 Synthase-1 Modulates the Response to Vascular Injury. *Circulation*. 2011;123:631–639.

2. Ray JL, Leach R, Herbert JM, Benson M. Isolation of vascular smooth muscle cells from a single murine aorta. *Methods Cell Sci*. 2001;23:185–188.