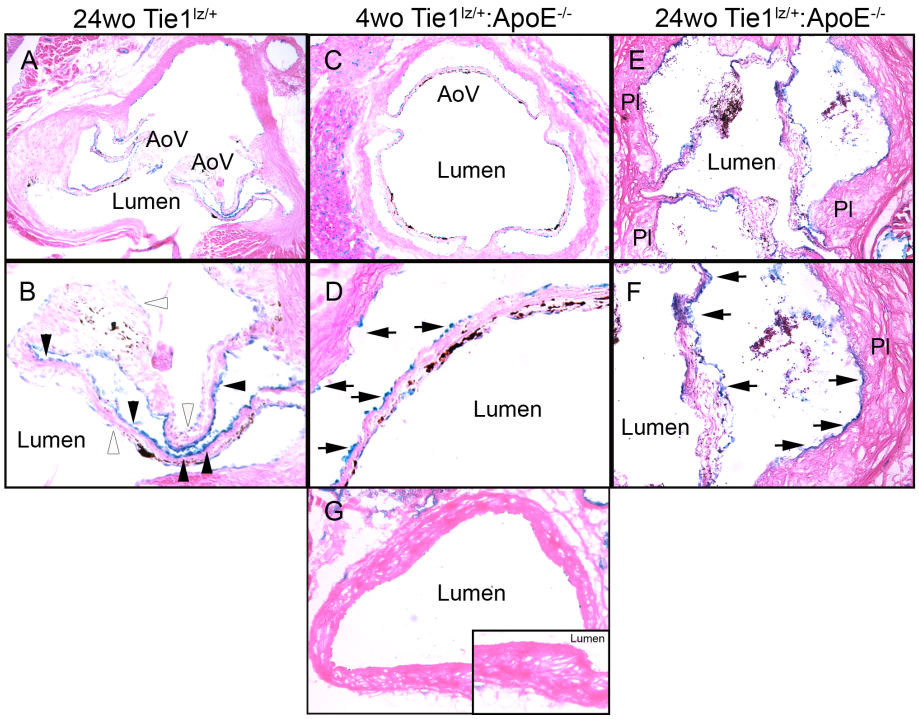
Supplemental Figure 1

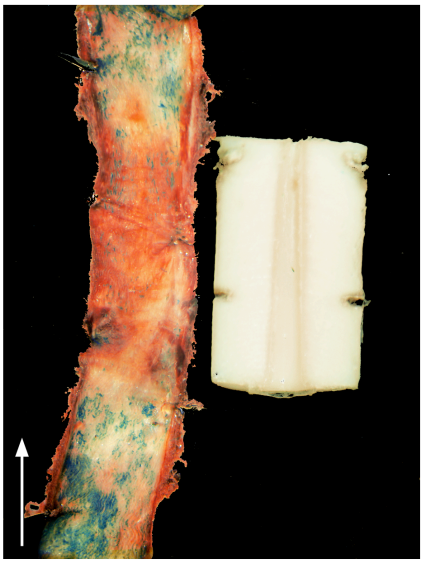


Supplemental Figure 1

Tie1 is Expressed in Regions of Disturbed Flow

(**A-G**) Cryosections of aortic valves from Tie1-LacZ mice, X-gal stained and H&E counterstained. (**A**) 24 week-old Tie1$^{lz/+}$ mouse aortic valves shown in high magnification (**B**), illustrating specific X-gal staining on fibrosa surface of aortic valve leaflet (black arrowheads) but not on ventricularis surface (white arrowheads). Tie1-LacZ expression (black arrows) persists on the fibrosa surface of the aortic valve leaflet and aortic wall of Tie1$^{lz/+}$:ApoE$^{-/-}$ mouse, prior to atherosclerotic lesion formation at (**C**) 4 weeks, (**D**) high magnification and during advanced disease progression at (**E**) 24 weeks, (**F**) high magnification. (**G**) Cryosection of ascending aorta (immediately downstream of aortic valves) depicting absence of endothelial LacZ staining where laminar flow is predominant (inset, higher magnification of aorta wall and endothelium). (A,C,E,G – 50x magnification; B,D,F – 100x magnification; AoV, aortic valve leaflets; Pl, atherosclerotic plaques).

Supplemental Figure 2

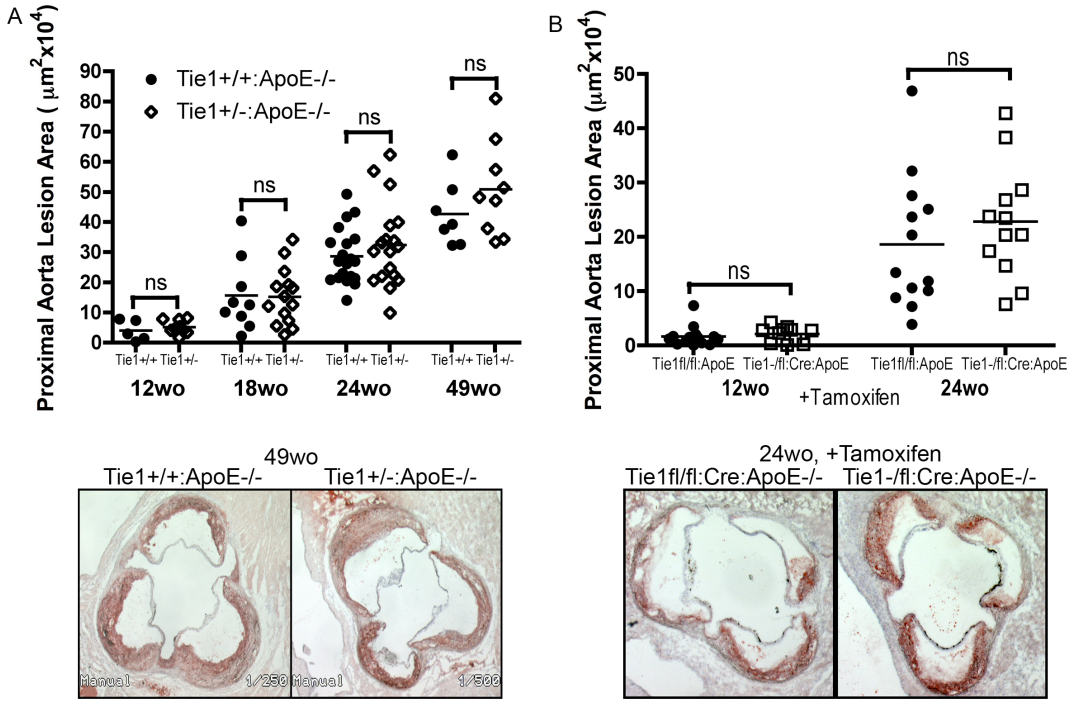


Supplemental Figure 2

Endothelial Immunostaining in Cast Implanted Carotid Artery

Carotid artery that was implanted with the cast was first processed for X-gal staining. Subsequently the artery was counterstained with VECadherin antibody and developed with DAB (region of staining was pseudo-colored to red; white arrow indicates direction of blood flow).

Supplemental Figure 3

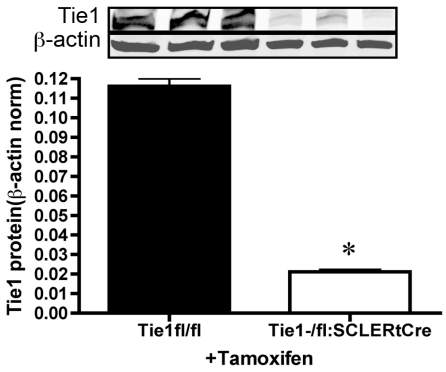


Supplemental Figure 3

Tie1 Reduction Does Not Alter Atherosclerosis in the Aortic Valves

(**A**) Graphical analyses of atherosclerosis in aortic sinuses of $Tie1^{+/+}$:$ApoE^{-/-}$ vs. $Tie1^{+/-}$:$ApoE^{-/-}$ mice at 12, 18, 24 and 49 weeks. (12 week-old: 40030±15311μm^2 vs. 51719±6094μm^2; 18 week-old: 156155±40275μm^2 vs. 152210±25157μm^2; 24 week-old: 286433±16160μm^2 vs. 334760±41861μm^2; 49 week-old: 426680±40877μm^2 vs. 529025±52711μm^2). Representative images of oil red o stained aortic sinuses from 49 week-old $Tie1^{+/+}$:$ApoE^{-/-}$ and $Tie1^{+/-}$:$ApoE^{-/-}$ mice. (**B**) Graphical analyses of atherosclerosis in aortic sinuses of $Tie1^{flox/flox}$:$ApoE^{-/-}$ vs. $Tie1^{-/flox}$: SCL-ER^T-Cre:$ApoE^{-/-}$ tamoxifen treated mice (12 week-old: 16607 ± 5004 vs. 21126 ± 4274 μm^2, NS ; 24 week-old: 22816 ± 3019 vs. 18587 ± 33856 μm^2, NS). Data points denote individual animals and horizontal bars indicate group average. Representative images of oil red o stained aortic sinuses from 24 week-old tamoxifen treated $Tie1^{-/flox}$:SCL-ER^T-Cre:$ApoE^{-/-}$ vs. $Tie1^{flox/flox}$:$ApoE^{-/-}$ mice.

Supplemental Figure 4

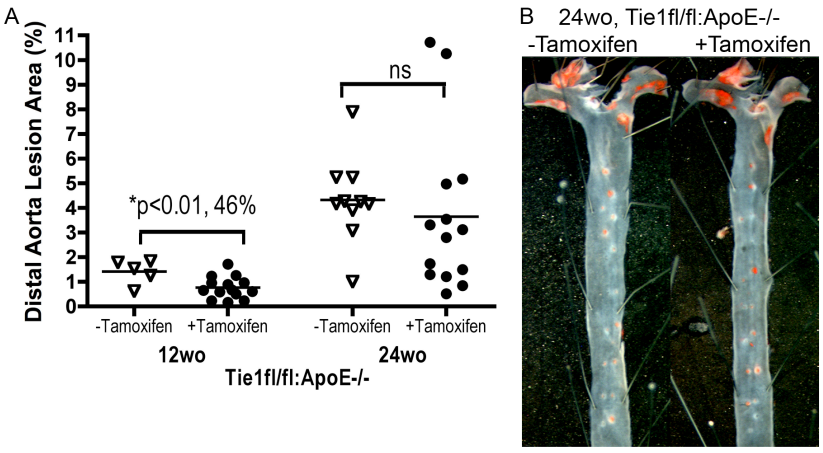


Supplemental Figure 4

Western blot Analysis of Pulmonary Tie1

Graphical analyses of Tie1 levels from tamoxifen treated $Tie1^{-/flox}$:SCL-ER^{T}-Cre mice (0.116 ± 0.003 vs. 0.0211 ± 0.001, 81% reduction, n=6, *p<0.0001).

Supplemental Figure 5

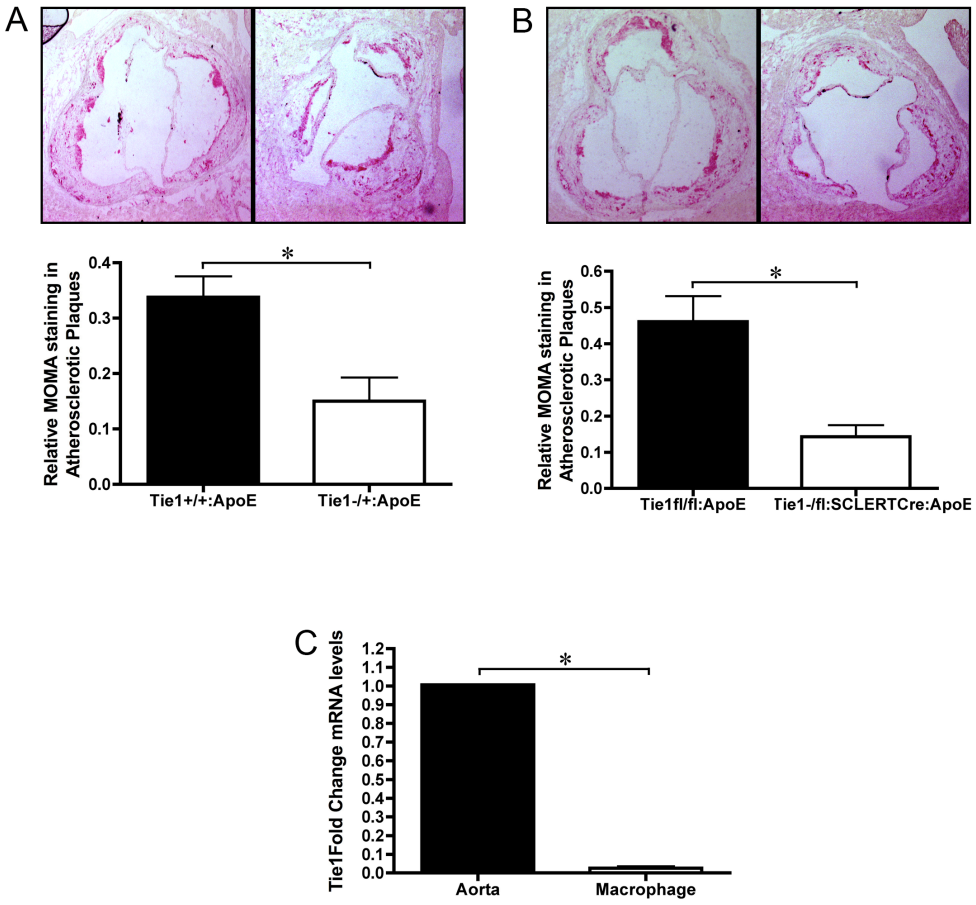


Supplemental Figure 5

Tamoxifen Treatment Temporarily Reduces Atherosclerosis in the Short Term

(**A**) Comparisons of atherosclerosis burden in untreated and tamoxifen treated Tie1$^{flox/flox}$:ApoE$^{-/-}$ mice 4 weeks post treatment (12 week-old: 1.41 ± 0.22% vs. 0.76 ± 0.11%, $p<0.01$,) and 16 weeks post treatment (24 week-old: 4.32 ± 0.54% vs. 3.64 ± 0.86%, NS). Data points denote individual animals and horizontal bars indicate group average. (**B**) Representative images of Sudan IV stained distal aortas from 24 week-old Tie1$^{flox/flox}$:ApoE$^{-/-}$ untreated and tamoxifen treated mice.

Supplemental Figure 6

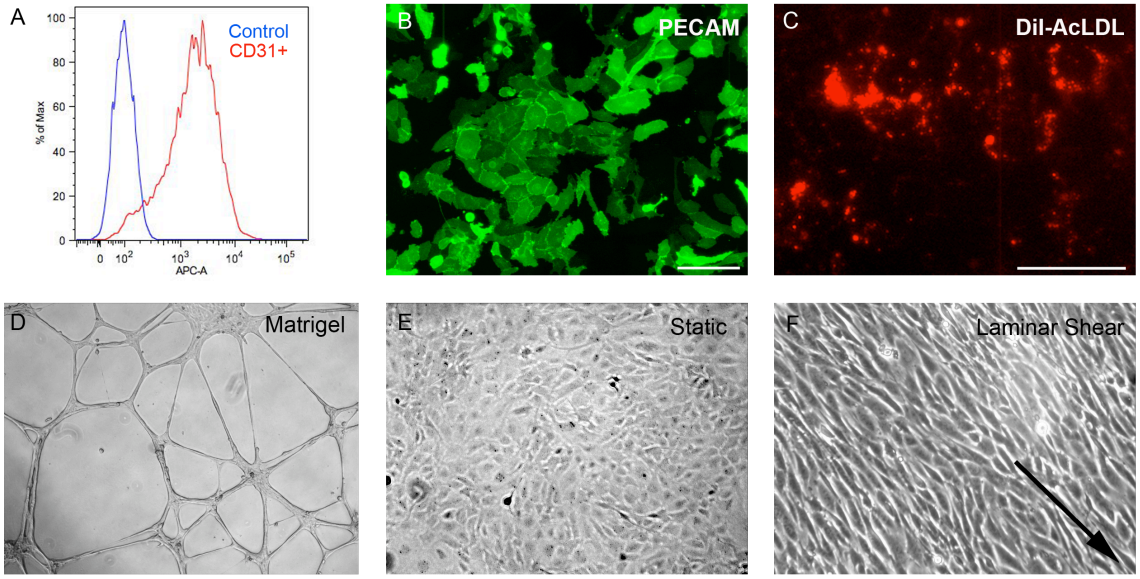


Supplemental Figure 6

Tie1 is Not Expressed in Macrophages and Tie1 Deletion Reduces Macrophage Infiltration

Representative images of cryosections from proximal aorta were stained with macrophage antibody (MOMA) and compared between (**A**) $Tie1^{+/+}$:$ApoE^{-/-}$ vs. $Tie1^{-/+}$:$ApoE^{-/-}$ (56% reduction, 0.33 ± 0.03 units vs. 0.07 ± 0.44 units, n=3, $p<0.01$) and (**B**) $Tie1^{flox/flox}$:$ApoE^{-/-}$ vs. $Tie1^{-/flox}$:SCL-ER^{T}-Cre:$ApoE^{-/-}$ (70% reduction, 0.46±0.07 units vs. 0.14 ± 0.03 units, n=3, $p<0.05$). (**C**) Semi-quantitative RT-PCR analysis of Tie1 mRNA in primary mouse macrophages (n=5, $p<0.0001$).

Supplemental Figure 7

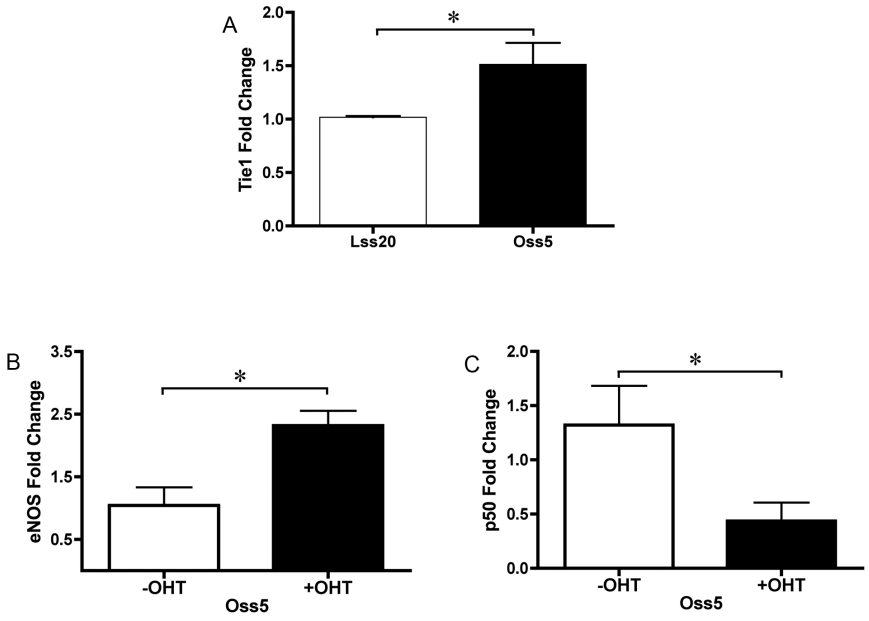


Supplemental Figure 7

Characterization of Murine Aortic Endothelial Cells

(**A**) Flow cytometry analysis of mouse aortic endothelial cell, 89.7% CD31+. (**B**) Representative photomicrograph of PECAM immunostained mouse aortic endothelial cells showing uniform cytoplasmic staining in individual cells and localization of PECAM to the cell border when in apposition with adjacent cells. (**C**) Representative image of DiI-AcLDL uptake *in vitro*. (**D**) Light microscopy images displaying network formation after 24 hours of culture on matrigel. (**E**) Phase contrast microscopy photographs illustrating cobblestone morphology. (**F**) Representative image showing alignment of mouse aortic endothelial cells to direction of flow after 24 hours 20 dynes/cm^2 laminar shear. (White bar represents 100μm scale)

Supplemental Figure 8



Supplemental Figure 8

Oscillatory Flow Increases Tie1 Expression and Tie1 Deletion Mediates Oscillatory Flow Induced eNOS Expression and p50 Activity

(**A**) Graphical representation of western blot analysis of relative Tie1 protein levels in wildtype mouse aortic endothelial cells under laminar flow or oscillatory flow. (**B**, **C**) Graphical representation of western blot analyses of eNOS protein expression and p50 nuclear translocation in 4OHT treated (+OHT) and untreated (-OHT), $Tie1^{flox/flox}$:SCL-ER^{T}-Cre mouse aortic endothelial cells in 24 hours of oscillatory flow. (n=4, $p<0.05$; Lss20, laminar flow 20 dynes/cm^2; Oss5, oscillatory flow 5 dynes/cm^2)