

Figure S1. Vessel phenotypes in *Smad4* deficient mice. (A) Western analysis of SMAD4 in embryo lysates shows a dose dependent depletion of SMAD4 in wild type, *Smad4 fl/+*, and *Smad4 fl/fl* animals. (B) Imaging of e12.5 *RosaCreER Smad4 floxed* animals. The coronary vascular plexus, including peritruncal vessels near the aorta, is comparable between mutant and control animals at e12.5, prior to blood flow. (C) Quantification of peritruncal vessel parameters (Controls: n=4 and *RosaCreER Smad4 fl/fl*: n=5). (D) Imaging of high magnification of arteries and capillaries at e15.5. Error bars are s.d. ns, non-significant. Cv, coronary vessels, Scale bars, B, 100 μ m and D, 50 μ m.

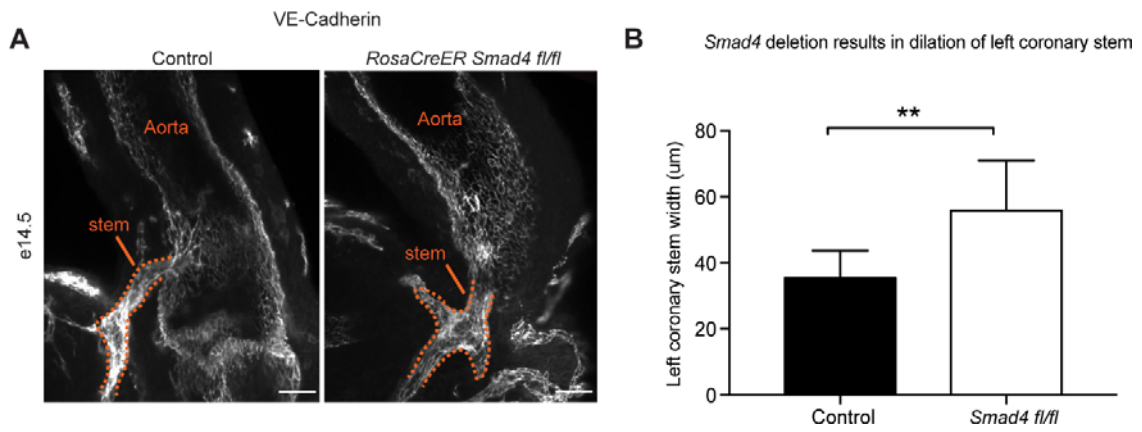


Figure S2. The left coronary artery stem is dilated in *Smad4* mutants. (A) Confocal image of a left coronary artery stem in a control and mutant heart. (B) Quantification of stem width (Controls: n=8 and *RosaCreER Smad4 fl/fl*: n=8). Error bars are s.d. **, $p \leq 0.01$. Scale bars, A, 50 μm .

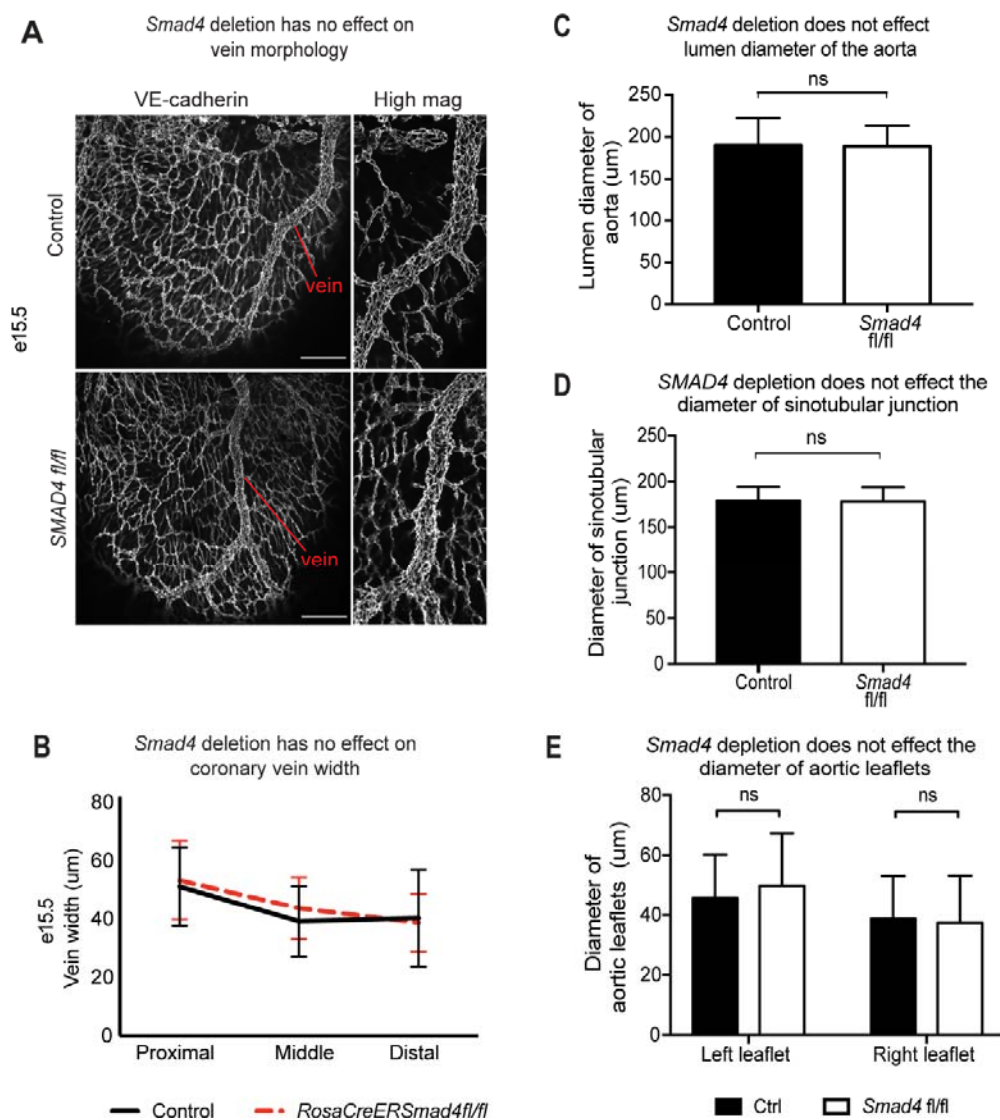


Figure S3. Various cardiac structures with no defects in *Smad4* mutants. (A and B) Confocal images (A) and quantification (B) of coronary veins (Controls: n=7 and *RosaCreER Smad4* fl/fl: n=8). (C-D) Measurements of the outflow tract (Controls: n=9 and *RosaCreER Smad4* fl/fl: n=10). Error bars are s.d. ns, non-significant. Scale bars, A, 100 μm .

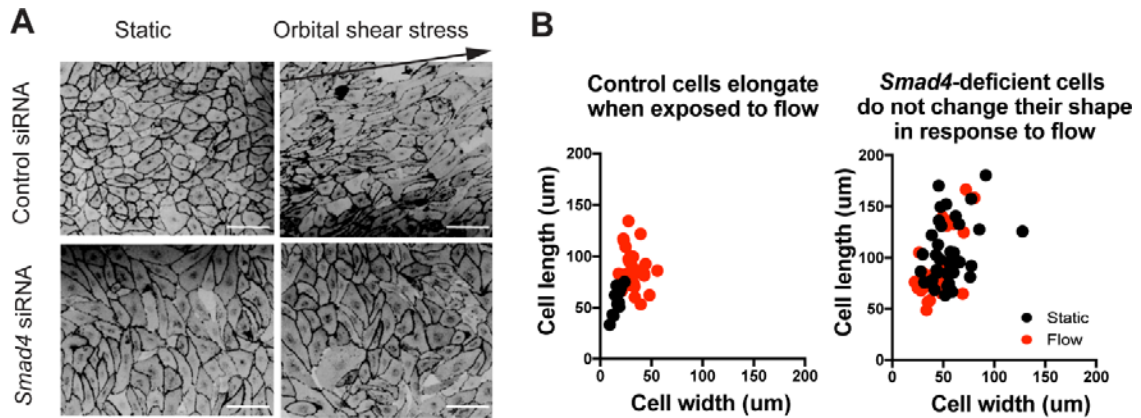
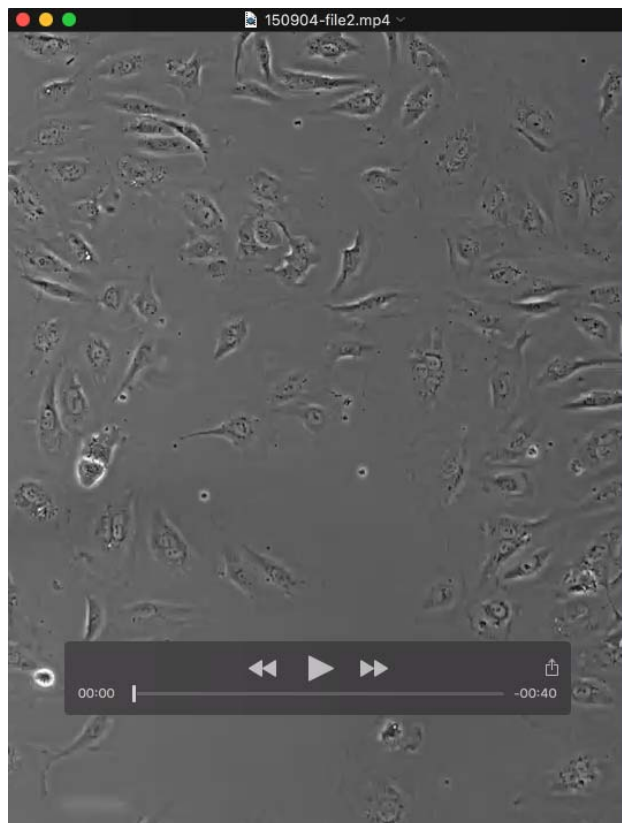
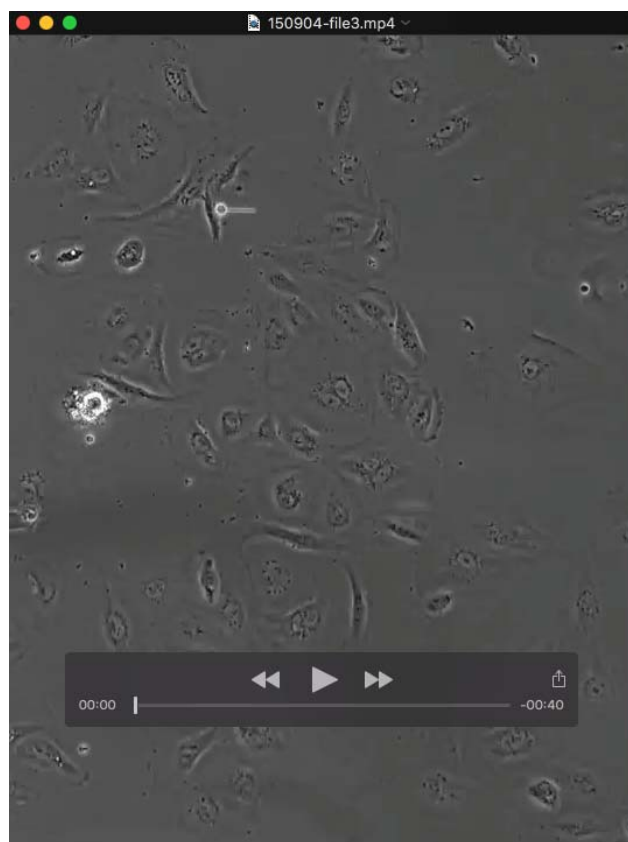


Figure S4. Alignment of *Smad4*-deficient cells in response to shear stress imparted by orbital flow. (A and B) Human coronary artery endothelial cells (HCAECs) exposed to orbital laminar shear stress (arrow indicates direction). (A) Cell-cell junctions are immunostained with VE-cadherin (black) showing the absence of alignment with flow when treated with *Smad4* siRNA. (B) The absence of shear stress-induced cell shape change is quantified by plotting cell length versus width. Each dot is an individual cell. Scale bar, 50 μm.



Movie S1. Endothelial cells treated with control siRNA align and migrate against the direction of flow. Time-lapse movie (1 frame every 15 minutes) of HCAECs treated with control siRNA subjected to shear stress at 35 dyne/cm² for 72 hours. The direction of flow is left to right.



Movie S2. Endothelial cells treated with *Smad4* siRNA do not align and migrate against the direction of flow. Time-lapse movie (1 frame every 15 minutes) of HCAECs treated with *Smad4*-targeted siRNA subjected to shear stress at 35 dyne/cm² for 72 hours. The direction of flow is left to right.