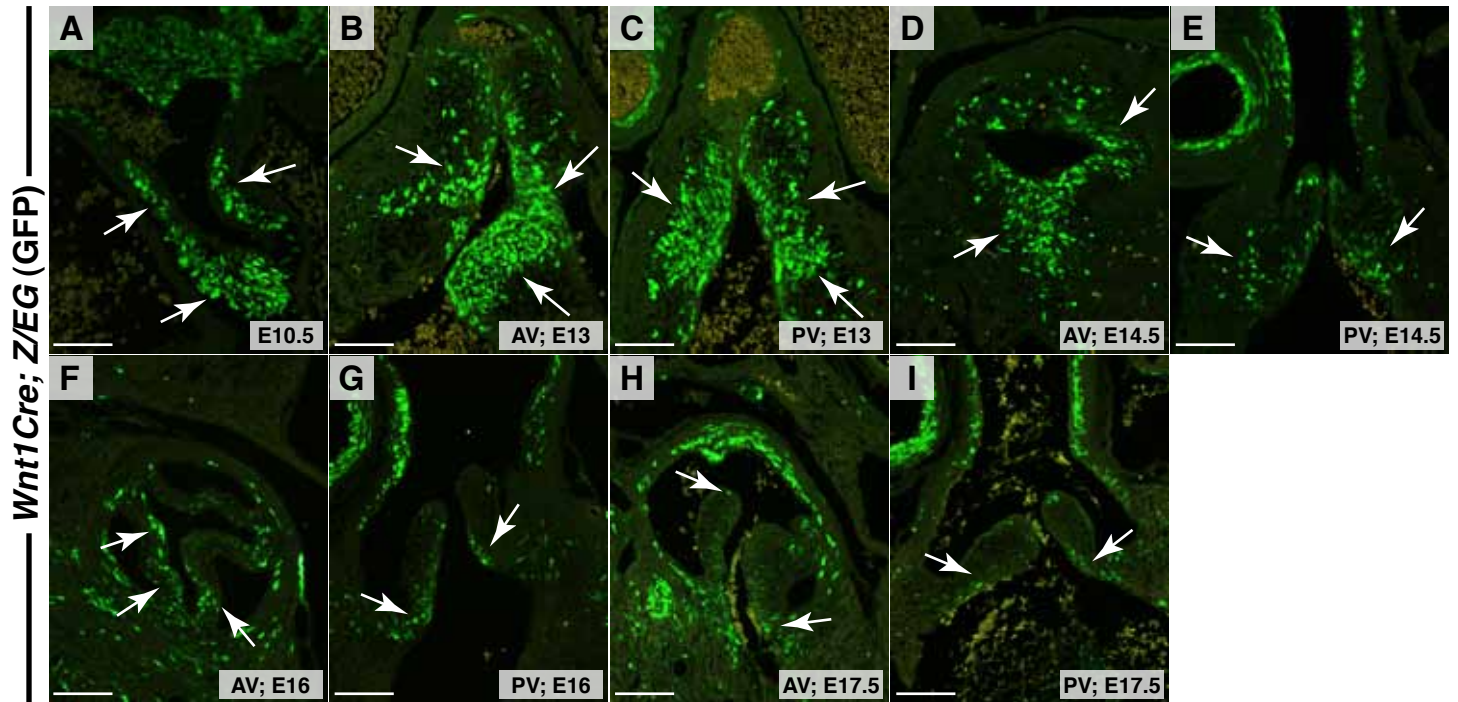


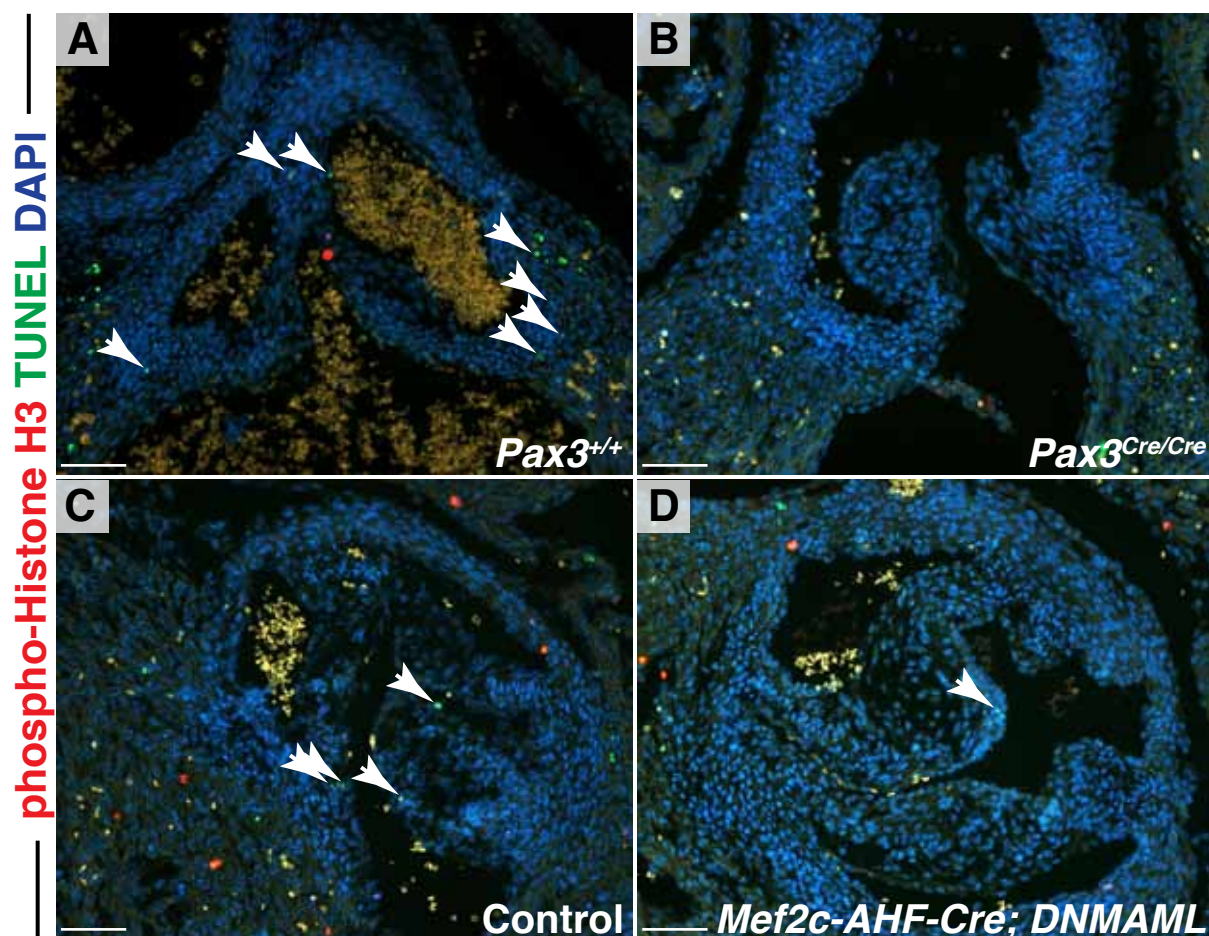
Supplementary Figure 1: Atrioventricular valves in *Pax3*^{Cre/Cre} and Notch-second heart field mutants are morphologically normal. Cross sections through the tricuspid and mitral valves of E16.5 *Pax3*^{+/+} (A), *Pax3*^{Cre/+} (B), *Pax3*^{Cre/Cre} (C) embryos. Cross sections through the tricuspid and mitral valves of E17.5 control (wildtype) (D) and *Mef2c-AHF-Cre; DNMAML* (E) embryos. Scale bars = 250 μm.

Supplementary Figure 2



Supplementary Figure 2: *Wnt1Cre* fate-mapping demonstrates a decrease of neural crest derivatives in the semilunar valves late in gestation. Cross sections of *Wnt1Cre; Z/EG* embryos were analyzed by GFP immunohistochemistry to detect neural crest derivatives. Outflow tract cushions of E10.5 (A), E13 (B, C), E14.5 (D, E) embryos and maturing semilunar valve leaflets of E16 (F, G), and E17.5 (H, I) embryos are shown. The white arrows point to the GFP staining within the outflow tract cushions and semilunar valve leaflets. AV, aortic valve; PV, pulmonic valve. Scale bars = 100 μm.

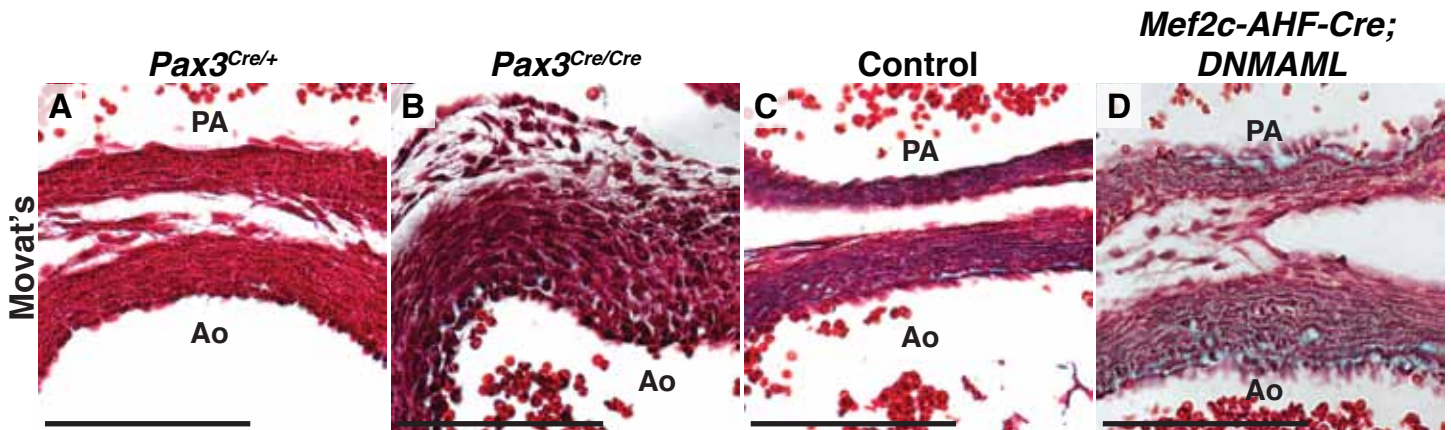
Supplementary Figure 3



Supplementary Figure 3: Neural crest and Notch mutants display a deficiency of apoptosis.

Representative cross sections of the semilunar valve leaflets of E16.5 *Pax3^{+/+}* (A) and *Pax3^{Cre/Cre}* (B) embryos and E17.5 control (wildtype) (C) and *Mef2c-AHF-Cre; DNMAML* (D) embryos used for quantitation of proliferation and apoptosis indices. Sections are stained with phospho-Histone H3, TUNEL, and DAPI and imaged on a triple filter. *Pax3^{Cre/Cre}* and *Mef2c-AHF-Cre; DNMAML* both display a lack of apoptosis compared to their respective controls. White arrows point to TUNEL+ cells in the valve leaflets. Scale bars = 50 μ m.

Supplementary Figure 4



Supplementary Figure 4: Increased ECM in aortic walls of *Pax3*^{Cre/Cre} and *Mef2c-AHF-Cre; DNMA ML* embryos. Sections of the aortic wall of E16.5 *Pax3*^{Cre/+} (A) and *Pax3*^{Cre/Cre} (B) embryos and E17.5 control (C) and *Mef2c-AHF-Cre; DNMA ML* (D) embryos are shown (Modified Movat's Pentachrome stain). Mutants demonstrate increased extracellular matrix deposition (blue) in the aortic wall. The control embryo (C) is an age-matched control for the *Mef2c-AHF-Cre; DNMA ML* embryo (D). Ao, aorta; PA, pulmonary artery. Scale bars = 100 μ m.