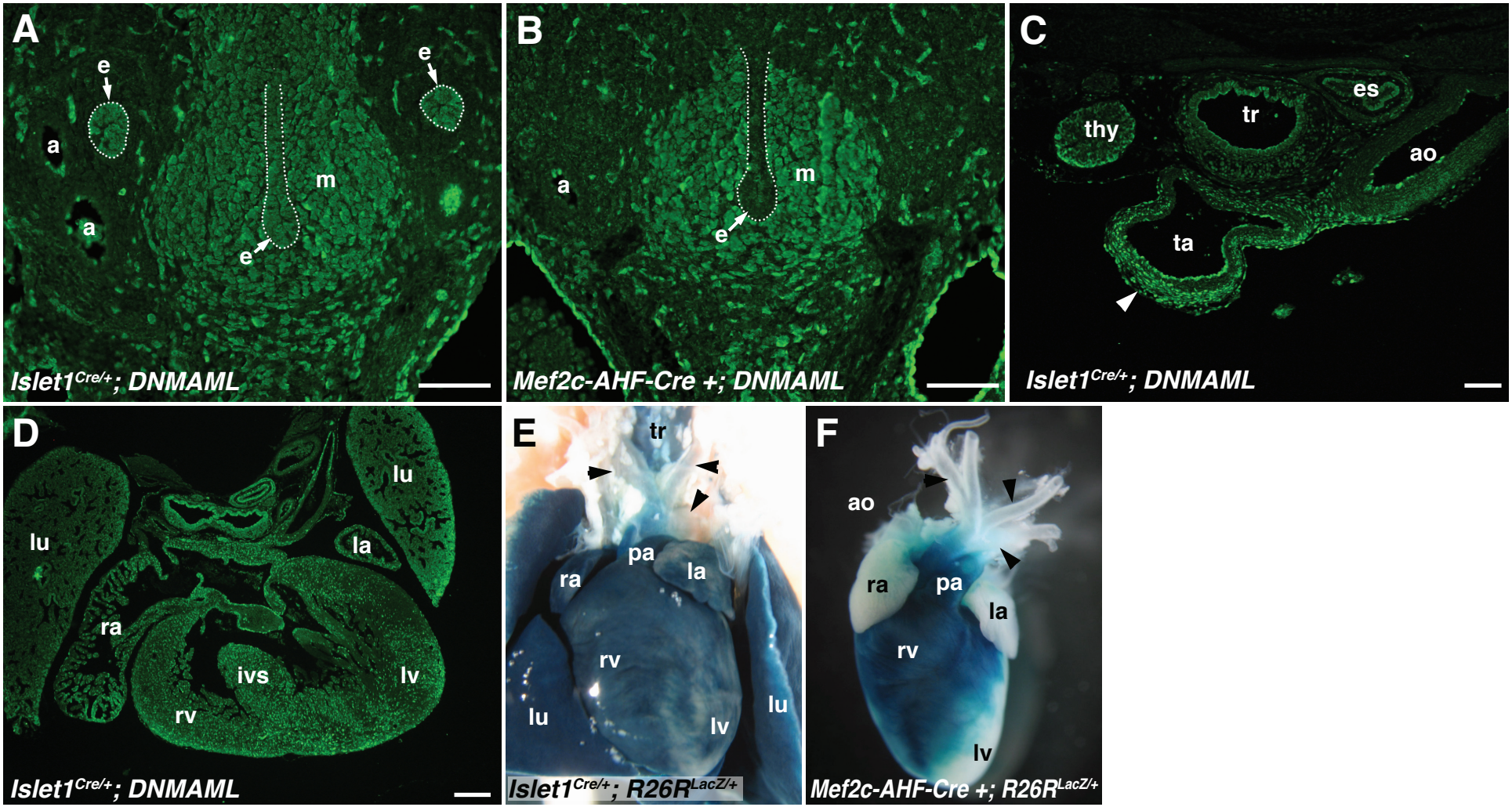
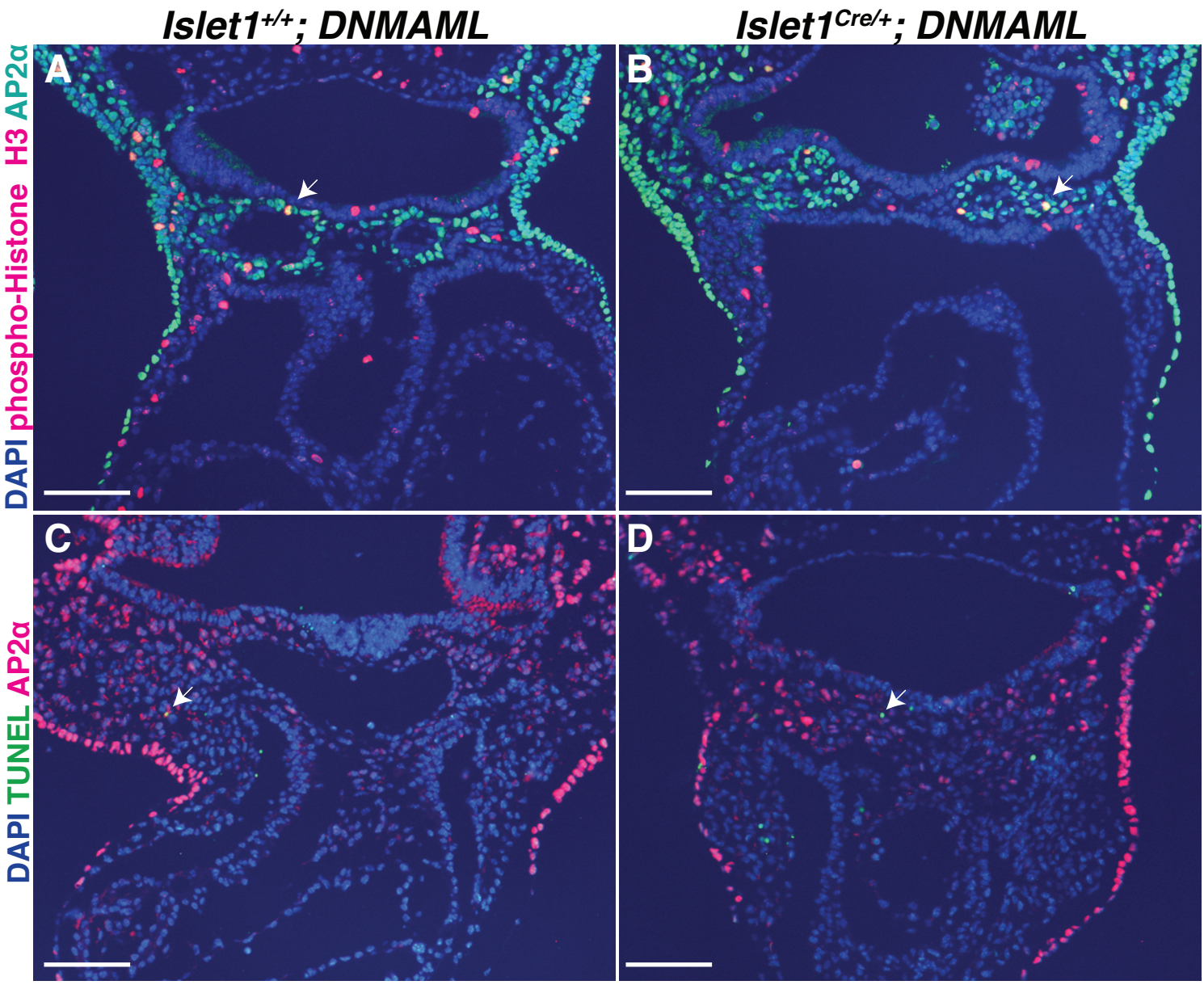
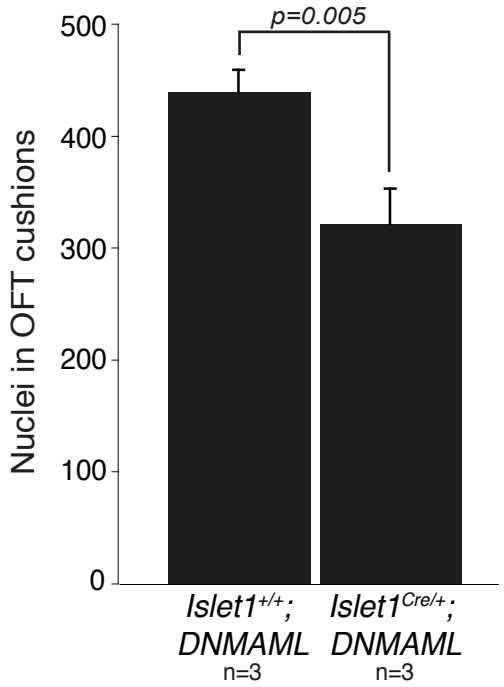


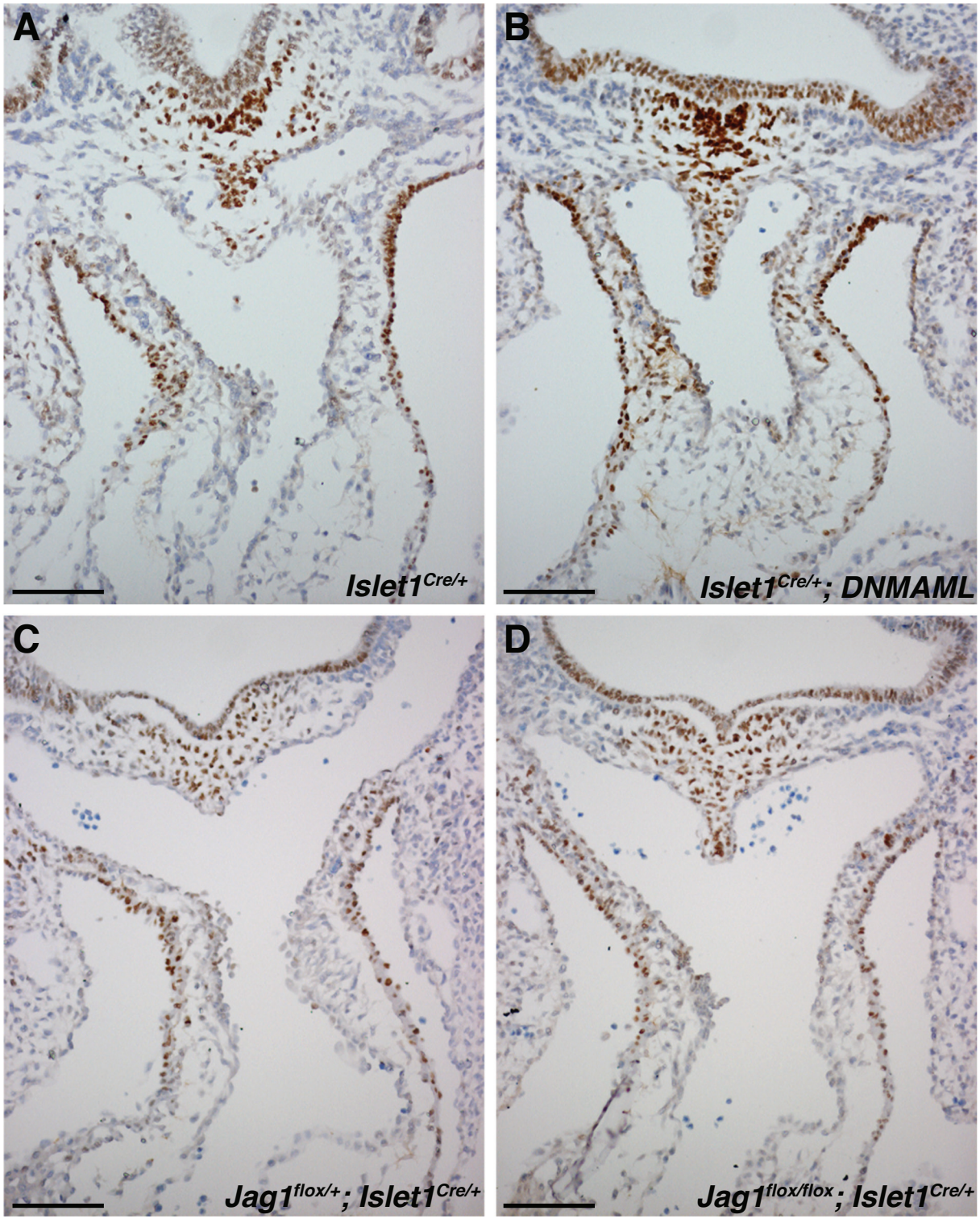
Supplementary Figure 1: Fate mapping of Islet1Cre and Mef2c-AHF-Cre derivatives. (A-D) Immunostaining for GFP to reveal DNMAML-GFP expression in mutant embryos. (A and B) Comparable frontal sections through E12.5 Islet1Cre/+; DNMAML (A) and Mef2c-AHF-Cre +; DNMAML (B) embryos. Note that both Cre lines result in DNMAML-GFP expression in the pharyngeal mesenchyme, while only Islet1Cre/+; DNMAML embryos show DNMAML-GFP expression in the pharyngeal endoderm. (C) Section through the aortic arch of an E17.5 Islet1Cre/+; DNMAML embryo, showing DNMAML-GFP expression in the second heart field-derived cells at the base of the truncus arteriosus (ta, arrowhead), while there is no significant contribution of DNMAML-GFP positive cells to the aortic arch (ao). Note also DNMAML-GFP expression in endoderm-derived tissues, including the esophagus and trachea. (D) Section through the heart of an E17.5 Islet1Cre/+; DNMAML mutant, showing broad distribution of DNMAML-GFP positive cells throughout the heart, including the right ventricle (rv), inter-ventricular septum (ivs), the majority of the left ventricle (lv), and regions of the right and left atria (ra, la). (E and F) Postnatal hearts from Islet1Cre/+; R26RLacZ/+ (E) and Mef2c-AHF-Cre +; R26RLacZ/+ (F) mice. Note that Islet1Cre results in Cre-mediated recombination throughout most of the heart, as well as the lungs and trachea. In contrast, Mef2c-AHF-Cre results in more restricted recombination in the right ventricle and OFT. Neither Islet1Cre nor Mef2c-AHF-Cre results in significant recombination in the aortic arch or its branches (arrowheads in E and F). Scale bars: 100μm (A-C), 250μm (D). Magnification: 30X (E) and 15X (F). Abbreviations: m: mesenchyme, e: pharyngeal endoderm, es: esophagus, tr: trachea, lu: lung, pa: pulmonary artery.



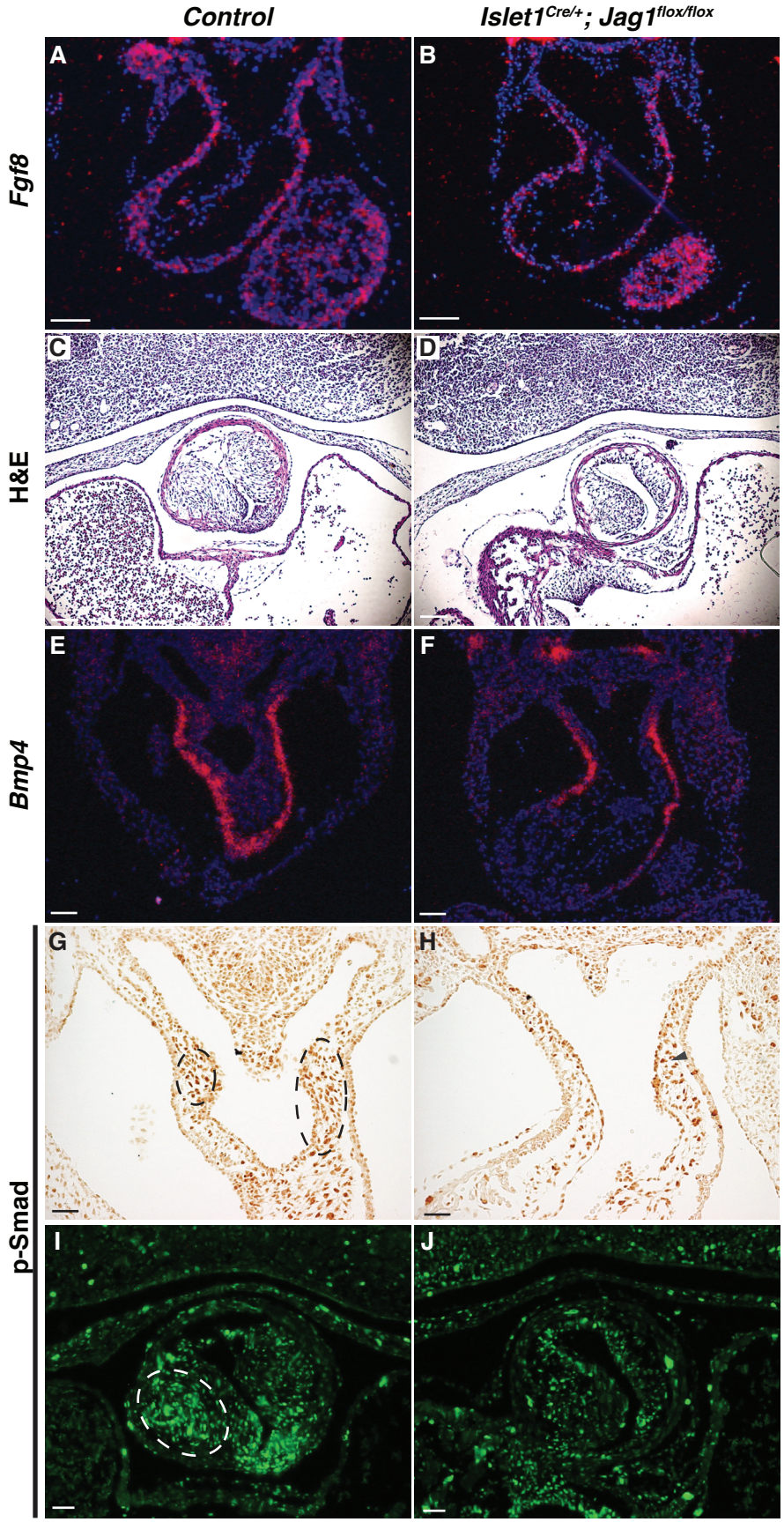
Supplementary Figure 2: Analysis of proliferation and apoptosis of neural crest.
A, B - Costaining of Phospho-histone H3 (to show proliferation) and AP2α (a marker of neural crest and ectoderm) at E9.5. C,D - Costaining for TUNEL (to show apoptosis) and AP2α at E10. Arrows indicate co-stained cells. As discussed in the results section, no statistically significance was seen in the percentage of p-Histone3 positive or TUNEL positive neural crest cells between Islet1+/+; DNMAML and Islet1Cre/+; DNMAML in the ventral pharynx after analysis of serial sections of multiple litters. Scale bars: 100µm.



Supplementary Figure 2: Quantification of Islet1Cre/+; DNMAML outflow tract cushion cells. Nuclei were counted in outflow tract cushions, both mutant and control, from 3 different E10.5 litters. There was a statistically significant difference in number of nuclei, p=0.005. Error bars represent one standard deviation.



Supplementary Figure 4: Islet1 Immunohistochemistry (A and B) Representative cross sections of Islet1 IHC of *Islet1*$^{Cre/+}$ (A) and *Islet1*$^{Cre/+}$*; DNMAML* (B) embryos (E10.5). (C and D) Representative cross sections of *Jag1*$^{flox/+}$*; Islet1*$^{Cre/+}$ (C) and Jag1$^{flox/flox}$; Islet1$^{Cre/+}$ (D) littermates (E10). Quantitation of serial sections did not reveal a statistically signficant difference in number of Islet1+ cells (see Results). Scale bars: 100µm.



Supplementary Figure 5: Cardiovascular defects and diminished Fgf8 expression and Bmp4 signaling in Islet1Cre/+;Jag1flox/flox mutants. (A and B) In situ hybridizations for Fgf8 on transverse sections of E9.5 control (A) and mutant (B) embryos. (C and D) Coronal H&E sections of E10.5 littermate embryos demonstrate normal OFT cushion cellularity in control embryos (C), but hypocellularity in the mutant OFT cushions (D). (E and F) In situ hybridization for Bmp4 using transverse sections of control (E) and mutant (F) E10.5 embryos. (G-J) Phospho-Smad immunohistochemistry of E10.5 embryos of control (G and I) and mutants (H and J). Phospho-Smad positive cells are indicated by the dotted circle in I. Scale bars: 100 μm.

Supplementary Table 1: Genotyping Data

Cross	Genotype	E9.5-E14.5	E17.5-E18.5	P0	P1-P7
Islet1$^{Cre/+}$ *x* *R26R*$^{DNMAML/DNMAML}$	*R26R*$^{DNMAML/+}$	127	11	3	18
	Islet1$^{Cre/+}$; *R26R*$^{DNMAML/+}$	119	10	3*	0[1]
Mef2c-AHF-Cre + *x* *R26R*$^{DNMAML/DNMAML}$	*R26R*$^{DNMAML/+}$	20	-	6	14
	Mef2c-AHF-Cre+; *R26R*$^{DNMAML/+}$	16	-	9*	0[2]
Islet1$^{Cre/+}$; *Jag1*$^{flox/+}$ *x* *Jag1*$^{flox/flox}$	*Jag1*$^{flox/+}$	33	21	4	-
	Jag1$^{flox/flox}$	40	24	8	-
	Islet1$^{Cre/+}$; *Jag1*$^{flox/+}$	28	25	9	-
	Islet1$^{Cre/+}$; *Jag1*$^{flox/flox}$	23	8	0[3]	-
Mef2c-AHF-Cre+; *Jag1*$^{flox/+}$ *x Jag1*$^{flox/flox}$	*Jag1*$^{flox/+}$	2	2	13	-
	Jag1$^{flox/flox}$	2	6	7	-
	Mef2c-AHF-Cre+; Jag1$^{flox/+}$	3	1	15	-
	Mef2c-AHF-Cre+; Jag1$^{flox/flox}$	1	2	7*	-

[1]$p<0.0001$; [2]$p=0.0002$; [3]$p=0.01$

* At P0 all mutants were dead or cyanotic