

# Supplement Material

## Supplemental Figure Legends

**Supplemental Figure I** Tissue-specific deletion of *Dicer* in NCCs. (A) The gene targeting strategy for neural-crest-specific deletion of *Dicer*. E: Exon. (B) *Wnt1-Cre* mediated robust recombination in NCCs in E9.5 *Rosa-LacZ* reporter embryos. Pharyngeal arches are numbered. Migrating neural crest cells are indicated by arrowheads. The migration of cardiac NCCs into outflow tract is indicated by the white arrow. H: heart. (C-D) Detection of *Dicer* expression in neural-crest-derived pharyngeal arch tissue samples with quantitative RT-PCR (C, n=3, \*: P=0.0001) and Western blot (D).

**Supplemental Figure II** Immunohistochemistry detecting a proliferation marker, phospho-histone H3 (pHH3), showed no significant difference of cranial NCCs proliferation in 1<sup>st</sup> pharyngeal arch between mutant embryos and control littermates. Three incontinous sections containing 1<sup>st</sup> pharyngeal arch were stained and counted. 1PA: 1<sup>st</sup> pharyngeal arch.

**Supplemental Figure III** Immunohistochemistry detecting an apoptosis marker, cleaved-caspase-3 (cCasp-3), indicated an abnormal apoptosis in 1<sup>st</sup> pharyngeal arch in E10.5 *Dicer* mutant embryos. 1PA: 1<sup>st</sup> pharyngeal arch.

**Supplemental Figure IV** Three pairs of pharyngeal arch arteries were shown by whole mount immunochemistry detecting an endothelial cell marker, PECAM-1, and numbered. No obvious developmental defect of 4<sup>th</sup> (4) pharyngeal arch artery was found in *Dicer* mutant embryos.

**Supplemental Figure V** (A-B) Whole mount immunostaining detecting the neurofilament (with the 2H3 antibody) shows the impaired development of nerve in E10.5 (A) and E13.5 (B) *Dicer* mutant embryos. Vmb: mandibular branch of cranial nerve V; Vmx: maxillary branch of cranial nerve V; Vop: ophthalmic branch of cranial nerve V.

**Supplemental Figure VI** The migration, proliferation and apoptosis of cardiac NCCs in

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outflow tract. (A-B) Whole mount  $\beta$ -gal staining of Dicer mutant embryos and control littermates. Cardiac NCCs were shown to migrate into outflow tract, which is indicated by arrows. (C-F) Immunohistochemistry detecting a proliferation marker, phospho-histone H3 (pHH3), an apoptosis marker, cleaved-caspase-3 (cCasp-3), and a smooth muscle cell marker, smooth muscle actin (SMA), to examine the proliferation and apoptosis of cardiac NCCs in outflow tract. No significant difference was found between Dicer mutant embryos and control littermates. An abnormal apoptosis was shown in the pharyngeal arch of mutant embryos (F). H: heart; PA: pharyngeal arch; OFT: outflow tract.

**Supplemental Figure VII** Histological examination showed the defect in the developing aortic arch of NCC-Dicer mutant mice. The lumen of aortic arch is narrowed (arrowheads) and the vessel wall of segment B (dot boxes, enlarged pictures are shown in Figure 3E) is thinner in mutant embryos. The dashed line in cartoon indicates the orientation of the sections and the arrow indicates the segment B of aortic arch (in purple) and the region shown in dot boxes. AAO: ascending aorta; AoA: aortic arch; E: esophagus; PT: pulmonary truck; T: trachea.

**Supplemental Figure VIII** Smooth muscle differentiation of cardiac NCCs in ascending aorta and aortic arch. (A-D) Immunohistochemistry detecting smooth muscle markers, smooth muscle  $\alpha$  22 (SM-22; A, B) and smooth muscle actin (SMA; C, D), showed no significant difference in smooth muscle differentiation of cardiac NCCs between mutant embryos and control littermates. AAO: ascending aorta; AoA: aortic arch; E: esophagus; T: trachea.

**Supplemental Figure IX** Smooth muscle assembling in ascending and descending aorta. (A-B) Elastic fiber staining of sections from ascending aorta. The ascending aorta of E17.5 mutant embryos (B) displayed fewer layers of smooth muscle cells, which were not well organized. (C-D) The elastic fibers in descending aorta shown by fluorescent microscopy (C', D') revealed the assembly of non-neural-crest-derived smooth muscle cells was unaffected in mutant embryos. AAO: ascending aorta; DAO: descending aorta.

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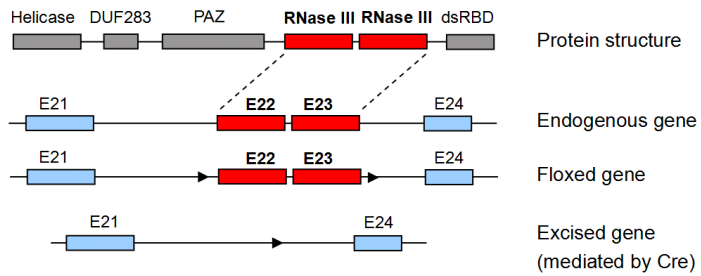
## Supplemental Table I

**Genotypes of embryos from the crossing of  $Dicer^{lox/lox}$  mice to  $Wnt1-Cre/Dicer^{lox/+}$  mice.**

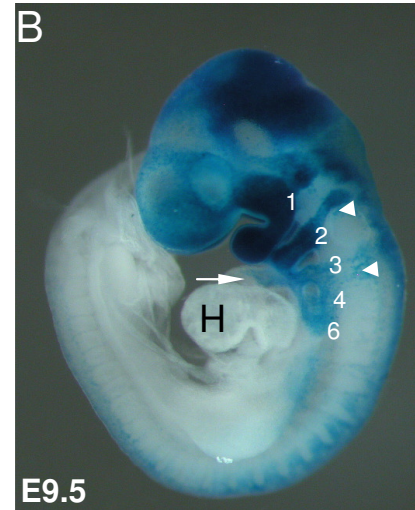
Embryonic day (# of embryos)	$Dicer^{lox/+}$	$Dicer^{lox/lox}$	$Wnt1-Cre/Dicer^{lox/+}$	$Wnt1-Cre/Dicer^{lox/lox}$
E9.5-E11.5 (141)	33	41	33	34
E12.5-E15.5 (62)	15	14	19	14
E16.5-E18.5 (25)	6	6	6	7
Total (228)	54 (23.7%)	61 (26.8%)	58 (25.4%)	55 (24.1%)

## Supplemental Figure I

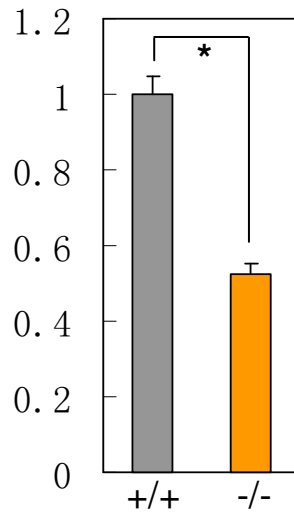
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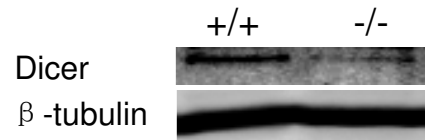
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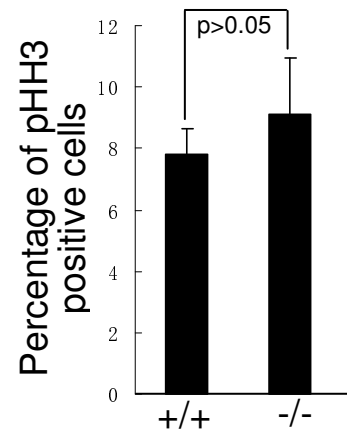
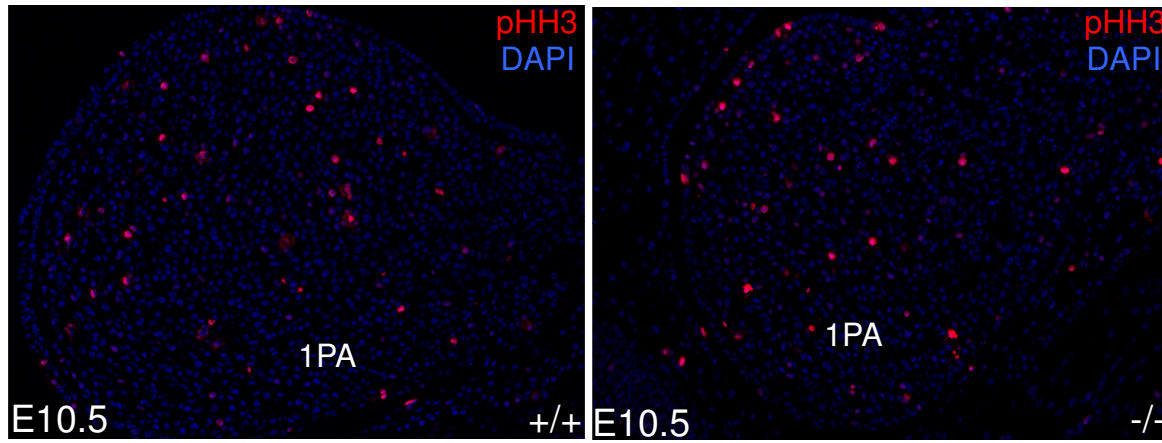
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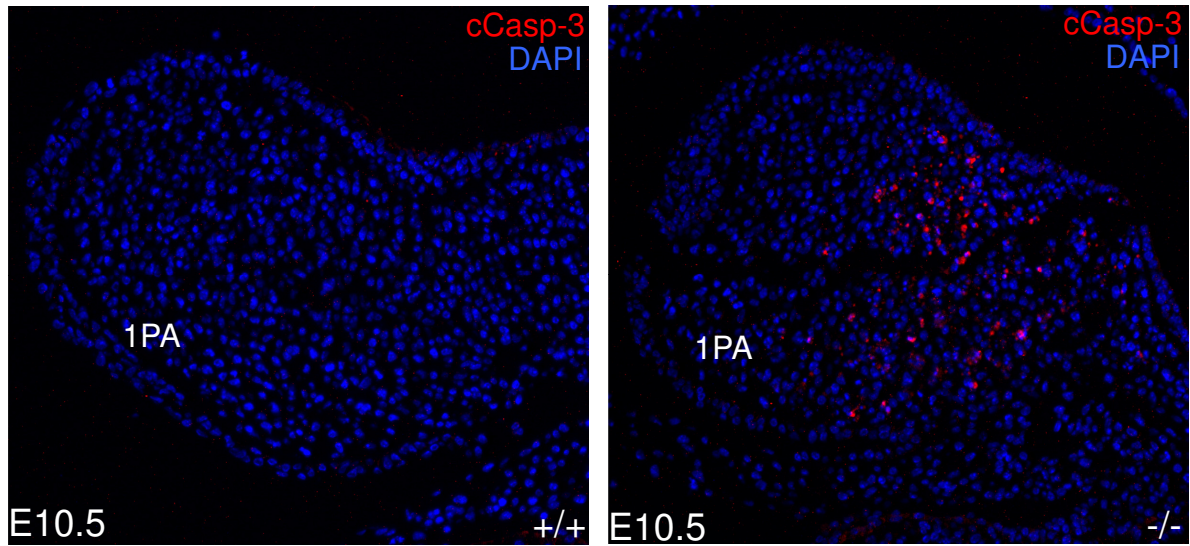
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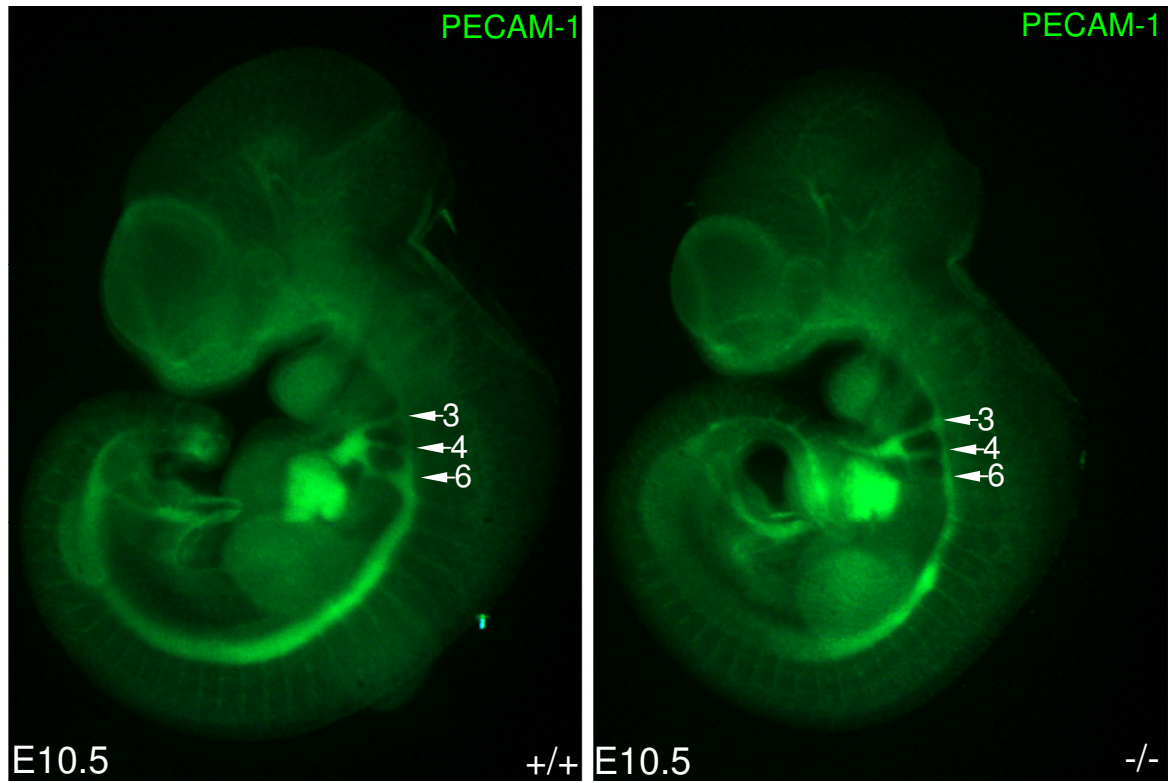
## Supplemental Figure II



## Supplemental Figure III

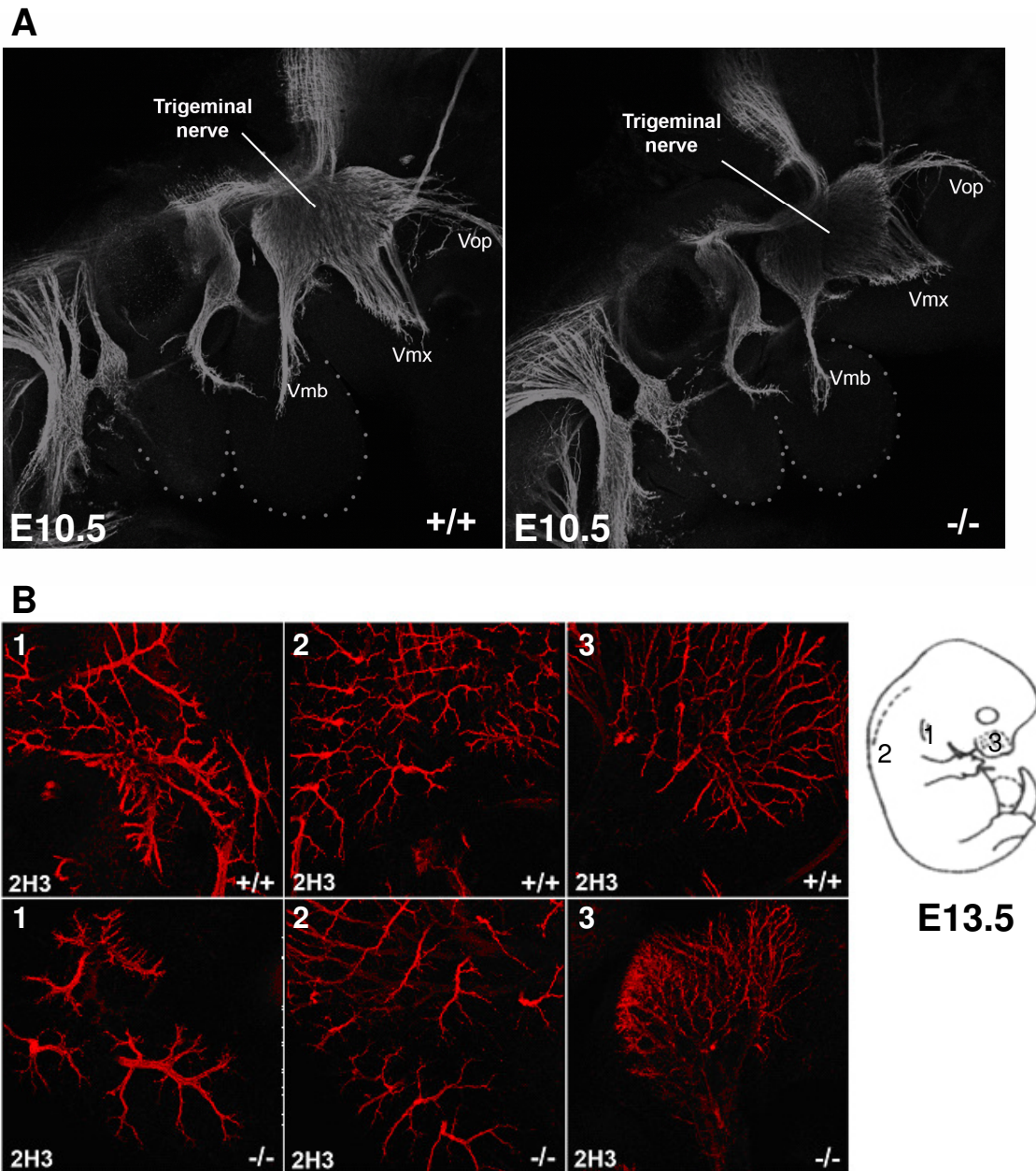


## Supplemental Figure IV





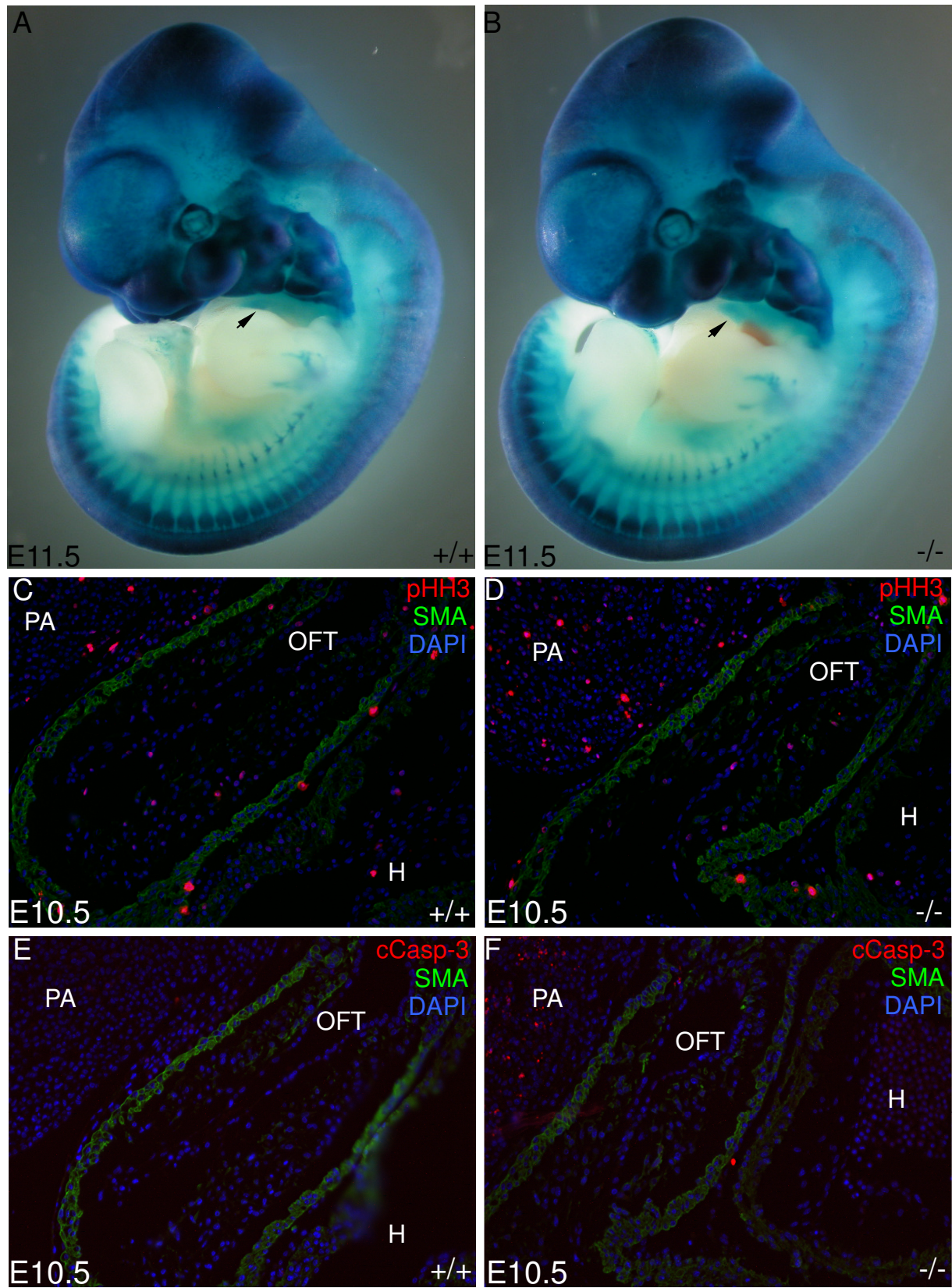
## Supplemental Figure V



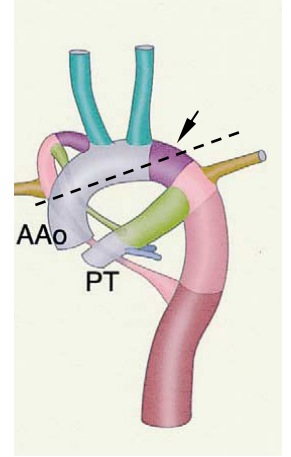
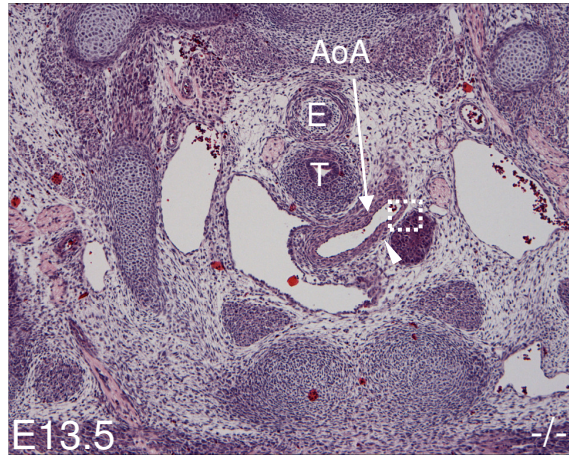
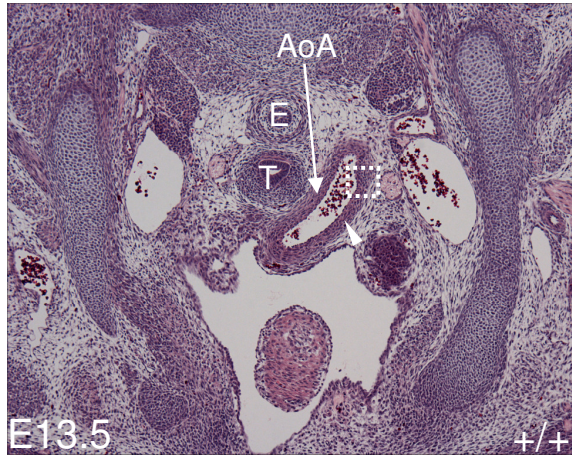


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## Supplemental Figure VI

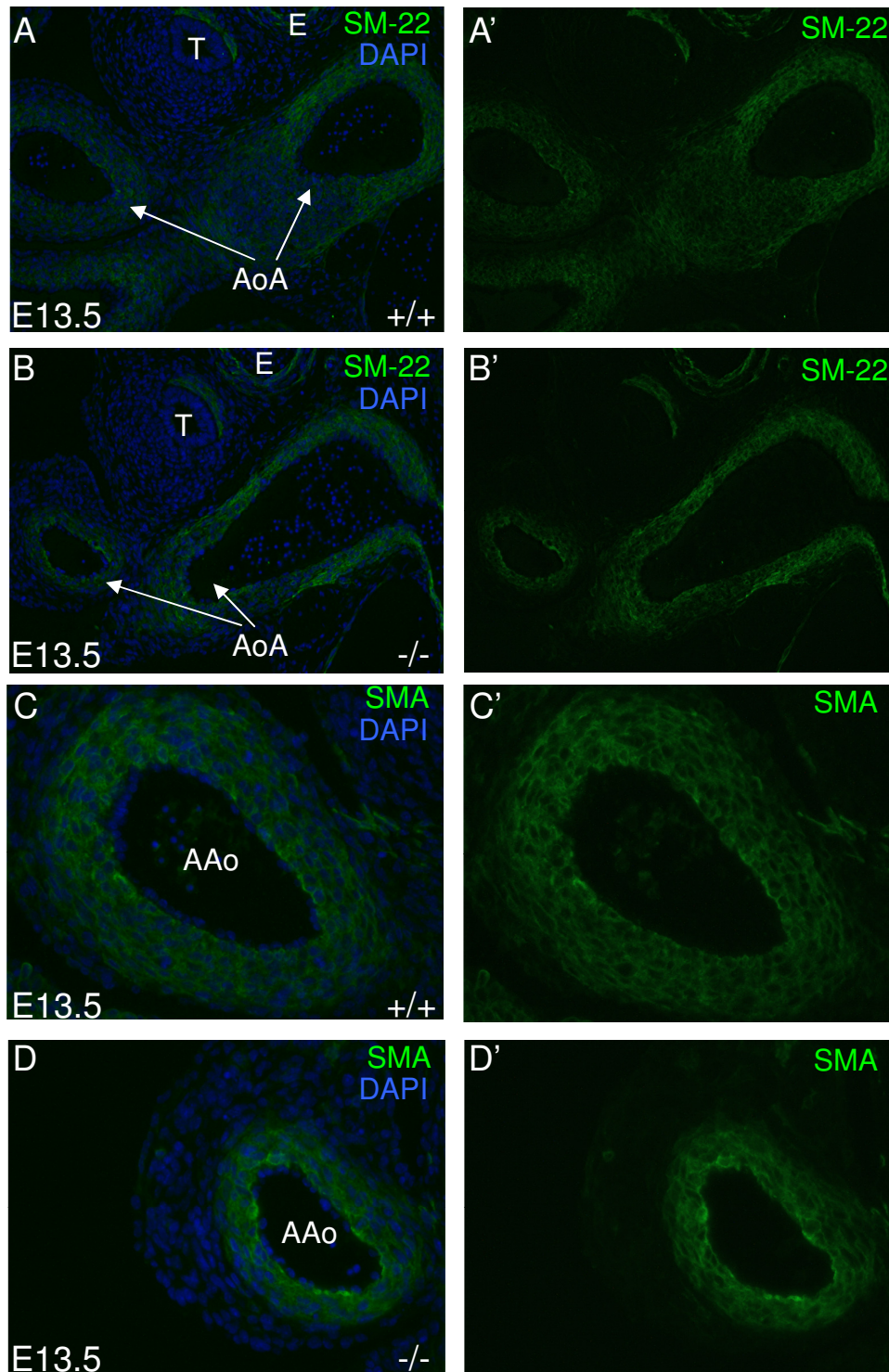


## Supplemental Figure VII





## Supplemental Figure VIII



## Supplemental Figure IX

