SUPPLEMENTAL MATERIAL

Detailed Methods

Mouse husbandry

Mice used in these experiments were mated to collected embryos following standard techniques [1]. All husbandry was carried out under the supervision of veterinarians at UT Southwestern and follow guidelines established by the Institutional Animal Care and Use Committee and is consistent with the Animal Welfare Act and the NIH *Guide for the Care and Use of Laboratory Animals*.

Mouse lines and genotyping primers

Foxh1 mice were maintained on CD1 backgrounds and genotyped as previously described [2]; *Foxa2*$^{flox/flox}$ mice [3] on 129/Sv backgrounds, were crossed with *Foxa3-Cre* [4] and *Tie2-Cre* [5]. *Foxa2*$^{flox/+}$ mice were generated by *Foxa2*$^{flox/flox}$ mice crossed with *Sox2-Cre* mice [6]. Genotyping PCR primers are as follows: (Forward) 5'-GCGCCTGAGTTGGCGGTGGT-3; (Mut) 5'-GGGAGACACCATTCCTGAGA-3'; (WT) 5'-GCGGACATGCTCATGTATGTG-3', yield a 307 bp wild-type and a 485 bp mutant allele product. *Sema3E* mice were maintained on C57BL/6-CD1 backgrounds and genotyped as previously described [7].

Statistical analyses

Sema3E$^{+/-}$ and $^{-/-}$ vasculature was assessed for total branch points, lateral branch points and avascular midline area using ImageJ. In all cases, 3-4 embryos per somite stage (5-9S) were counted. Counts of total number of branch points: from left and right of the midline to extra-embryonic ECs. Counts of lateral branch points: a 100 sq μm box was placed into representative right and left lateral areas and branch points were averaged. Counts in EC-free midline: avascular midline was outlined three times per embryo to give the average area (sq μm). Data represents average area (sq μm/height) for 3-4 embryos per somite stage.

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SUPPLEMENTAL FIGURE LEGENDS

Online Figure I. Expression analysis of NGC receptors during embryonic vasculogenesis. (**A,B**) Cartoon depiction of an E8.25 embryo; (**A**) anterior and (**B**) ventral views showing DA (red) and surrounding tissues. (**C-H**) In situ hybridization for *plexinD1*, *robo4* and *unc5b* at E8-8.25. (**C**,**E,G**) Anterior and (**D,F,H**) ventral views are shown. Arrowheads mark the DA. Scale bar: 200 μm (**C-H)**.

Online Figure II. *Foxh1*$^{-/-}$ embryos exhibit dramatic vascular defects. (A-H) Expression of *plexind1* and *rasip1* transcripts in *Foxh1* heterozygous and null embryos at E8-8.25; ventral (**A,B,E,F**) and cross-section (**C,D,G,H**) views. (**B',D',F',H'**) Cartoon depiction of stained ECs (red) in **B,D,F,H** respectively. (**I-N**) Anterior views of E8-8.25 *Foxh1*$^{+/-}$*; Flk1-LacZ* (**I, L**) and *Foxh1*$^{-/-}$*;Flk1LacZ* (**J,K,M,N**) embryos stained for β-galactosidase (light blue). Scale bar: 200 μm (**A,B,E,F,I-N**) and 25 μm (**C,D,G,H**).

Online Figure III. *Foxa2* deletion in the endoderm and ECs results in normal DA patterning. (A-D) Expression of *rasip1* in *Foxa2*$^{f/f}$ (control), *Foxa2*$^{f/f}$*;Foxa3-cre* (endoderm-specific expression of cre) and *Foxa2*$^{f/f}$*;Tie2-cre* (EC-specific expression of cre) E8.0 embryos. Anterior views are shown. Scale bar: 200 μm.

Online Figure IV. Notochordless embryos lack midline repulsive guidance cues. (**A-L**) In situ hybridization for *chordin, netrin1* and *slit2* transcripts in E8-8.25 *Foxh1*$^{+/-}$ and *Foxh1*$^{-/-}$ embryos: (**A,C,E,G,I,K,** scale bars: 200 μm) anterior and (**B,D,F,H,J,L,** scale bars: 25 μm) cross-section views. Arrows mark the notochord and DA are outlined in black. (**B',D',F',H',J',L'**) Cartoon depiction of stained tissues (red) in **B,D,F,H,J,L** respectively. Scale bars: 200 μm .

Online Figure V. *Sema3E* transcripts are detected in tissues adjacent to the dorsal aortae but are lost as the aortic vessels fuse. (A-P) *In situ* hybridization for *sema3E* transcripts in E7.5 to E11.5 mouse embryos**;** anterior (**A,C,E,G,I,K,M,O,P**) and cross-section (**B,D,F,H,J,L,N**) views. Dotted lines indicate respective sections. After E9.0 *sema3e* expression is detected in the somites (arrows). Scale bars; (A,C,D): 200 μm, (B,D,F,H, J,L,N): 50 μm, (G,I,K): 500 μm, (M,O): 500 μm and (P): 200 μm.

Online Figure VI. Expression levels of blood vessel promoting factors are unperturbed in *sema3E* mutants. (**A,B**) E8.25 *sema3E*$^{+/-}$*;VEGF-LacZ* and *sema3E*$^{-/-}$*;VEGF-LacZ* embryos stained for β-galactosidase (green, cross-section views, scale bar: 100 μm). **(C-X)** Expression of *shh, patched, smoothened, bmp2,4,7, bmp receptor 1a*, *fgf receptors 1,2,3* and *4* transcripts in *sema3E* heterozygous and null embryos at E8-8.25 (anterior views, scale bar: 100 μm).

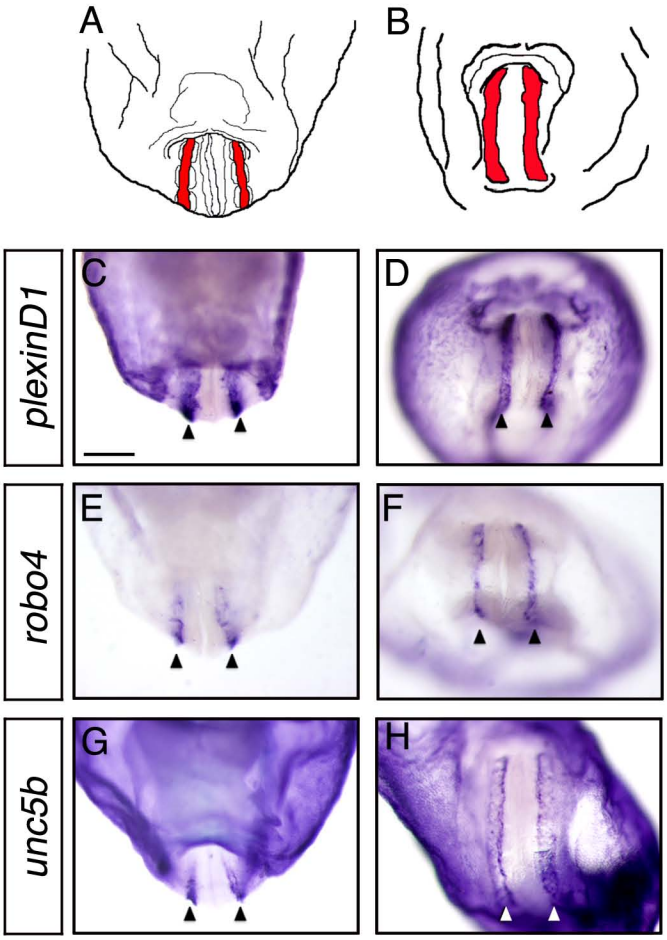
Online Figure VII. Expression of artery markers and repulsive guidance cues are normal in *sema3E* mutants; Sema3E creates avascular zones in vivo. (A,B) E8.25 *sema3E*$^{+/-}$*;Flk1-LacZ* and *sema3E*$^{-/-}$*;Flk1-LacZ* embryos stained for β-galactosidase (light blue, ventral views). (**C,D**) Cross-section of embryos similar to **A,B**, showing proximity of ECs to notochord in *sema3E*$^{-/-}$ embryos. (**E,F**) *Sema3E*$^{+/-}$ *and sema3E*$^{-/-}$ E8.25 embryos stained for PECAM (brown, anterior view). Expression of artery-specific genes *cx40*

(**G,H**) and *dll4* (**I,J**) in *sema3E*$^{+/-}$ *and sema3E*$^{-/-}$ embryos. (**K-R**) Expression of *chordin*, *noggin*, *netrin1* and *slit2* in E8.25 *sema3E*$^{+/-}$ *and sema3E*$^{-/-}$ embryos (ventral views); anterior views are shown. (**S,T**) Chick embryos implanted with control HEK293T (**S**) or HEK293T-Sema3E (**T**) cells; (**T**) example of strong repulsion. Cell aggregates are outlined in black. Note the lack of ECs near the HEK293T-Sema3E cell implant. Scale bars: 200 μm.

Online Figure VIII. Embryonic ECs arise in wider intraembryonic domains in *sema3E*$^{-/-}$ aortic vessels. (A-J) E7.5 to E8.0 *sema3E*$^{+/-}$*;Flk1-LacZ* and *sema3E*$^{-/-}$*;Flk1-LacZ* embryos stained for β-galactosidase (light blue, anterior views). Note that angioblasts do not arise preciously, and mutant branched aortae do not arise from branching of initially normal aortic vessels. Scale bar: 100 μm.

Online Figure IX. *Sema3E* is expressed in the notochord, but not the LPM during chick DA development. (A-D) Expression of *plexinD1* and *sema3E* in Hamburger-Hamilton (HH) stage 12 embryos: ventral views. (**C, D**) Magnified images of embryos in (**A**) and (**B**) respectively. The bracket denotes blood vessels adjacent to the DA. *Sema3E* expression is only detected in the notochord.

Online Figure I



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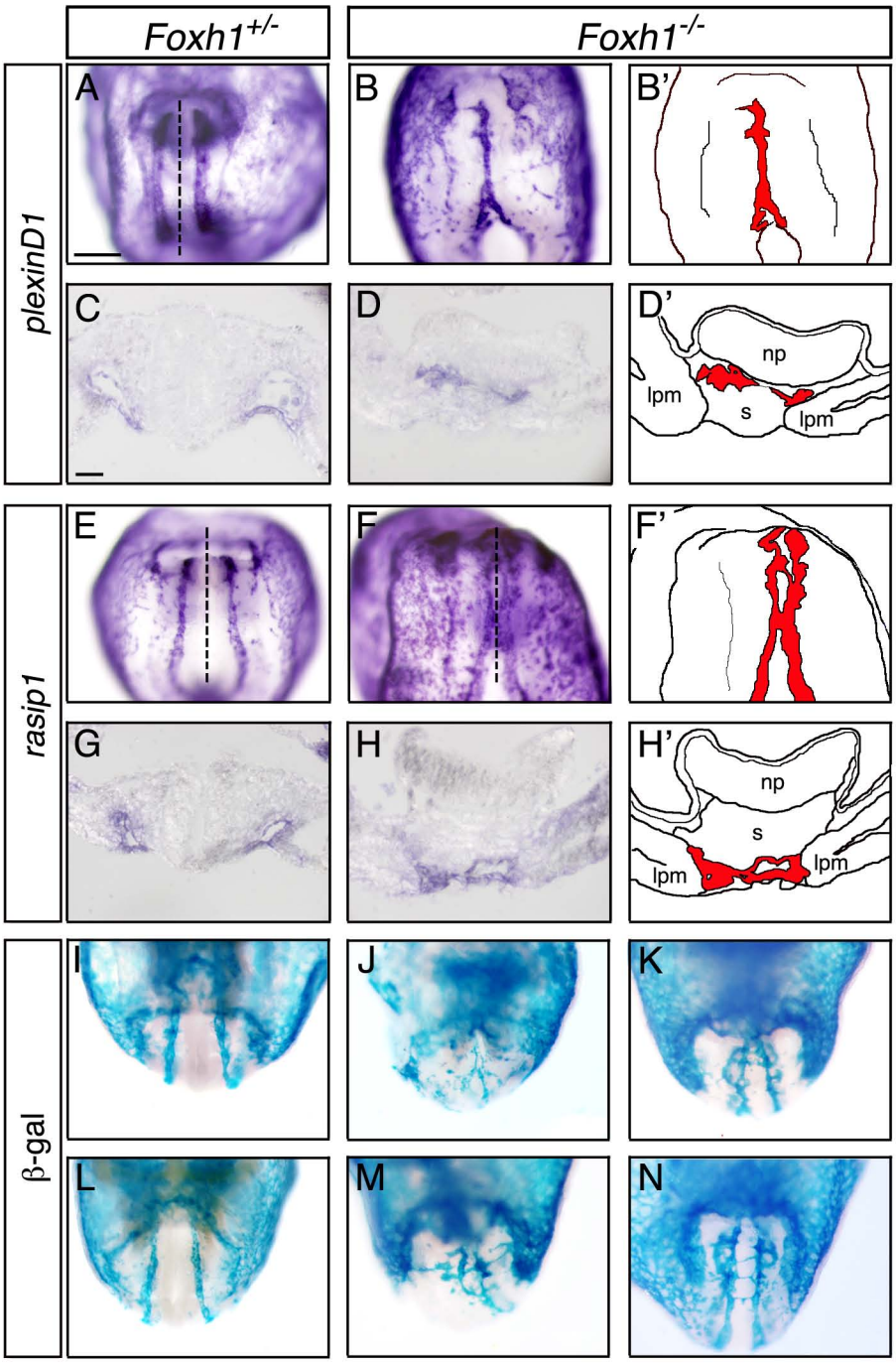

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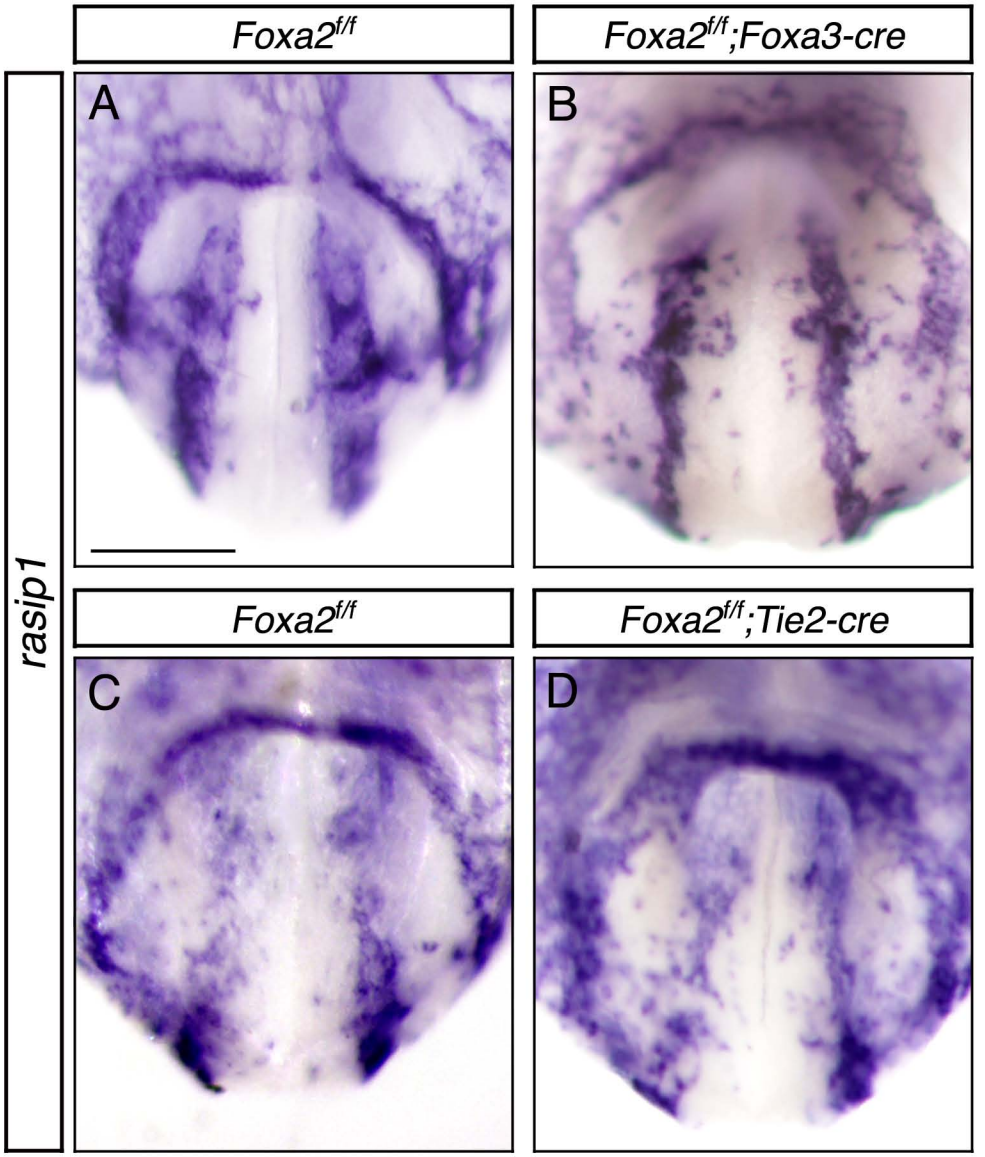

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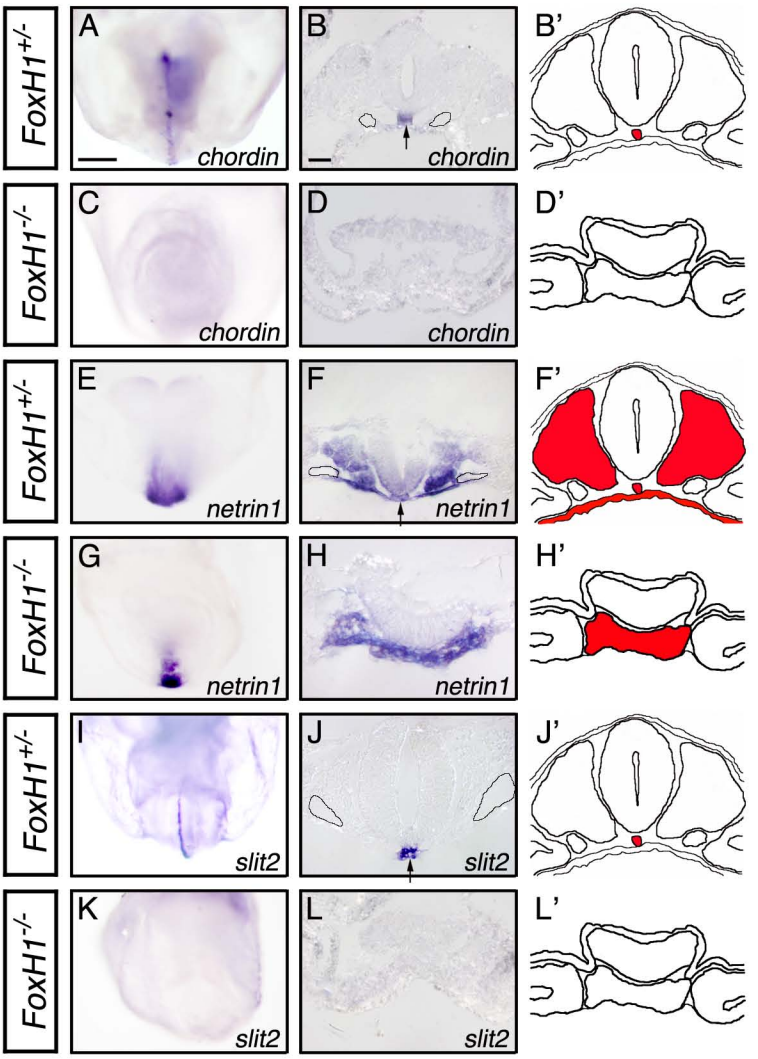

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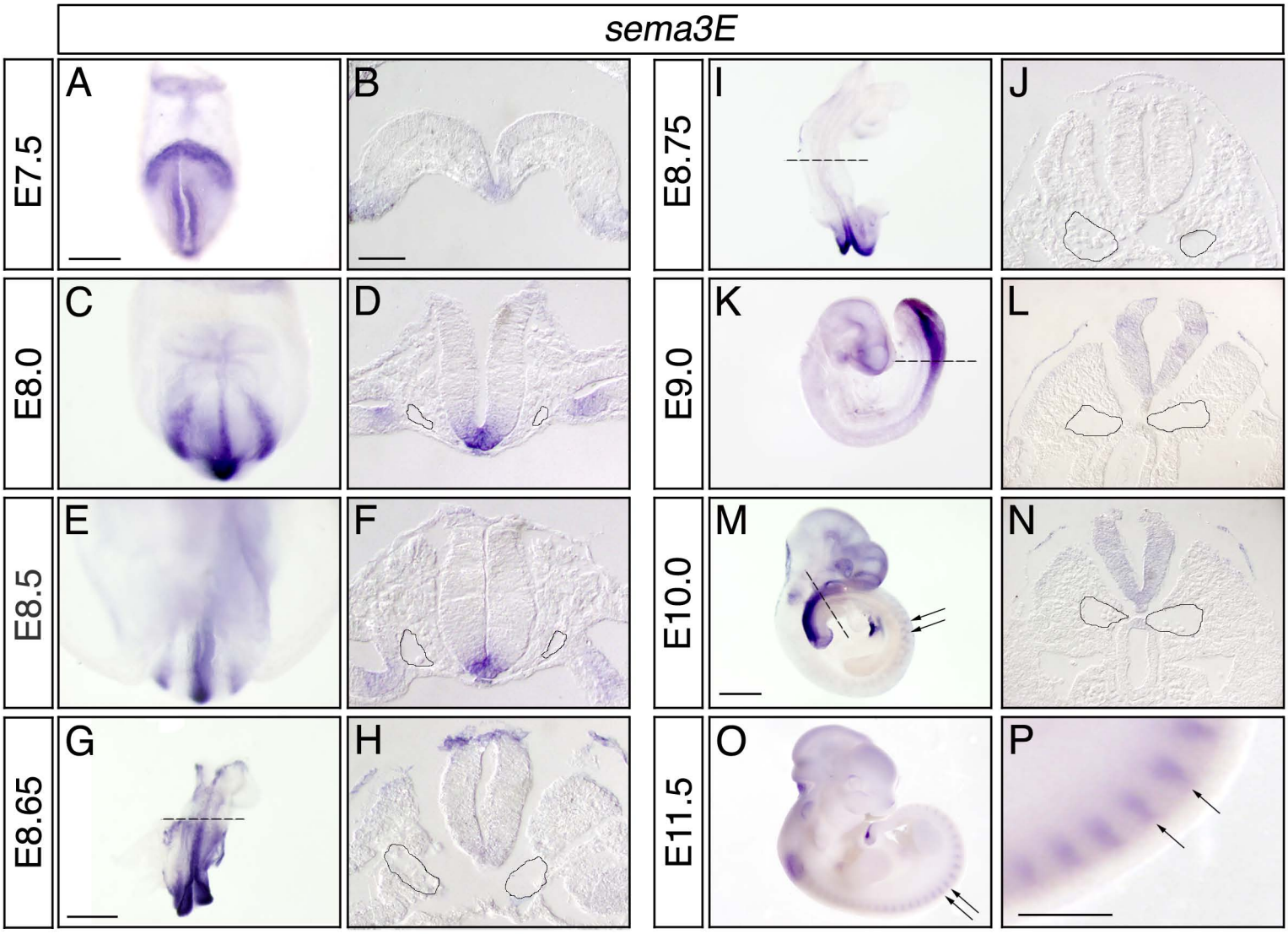

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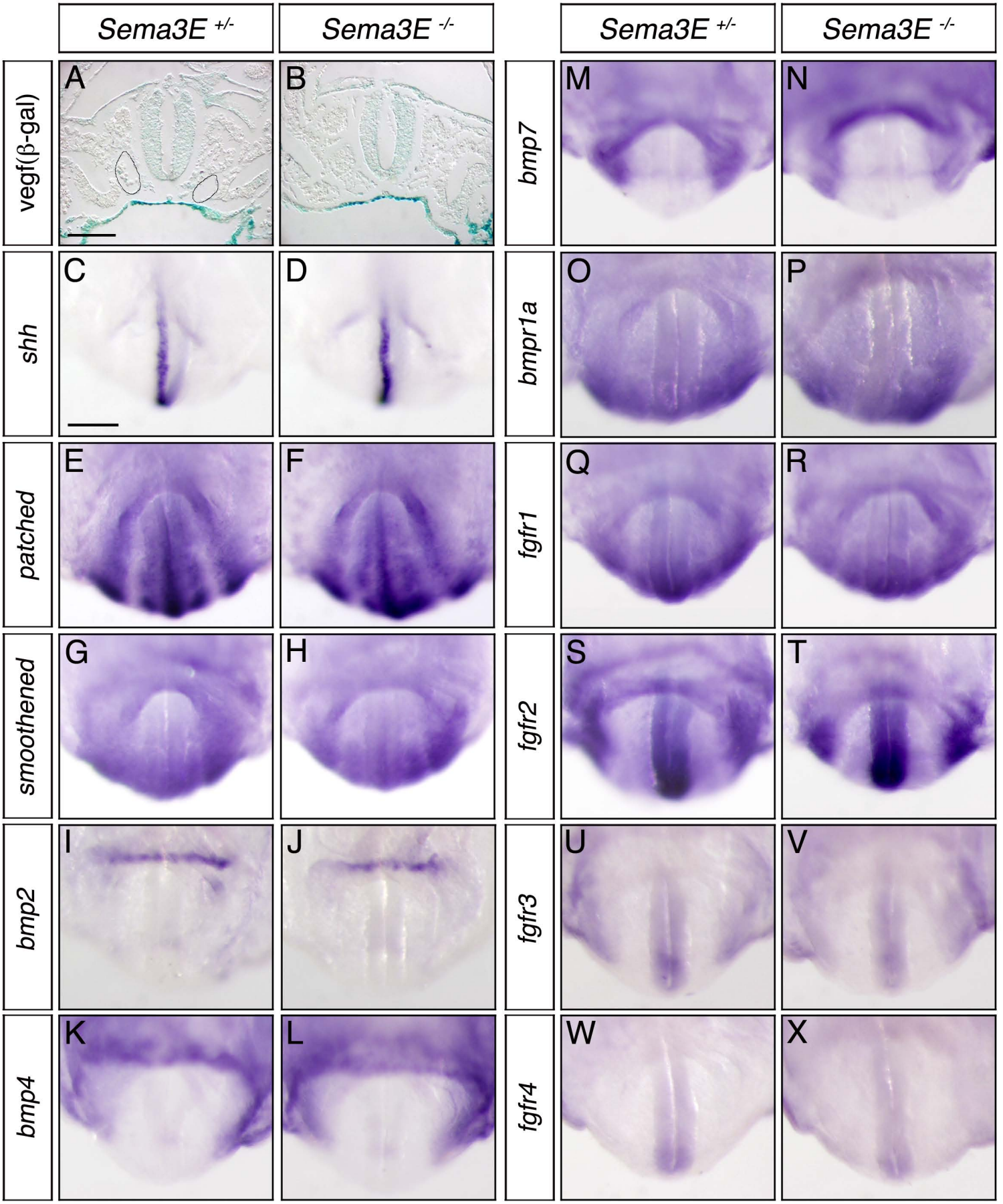

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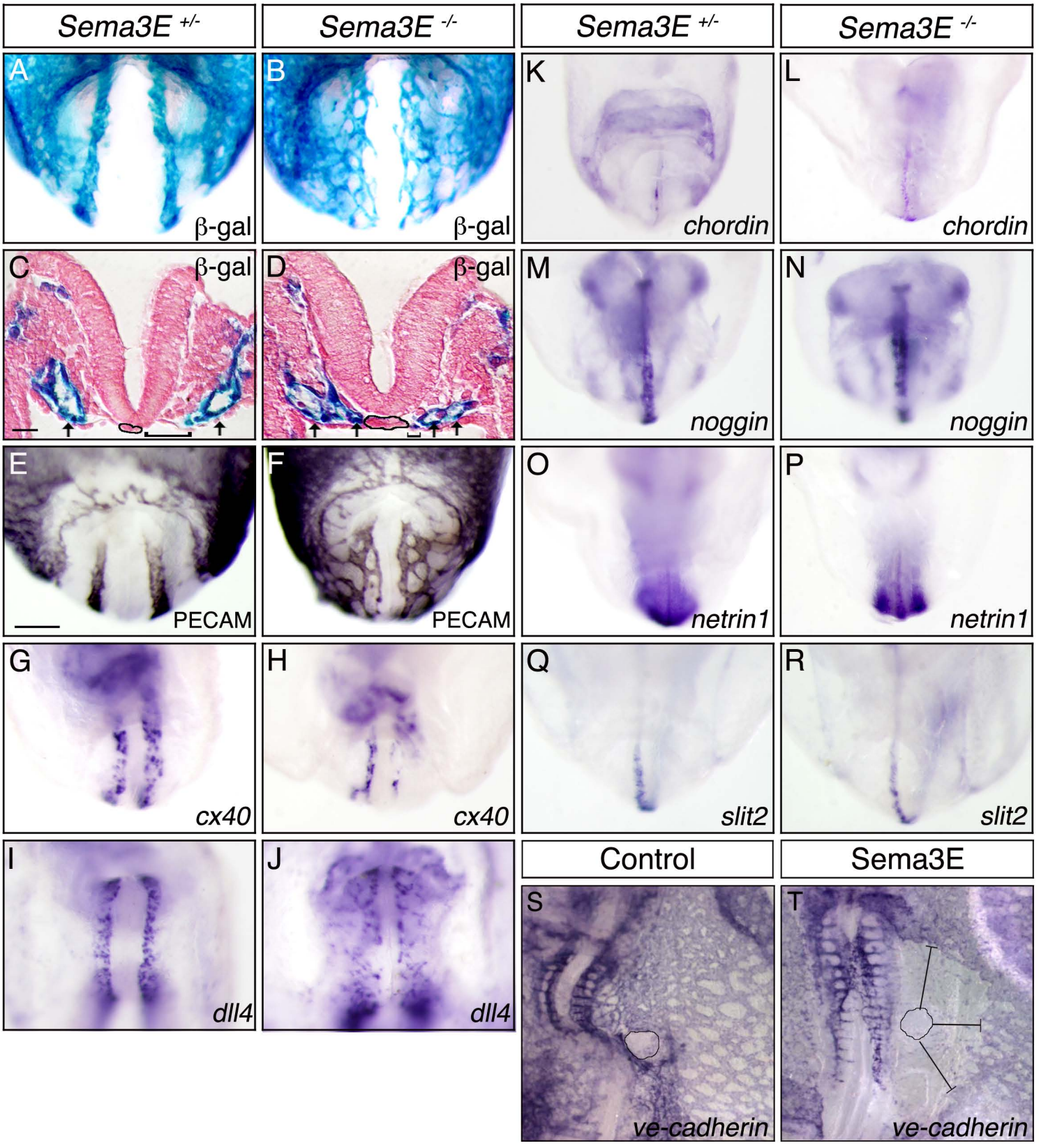

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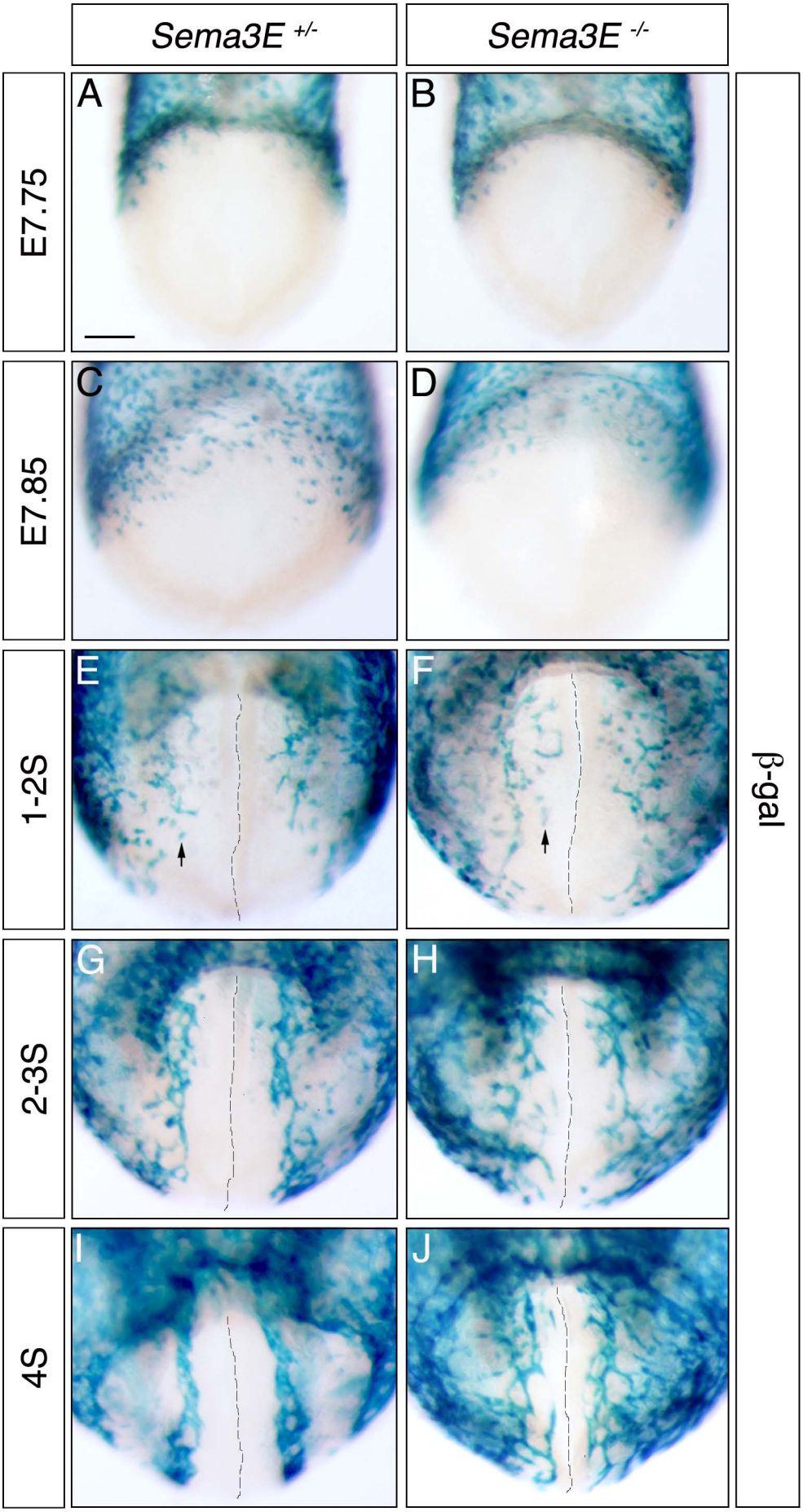

Online Figure IX