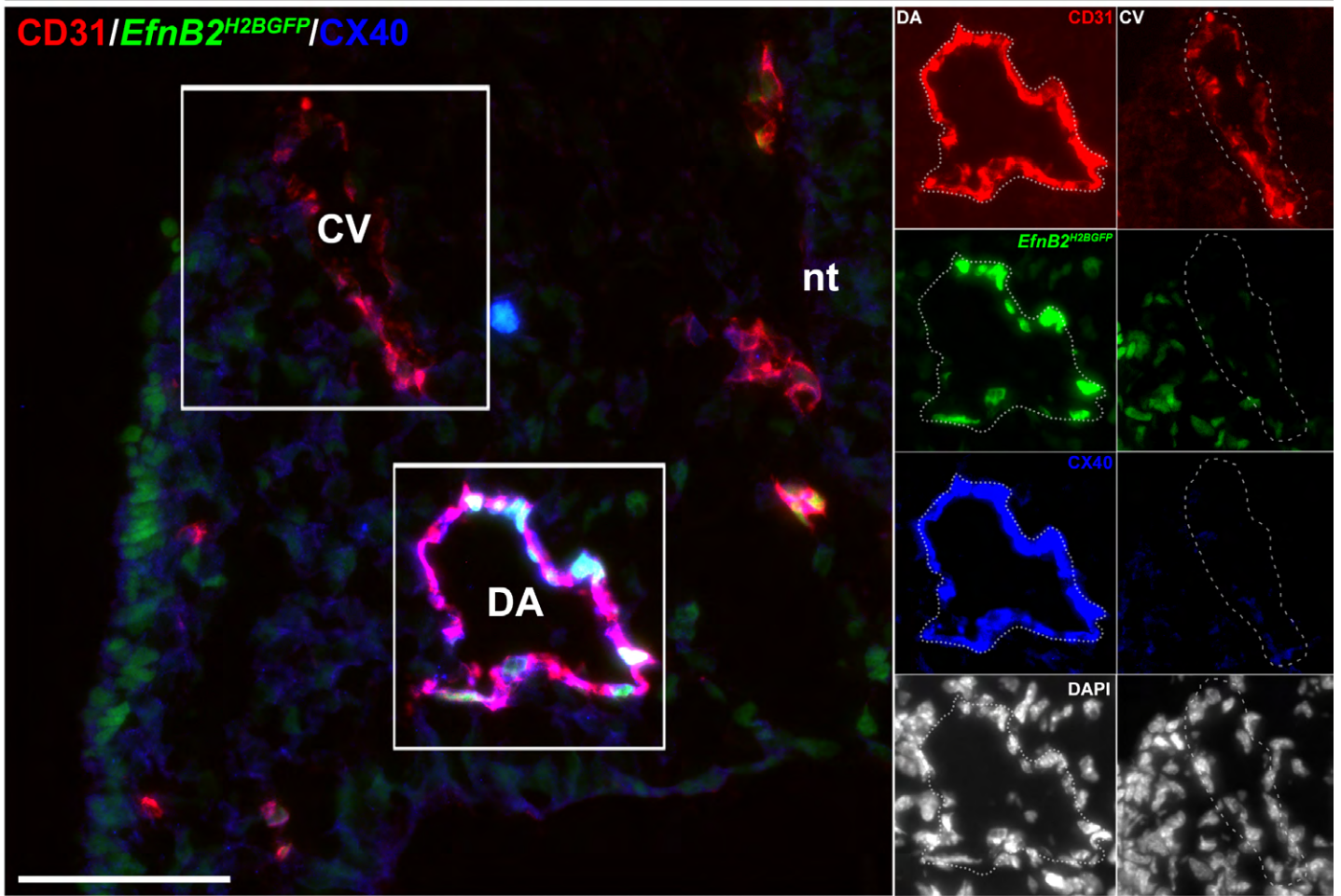


Supplementary figure 1

Notch1^{β-Gal} is a faithful reporter for Notch1, judged by known expression in the presomitic and somitic mesoderm, and the endocardium.

(A-C) To validate the *Notch1*^{+/LacZ} line, we examined the expression pattern of β-Gal in 5ss whole-mount X-Gal-stained embryos. (A) Ventral view. (B, C) Cross-sections. As previously reported (Conlon et al., 1995; Williams et al., 1995), we detected expression in the somites and presomitic mesoderm (A, B, red arrows) as well as in the endocardium and sinus venosus (A, C, red arrows). (D-E) *Notch1*^{β-Gal} expression in the DA between 4 and 7ss. (D) At 4ss, X-Gal positive cells (red arrow) were found in the endocardium. Only weakly X-Gal positive ECs were occasionally found in the DA; most cells in the DA were negative for X-Gal (open arrows). (E) At 7ss, both X-Gal positive (red arrows) and negative (open arrows) ECs were found in the DA. psm: pre-somitic mesoderm; ecm/sv: endocardium/sinus venosus; nt: neural tube. DA: dorsal aorta, CV: cardinal vein. Scale bars 25 μm.

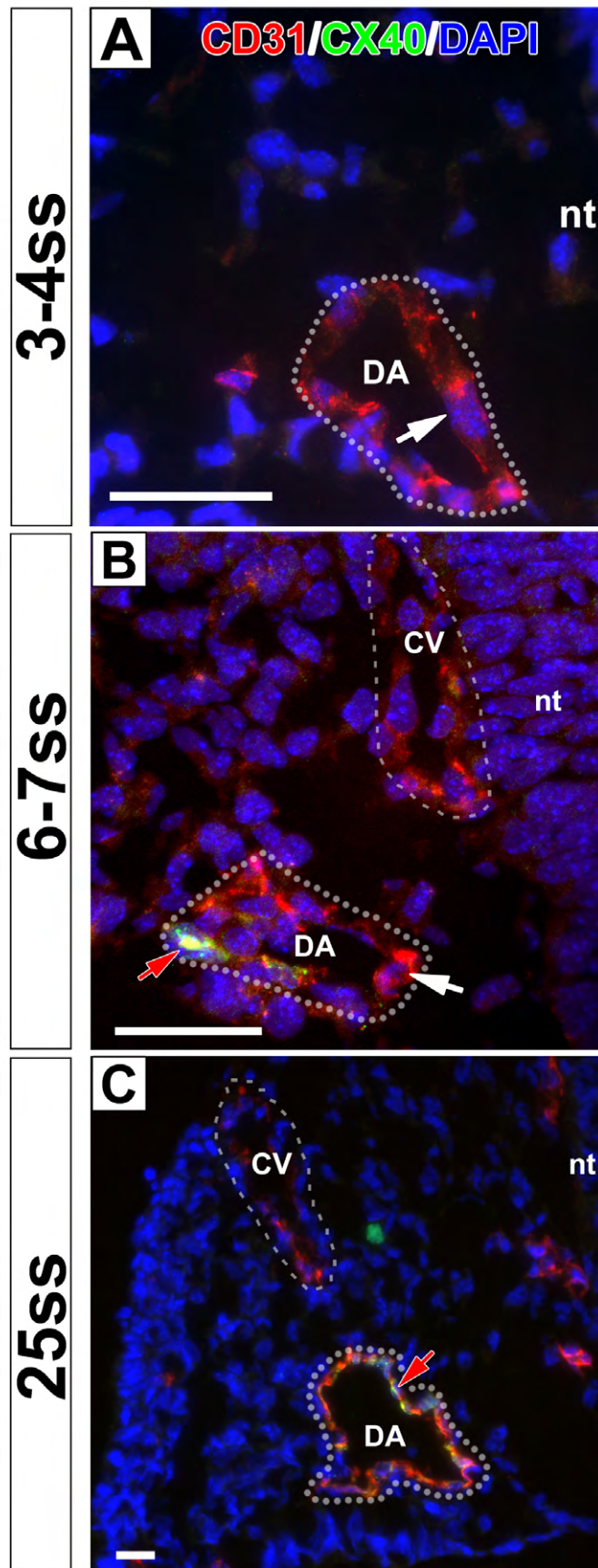
25ss



Supplementary figure 2

Validation of arterial marker Connexin 40 by its expression in the dorsal aorta but not in the cardinal vein at 25ss.

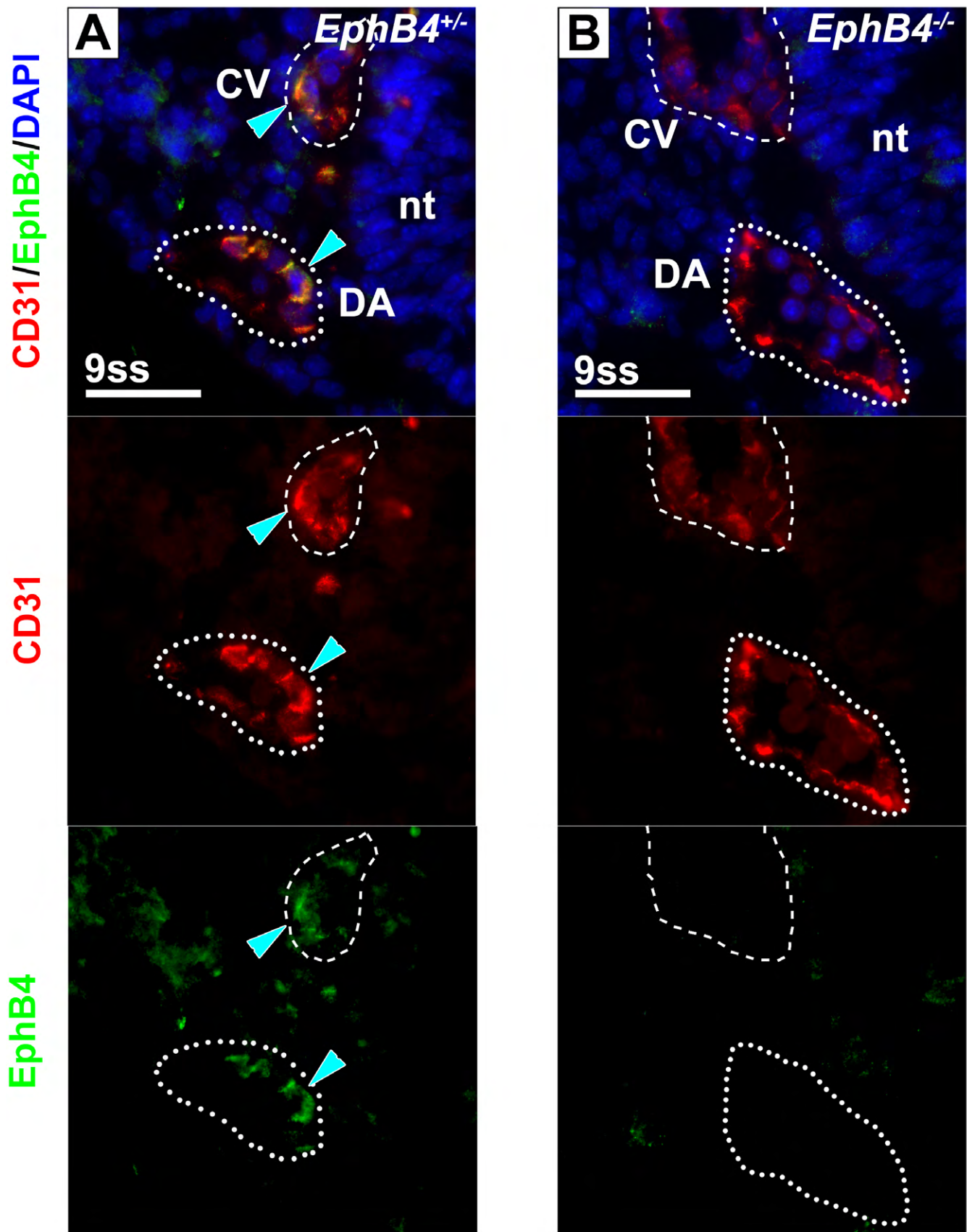
CD31 (red) and Connexin 40 (CX40, blue) staining in a 25ss *Efnb2*^{H2BGF/+} (green) embryo. Dotted line indicates the DA. At this stage, all ECs in the DA express Connexin 40, whereas no expression was detected in the CV (dashed line). DAPI (white). nt: neural tube. DA: dorsal aorta, CV: cardinal vein. Scale bar 50 μ m.



Supplementary figure 3

Connexin 40 expression in the dorsal aorta and cardinal vein between 3-4 and 25ss.

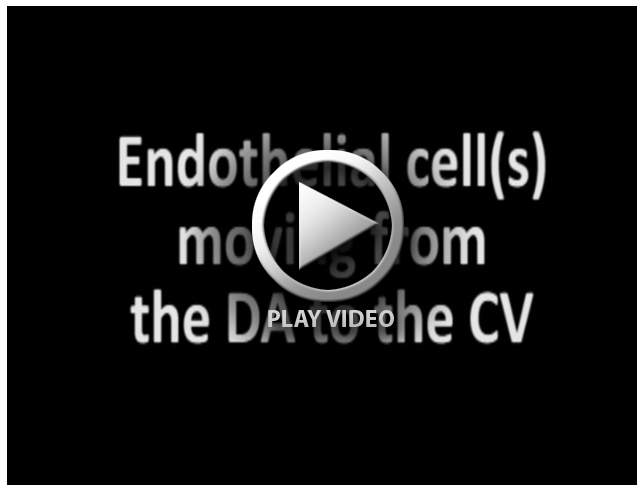
ECs in the DA were primarily negative for CX40 expression (white arrows) at 3-4ss, but at 6-7ss, both positive (red arrows) and negative ECs were present in the DA. At 25ss, all ECs were positive for CX40. At all times, the ECs of the CV were negative for CX40. CD31 (red), CX40 (green), DAPI (blue). nt: neural tube. DA: dorsal aorta, CV: cardinal vein, ECs: endothelial cells. Scale bars 25 μ m.



Supplementary figure 4

EphB4 was expressed in the dorsal aorta.

To validate the EphB4 antibody, we stained *Ephb4*^{+/+} and *Ephb4*^{-/-} embryos. EphB4 expression was detected in the DA and CV (arrowheads) in 9ss *Ephb4*^{+/+} (A) but not in *Ephb4*^{-/-} (B) embryos. DAPI (blue). nt: neural tube. DA: dorsal aorta (dotted line), CV: cardinal vein (dashed line). Scale bars 25 μ m.



Supplementary movie 1

Endothelial cells move from the dorsal aorta to the cardinal vein as shown by wide field fluorescent microscopy.

Time-lapse movie showing moving EC(s) from the DA to the CV at 10ss in a *Tie2-Cre;mT/mG* embryo.



Supplementary movie 2

Endothelial cells move from the dorsal aorta to the cardinal vein as shown by two photon microscopy.

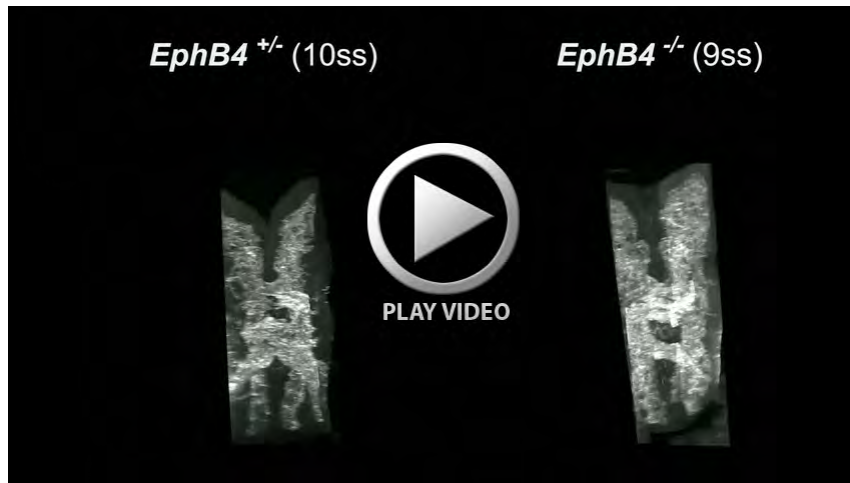
Three-dimensional two-photon time-lapse movie showing moving EC(s) from the DA to the CV at 10ss in a *Tie2-Cre;mT/mG* embryo.



Supplementary movie 3

No apparent difference in dorsal aorta size at 8-9ss in *EfnB2*^{-/-} embryos.

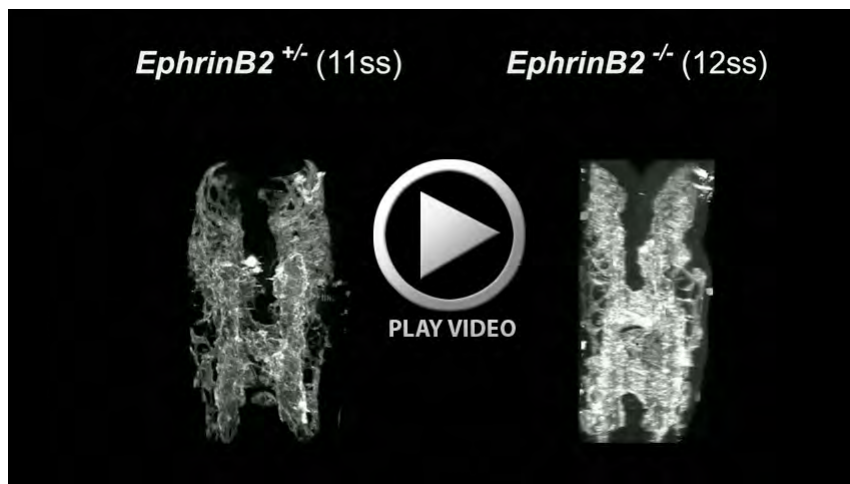
High resolution optical sectioning and 3D reconstruction of two-photon images from CD31 stained 8-9ss *EfnB2*^{+/-} and *EfnB2*^{-/-} embryos. No apparent difference in the size of the dorsal aorta was detected.



Supplementary movie 4

No apparent difference in dorsal aorta size at 9-10ss in *EphB4*^{-/-} embryos.

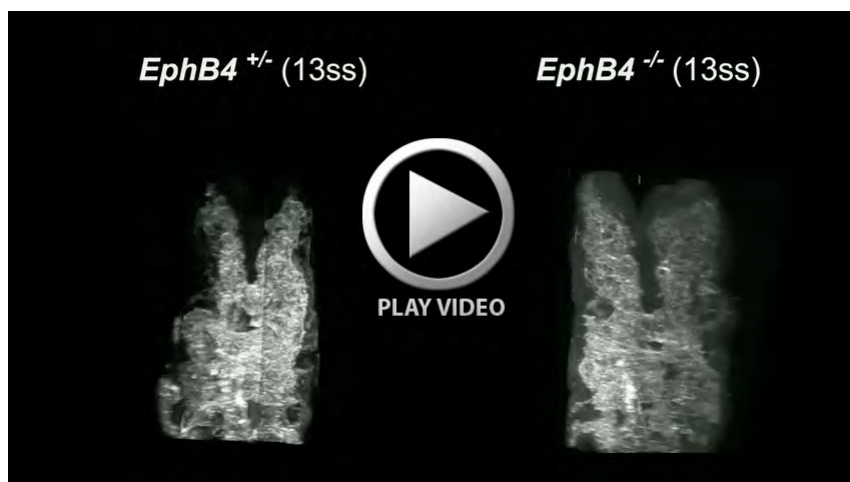
High resolution optical sectioning and 3D reconstruction of two-photon images from CD31 stained 9-10ss *EphB4*^{+/-} and *EphB4*^{-/-} embryos. No apparent difference in the size of the dorsal aorta was detected.



Supplementary movie 5

The dorsal aorta is bigger and the cardinal vein smaller in 11-12ss *EfnB2*^{-/-} embryos.

High resolution optical sectioning and 3D reconstruction of two-photon images from CD31 stained 11-12ss *EfnB2*^{+/-} and *EfnB2*^{-/-} embryos. The DA is bigger and the CV smaller in *EfnB2*^{-/-} embryos.



Supplementary movie 6

The dorsal aorta is bigger and the cardinal vein smaller in 13ss *EphB4*^{-/-} embryos

High resolution optical sectioning and 3D reconstruction of two-photon images from CD31 stained 13ss *EphB4*^{+/-} and *EphB4*^{-/-} embryos. The DA is bigger and the CV smaller in *EphB4*^{-/-} embryos.

Supplementary Table S1 – Statistical analysis for data in Figure 1B: *Efnb2*-positive endothelial cells in the DA

Supplementary table S1A

Efnb2-H2BGFP expression in 3-28ss embryos

	3-4ss	5-7ss	8-10ss	12-13ss	24-28ss
Embryos analyzed	DA 4	DA 3	DA 7	DA 3	DA 2
Average % positive cells	2.1	34.9	57.8	95.2	92.7
± SEM	1.2	14.2	6.4	1.5	3.8

Supplementary table S1B

Statistical significance between average *Efnb2*-H2BGFP expression at various somite stages in the DA

DA vs DA	5-7ss	8-10ss	12-13ss	24-28ss
3-4ss	ns	***	***	***
5-7ss		ns	**	**
8-10ss			*	ns
12-13ss				ns

One-way ANOVA analysis with Bonferroni's multiple comparison test

ns = not significant

* = p<0.05

** = p<0.01

*** = p<0.001

Supplementary Table S2 – Statistical analysis for data in Figure 2B: COUP-TFII positive endothelial cells in the DA

Supplementary table S2A

COUP-TFII positive ECs in 4-12ss embryos

	4-5ss	7-8ss	10-12ss
	DA	DA	DA
Embryos analyzed	4	3	3
Average % positive cells	17.3	11.4	3.8
± SEM	3.6	1.1	0.7

Supplementary table S2B

Statistical significance between number of COUP-TFII positive ECs at various somite stages in the DA

DA vs DA	7-8ss	10-12ss
4-5ss	ns	*
7-8ss		ns

One-way ANOVA analysis with Bonferroni's multiple comparison test

ns = not significant

* = p<0.05