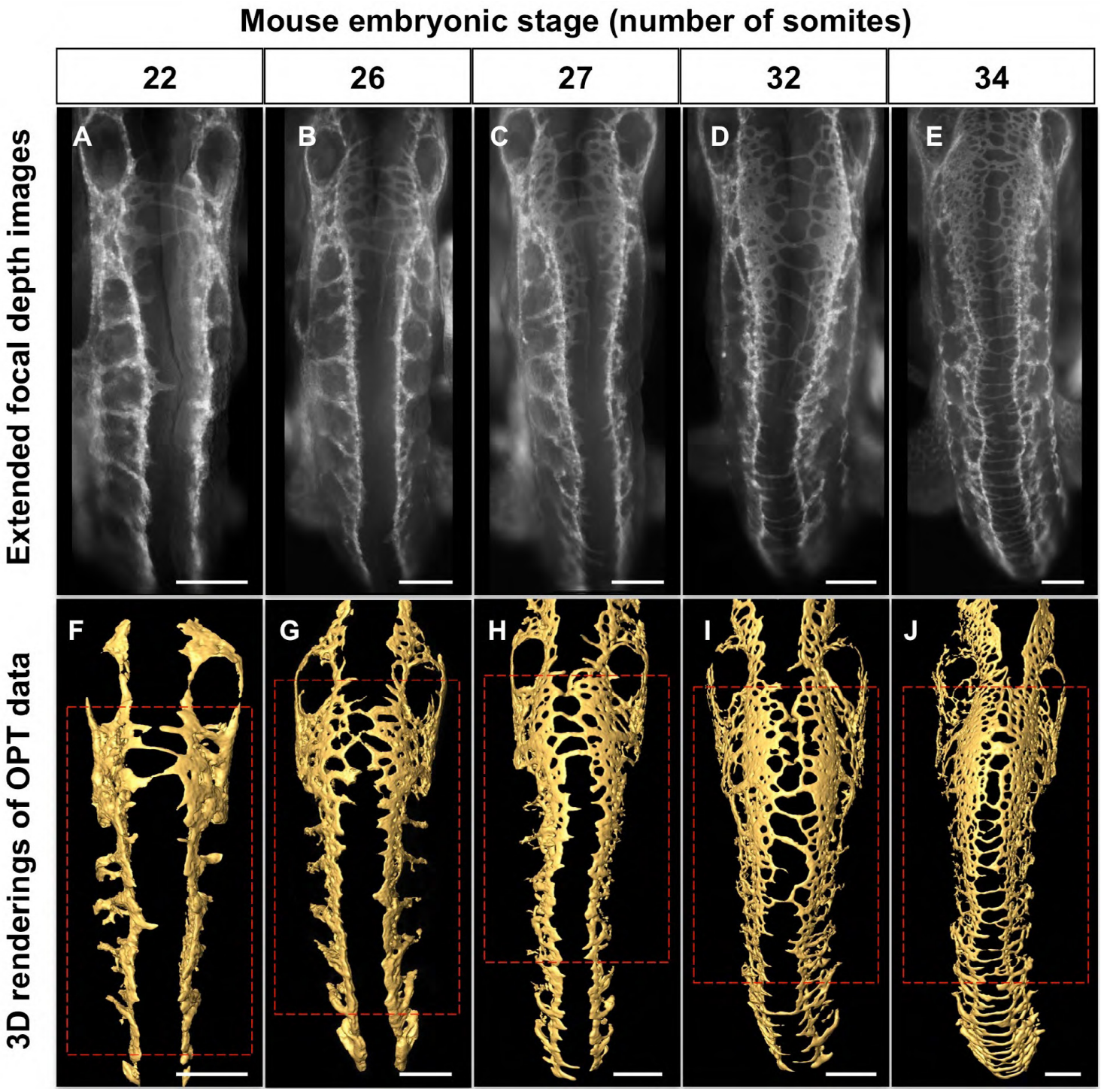



**Fig. S1. DLAV plexus morphogenesis in mouse embryos.** (**A-J**) Dorsal views of DLAV plexus development in 22 (A,F), 26 (B,G), 27 (C,H), 32 (D,I) and 34 (E,J)-somite stage Swiss-Webster wild-type mouse embryos. Anterior, top. Scale bars: 250 μm. (**A-E**) PECAM-1 immunofluorescence. Endothelium, white. (**F-J**) 3D-rendered OPT data (see Materials and Methods) collected from the same embryos. Endothelium, gold. Red dashed lines denote cropped regions displayed in Fig. 1Q-U.

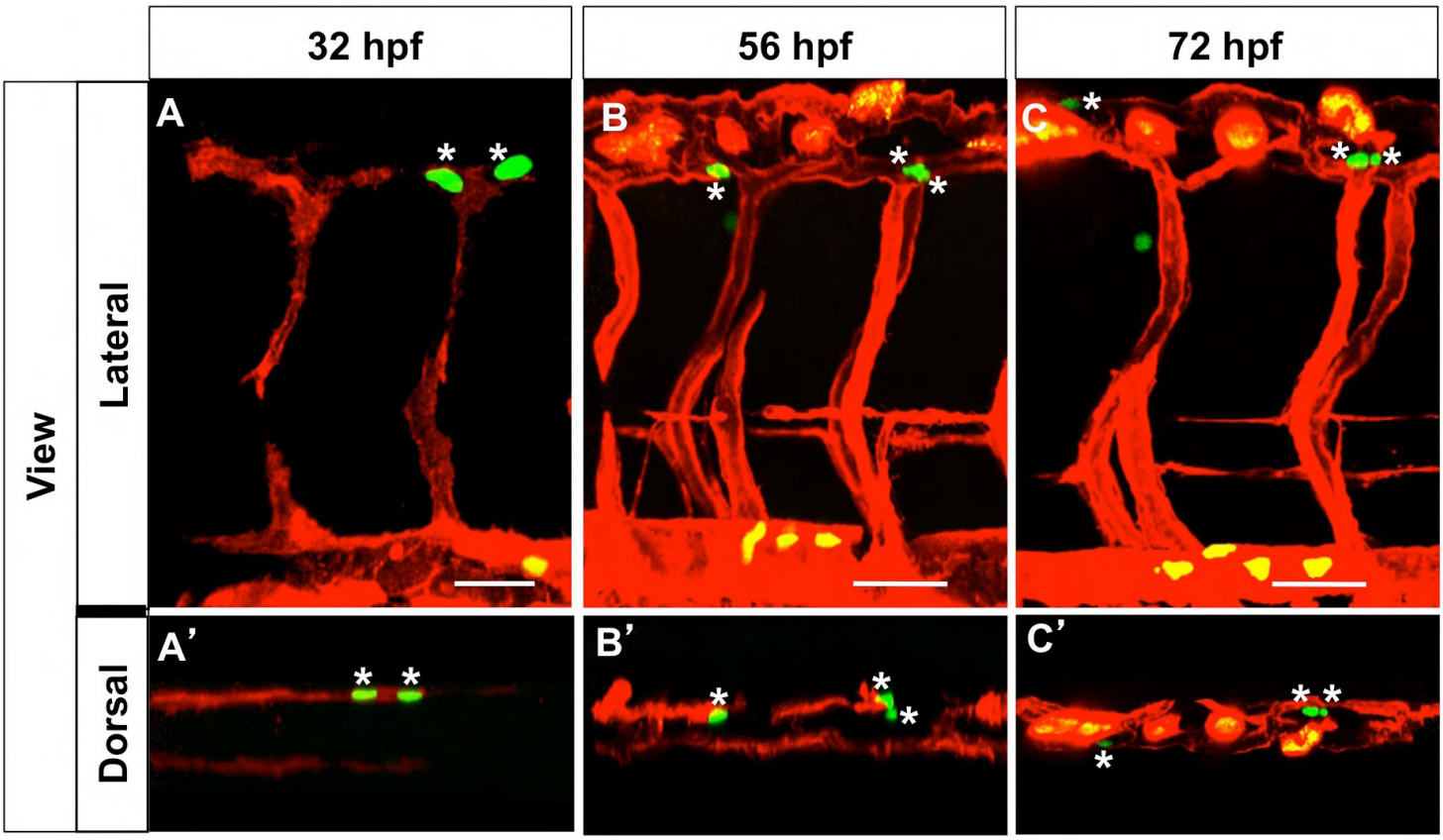
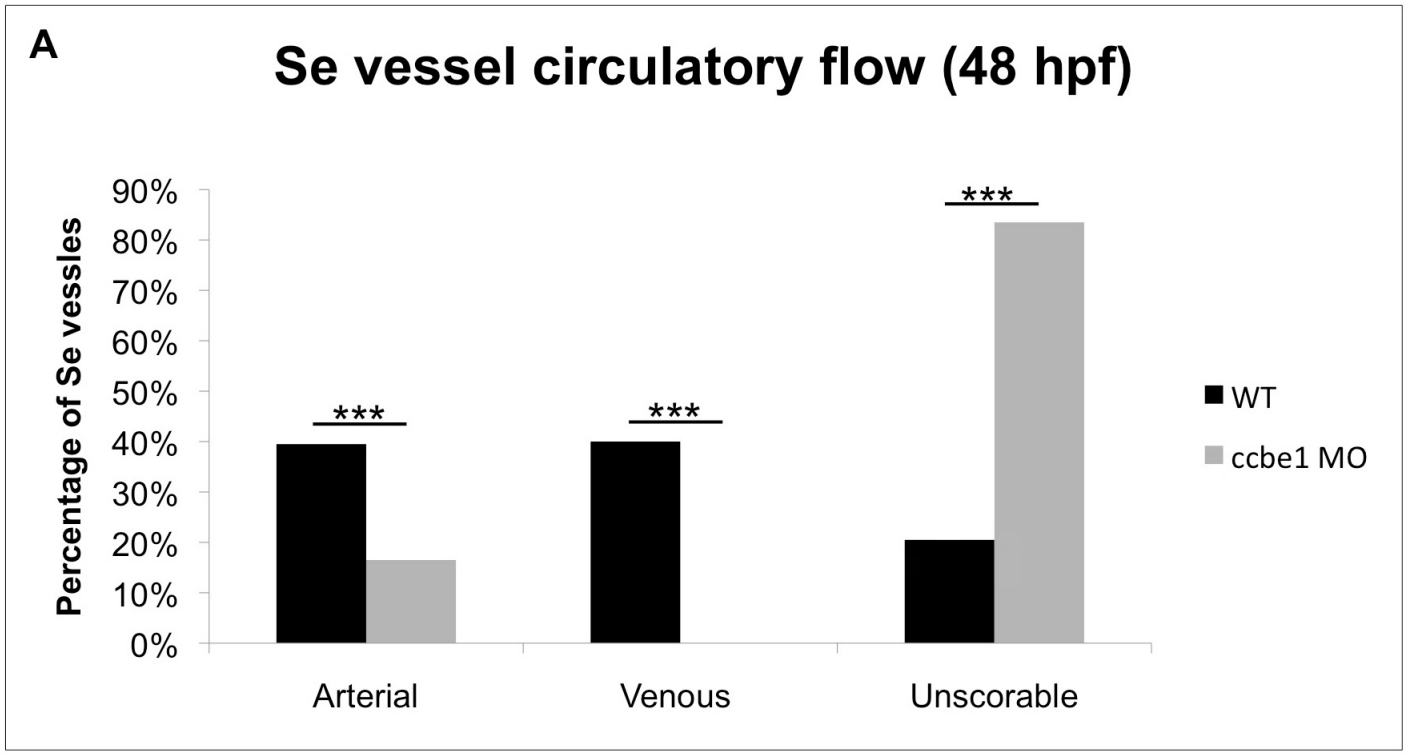



**Fig. S2. Endothelial cell proliferation contributes to DLAV plexus morphogenesis.** Lateral (**A-C;** dorsal, up) and dorsal (**A′-C′**) images of the trunk vasculature of a chimeric embryo made from a *Tg(flk1:EGFP-NLS)* donor and a *Tg(flk1:ras-mCherry)*$^{s896}$ host at 32 (A-A′), 56 (B-B′), and 72 (C-C′) hpf. Anterior, left. White asterisks, endothelial cell nuclei of donor origin found within the DLAVs and/or DLAV plexus. (A-C′) Endothelium, red. Endothelial cell nuclei, green. Scale bars: 30 μm.



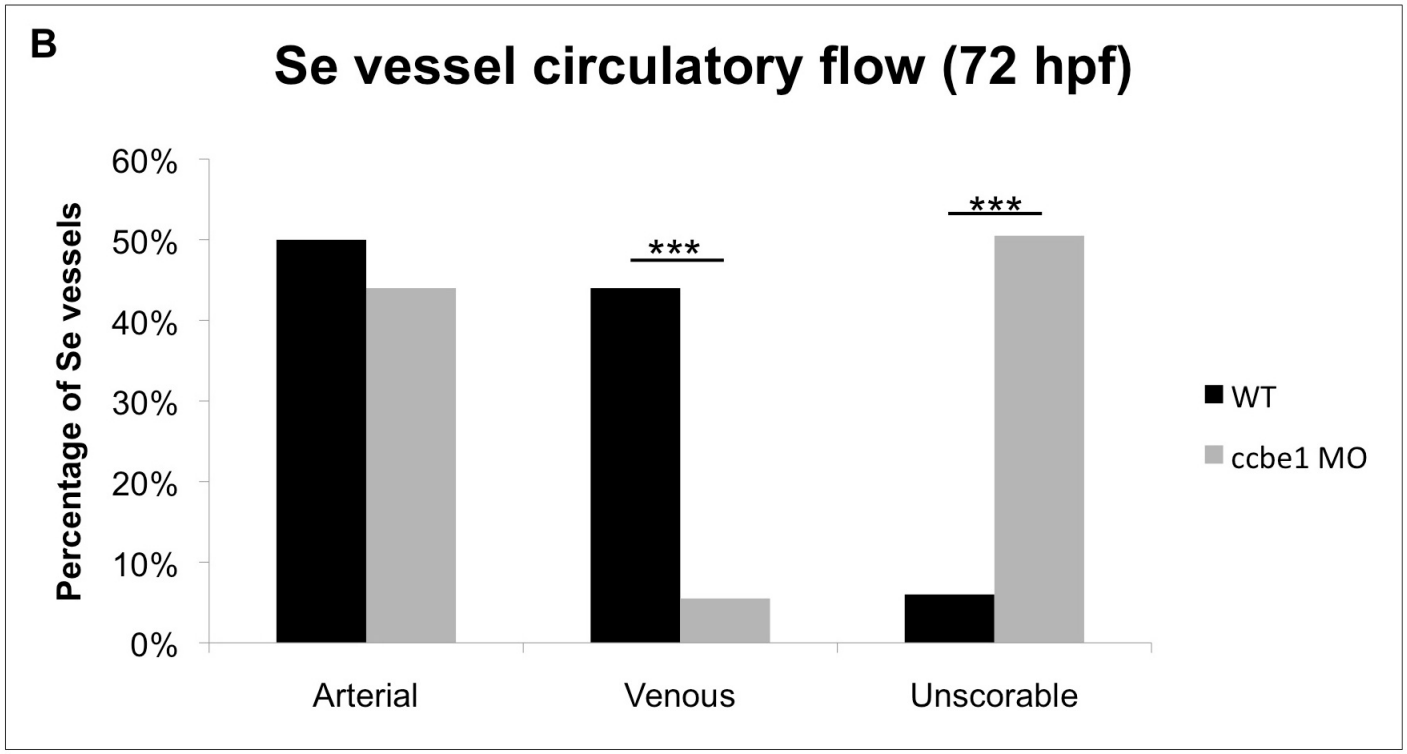




**Fig. S3. The percentage of Se vessels carrying venous flow is dramatically reduced in *ccbe1* morphants.** Quantification. Arterial and venous flow through Se vessels in WT and *ccbe1* morphants (ccbe1 MO) at 48 (**A**) and 72 (**B**) hpf. *n*=20 animals per condition and stage. ***$P<0.001$.

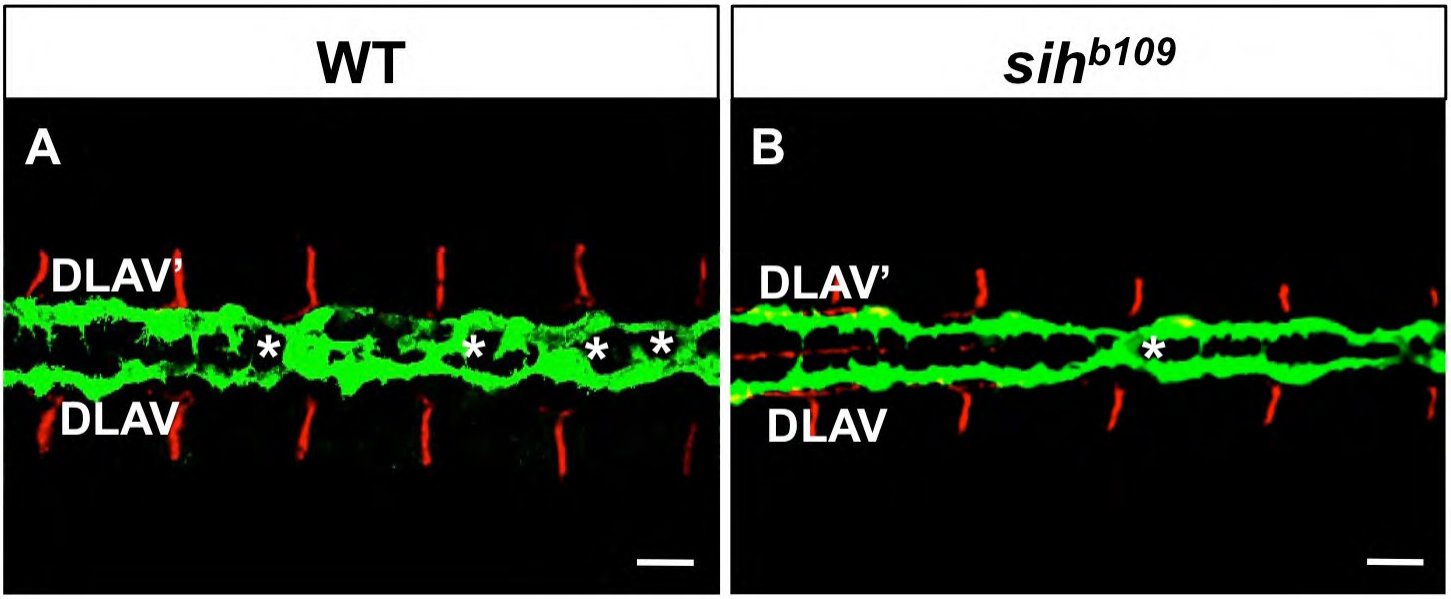



**Fig. S4. Circulatory flow promotes DLAV plexus formation.** (**A,B**) Dorsal views of the trunk vasculature of 48 hpf WT (A) and *sih*$^{b109}$ mutant (B) *Tg(fli:EGFP)*$^{y1}$ embryos. Anterior, left. White asterisks, contra-lateral DLAV interconnections. Endothelium, green. Somite boundaries, red. Scale bars: 30 μm.

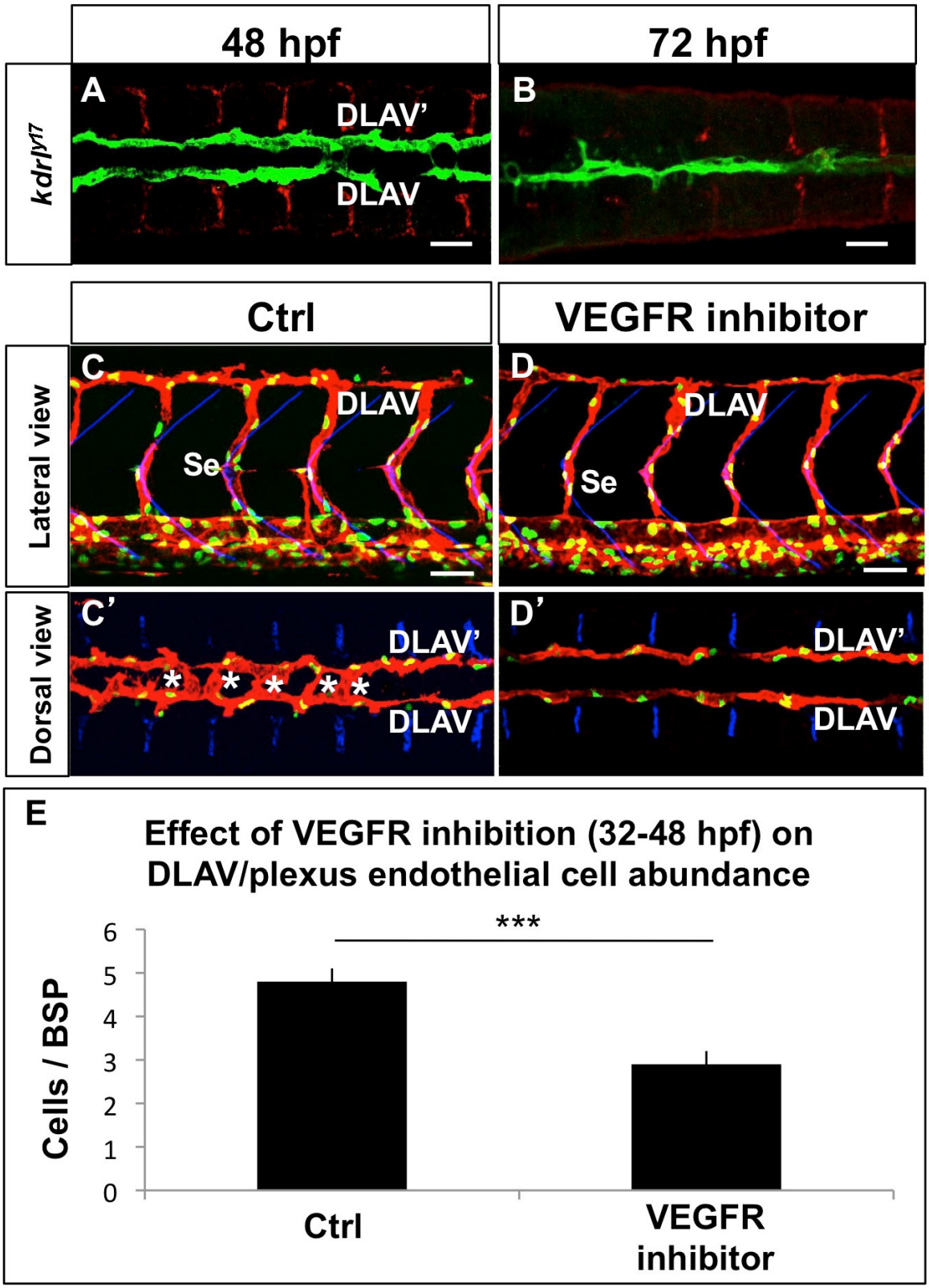



**Fig. S5. Lack of VEGF signaling prevents the initiation of DLAV plexus formation.** (**A,B**) Dorsal views of the trunk vasculature of 48 (A) and 72 (B) hpf *kdrl*$^{y17}$; *Tg(fli:EGFP)*$^{y1}$ mutant embryos. Note lack of DLAV plexus formation. Anterior, left. (**C-D**′) Lateral (C,D) and dorsal (C′,D′) views of the mid trunk vasculature of 48 hpf *Tg(flk1:EGFP-NLS); Tg(flk1:ras-mCherry)*$^{s896}$ embryos treated post-DLAV formation (32 hpf onwards) with either vehicle (Ctrl; DMSO) or with VEGFR inhibitor (SU5416). (A-D′) Anterior, left; dorsal, up; endothelium, green (A,B) or red (C-D′); endothelial cell nuclei, green (C-D′); somite boundaries, red (A,B) or blue (C-D′); scale bars: 30 μm. (**E**) Quantification of the effect of VEGFR inhibition (32-48 hpf) on DLAV and/or DLAV plexus endothelial cell abundance. *n*=10 animals per treatment. Error bars, s.e.m. ***$P$<0.001.

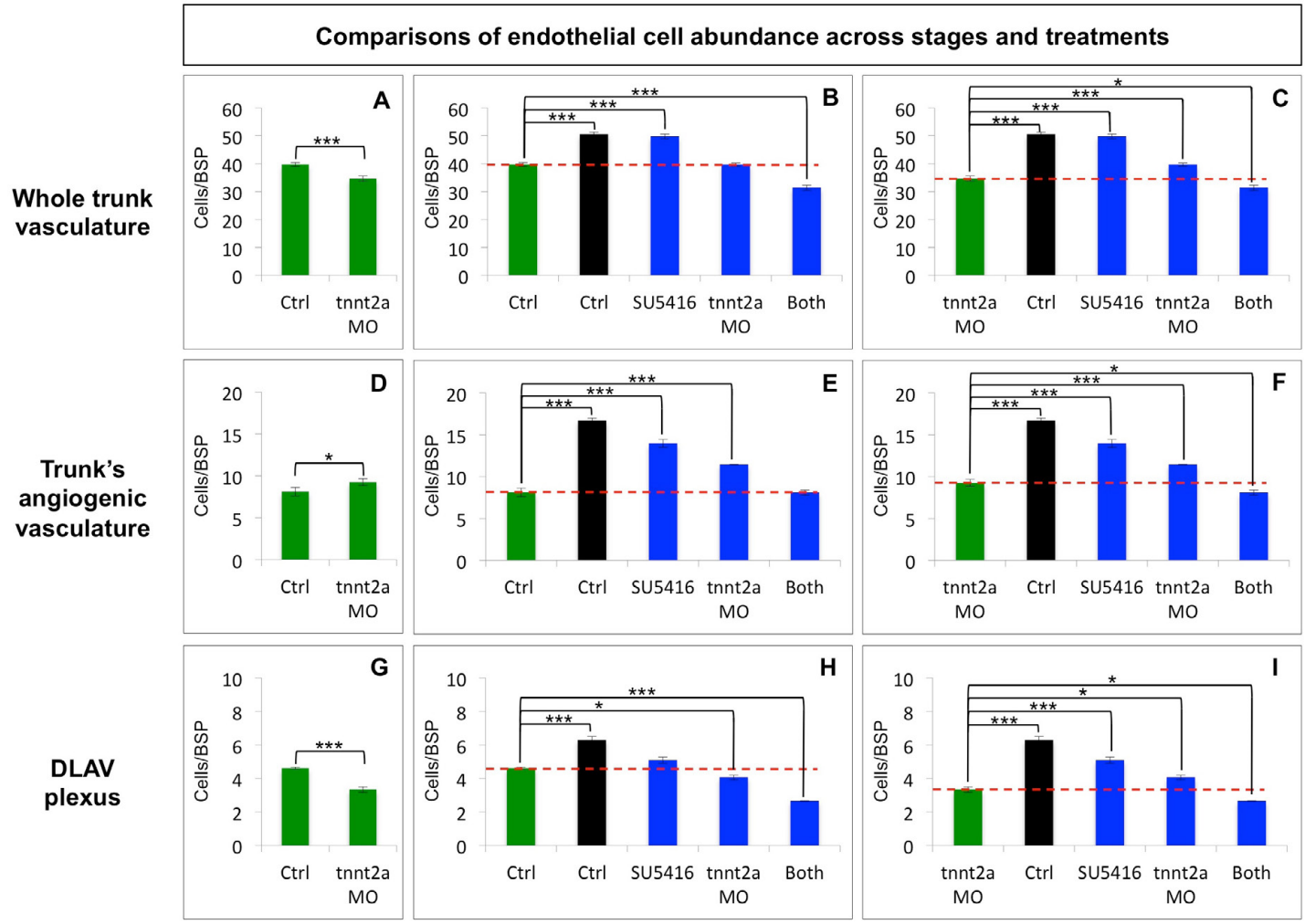



**Fig. S6. Additive effects of VEGF signaling and circulatory flow on DLAV plexus development and maintenance. (A-I)** Quantifications of the vasculature of *Tg(flk1:EGFP-NLS); Tg(flk1:ras-mCherry)*$^{s896}$ embryos. 43 hpf, green bars (ctrol and *tnnt2a* morphants); 56 hpf, black (ctrol) or blue bars (experimental conditions); as in Fig. 4 (see Materials and Methods). The red dotted lines (at the level of the corresponding 43 hpf value) are included to facilitate comparisons. *n*=6-10 animals per treatment and stage. Error bars, s.e.m. **P*<0.5; ****P*<0.001.