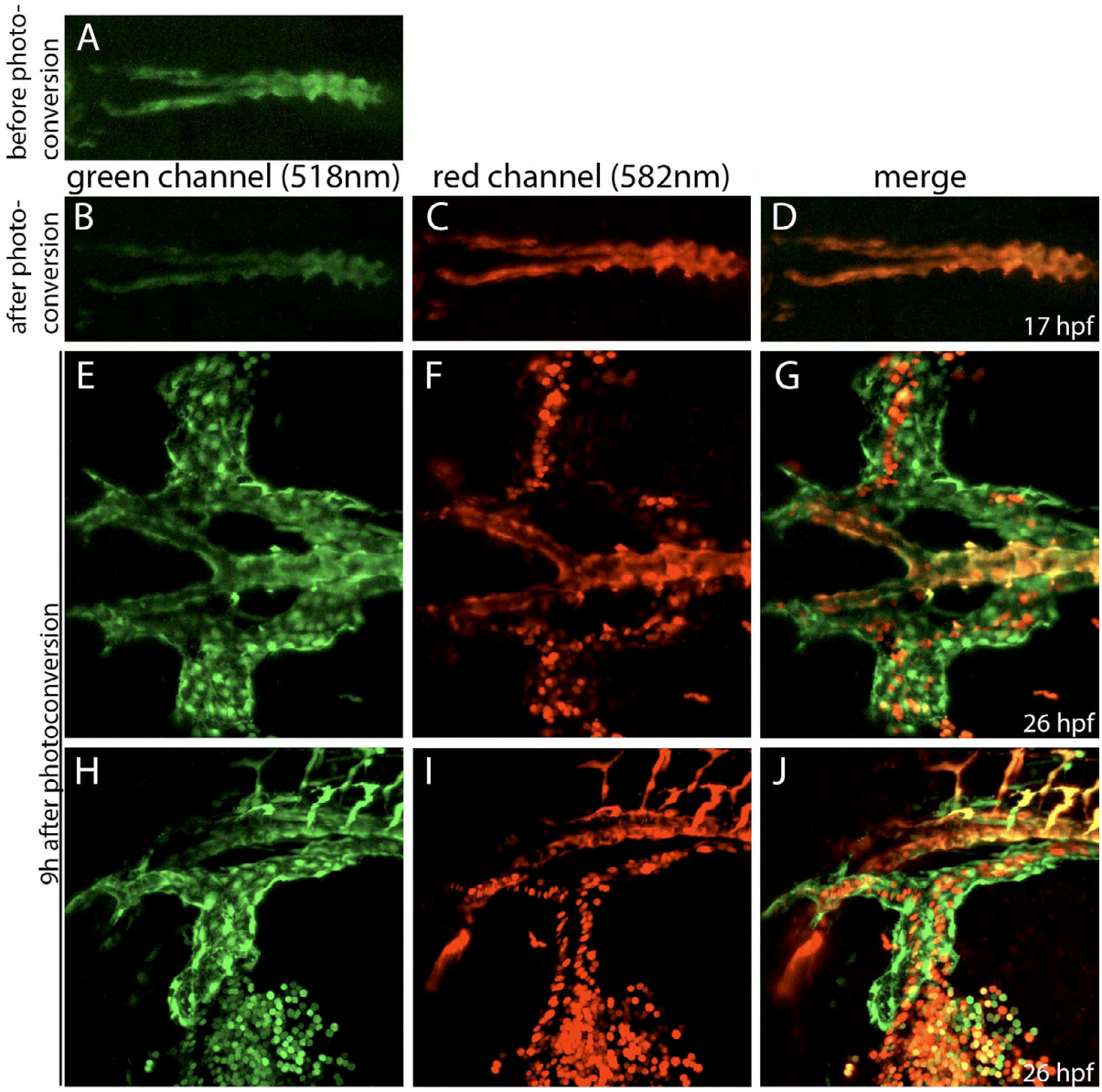


Fig. S1. The angioblasts forming the bilateral cardinal veins and the common cardinal veins (CCVs) are specified as a second population and at a separate time point to the population forming the lateral dorsal aortae. (A-J) Confocal projections of *Tg(fli1ep:GAL4FF)*^{ubs4} *(UAS:KAEDE)*^{rk8} embryos at the indicated time points. Endothelial cells (ECs) (and blood cells) were visualized by transgenic Kaede expression. (A-G) Dorsal views; (H-J) lateral views. (**A**) EC-specific Kaede expression before photoconversion at 17 hpf, before the second population of angioblasts starts to express Kaede. (**B**) EC-specific non-converted Kaede (green) expression after photoconversion, indicating that not all Kaede protein was converted. (**C**) EC-specific converted Kaede (red) expression after photoconversion. (**D**) Merged image of converted and non-converted Kaede expression after photoconversion. (**E**) Non-converted Kaede expression, reflecting newly synthesized Kaede protein, imaged 9 hours after photoconversion. Green Kaede protein can be detected in the LDA and the bilateral cardinal veins as well as in the CCVs. (**F**) Nine hours after photoconversion, converted Kaede (red) expression can only be detected in the LDA, but not in the veins. (**G**) Merged image of the red and green channel showing Kaede expression 9 hours after photoconversion. Note that the transgenic Kaede expression can also be detected in blood cells. (**H**) Non-converted Kaede expression 9 hours after photoconversion is detected in the LDA and the bilateral cardinal veins as well as in the CCV. (**I**) Converted Kaede expression 9 hours after photoconversion is only detected in the LDA. (**J**) Merged image of the red and green channel showing Kaede expression 9 hours after photoconversion. Note that the transgenic Kaede expression can also be detected in blood cells.

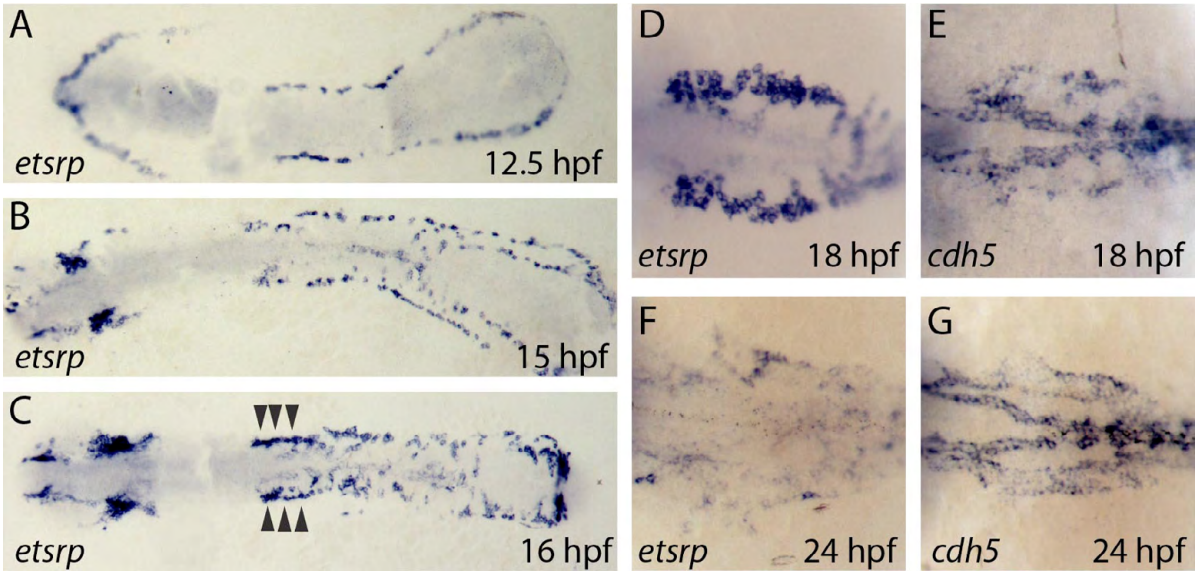


Fig. S2. *etsrp* is strongly expressed in newly specified angioblasts but becomes downregulated after angioblast migration. (A-D,F) Brightfield images showing expression of *etsrp* (etv2) detected by *in situ* hybridization in embryos at the indicated time points (dorsal views). (A-C) Flat-mounted embryos; (D-G) higher magnification of the trunk region. (E,G) Brightfield images showing expression of *cadherin 5* (*cdh5*) detected by *in situ* hybridization in embryos at the indicated time points (dorsal views). (**A**) *etsrp* mRNA can be detected in angioblasts within the lateral plate mesoderm. (**B**) *etsrp* mRNA levels are lower in angioblasts that have already migrated to the midline. (**C**) High *etsrp* expression in angioblasts that will form the bilateral cardinal veins/CCVs (arrowheads). (**D**) Cells forming the bilateral cardinal veins/CCVs show high levels of *etsrp* expression, whereas *etsrp* expression is very weak in the ECs of the LDA. (**F**) *etsrp* expression in the bilateral cardinal veins/CCVs decreases over time until it is no longer detectable. (**E**,**G**) *cdh5* expression labels all angioblasts. CCV, common cardinal vein; LDA, lateral dorsal aortae.

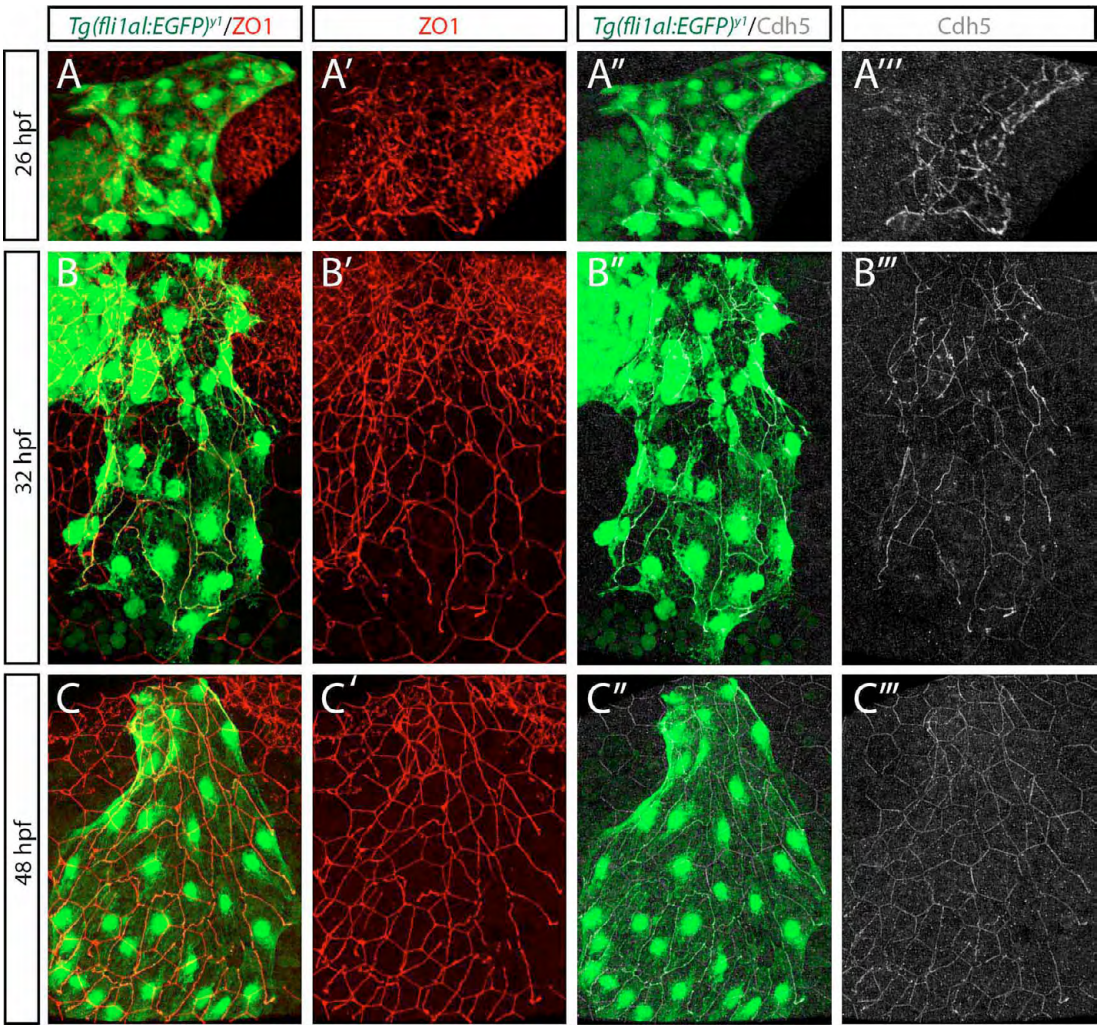


Fig. S3. ECs of the CCV form tight and adherens junctions. (A-C) Fluorescent confocal images of immunostainings of the junctional proteins ZO-1 and Cdh5 at the indicated time points; lateral views. The developing vasculature is visualized by transgenic EGFP expression of *Tg(fli1a:EGFP)^{y1}*. (**A-A‴**) ECs of the CCV express the tight junction protein ZO-1 and the adherens junction protein Cdh5 at 26 hpf. (**B-B‴**) ECs of the CCV express the tight junction protein ZO-1 and the adherens junction protein Cdh5 at 32 hpf. (**C-C‴**) ECs of the CCV express the tight junction protein ZO-1 and the adherens junction protein Cdh5 at 48 hpf. Note that the epidermis shows nonspecific Cdh5 staining at 48 hpf.

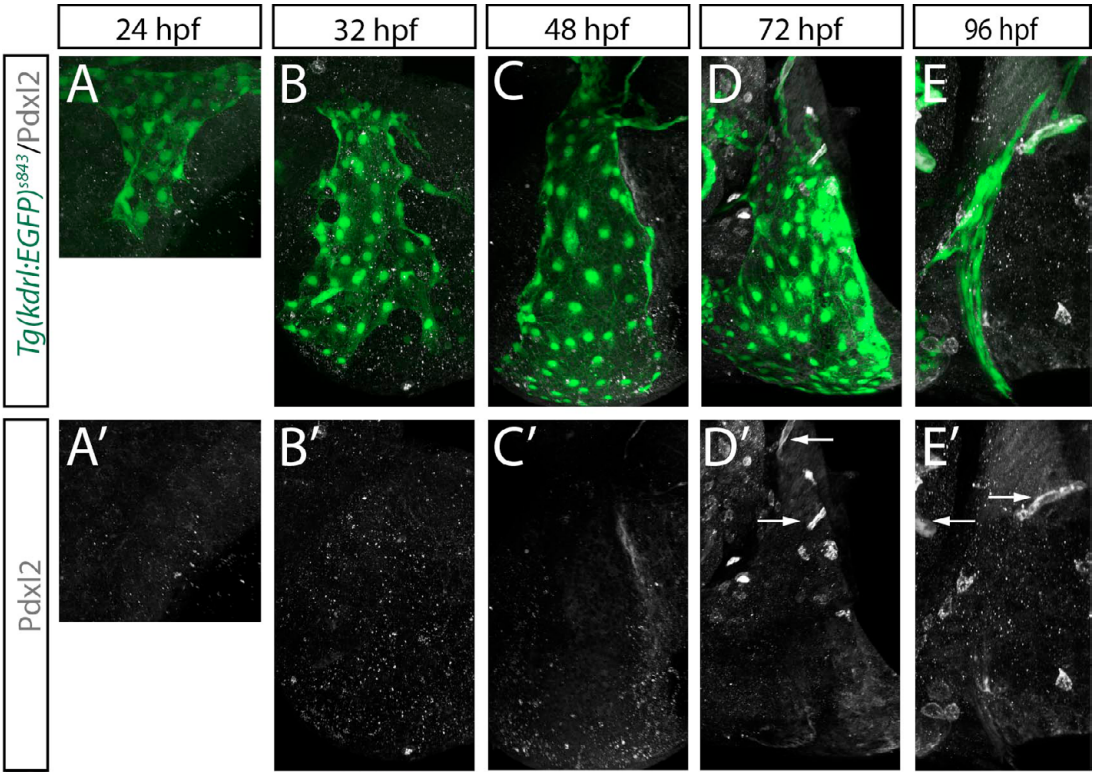


Fig. S4. ECs of the CCV have no apical polarization (analyzed by Podocalyxin-like 2 expression). (**A-E′**) Fluorescent confocal images of immunostainings of Podocalyxin 2 (Pdxl2) at the indicated time points; lateral views. The developing vasculature is visualized by transgenic EGFP expression of *Tg(kdrl:EGFP)*^{s843}. ECs of the CCV do not form Pdxl2-positive membrane compartments, whereas ECs in the fin bud (arrows in D′ and right arrow in E′) and ECs in the aortic arches (left arrow in E′) form apical membrane compartments as indicated by Pdxl2 staining.

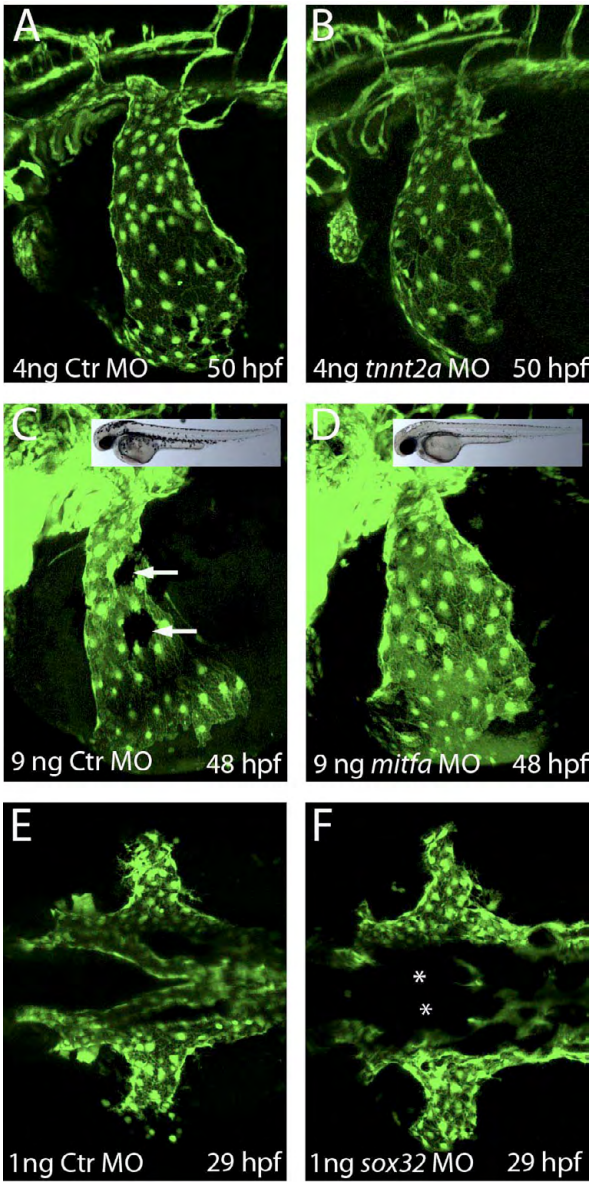


Fig. S5. CCV development is not affected by blood flow, melanocytes or endoderm. (A-F) Confocal fluorescent images of transgenic zebrafish embryos at the indicated time points. ECs were visualized by transgenic EGFP expression [*Tg(kdrl:EGFP)*^{s843}]. (A-D) Lateral views; (E,F) dorsal views. (**A**,**B**) Embryos injected with control MO (A) or with *tnnt2a* MO (B). Note that the CCV developed normally in the absence of blood flow in *tnnt2a* MO-injected embryos (B). (**C**,**D**) Embryos injected with control MO (C) or *mitfa* MO (D). Note that although *mitfa* MO-injected embryos had no melanocytes (compare brightfield images, insets in C and D) the CCVs developed normally. Arrows indicate melanocytes in C. (**E**,**F**) Embryos injected with control MO (E) or with *sox32* MO (F). Note that in the absence of endoderm the LDA fail to form (asterisks), whereas the CCVs develop (F). CCV, common cardinal vein; Ctr, control; LDA, lateral dorsal aortae; WT, wild type.

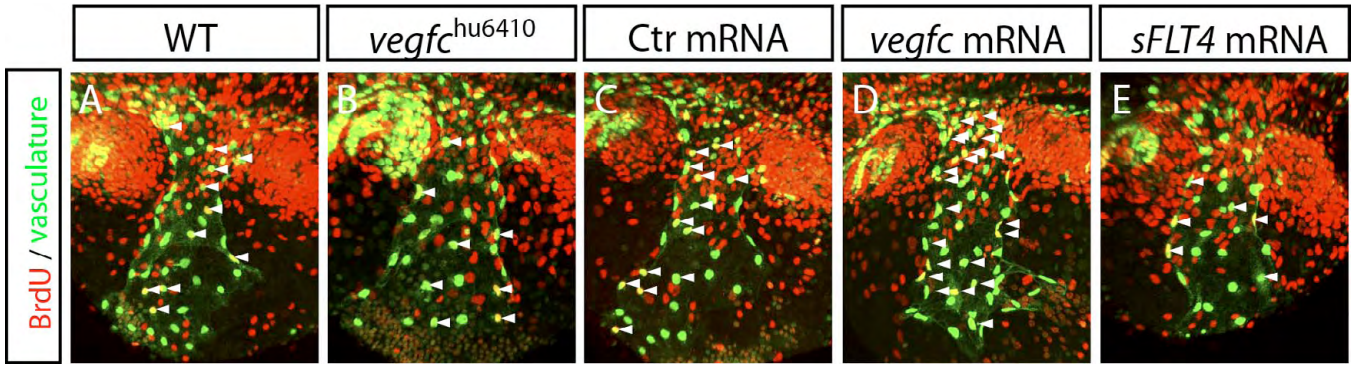
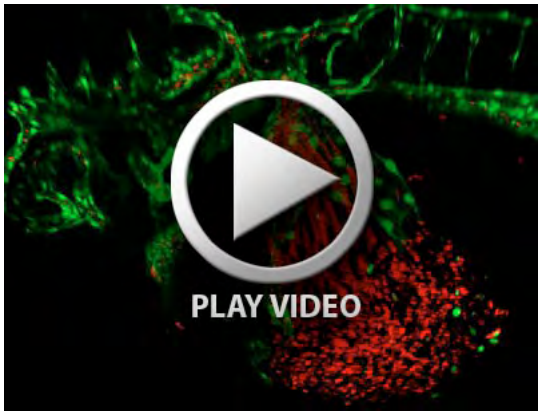
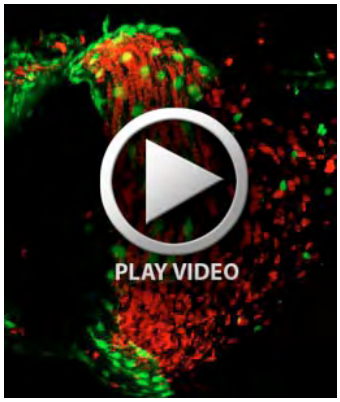


Fig. S6. Vegfc positively regulates EC proliferation in the CCVs. Confocal projections of 32 hpf *Tg(kdrl:EGFP)*^{s843} wild-type siblings (**A**), homozygous *vegfc*^{hu6410} mutant embryos (**B**) or embryos injected with *H2B-cherry* mRNA (control, **C**), *vegfc* mRNA (**D**) or *sFLT4* mRNA (**E**), all pulsed with BrdU from 24-32 hpf. BrdU staining is shown in red; vascular ECs are visualized in green by staining for GFP expression [of *Tg(fli1al:EGFP)*^{y1} in A,B and of *Tg(kdrl:EGFP)*^{s843} in C-E]. EC proliferation is decreased in *vegfc*^{hu6410} (B) or *sFLT4* mRNA-injected (E) embryos and increased upon *vegfc* overexpression (D). Arrowheads mark BrdU-positive ECs in the CCV.

Movie 1. Angioblasts forming the bilateral cardinal veins and the CCVs become specified at a different time point than the cells forming the arteries. Confocal time-lapse movie of the trunk of a transgenic zebrafish embryo imaged in dorsal view. The vasculature was visualized by transgenic EGFP expression [*Tg(fli1al:EGFP)*^{y1}]. This movie shows that an initially specified population of angioblasts forms the lateral dorsal aortae, whereas a second, later-specified population of angioblasts will form the bilateral cardinal veins and the CCVs. Starting at 14 hpf, the time-lapse series covers a period of 8 hours with one confocal *z*-stack recorded every 15 minutes.



Movie 2. ECs in the CCV migrate around the flowing blood to form the CCV. Confocal time-lapse movie of a double-transgenic embryo. The developing vasculature is shown in green [visualized by transgenic EGFP expression of *Tg(kdrl:EGFP)*^{s843}] and the blood is shown in red [visualized by transgenic dsRed expression of *Tg(gata1:dsRed)*^{sd2}]; lateral views. Starting at 33 hpf, this time-lapse series covers a period of 20 hours with one confocal *z*-stack recorded every 15 minutes. Note that blood flows out of the open-ended CCV, while the ECs migrate towards the endocardial cells at the sinus venosus of the heart.



Movie 3. ECs in the CCV connect to the endocard. Confocal time-lapse movie of a double-transgenic embryo. The developing vasculature is shown in green [visualized by transgenic EGFP expression of *Tg(kdrl:EGFP)*^{s843}] and the blood is shown in red [visualized by transgenic dsRed expression of *Tg(gata1:dsRed)*^{sd2}]; lateral views. Starting at 33 hpf this time-lapse series covers a period of 20 hours with one confocal *z*-stack recorded every 15 minutes. Note that that the endocardial cells do not migrate, while the ECs of the CCV migrate towards the endocardial cells at the sinus venosus of the heart.

Movie 4. ECs of the CCV actively migrate. Confocal time-lapse movie of a double-transgenic embryo. The actin cytoskeleton of the ECs is labeled by transgenic GFP expression in *Tg(UAS:lifeact-GFP)*^{mu271} *Tg(fli1ep:GAL4FF)*^{ubs4} double-transgenic embryos; lateral views. Starting at 41 hpf this time-lapse series covers a period of 5 hours with one confocal *z*-stack recorded every 2 minutes. Note that ECs migrate with Actin-rich lamellipodia in the front.

Movie 5. ECs of the CCV fuse distally to close the lumen of the CCV. Confocal time-lapse movie of a double-transgenic embryo. The developing vasculature is shown in green [visualized by transgenic EGFP expression of *Tg(etsrp:EGFP)*^{ci1}]; lateral views. Starting at 58 hpf this time-lapse series covers a period of 12 hours with one confocal *z*-stack recorded every 7 minutes. After ECs ensheath the blood stream, they close the lumen at the YSL side from dorsal to ventral. ECs at the epidermal side are cropped away.