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Supplemental Information

Arterial and Venous Progenitors of the Major Axial Vessels Originate at Distinct Locations

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INVENTORY OF SUPPLEMENTAL INFORMATION

Figure S1, related to Figure 1. In situ hybridization analysis for *etv2, ephrinB2a,* and *ephB4*.

Figure S2, related to Figure 3. The intersomitic migration of angioblasts in photoactivated Kaede mRNA injected embryos and Tg(*fli1a:*GFP) embryos.

Figure S3, related to Figure 4. *etv2*:Kaede fluorescence in trunk angioblasts at the 15-somite stage.

Figure S4, related to Figure 6. The effects of photoactivatable *etv2* MO on Tg(*etv2*:GFP) fluorescence and *etv2* expression.

Movie S1, related to Figures 1, 2, 3. Time lapse confocal movie depicting *etv2*:GFP expression in the trunk medial angioblasts, and their migration towards the midline in the anterior to posterior sequence in three separate embryos. Embryos 2 and 3 are dorsally mounted.

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Movie S7, related to Figure 5. Timelapse movie of *etv2:*Kaede expression in two different embryos visualizing migration of the medial angioblasts to the DA and lateral angioblasts to the PCV.

Movie S8, related to Figure 5. Time lapse confocal imaging of a dorsolaterally tilted Tg(*etv2*:GFP; *kdrl*:mcherry) embryo showing angioblast migration, and the assembly of the DA and PCV.

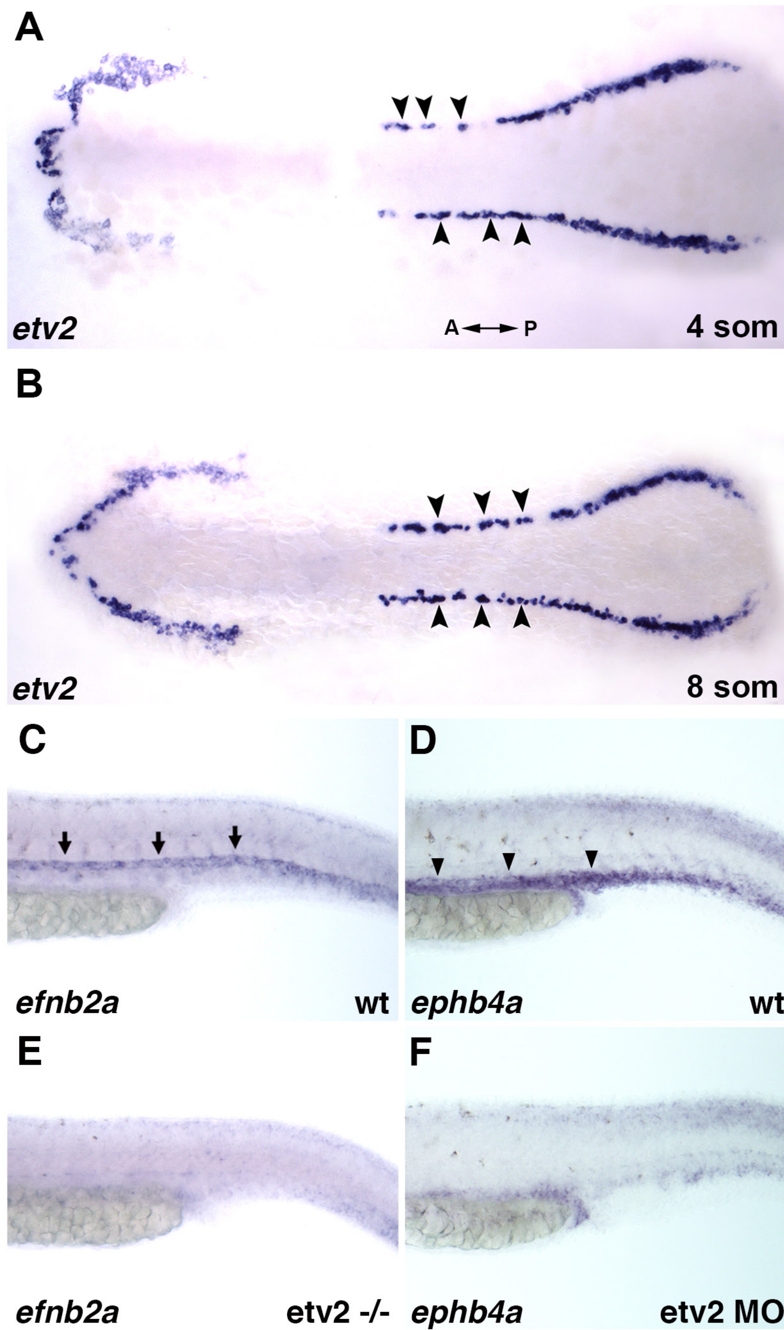


Figure S1, related to Figure 1. In situ hybridization analysis for *etv2, ephrin B2a,* and *ephB4.* (A,B) *Etv2* expression in wild type embryos at 4 somite (A) and 8 somite stages (B). Note that *etv2* expression is observed in the medial angioblasts (arrowheads) only. Flat mounted embryos, dorsal view, A – anterior; P – posterior. (C-F) Arterial expression of *ephrin B2a* (C,E) and venous expression of *ephb4a* (D,F) is absent in $etv2^{y11-/-}$ mutant (E) or morphant (F) embryos at 24-27 hpf. Arrows in (C) indicate expression in the dorsal aorta. Arrowheads in (D) indicate expression in the posterior cardinal vein.

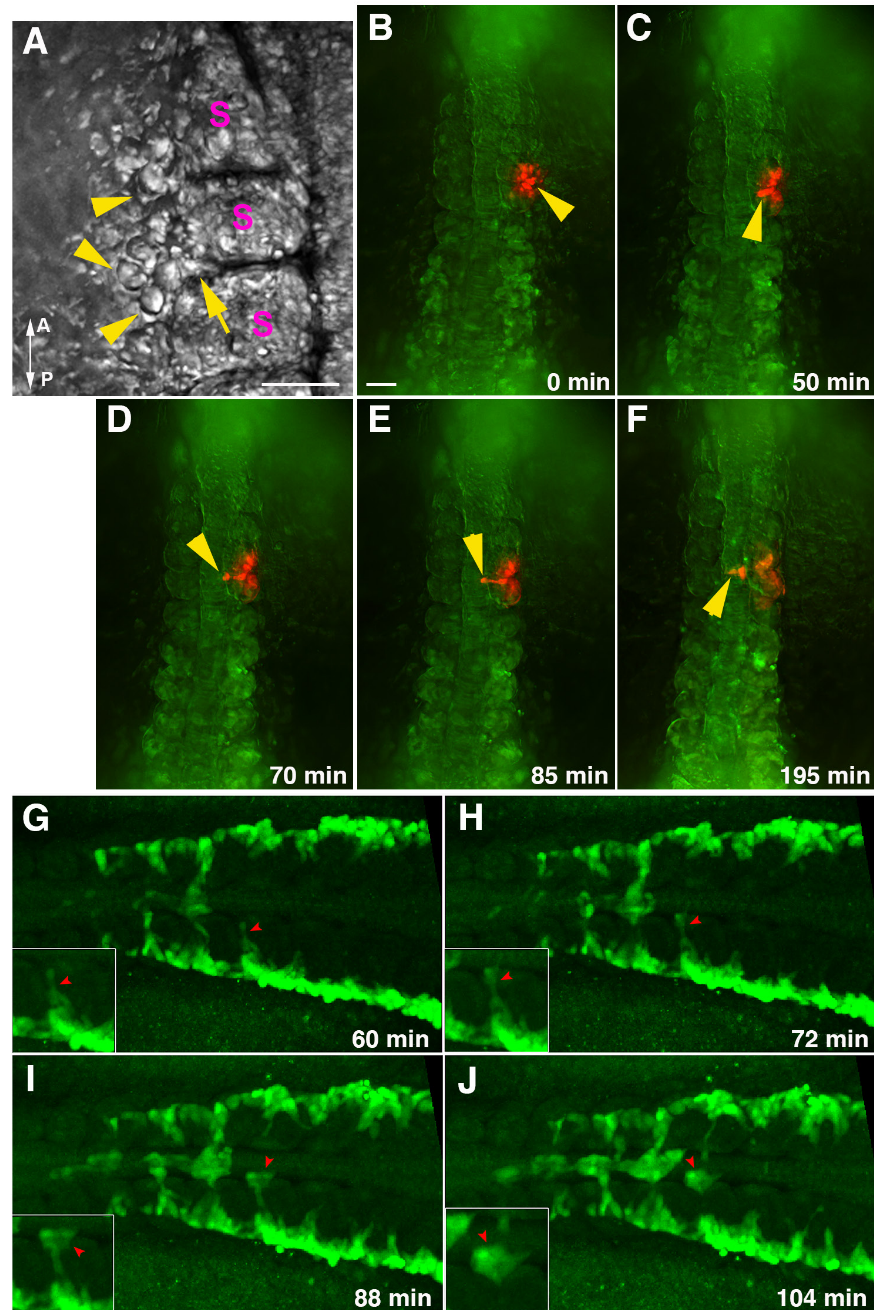


Figure S2, related to Figure 3. The intersomitic migration of angioblasts in photoactivated Kaede mRNA injected embryos and Tg(*fli1a*:GFP) embryos. (A-F) Images extracted from time lapse video depict the intersomitic midline migration of photoconverted angioblasts. (A) Brightfield image before photoactivation showing angioblasts (arrowheads) clustering at the boundary of adjacent somites (S). Arrow depicts angioblasts ingressing into the somitic boundary. (B-F) In Kaede mRNA injected embryos, a cluster of cells that included anterior trunk angioblasts were photoconverted for differential labeling. Following photoconversion, the time progression shows activated angioblasts migrating between somites (C-E; arrowhead) towards the midline (F). Some cells in the somitic mesoderm were labeled as well during photoconversion. Scale bar for (A) and (B-F) represent 50 and 100 μm, respectively. A – anterior; P – posterior; S – somites**.** (G-J) Images extracted from time lapse confocal imaging of a dorsally mounted *fli1a*:GFP embryo showing angioblast and erythroid cell migration. Red arrows depict the intersomitic migration of angioblasts towards the midline. Insets show magnified views of migrating angioblasts.

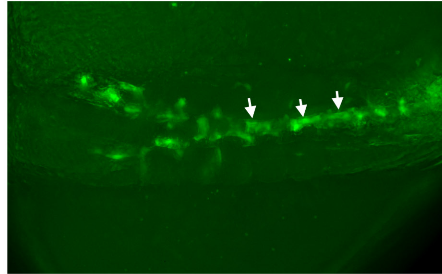
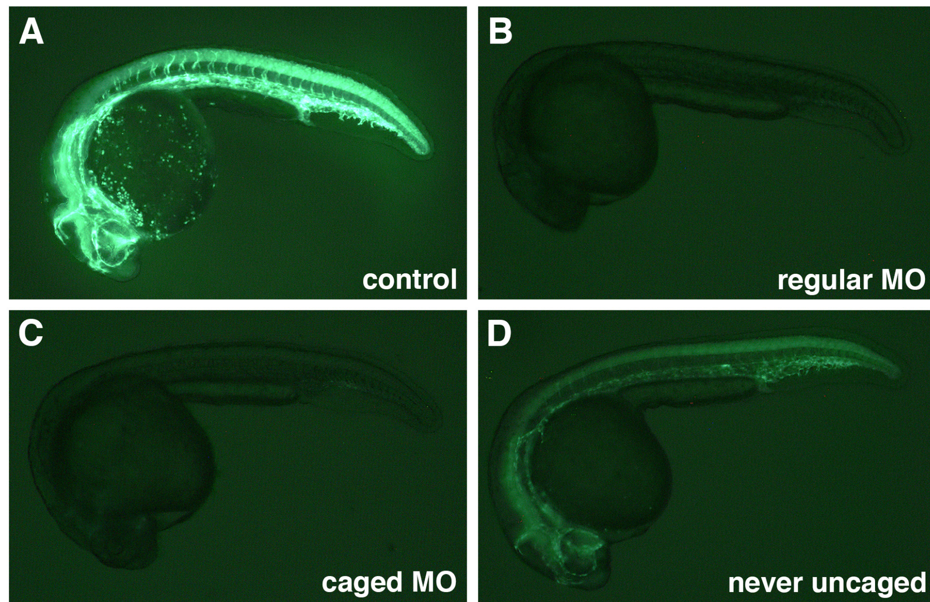


Figure S3, related to Figure 4. Tg(*etv2*:Kaede) fluorescence in trunk angioblasts at the 15-somite stage. Note that Kaede expression is only apparent in medial angioblasts most of which are at the midline at this stage. Dorsal view, anterior is to the left.



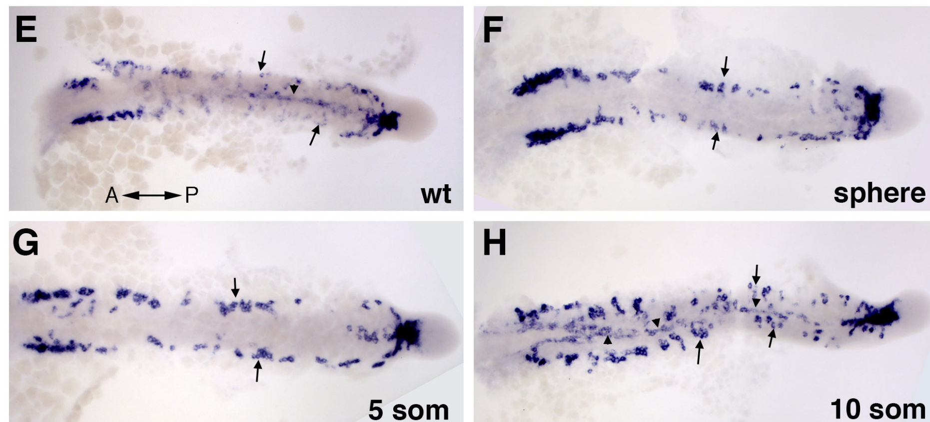


Figure S4, related to Figure 6. The effects of photoactivatable *etv2* MO on Tg(*etv2*:GFP) fluorescence and *etv2* expression. (A-D) Photoactivatable *etv2* MO inhibits *etv-2:*GFP fluorescence. (A) Control uninjected, (B) regular *etv2* MO2 injected embryo, (C) caged *etv2* MO injected embryo uncaged at the sphere stage (4 hpf), (D) caged *etv2* MO injected embryo never uncaged. All embryos were analyzed at 24 hpf with the same imaging and acquisition settings. (C) Embryos that were uncaged at the sphere stage resulted in nearly complete inhibition of GFP expression, showing a similar phenotype to standard *etv2* MO injected embryos. (D) Caged MO injected embryos that were never uncaged showed higher GFP fluorescence than (C) uncaged embryos. While caged MO injected embryos that were never uncaged (D) showed a decrease in GFP expression in comparison to (A) uninjected embryos, these embryos were phenotypically normal with no apparent vascular defects. (E-H) *etv2* expression analyzed in flat mounted embryos at the 22 to 24-somite stage in (E) control uninjected, (F) caged MO injected embryos that were uncaged at the sphere, (G) 5-somite and (H) 10-somite stage. Dorsal view, trunk and tail region is shown. (E) Medial (arrowhead) and lateral (arrows) angioblasts are shown migrating, and many cells have reached the midline. (F, G) Midline migration is absent in embryos that were uncaged at the sphere, and the 5-somite stage. (H) In embryos uncaged at the 10-somite stage, the medial angioblasts migrate to the midline while the migration of lateral angioblasts is inhibited.

Movie Legends

Movie S1. Time lapse confocal movies of three different embryos depicting *etv2*:GFP expression in the trunk medial angioblasts, and their intersomitic migration towards the midline in the anterior to posterior sequence in three separate embryos. Embryos were mounted in the dorsal orientation. Time lapse imaging was started at approximately the 10-somite stage and time frames were captured every 10 min. As seen in the video, angioblasts in the trunk region migrate between the somites towards the midline. Embryo 3 is presented at a higher magnification.

Movie S2. Time lapse confocal movie depicting angioblast migration in the same dorsally mounted embryo at two different z-planes. (a) At a z-plane at the level of the somites angioblasts are shown clustering around the somites, and seen ingressing into the somitic boundary to migrate intersomitically to the midline. As the angioblasts disappear through the somitic boundary, (b) the angioblasts continue to migrate intersomitically to the midline as shown in the movie with a chosen z-plane deeper within thesomites. Notice the consistent anterior to posterior migration of trunk angioblasts to the midline. Both (a) and (b) are identically time indexed. Imaging was started at approximately the 10-somite stage, and time frames were acquired every 12 min.

Movie S3. Time lapse movie depicting the intersomitic midline migration of photoconverted Kaede positive angioblasts. Wild-type embryo was injected with Kaede mRNA at the 1-2-cell stages. After photoactivation, angioblasts (photoconverted from green to red fluorescence) are shown migrating between the boundaries of adjacent somites. The embryo is shown mounted in the dorsal orientation. Imaging was started at approximately the 10-somite stage, and time frames were acquired every 5-10 min. Some labeled cells are in the somitic mesoderm and do not migrate.

Movie S4. Time lapse confocal imaging depicting the midline intersomitic migration of GFP positive angioblasts in live Tg(*fli1a*:GFP) embryos. Arrows locate angioblasts migrating intersomitically to the midline. Imaging was started at approximately the 10-somite stage, and time frames were acquired every 4 min.

Movie S5. A deconvolved time lapse confocal imaging of a dorsolateral oriented embryo depicting angioblast migration and trunk axial vessel formation in live Tg(*etv2*:GFP) embryos. Medial angioblasts can be seen migrating from the LPM to the dorsal position where the DA will

develop. Several migrating medial angioblasts are labeled with arrows. Imaging was started at the 10-somite stage, and time frames were acquired every 7 min. Anterior is to the left, dorsal is up.

Movie S6. Time lapse confocal imaging of a dorsolaterally tilted embryo depicting angioblast migration and trunk axial vessel formation in live Tg(*etv2*:GFP) embryos. Individual medial angioblasts can be seen migrating from the LPM to a dorsal position where the DA will develop. Arrow locates an LPM medial angioblast migrating to a dorsal position to join the forming DA. Imaging was started at approximately the 12-somite stage, and time frames were acquired approximately every 8 min.

Movie S7. Timelapse movies of *etv2:*Kaede expression in two different embryos visualizing migration of the medial angioblasts to the DA and lateral angioblasts to the PCV. Imaging started at approximately the 13-somite stage. Time frames were captured every 15 minutes prior to photoconversion and every 30 minutes post-photoconversion. Whole embryos were photoconverted from green to red at the 14-15-somite stages. Arrows indicate lateral angioblasts that contribute directly to the PCV. Embryos were mounted in a dorsolateral position, anterior is to the left and dorsal is to the top.

Movie S8. Time lapse confocal imaging of a dorsolaterally tilted Tg(*etv2*:GFP; *kdrl*:mcherry) embryo showing angioblast migration, and the assembly of the DA and PCV. Arrow locates a tracked lateral angioblast migrating to join the forming PCV. Imaging was started approximately at the 15-16-somite stage, and time frames were acquired every 10-12 min.