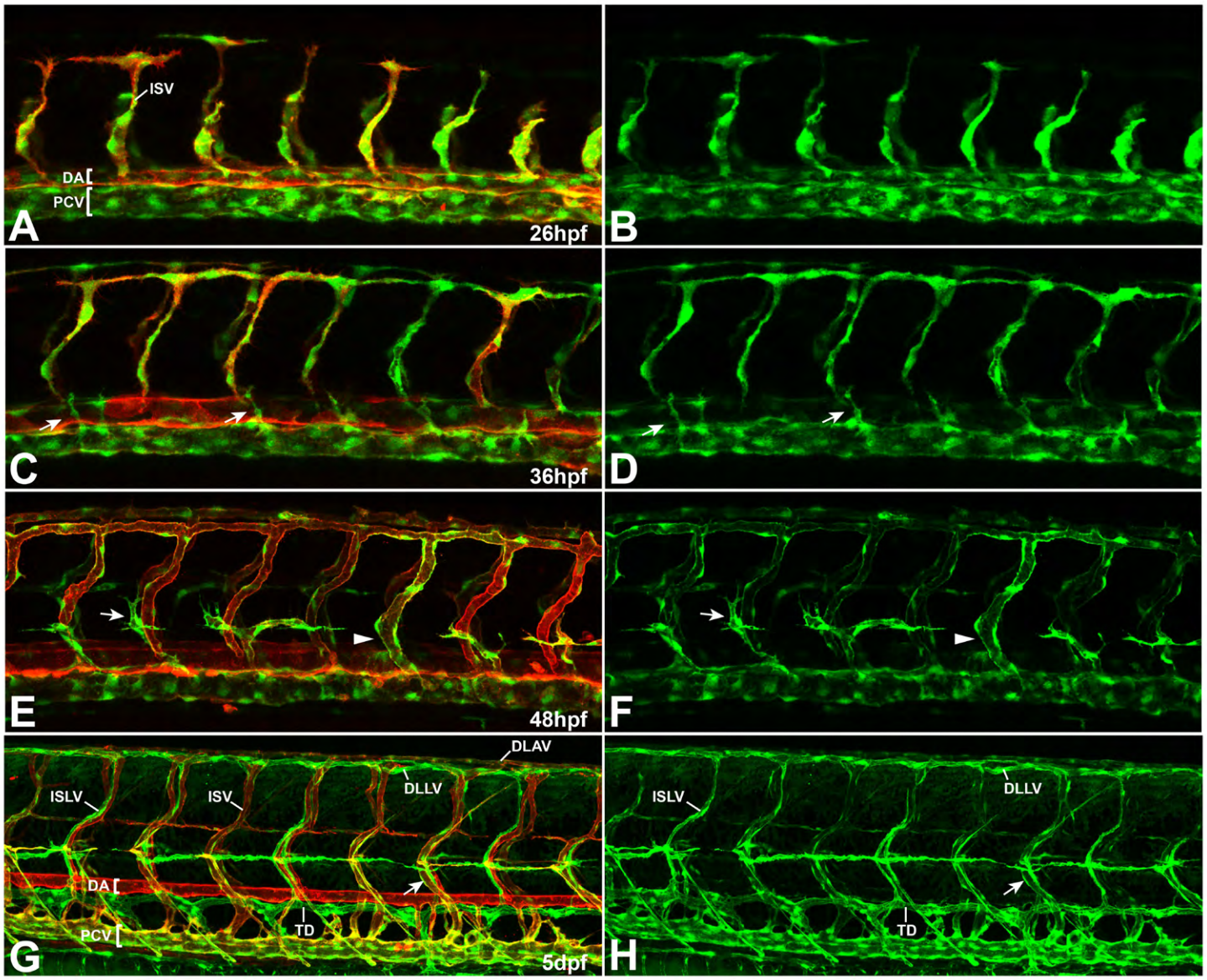

Fig. S1. Expression pattern of *flt4*^{BAC}*:mCitrine* reporter line at different stages of vascular development.

(A-H) Double transgenic embryos for *flt4:mCit* (green) and *kdrl:mCherry-Caax* (red) at different stages of vascular development. (A, B) Initially, the *flt4* reporter shows expression in both, arterial and venous ECs with an enriched signal within the venous compartment (26 hpf). (C, D) From about 26 hpf onwards, arterial expression of the construct decreases, so that emerging secondary sprouts can be easily followed at around 36 hpf (arrows). (E, F) At 2 dpf, the *flt4* reporter expression is strongly confined to venous derived structures (venous ISV marked by arrowhead) and the signal gradually increases in the lymphatic lineage (see PLs highlighted by arrows). (G, H) By day 5, *flt4:mCit* expression is still evident in the PCV and venous ISVs (arrow). In addition, lymphatic structures including the TD, ISLVs as well as the DLAV are clearly highlighted throughout the trunk. [DA-dorsal aorta, PCV-posterior cardinal vein, TD-thoracic duct, ISV-intersegmantal vessel, ISLV-intersegmental lymphatic vessel, DLAV-dorsal longitudinal anastomotic vessel, DLLV-dorsal longitudinal lymphatic vessel]

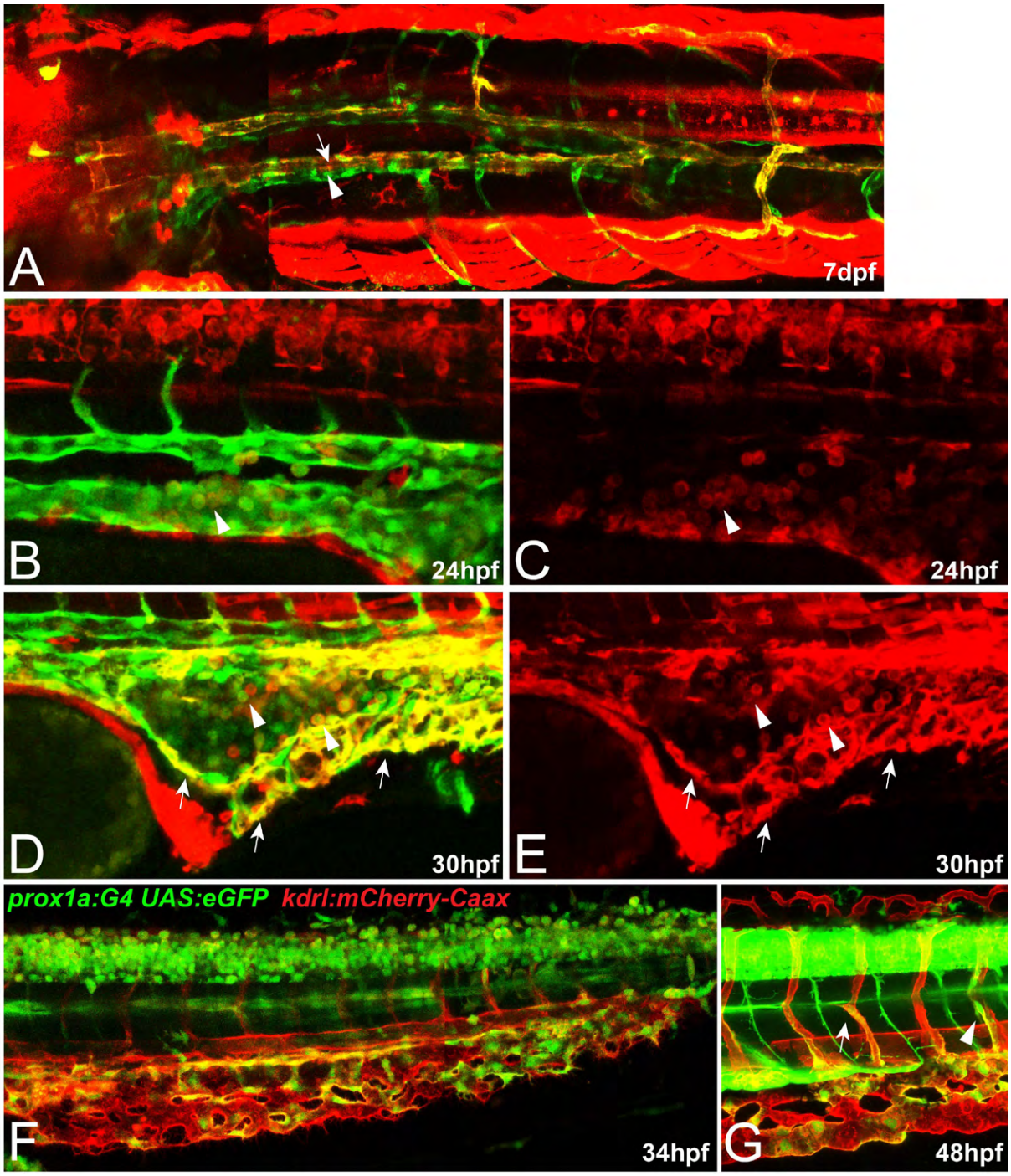

Fig. S2. Additional expression domains of the *prox1a* reporter line

(A-E) *Prox1a* reporter expression is shown in red, *flt4:mCit* expression is marked in green. (F, G) *kdr-l* expression domains are highlighted in red while expression of the *prox1a* reporter line is shown in green.

(A) Dorsal view on the anterior part of the TD which splits up in two vessels (arrow) closely aligning to the likewise bilateral PCV (arrowhead) in a 7 dpf embryo. The image has been assembled from two overlapping partial z-projections (see dotted line).

(B-E) At 24 hpf, before the onset of circulation, as yet undefined round cells within the axial vessels display expression of both markers (arrowheads in B, C). At 30 hpf, similar cells can still be seen with in the cardinal vein region (arrowhead in D, E). In addition, strong expression of the *prox1a* reporter is evident in a subpopulation of caudal vein cells (arrows in D, E).

(F, G) *prox1a* is expressed within a subgroup of caudal vein cells at 34 hpf which most likely results in the higher amounts of *prox1a* positive venous (arrowhead) and lymphatic secondary sprouts (arrow) in this area at 48 hpf (G). Image F is composed of two overlapping z-projections.

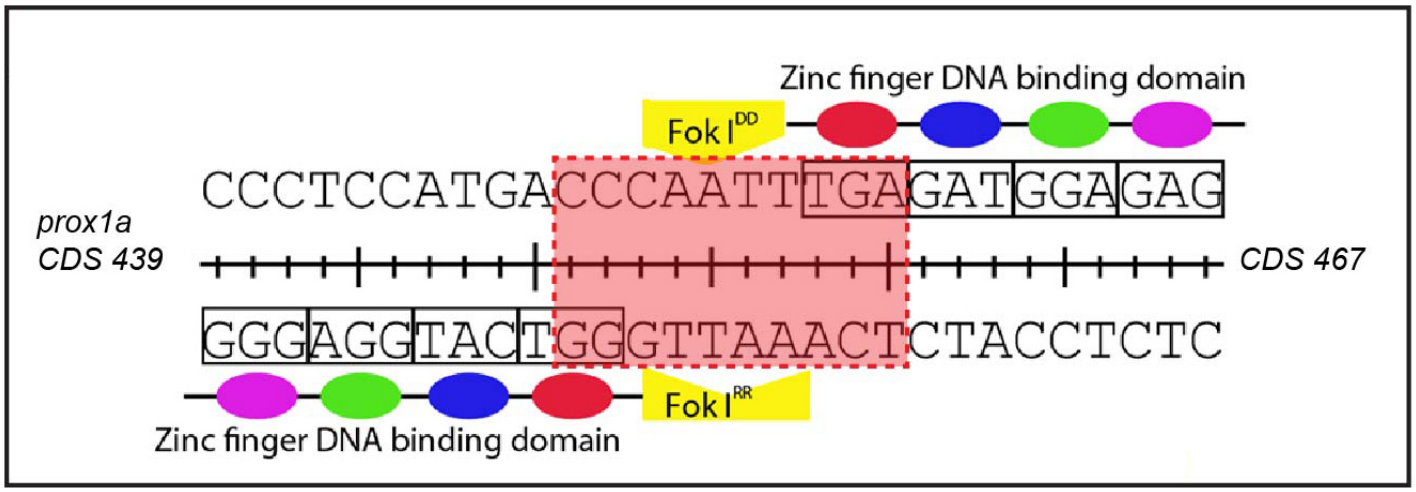

Fig. S3. Zinc-finger nuclease mediated generation of a *prox1a* mutant allele.

(A) Schematic representation of the zinc-finger nuclease target area in exon 1 of the *prox1a* gene. The red square indicates the deleted 10bp in the *prox1a*^{i278} allele.

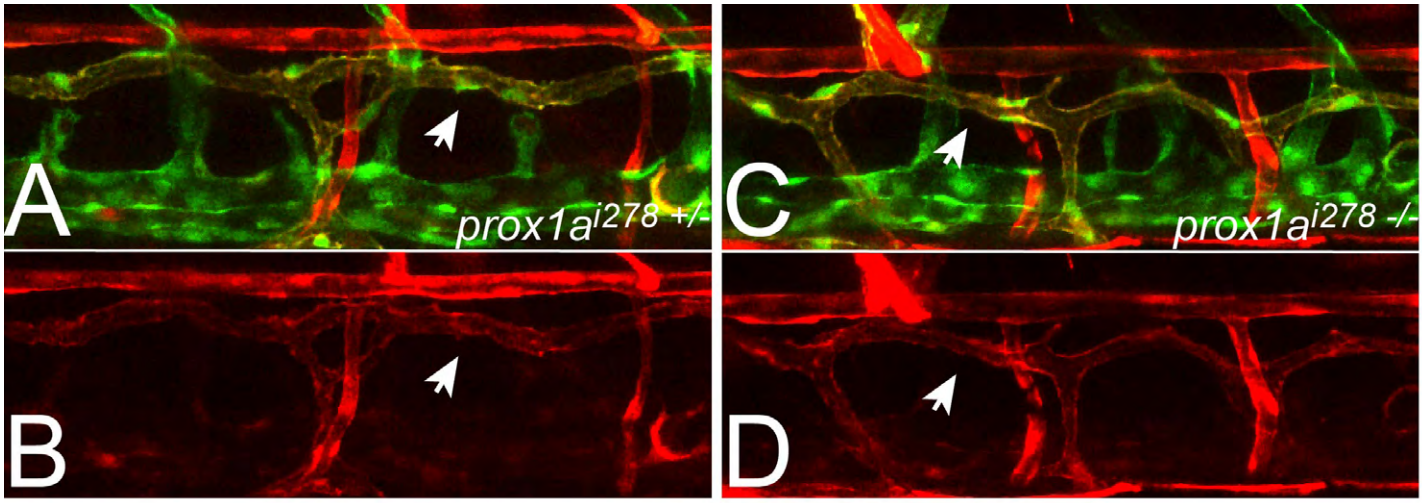

Fig. S4. Expression of the *prox1a* reporter line in *prox1a*^{i278} mutants.

(A-D) *prox1a:KalTA4,UAS:tagRFP* (TD in red), *flt1*^{enh}*:tdTom* (DA and arterial ISVs in red) and *flt4:mCit* positive triple transgenic embryos at 5 dpf. As in heterozygous siblings (A, B) *prox1a* reporter expression also marks the TD (see arrows) in homozygous *prox1a*^{i278} mutants (C, D), suggesting that Prox1a is not required for the maintenance of its own expression.

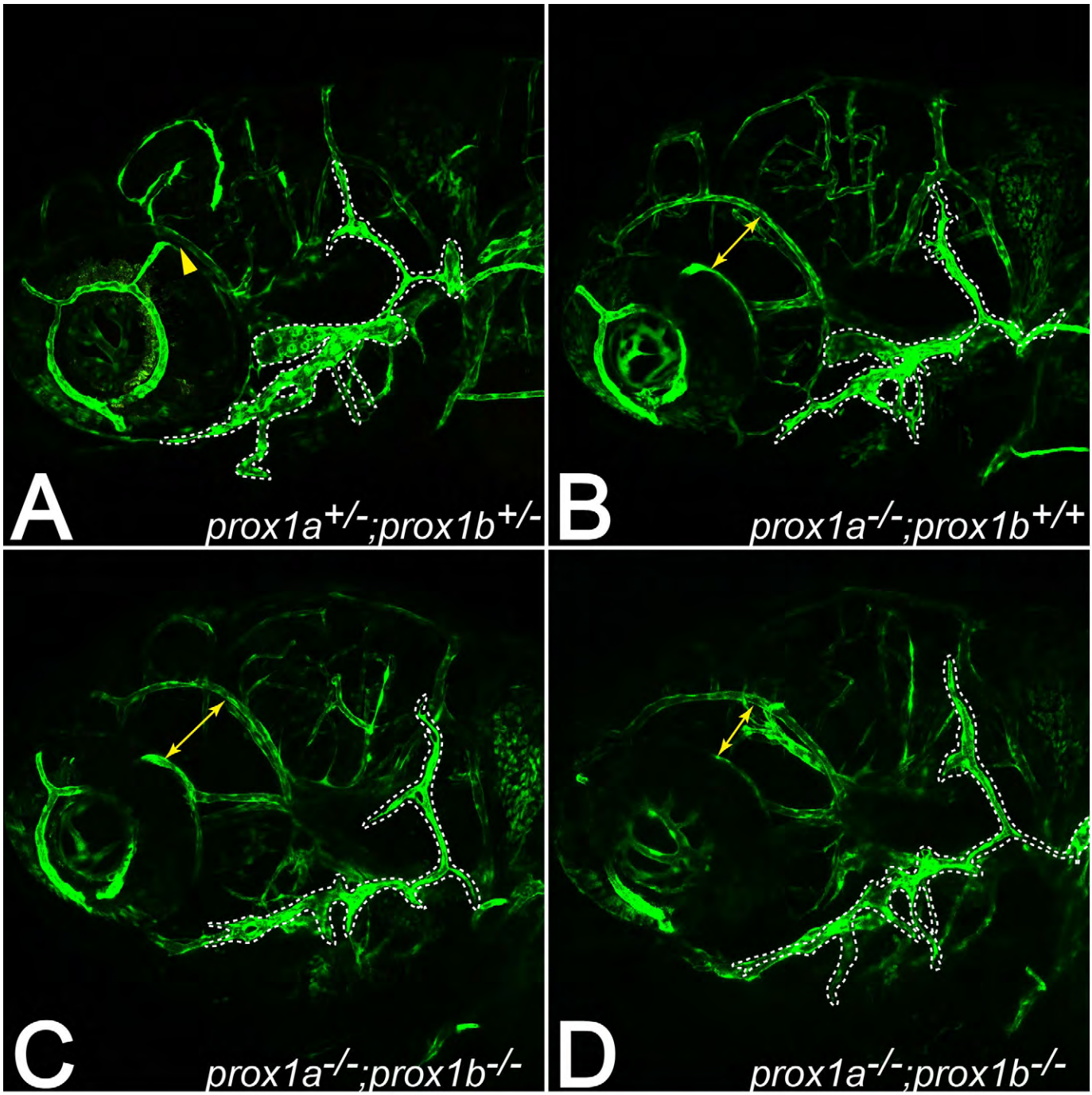

Fig. S5. Facial lymphatics are formed in *prox1a*^{i278} and *prox1a*^{i278}*;prox1b*^{SA0035} double mutants.

(A-D) Facial lymphatic network in *flt4:mCit* expressing embryos at 5dpf. As in wild-type siblings (A), the facial lymphatics (outlined by dotted lines) also emerge in *prox1a* single (B) and *prox1a;prox1b* double mutant embryos (C-D), but seem to be less developed compared to wild-type structures at the same age. Yellow arrows indicate the distance between the dorsal ciliary vein and the primordial midbrain channel that is enlarged in *prox1a*$^{-/-}$ embryos due to massive eye edema (also see Fig. 2E).

Table S1: Primers used in this study

primer name	Sequence
flt4_3.8kb_F	5'-CCGGAATTCCGCAGTCCGTCAATACTGAGG-3'
flt4_3.8kb_R	5'-GCGGAATTCCTCCAGATCTCCAGTCCAGAA-3'
prox1a_wt	5'-GAAGGTGACCAAGTTCATGCTACCATTCAGCAGGCCCTCCATGAC-3'
prox1a_mut	5'-GAAGGTCGGAGTCAACGGATTACCATTCAGCAGGCCCTCCATGAG-3'
prox1a_common	5'-GCCCTTAGATGCTCATCTGTTAGCCT-3'
Sox18_wt	5'-GAAGGTGACCAAGTTCATGCTCGCTGCTGTTCGAGACAC-3'
Sox18_mut	5'-GAAGGTCGGAGTCAACGGATTCGCTGCTGTTCGAGACAG-3'
Sox18_common	5'-GCACCAGTGCCTGGGTCTGGAA-3'
CoupTFII_wt	5'-GAAGGTGACCAAGTTCATGCTACACCCGTTCAAGGACCC-3'
CoupTFII_mut	5'- GAAGGTCGGAGTCAACGGATTACACCCGTTCAAGGACCC-3'
CoupTFII_common	5'-CGGGGTTGACTGTGTGTTGTT-3'