

Fig. S1 Sequence of the *sox7^{hu5626}* allele. Chromatogram of part of the *sox7* sequence comprising the site of the non-sense mutation (GTG>GTA, red arrow) in a heterozygous (middle panel) and homozygous mutant *sox7^{hu5626}* embryo (bottom panel).

Fig. S2 RT-PCR of the *sox7^{hu5626}* allele (S2A) *sox7* cDNA fragment of 10 pooled embryos with normal circulation (Sib.) and 10 pooled embryos with short loop phenotype (Mut.) at 2dpf. Left lane is 1kb DNA Ladder. Fragment size 1274 basepairs. (S2B) Part of *sox7^{hu5626}* cDNA sequence, upper (white) panel *sox7^{hu5626}* mutants and lower (yellow) panel *sox7^{hu5626}* siblings. Red arrow indicates G to A mutation site in *sox7* mutants.

Fig. S3 Indication of the relative positions of cross-sections shown in Fig.2B. Dorsal view of the head region of a *kdrl:eGFP* wild-type embryo highlighting (dotted red lines) the relative positions of cross-sections in Fig. 2B. First cross-section at location of bilateral aorta, second cross-section at location of bilateral aortae fusion in a single aorta, third section at the position just posterior to the aortae fusion and fourth section in the anterior part of the DA.

Fig. S4 Endothelial *sox17:eGFP* expression is indistinguishable between *sox7* mutants and siblings, and is detectable in the DA and ISVs but not in LDA cells. (S4A) Lateral view of trunk region of *kdrl:mCherry;sox17:eGFP*-positive *sox7* mutants and siblings at 2 dpf. *sox17:eGFP* is expressed in the DA and ISVs, but not in the PCV and is indistinguishable between *sox7* mutants and siblings. (S4B) Dorsal view of the head region of *kdrl:mCherry;sox17:eGFP*-positive *sox7* siblings and mutants at 2 dpf. LDA cells do not express detectable levels of the transgenic reporter line in both *sox7* siblings and mutants. Pictures in S4A and B are taken with same laser intensity and settings. (L)DA = (lateral) dorsal aorta, PCV = posterior cardinal vein, ISV = intersegmental vessel

Fig. S5 *dll4:Gal4FF;UAS:RFP* is expressed specifically in arterial endothelial cells. (S5A) Lateral view of the trunk region of *dll4:Gal4FF;UAS:RFP;flt4:mCitrine*-positive embryos at 25 hpf, 50 hpf and 5 dpf. (S5B) Dorsal view of the head region of *dll4:Gal4FF;UAS:RFP;flt4:mCitrine*-positive embryos at 25 hpf and 50 hpf. Right panels only *dll4:gal4FF;UAS:RFP* channel. Note *dll4:gal4FF;UAS:RFP* expression in the arteries (DA and LDA) but not within venous ECs (PCV and CCV). (L)DA = lateral dorsal aorta, PCV = posterior cardinal vein, CCV = common cardinal vein

Suppl. Movie 1 and 2 Brightfield movies of the head region (1A, 2A) and trunk region (1B, 2B) of *sox7* sibling (Movie 1) and mutant (Movie 2) embryos at 2.5dpf, timeframe of 5 seconds, 10x objective.

Suppl. Movie 3 and 4 Confocal time lapse movies of *kdr1:eGFP*-positive cells in the head region of a *sox7* sibling (Movie 3) and mutant (Movie 4) starting at the 20-somite stage; 1 timeframe per hour, 10x objective. Red lines outline the arterial cells, blue lines the venous cells. Green arrow indicates position of ectopic connection between arterial and venous ECs in *sox7* mutants.

Supplementary Table S1. Primers

Primer name	Sequence
Sox7_wt	GAA GGT GAC CAA GTT CAT GCT GAA CGC CTT CAT GGT GTG
Sox7_mut	GAA GGT CGG AGT CAA CGG ATT GAA CGC CTT CAT GGT GTA
Sox7_common	CGA GTC TCT TGC GCT CAT CTT
Efnb2a_wt	GAA GGT GAC CAA GTT CAT GCT CTC TGG AAC AGC TAA AGT CCTGT
Efnb2a_mut	GAA GGT CGG AGT CAA CGG ATT CTC TGG AAC AGC TAA AGT CCTGA
Efnb2a_common	AGT AGA GGC GTG TCT GCT TTT
Efnb2b_wt	GAA GGT GAC CAA GTT CAT GCT CGA GTT GTT CTT TGG GAA CAA GA
Efnb2b_mut	GAA GGT CGG AGT CAA CGG ATT CGA GTT GTT CTT TGG GAA CAA GT
Efnb2b_common	CTC CAC TGA GCA GAC GAA CAT
Hey2_ex2_Fw	TGTGAATGTGACGGATGTGA
Hey2_ex2_Rv	TCCGGTCCCTTCTTCTTTT
Sox7_5'UTR_Fw	GCCCGAGAAACAAAGTTTGAG
Sox7_3'UTR_Rv	CATGATGAGCCCACAGTCTC

Supplementary Figures

Fig. S1

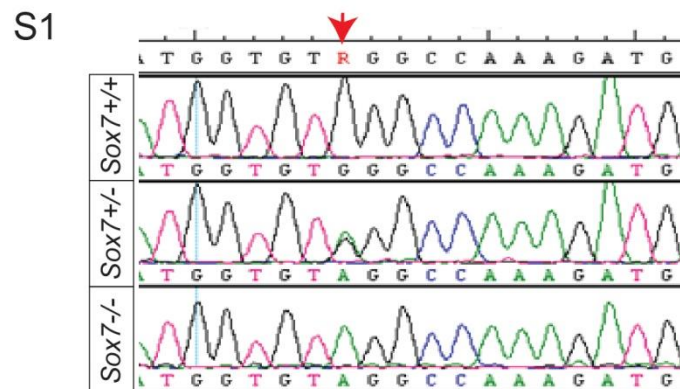


Fig. S2

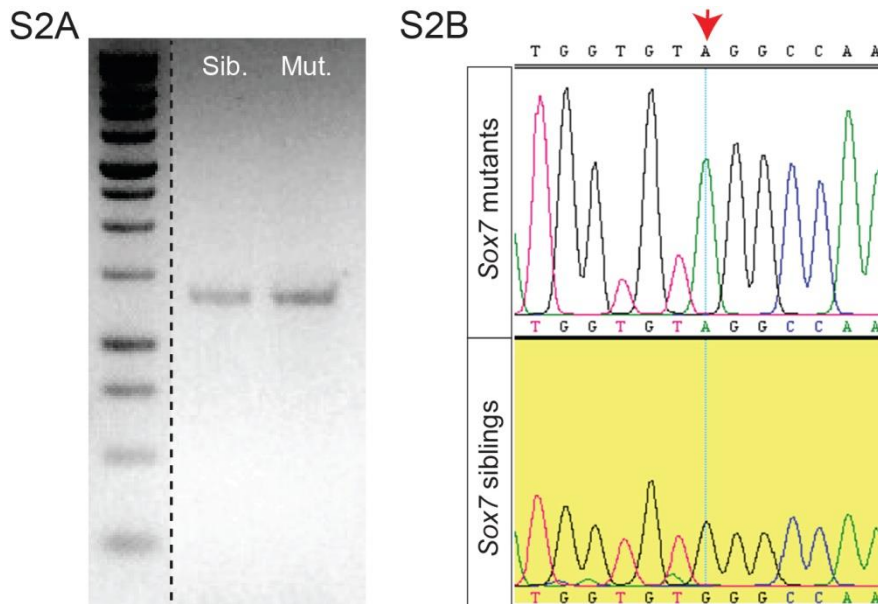


Fig. S3

S3

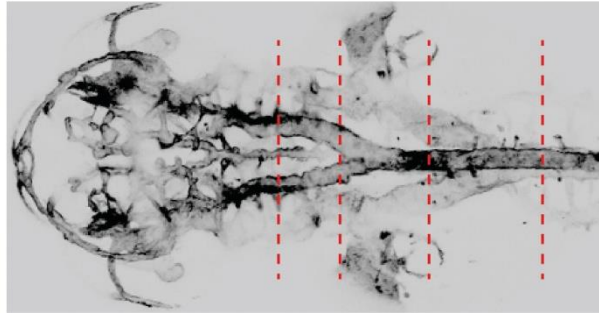


Fig. S4

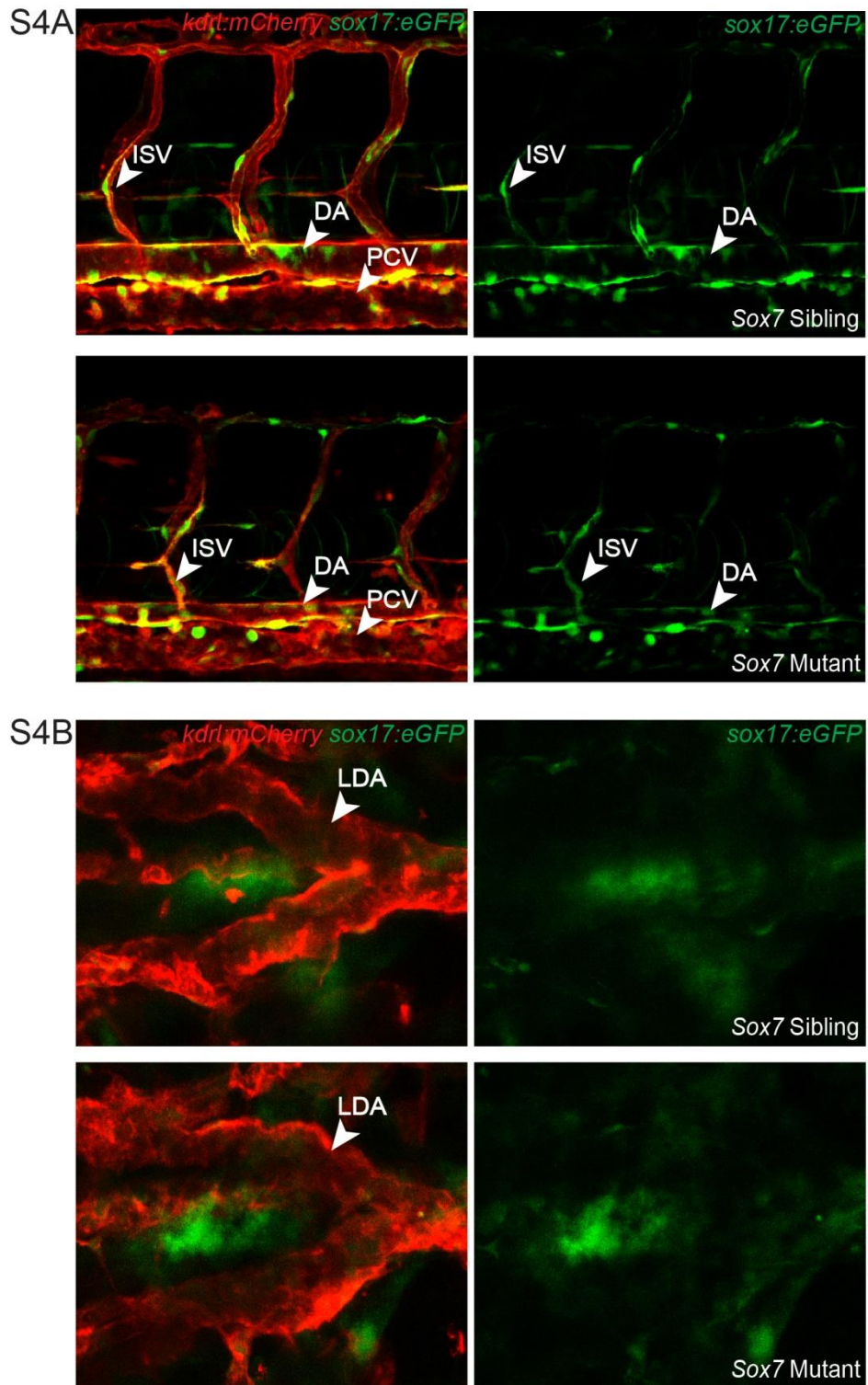
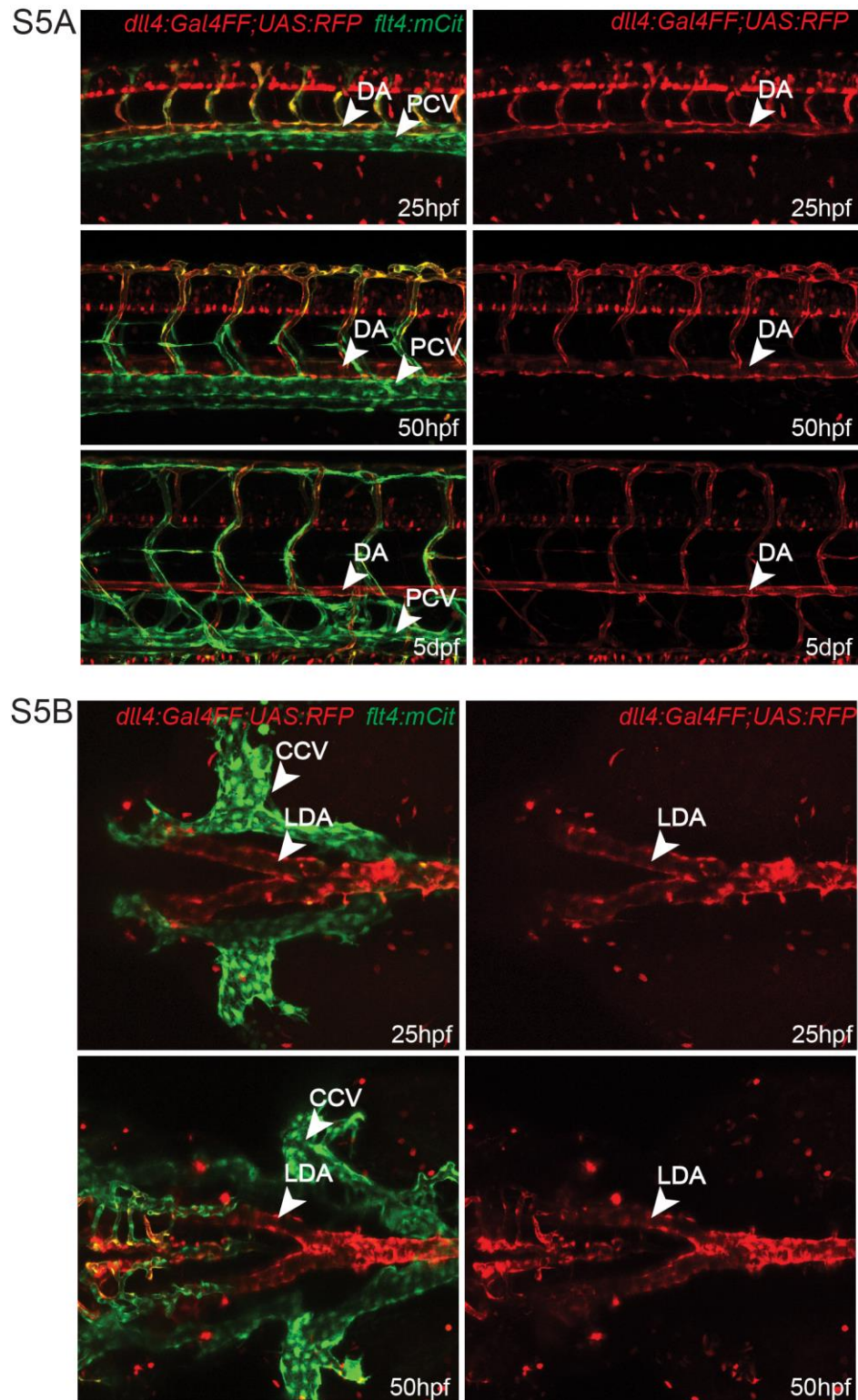


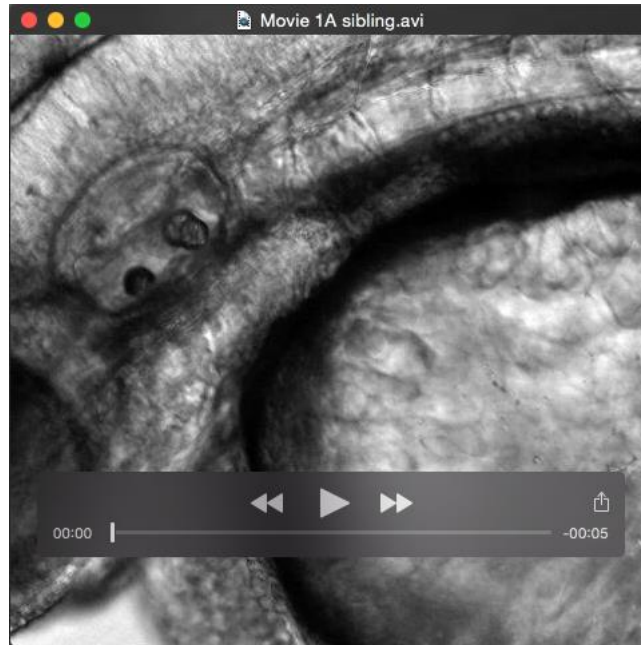
Fig. S5



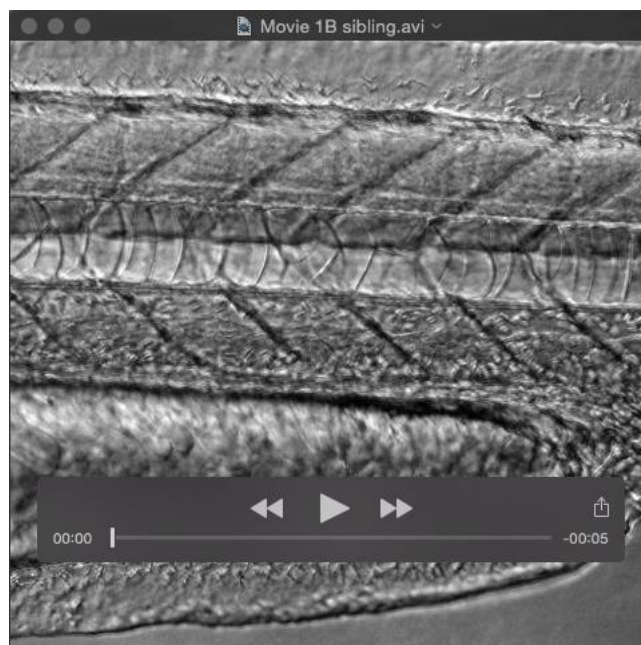
Supplementary Movies

Suppl. Movie 1

Movie 1A

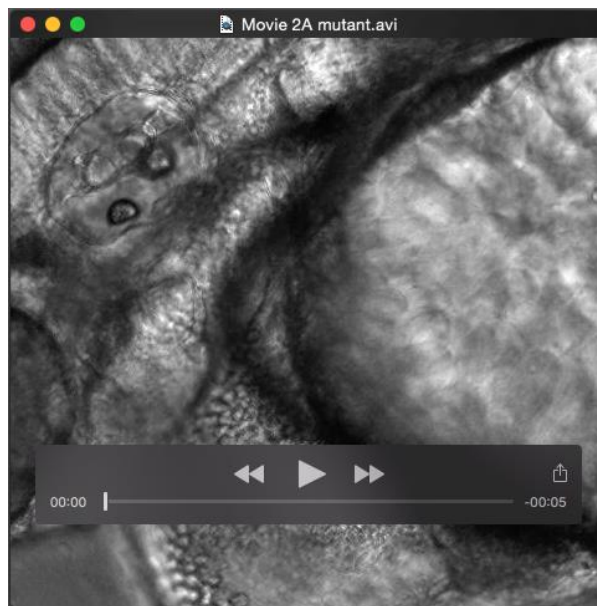


Movie 1B

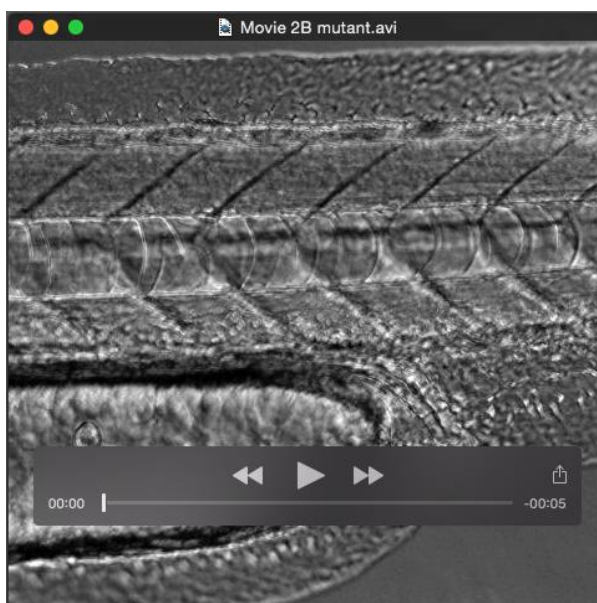


Suppl. Movie 2

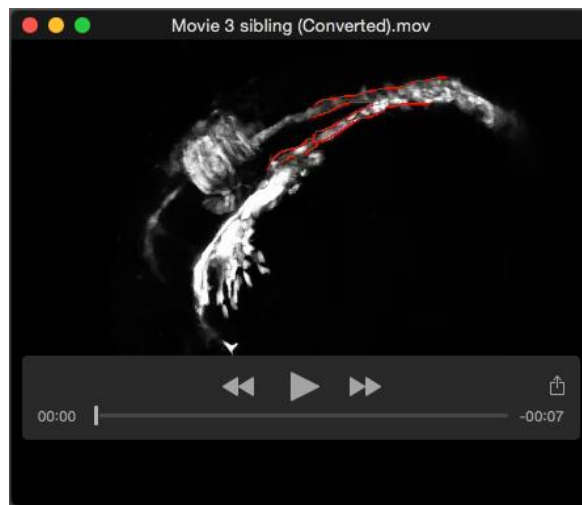
Movie 2A



Movie 2B



Suppl. Movie 3



Suppl. Movie 4

