

Fig. S1. The *reh* mutant intersegmental vessels start degenerating at 48 hpf and become absent or atretic by 72 hpf. Time-lapse confocal imaging of *Tg(kdrl:GFP) reh* and wild-type vasculature between 40-80 hpf shows that *reh* intersegmental vessels begin to degenerate at 48 hpf and are either absent or atretic by 72 hpf, whereas wild-type intersegmental vessels remain patent during the same time period. Time interval is every 15 minutes. Representative images are shown every 8 hours. Top, dorsal longitudinal anastomotic vessel; bottom, dorsal aorta/cardinal vein.

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|-------------------------------------|---|-----|
| Homo sapiens (NP_037451) | -----MAASQVLGEKINILSGETVKAGDRDPLGNDCEP-EQDRLPQRS--WRQ | 44 |
| Mus musculus (NP_082149) | -----MAAVQAPGEKINILAGETAKVGD--PQKNEWPE-QDRLPERS--WRH | 42 |
| Gallus gallus (NP_001026050) | -----MGPAELV-QKISINA-ESPRGGE----RNDGAGAERGPAGG--WRQ | 39 |
| Xenopus tropicalis (NP_001016538) | -----MKPDDCLNNIHIETENLDEVDLTKLTGAEHLSNGIVPPFTDKTFRKATSFQK | 53 |
| Danio rerio (NP_001186655) | -----MQEMKPAALSGSNGLNAGSGS--SVRVPCSR-LSRAGRMLDLQS | 42 |
| Drosophila melanogaster (NP_523581) | MATSSQLLPNGNLSRNGKTKTEDGEEVEAVVGARAAADAGVALTGRLLTGHPPSTSG-TFM | 59 |
| | . * * . * ↑ <i>reh</i> mutation site | |
| Homo Sapiens (NP_037451) | KCASYVLALRPWFSASLTPVALGSALAYRSHGV--LDPRLLVGCAVAVLAVHGAGNLVN | 102 |
| Mus musculus (NP_082149) | KCASYVLALRPWFSASLTPVALGSALAYRSQGV--LDPRLLLGCAVAVLAVHGAGNLVN | 100 |
| Gallus gallus (NP_001026050) | KCAAYVLALRPWFSASLTPVALGSALAYRAEGA--LDPRLLVGSAAVAVLAVHGAGNLVN | 97 |
| Xenopus tropicalis (NP_001016538) | KCATYVLALRPWFSASLIPVALGTAIAYRSGGS--LDLLLLFVVCVAVVAVLAVHGAGNLVN | 111 |
| Danio rerio (NP_001186655) | KCAAYVLALRPWFSASLTPVALGSALAYKLEGS--VDLLLLLVCAVAVLAVHGAGNLVN | 100 |
| Drosophila melanogaster (NP_523581) | KLKTYLLALRPWLSASLVPTLLGSALAYRSQWAEESLAFVFLTAFTVVTVHCAGNVVN | 119 |
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| Homo Sapiens (NP_037451) | TYYDFSKGIDHKKSDDRTLVDRILEPQDVVRFVGFYTLGCVCAACLYYLSPLKLEHLAL | 162 |
| Mus musculus (NP_082149) | TYYDFSKGIDHKKSDDRTLVDRILEPQDVVRFVGFYTLGCVCAACLYYLSALKLEHLAL | 160 |
| Gallus gallus (NP_001026050) | TYYDFSKGIDHKKSDDRTLVDQILEPQDVVRFVGFYTLVGCICAAGLYAVSTLKLEHLAL | 157 |
| Xenopus tropicalis (NP_001016538) | TYYDFSKGIDHKKSDDRTLVDHILEPQDVVRFVGFYTLGCLCAACLYFISKLEHLAL | 171 |
| Danio rerio (NP_001186655) | TYYDFSKGIDHKKSDDRTLVDQILKPDVVMFGAVLYSAGCLCATLLYFSSKLLEHLAL | 160 |
| Drosophila melanogaster (NP_523581) | TYFDKIKGIDKQKADDRTLVDHILTKDEVVSLGAILYMAGCGGFVLLAVLSPAKMEHLAL | 179 |
| | * * * * * * * * * * * * * * * * * * : : * : * . * * * * * * . * : * : * * * * * | |
| Homo Sapiens (NP_037451) | IYFGGLSGSFLYTTGGIGFKYVALGDLIILITFGPLAVMFAYAIQVGSIAIFPLVYAIPLA | 222 |
| Mus musculus (NP_082149) | IYFGGLSGSFLYTTGGIGFKYVALGDLVILITFGPLAVMFAYAVQVGSIAIFPLIYAIPLA | 202 |
| Gallus gallus (NP_001026050) | VYFGGLSSSFLYTTGGIGFKYVALGDVILITFGPLAVMFAHAVQVGYLSVSPLLYAVPLA | 217 |
| Xenopus tropicalis (NP_001016538) | IYFGGLSSSFLYTTGGIGFKYVALGDLVILITFGPLAVMFAHAVQVGYLAVTPLYAVPLA | 231 |
| Danio rerio (NP_001186655) | IYFGGLSSSFLYTTGGIGLKYVALGDVILITFGPLAVMFAHAVQVGYLSVPLVYAVPLA | 220 |
| Drosophila melanogaster (NP_523581) | IYFGGLSSSFLYTTGGIGFKYIALGDLVILILFPIISVLFAMFMSQTGHLDWTMMGYAIPLA | 239 |
| | : * * * * * . * * * * * * * * * * * * * * * * * * : : * * * . * : * : * * * * * | |
| Homo Sapiens (NP_037451) | LSTEAILHSNNTRDMESDREAGIVTLAILIGPTFSYILYNTLLFLPYLVFVSILATHCTIS | 282 |
| Mus musculus (NP_082149) | LSTEAILHSNNTRDMESDREAGIVTLAILIGPTFSYVLYNTLLFVPLYLIFTILATHCSIS | 280 |
| Gallus gallus (NP_001026050) | LSTEAILHSNNTRDMESDQAGIVTLAIIIGPAFVLYLTVLLFLPYLIFCVLATRYTIS | 277 |
| Xenopus tropicalis (NP_001016538) | LSTEAILHSNNTRDMESDRQAGIVTLAILVGPMSYMLYNLLVFLPYLIFSILATRYTIS | 291 |
| Danio rerio (NP_001186655) | LNTEAILHSNNTRDMDSKQAGIVTLAIIIGPTLSYVIYNLLFVPLYLLFCILATRYTIS | 280 |
| Drosophila melanogaster (NP_523581) | LNTEAILHSNNTRDADNDRRAGIVTLAIIIGRTASHVLYAMLLFAPYSLFFIFGLKYSVW | 299 |
| | * . * * * * * * * * * * . : * : * . * * * * * * * * * * * * * * * * * : : : : : | |
| Homo Sapiens (NP_037451) | LALPLLTIPLAFSLERQFRS-QAFNKLQRTAKLNLLLGLFVYVFGIILAPAGSLPKI--- | 338 |
| Mus musculus (NP_082149) | LALPLLTIPLAFSLERQFRS-QAFNKLQRTAKLNLLLGLFVYVFGIILAPAGSLPRL--- | 336 |
| Gallus gallus (NP_001026050) | MALPLLTIPLAFSLERQFRS-QNFNKLQRTAKLNLLLGLFVYVFGIILAPAGALPKL--- | 333 |
| Xenopus tropicalis (NP_001016538) | MALPLLTIPLAFSLERQFRS-QNFNKLQRTAKLNLLVGLFVYVFGIIVLAPAGSLP---- | 345 |
| Danio rerio (NP_001186655) | MALPLLTLPLAFPLERQFRC-RCYAKIPQRTAKLNLLMGLFVYVFGIILAPQGSPLLL--- | 336 |
| Drosophila melanogaster (NP_523581) | FLLPLVTLPLQAFQIEKFRFNEQTMHLVPRQTAKLNFVFFGILYVAVACCAHQLPVTFGLRRN | 359 |
| | : * * * * * : * * * * * : : * * * * * * * * * * * * . * . . | |

An * (asterisk) indicates positions which have a single, fully conserved residue.
 A : (colon) indicates conservation between groups of strongly similar properties
 A . (period) indicates conservation between groups of weakly similar properties

Fig. S2. Ubiad1 is conserved across vertebrate and invertebrate species. Alignment of human, mouse, chick, frog, zebrafish and fly UBIAD1 sequences. Underneath the protein sequences, identical and similar amino acids are labeled by an asterisk or a colon/period, respectively. Zebrafish UBIAD1 and human UBIAD1 proteins share 78.9% homology. The *reh* mutation results in the amino acid change of a highly conserved amino acid (boxed amino acid) in the second transmembrane domain of UBIAD1. GenBank accession numbers used for the analysis are as follows: human UBIAD1 (NP_037451), mouse UBIAD1 (NP_082149), chick UBIAD1 (NP_001026050), frog UBIAD1 (NP_001016538), zebrafish UBIAD1 (NP_001186655) and fly UBIAD1 (NP_523581). Human, mouse, chick, frog, and fly UBIAD1 proteins are highly similar to zebrafish UBIAD1 protein.

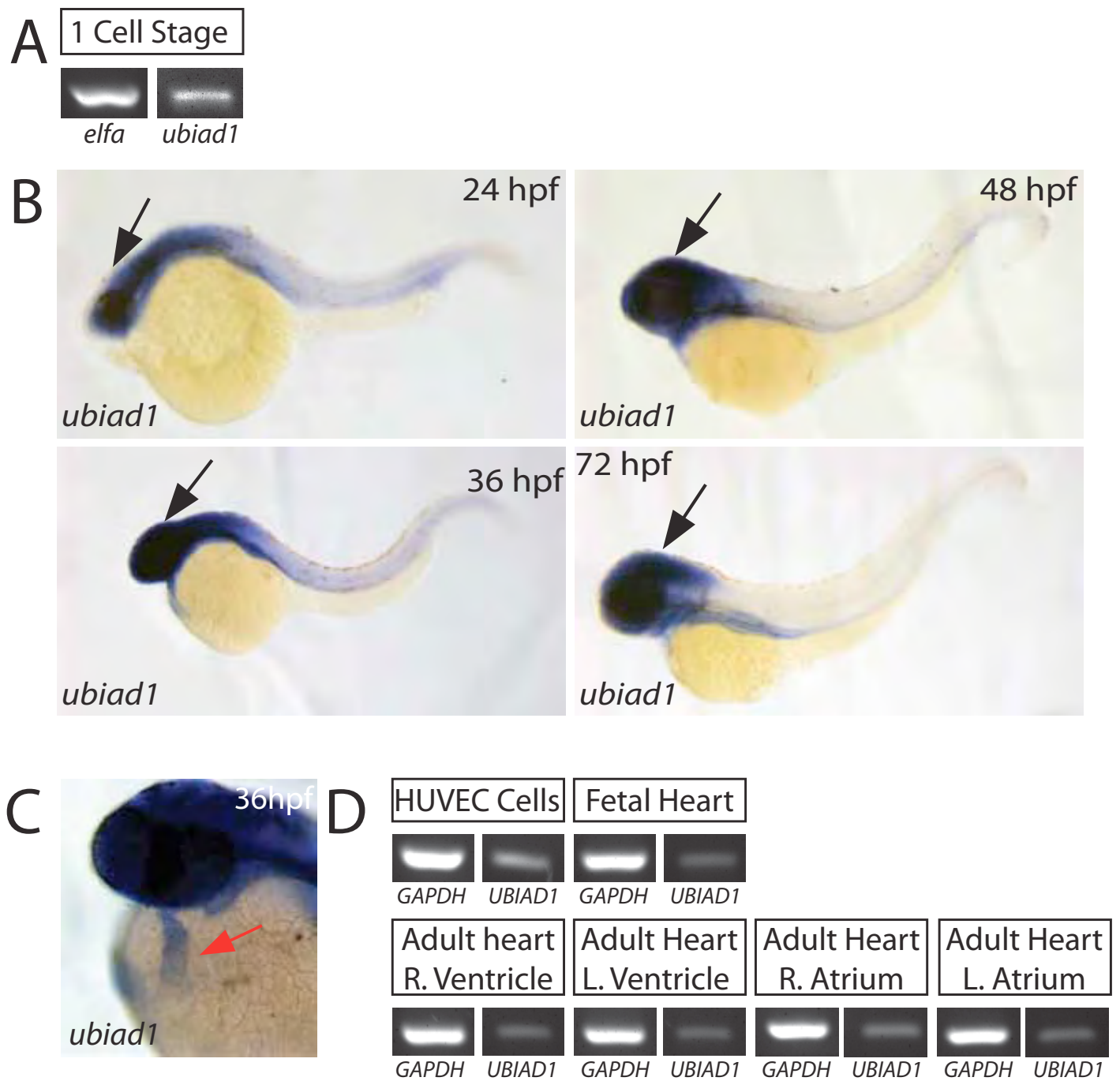


Fig. S3. UBIAD1 is expressed in the heart and vasculature. (A) RT-PCR shows that UBIAD1 is expressed at the one-cell stage of zebrafish embryos. (B,C) Whole-mount RNA in situ hybridization reveals UBIAD1 expression in the heart and brain. UBIAD1 is expressed in (B) the brain from 24 to 72 hpf (black arrows) and (C) the heart by 36 hpf (red arrow). (D) RT-PCR shows that UBIAD1 is expressed in human umbilical vein endothelial cells (HUVEC) and human hearts.

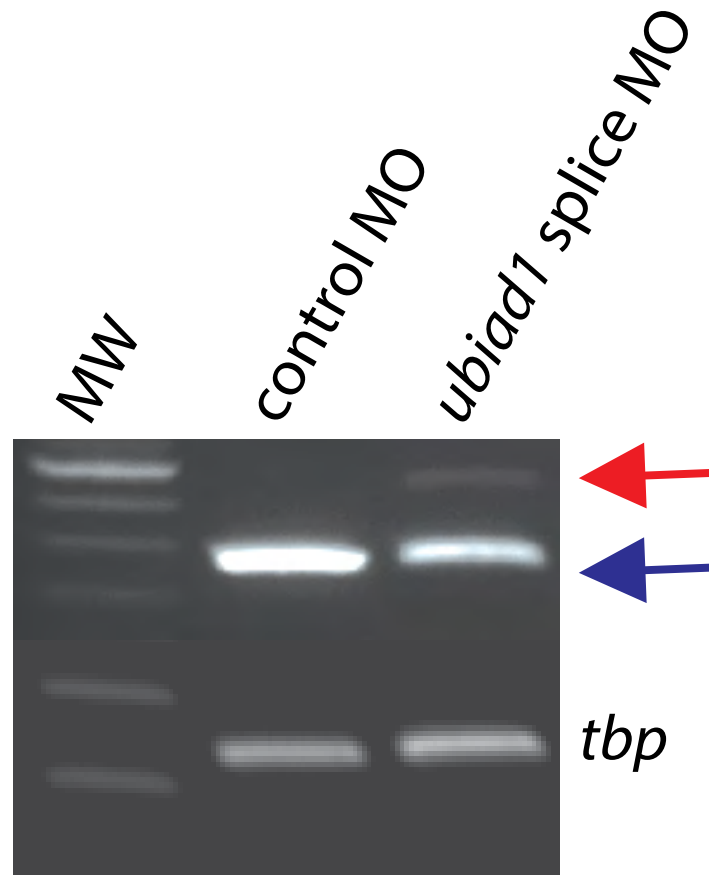


Fig. S4. Injection of *ubiad1* splice morpholino into wild-type zebrafish embryos blocks splicing of *ubiad1* RNA. Using forward 5'-ACACCTACTACGACTTCTCCA-3' and reverse 5'-GTGATGAGGATCACCACGTCT-3' primers to *ubiad1*, RT-PCR reveals that *ubiad1* splicing is altered in *ubiad1* splice morpholino (*ubiad1* splice MO)-injected zebrafish, but not in control morpholino (control MO)-injected zebrafish. This leads to a reduction in the levels of the correctly spliced *ubiad1* mRNA. Red arrow indicates altered splicing product; blue arrow indicates correctly spliced mRNA. *tbp* expression was used to control for *ubiad1* expression levels. MW, molecular weight marker.

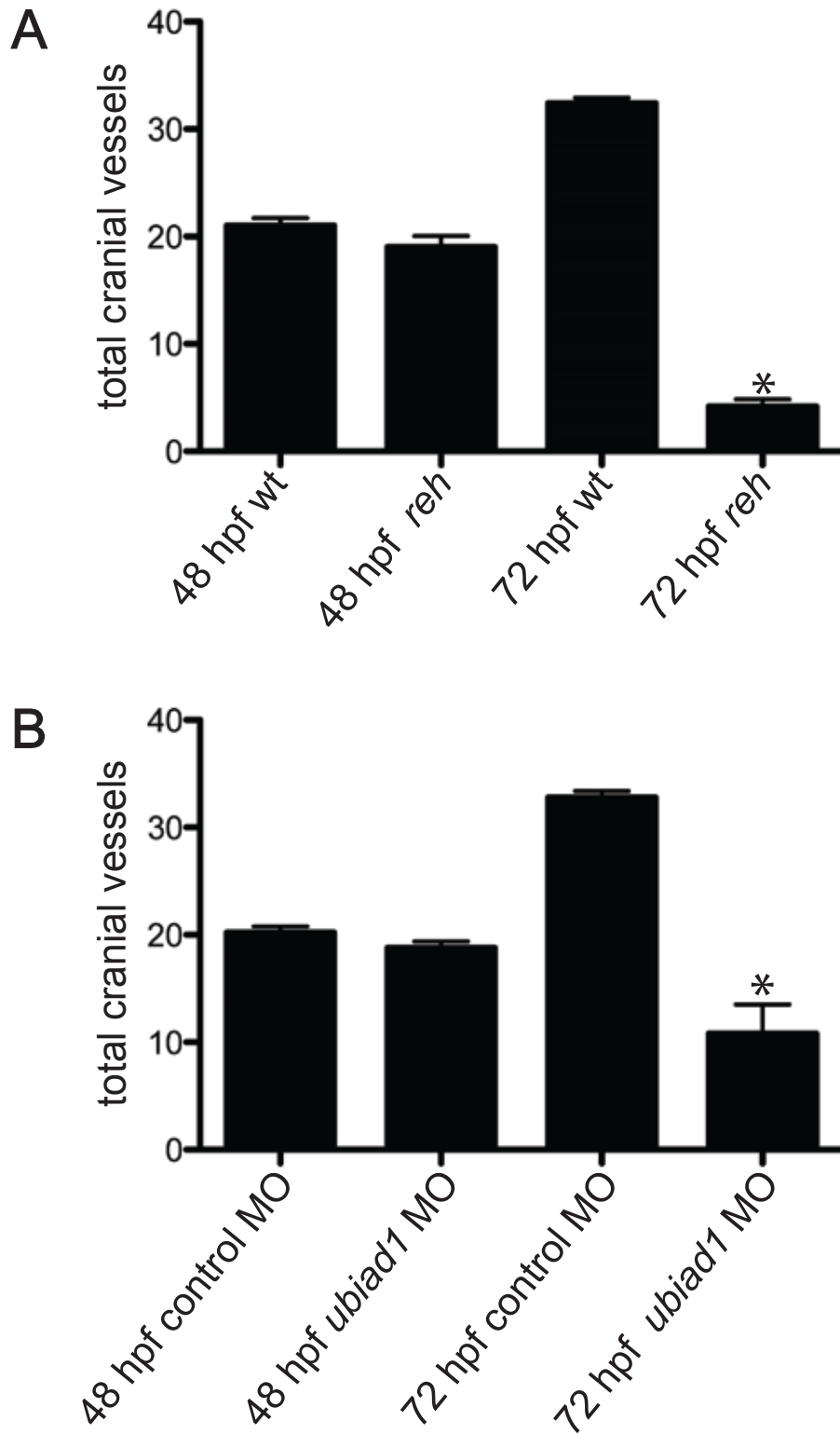


Fig. S5. *reh* mutants and *ubiad1* morpholino-injected zebrafish exhibit fewer cranial vessels than age-matched controls. Quantification of the number of cranial vessels detected on confocal projections of (A) *reh* and wild-type sibling control, as well as (B) *ubiad1* splice- and control MO-injected zebrafish at 48 and 72 hpf. Mean±s.e.m. Student's *t*-test, * $P < 0.05$ ($n=10$ for each condition).

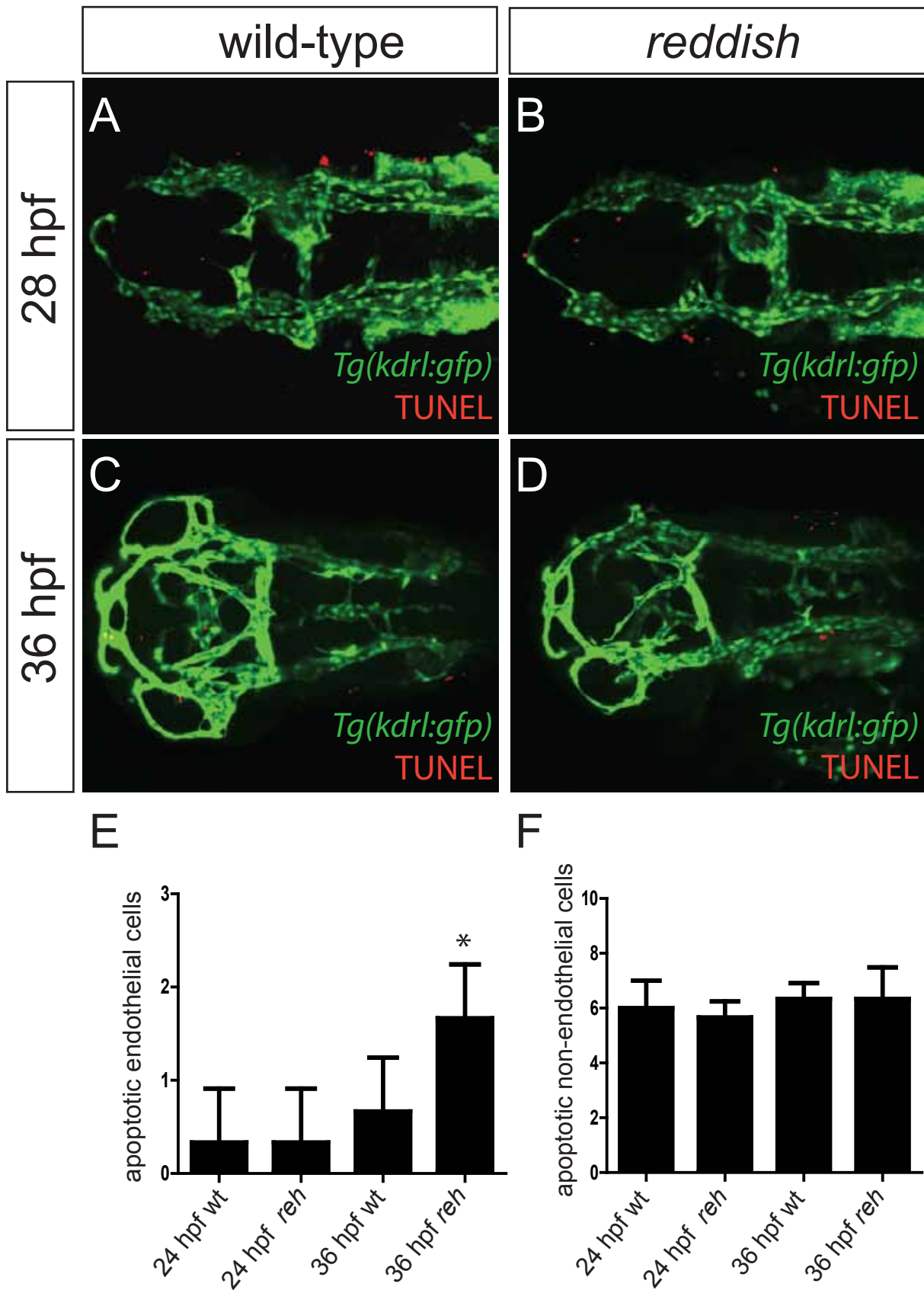


Fig. S6. Endothelial survival is compromised in *reh* mutants owing to increased apoptosis by 36 hpf. (A-D) Confocal projections of *Tg(kdrl:gfp)* wild-type (wt) and *reh* mutant zebrafish that were TUNEL stained (red) reveal that (D) *reh* endothelial cells exhibit increased apoptosis at 36 hpf when compared with (C) wild-type endothelial cells. However, there appeared to be no significant difference in endothelial cell death between (A) wild-type and (B) *reh* embryos at 28 hpf. (E,F) The number of apoptotic cells observed per high-power field for each condition. Mean±s.e.m. Student's *t*-test, * $P < 0.1$ ($n = 15$ *reh* and wild-type zebrafish).

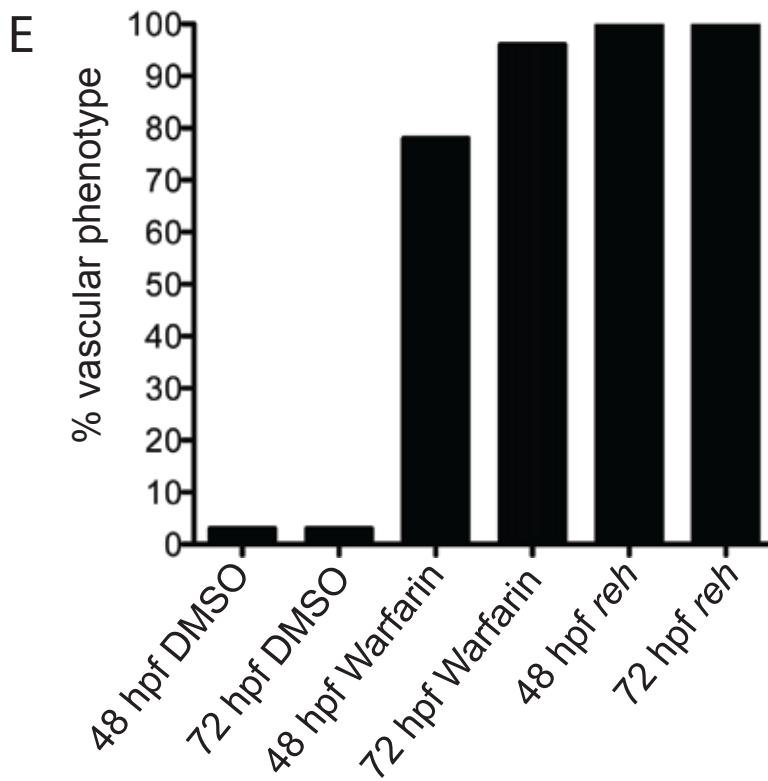
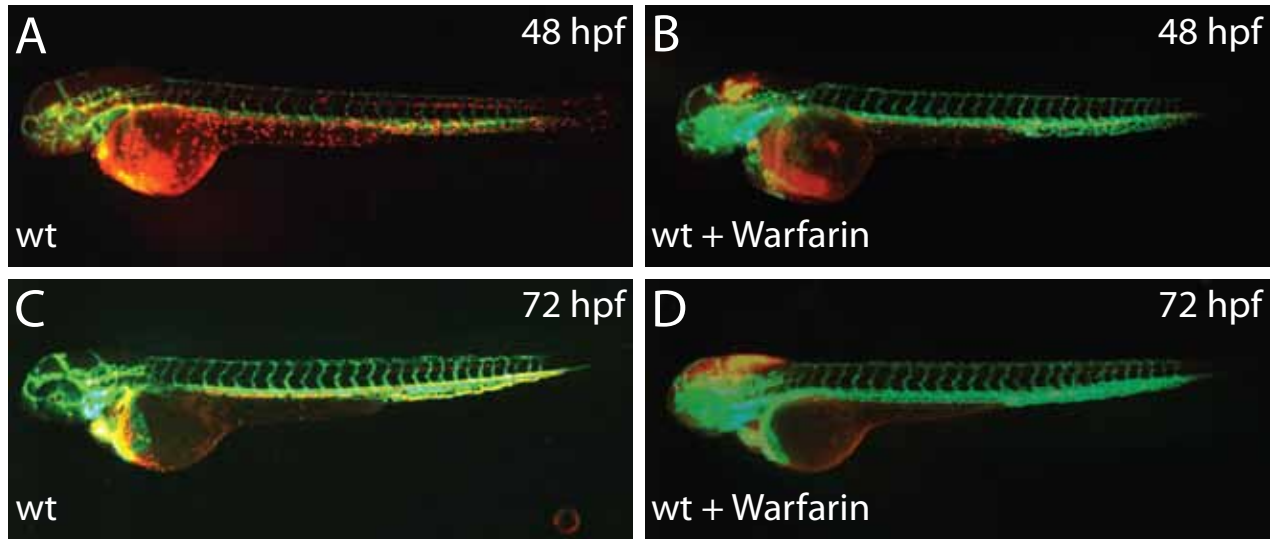


Fig. S7. Warfarin treatment of zebrafish at 24 hpf results in cranial hemorrhaging. (A-D) Micrographs of (A,C) DMSO- and (B,D) warfarin-treated *Tg(gata1:dsRed);Tg(kdr1:GFP)* zebrafish shows that warfarin exposure at 24 hpf can cause cranial hemorrhages by 48 and 72 hpf. (E) 78% of warfarin-treated zebrafish at 24 hpf displayed cranial hemorrhaged by 48 hpf, which increased to 96% by 72 hpf. All *reh* mutants but no DMSO-treated zebrafish exhibited cranial hemorrhage at 48 and 72 hpf.

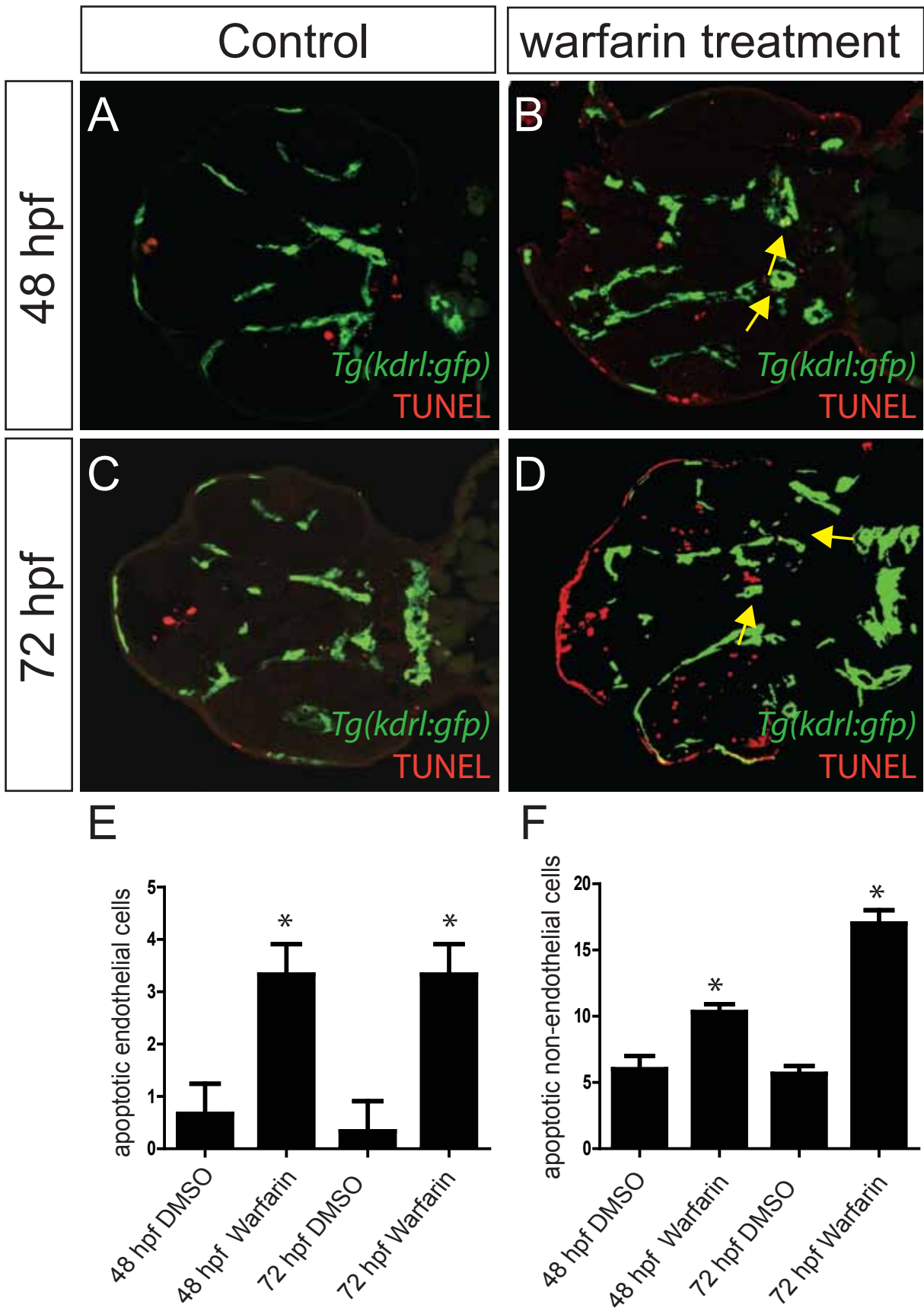
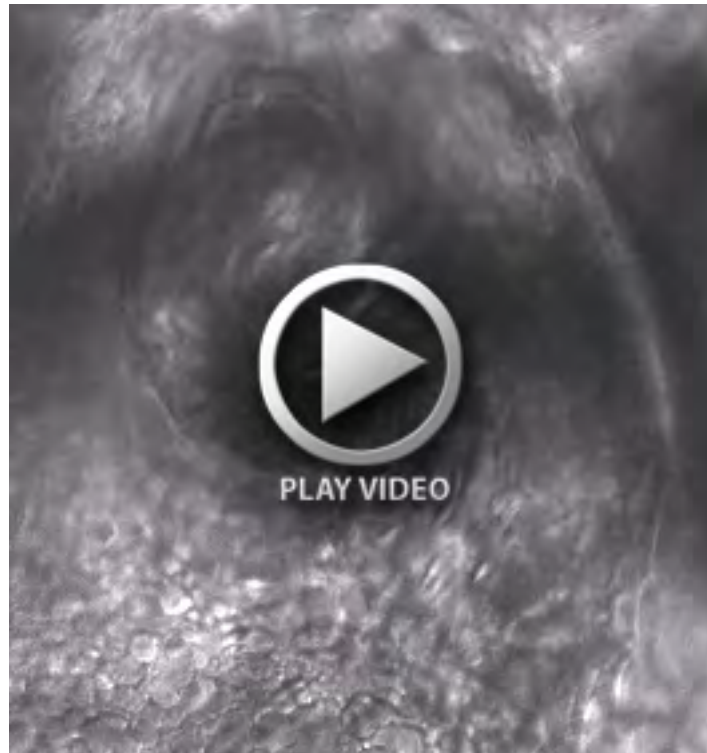


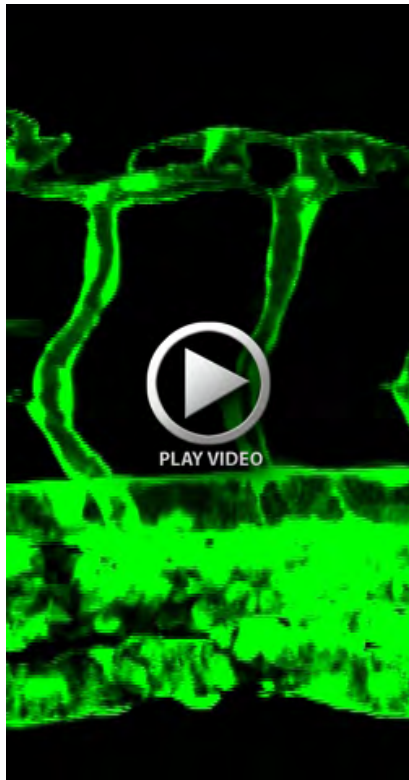
Fig. S8. Warfarin treatment of zebrafish at 24 hpf results in cranial endothelial cell apoptosis. (A-D) Confocal sections of 48 and 72 hpf *Tg(kdrl:gfp)* DMSO (control)- and warfarin-treated zebrafish that were TUNEL stained (red) reveal that (B,D) warfarin-treated endothelial cells exhibit increased apoptosis (yellow arrows) when compared with (A,C) control endothelial cells. (E,F) The number of apoptotic cells observed per high-power field for each condition. Mean±s.e.m. Student's *t*-test, * $P < 0.05$ ($n = 15$ *reh* and wild-type zebrafish).



Movie 1. Cardiac function is robust in 48 hpf age-matched control. Wild-type zebrafish (48 hpf) have robust contractile function and no cardiac edema. Ventral view, anterior towards the top. Ventricle on the left and atrium on the right.



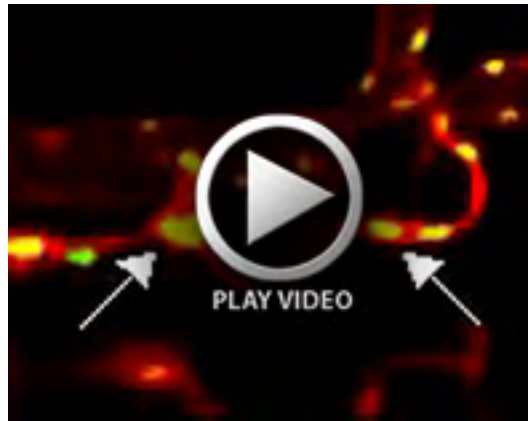
Movie 2. Cardiac function is significantly reduced in 48 hpf *reh* mutant. *reh* mutants (48 hpf) have significantly reduced contractile function leading to cardiac edema. Ventral view, anterior towards the top. Ventricle on the left and atrium on the right.



Movie 3. Time-lapse imaging of wild-type trunk vasculature reveals normal vessel development from 48-72 hpf. *Tg(kdrl:GFP)* fish were time-lapse imaged by confocal microscopy from 48-72 hpf. Time-lapse movie shows normal vascular maintenance and development. Time interval is every 15 minutes. Top, dorsal longitudinal anastomotic vessel; bottom, dorsal aorta/cardinal vein.



Movie 4. Time-lapse imaging of *reh* mutant vasculature reveals vessel degeneration from 48 to 80 hpf. *Tg(kdrl:GFP); reh* fish were time-lapse imaged by confocal microscopy from 40-80 hpf. Time-lapse movie shows degeneration of the *reh* trunk vasculature. Time interval is every 15 minutes. Top, dorsal longitudinal anastomotic vessel; bottom, dorsal aorta/cardinal vein.



Movie 5. Time-lapse imaging of *reh* mutants reveals increased endothelial nuclear fragmentation (karyorrhexis) from 56 to 72 hpf. *Tg(fli1a:nEGFP);Tg(kdrl:cherry-ras); reh* fish were time-lapse imaged by confocal microscopy from 48 to 72 hpf. Time-lapse movie shows endothelial nuclei undergoing karyorrhexis (white arrows). Time interval is every 15 minutes.