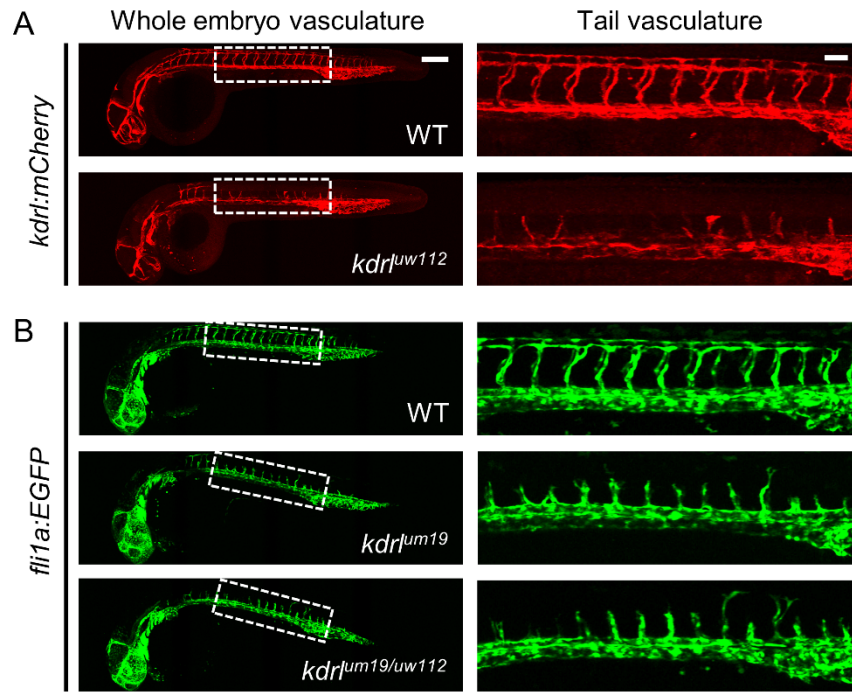
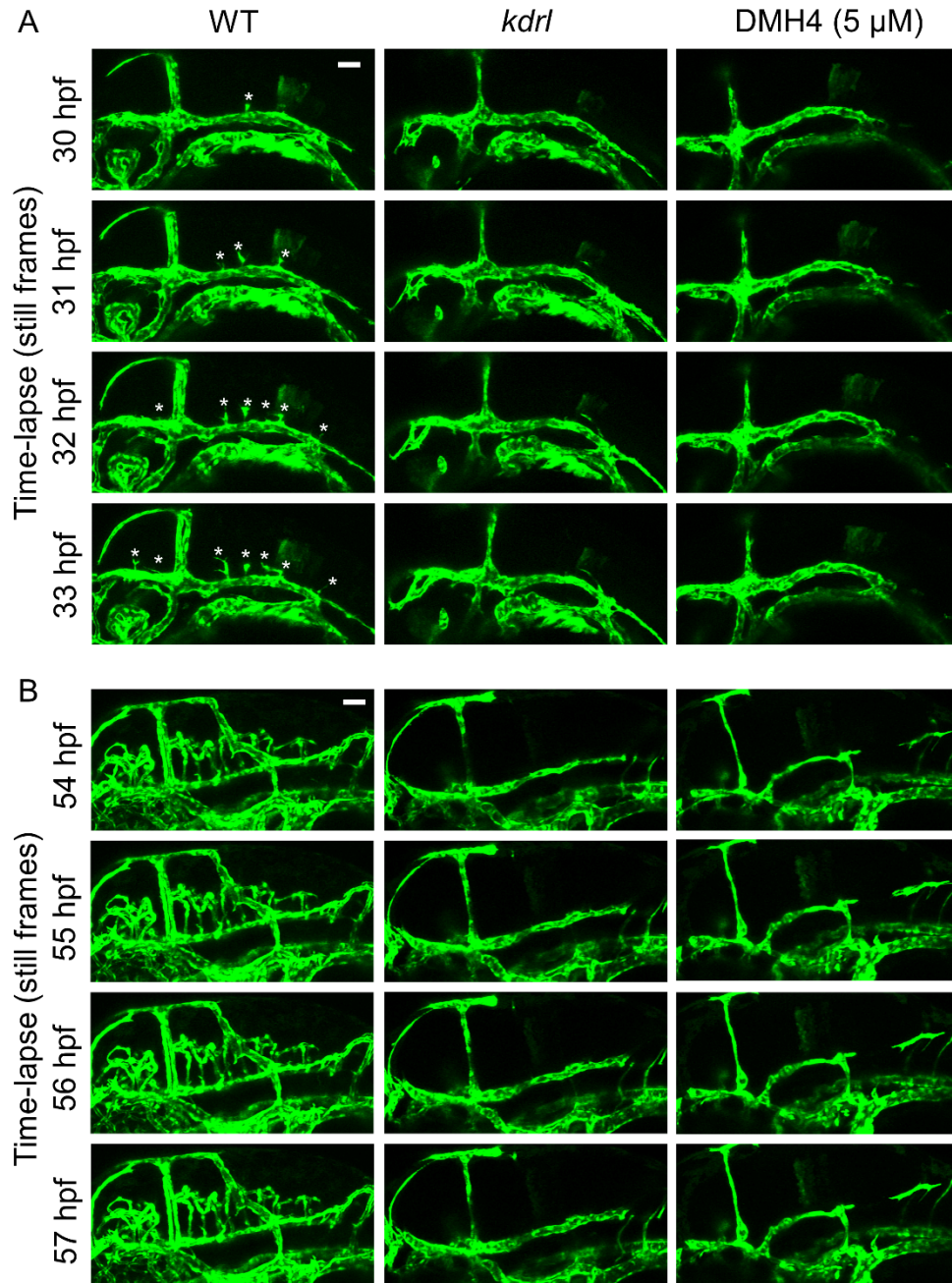


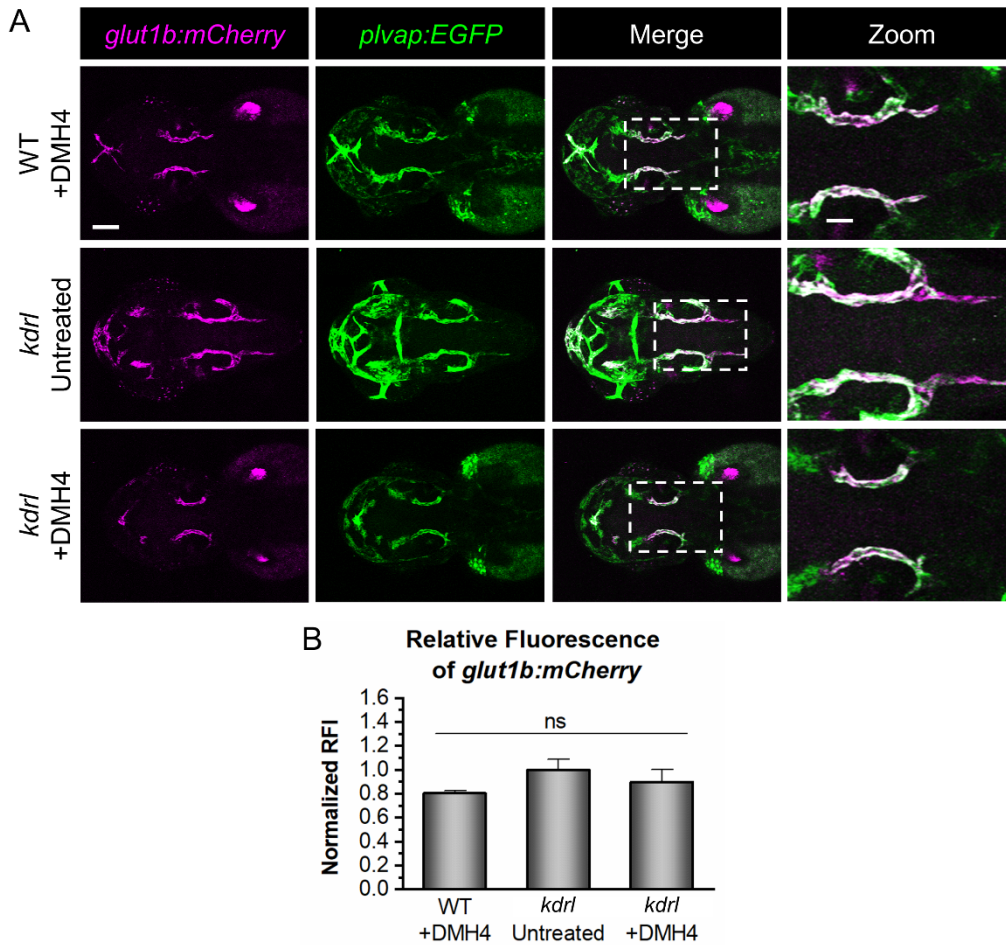
**Fig. S1. Relative fluorescence of *glut1b:mCherry*.** (A) Quantification of the normalized relative fluorescence intensity (RFI) of *glut1b:mCherry* in WT, *uw112* mutants, and *gpr124* mutants at 2 dpf. (B) Quantification of the normalized RFI of *glut1b:mCherry* in WT, *uw112* mutants, and *gpr124* mutants at 3 dpf. Data are presented as means ( $n = 3$ )  $\pm$  SEM (\* $p < 0.05$ ; \*\* $p < 0.01$ ; ns = not significant).



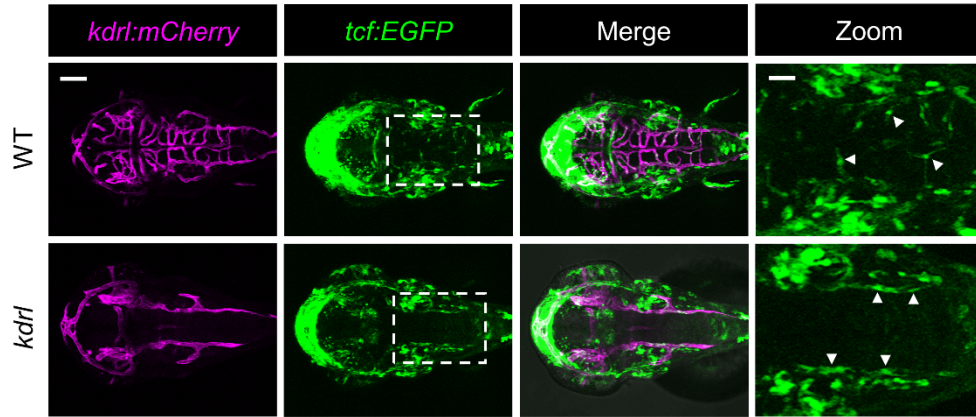
**Fig. S2. Vasculature in *kdr1* mutants.** (A) Representative confocal images of a WT and *kdr1<sup>uw112</sup>* mutant in the transgenic *kdr1:mCherry* background at approximately 28-30 hpf (lateral views; anterior left). (B) Representative confocal images of a WT, *kdr1<sup>um19</sup>*, and *kdr1<sup>um19/uw112</sup>* mutants in the transgenic *fli1a:EGFP* background at approximately 28-30 hpf (lateral views; anterior left). All embryos showed normal heartbeat and circulation and no gross abnormalities. Note that *kdr1<sup>uw112</sup>*, *kdr1<sup>um19</sup>*, and *kdr1<sup>um19/uw112</sup>* mutants show similar defects in tail vasculature. Scale bars are 100  $\mu$ m (left panels) and 50  $\mu$ m (right panels).



**Fig. S3. Time-lapse still frames of CNS angiogenesis.** (A) Representative confocal images of WT, *kdrl* mutant, and DMH4-treated (5 μM) embryos in the *kdrl:mCherry* transgenic background at 1-hour intervals beginning at 30 hpf (lateral views; anterior left). Note that *kdrl* mutant and DMH4-treated embryos lack endothelial tip cell spouting from the PHBCs, whereas WT embryos show normal spouting of the CtAs (white asterisks) (B) Representative confocal images of WT, *kdrl* mutant, and DMH4-treated embryos at 1-hour intervals beginning at 54 hpf (lateral views; anterior left). Note extensive CNS angiogenesis in WT and absence of CNS angiogenesis in both the *kdrl* mutant and the DMH4-treated embryos. Scale bar is 50 μm (top left panel).



**Fig. S4. *kdrl* mutants in combination with DMH4.** (A) Representative confocal images of WT treated with DMH4 (5  $\mu$ M), untreated *kdrl* mutants, and *kdrl* mutants treated with DMH4 (5  $\mu$ M) at 2 dpf (dorsal views; anterior left). Note that *glut1b:mCherry* is similarly expressed in the PHBCs of all embryos. Scale bar is 100  $\mu$ m for the first three columns and 40  $\mu$ m for the zoomed images (right panels). (B) Quantification of the normalized RFI of *glut1b:mCherry* in WT treated with DMH4, untreated *kdrl* mutants, and *kdrl* mutants treated with DMH4 at 2 dpf ( $n=3$ ). Data are presented as means  $\pm$  SEM (ns = not significant).



**Fig. S5. *tcf:EGFP* expression in *kdrl* mutants.** Representative confocal images of WT and *kdrl* mutants in the *tcf:EGFP*, *kdrl:mCherry* transgenic background at 2 dpf (dorsal views; anterior left). *tcf:EGFP* signal is present in the brain vasculature of WT (top panels; white arrows) and in the PHBCs of the *kdrl* mutant (bottom panels; white arrows). Scale bar is 100  $\mu$ m for the first three columns and 40  $\mu$ m for the zoomed images (right panels).