nature portfolio

https://doi.org/10.1038/s41591-021-01572-7

Supplementary information

PPIL4 is essential for brain angiogenesis and implicated in intracranial aneurysms in humans

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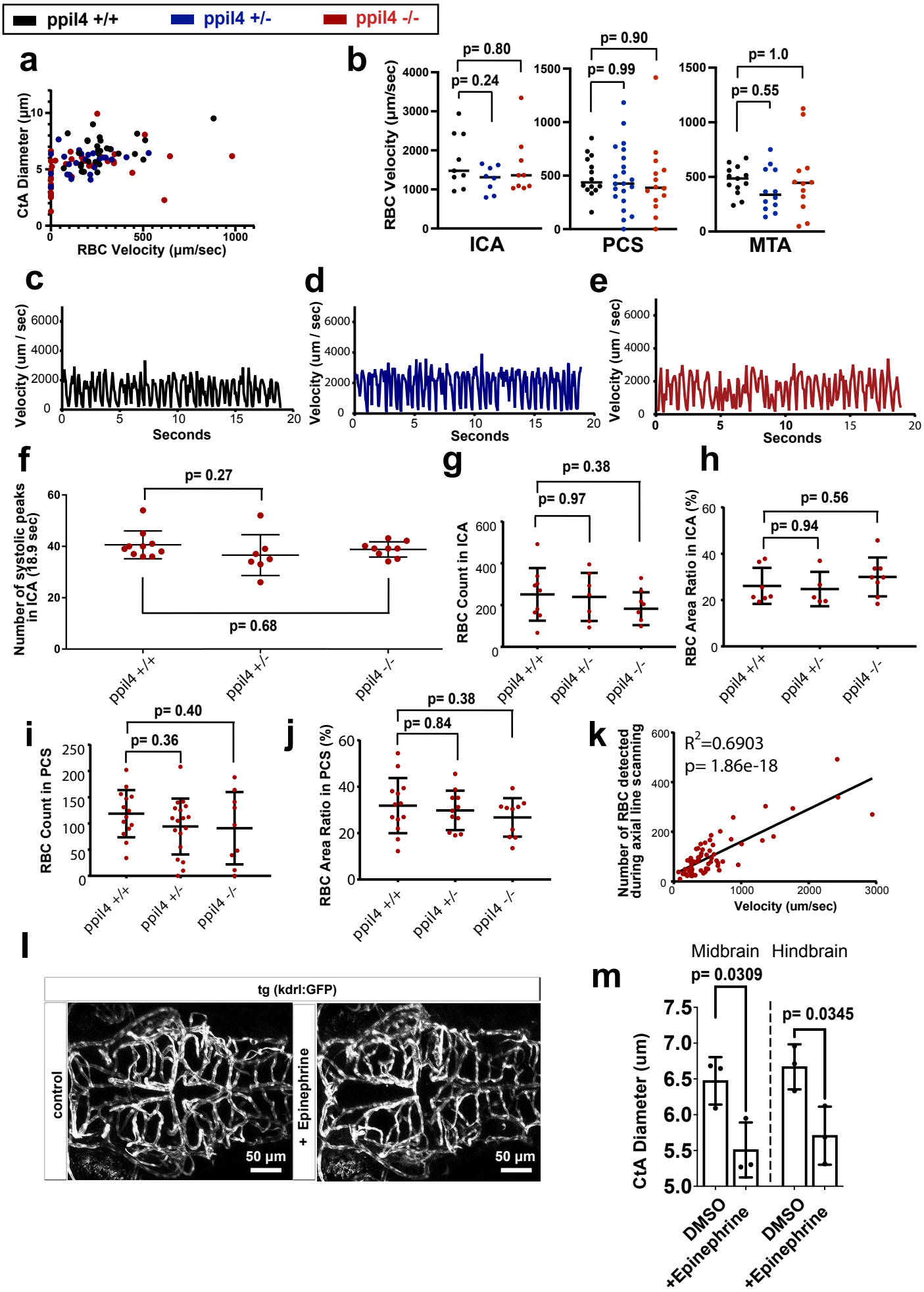
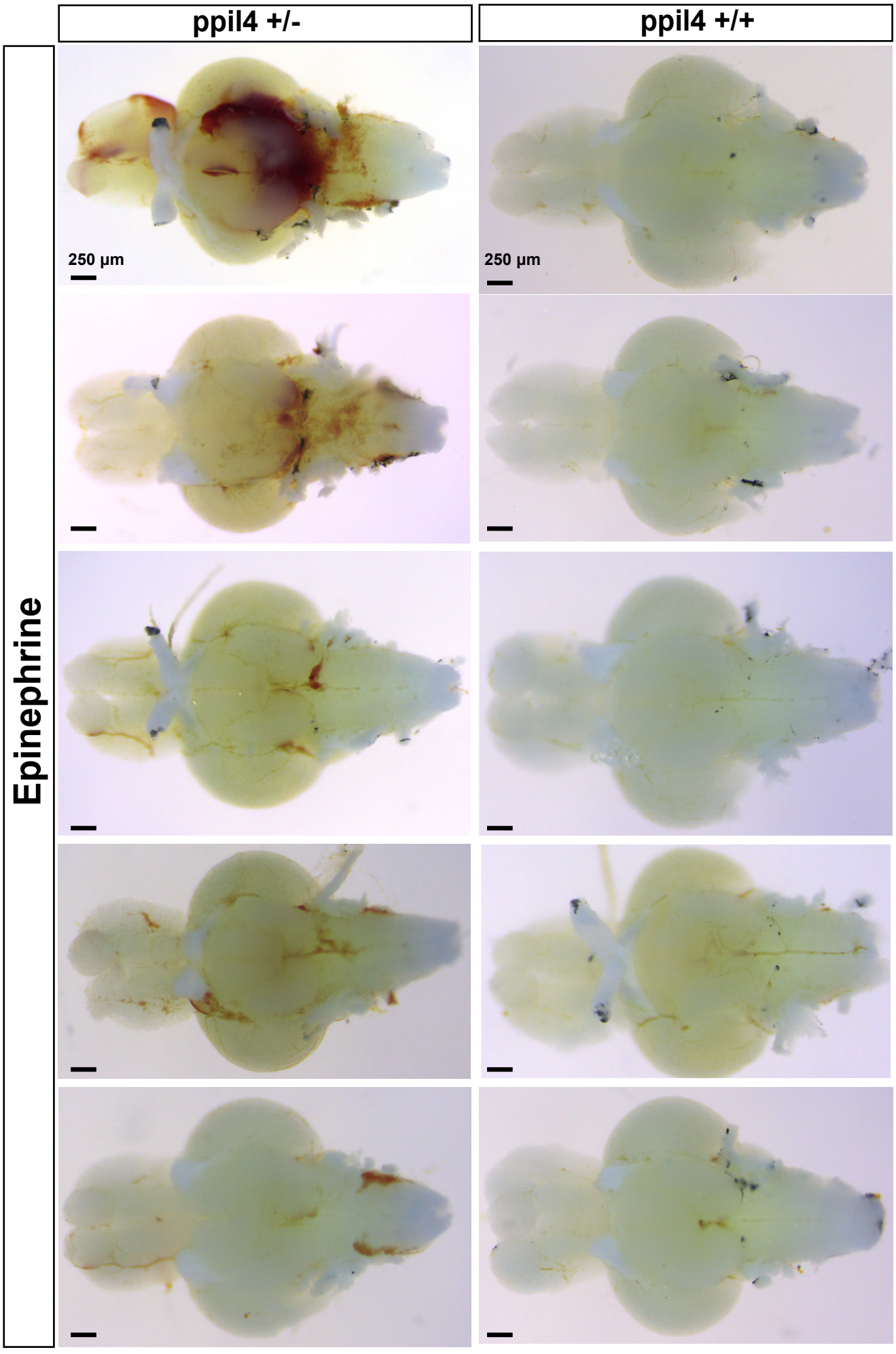
Supplementary Fig.1



Supplementary Figure 1: Impaired blood flow in central arteries is not due to systemic hemodynamic defects.

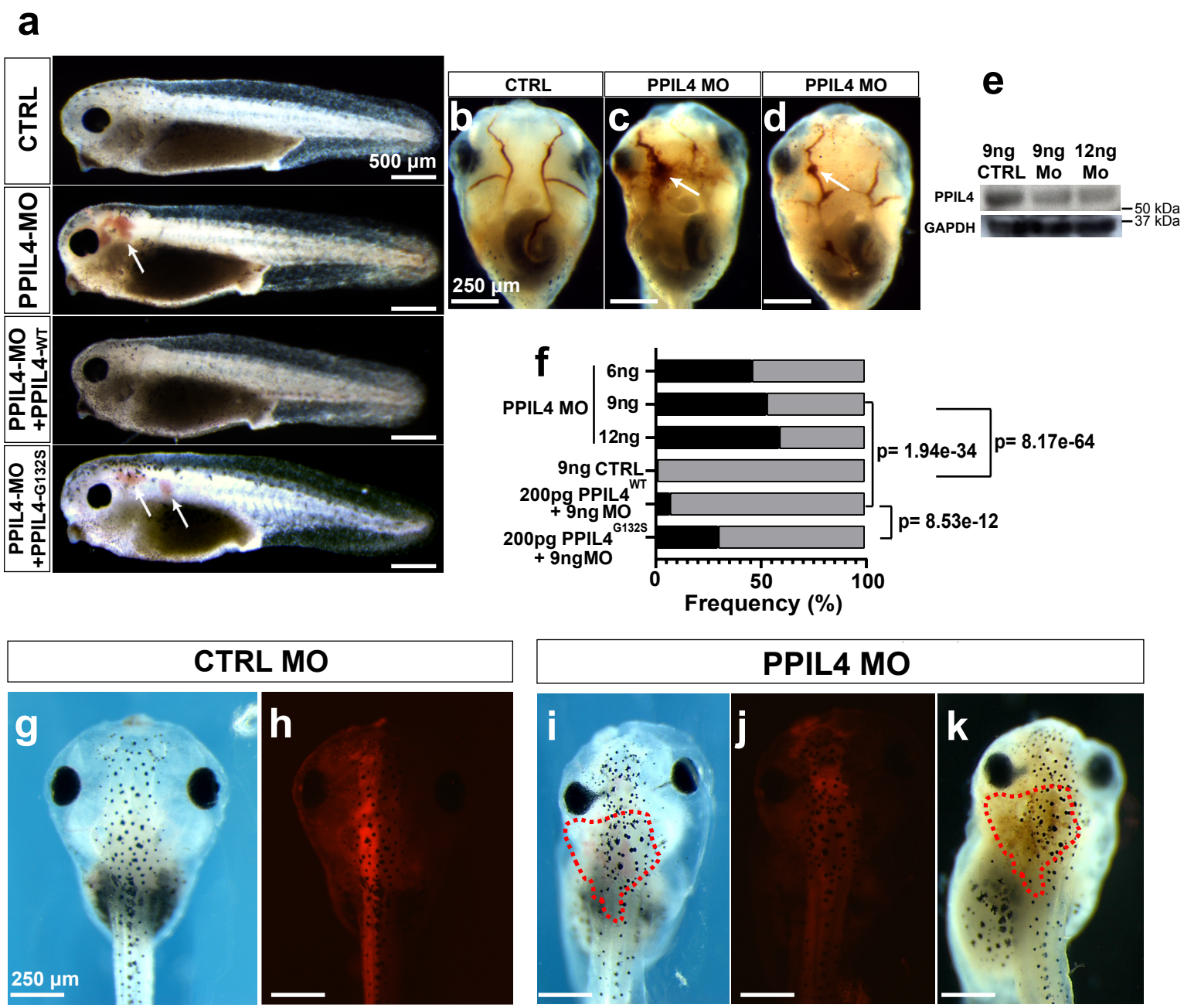
a, Scatter plot demonstrating the relationship between diameter and red blood cell (RBC) velocity in midbrain CtAs of 2.5 dpf *ppil4*$^{+/+}$(n=28), *ppil4*$^{+/-}$(n=37), *and ppil4*$^{-/-}$ (n=26). **b,** RBC velocity in internal carotid artery (ICA), n= 9, 8, 9; posterior communicating segment (PCS), n=14, 21, 14; and metencephalic artery (MTA), n= 13, 12, 12 for *ppil4 +/+, ppil4+/- and ppil4-/-* respectively. **c-e** Representative time-velocity plots showing RBC-velocity in ICA. **f,** Systolic peaks in ICA, n= 10, 7, 9 for *ppil4 +/+, ppil4+/- and ppil4-/-* respectively. **g,** Comparison of the RBC count in ICA, n= 9, 7, 7; **h,** RBC area ratio in ICA n= 7, 5, 8; **i**, RBC count in PCS, n= 14, 18, 9; **j**, RBC area ratio in PCS, n= 13, 11, 10 for *ppil4 +/+, ppil4+/- and ppil4-/-* respectively. **k,** Linear regression analysis demonstrating the relationship between the RBC-velocity and number of RBCs identified in the kymograph in *ppil4*$^{+/+}$ embryos, n= 68. **l,m**, Brain vasculature of 3dpf wildtype control and embryos treated with 0.2mM epinephrine (**l**), and comparison of the average diameter of the midbrain and hindbrain central arteries **(m)**, n=3 per genotype. Individual values shown with scatter dot plot and median in **b**. Data presented as individual values and mean with standard deviation (SD) in **f-j**; and scatter plot with mean and SD in **m**. Statistical tests: (**b, f-j)** One-way ANOVA with Dunnett's multiple comparison, **(k)** Linear regression, (**m**) Two-tailed t-test.

Supplementary Fig.2



Supplementary Figure 2: Hemodynamic stress leads to intracranial hemorrhage in adult *ppil4+/-* zebrafish.
Bright-field images of 5 representative wild type (n=19) (left) and *ppil4*$^{+/-}$ (n=51) (right) zebrafish brains, ventral view. Administration of 0.5 mg/kg epinephrine in Ringer solution via retro-orbital injection in 3-month-old zebrafish in the *tg(kdrl:gfp;gata1:dsred)* background resulting in intracranial hemorrhage (black arrows) and dilation in circle of Willis vessels. Scale bar: 250 μm.

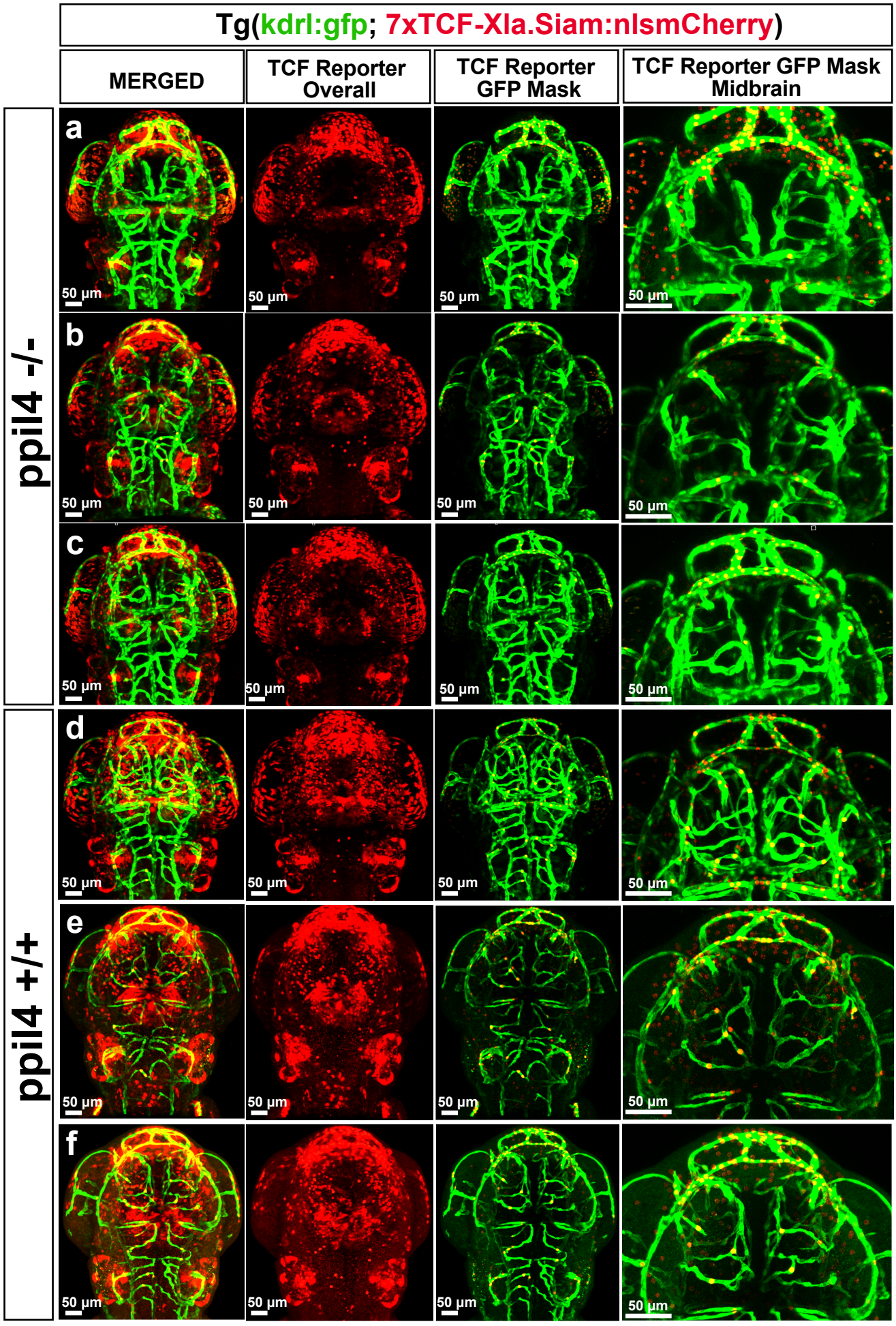
Supplementary Fig.3



Supplementary Figure 3: Downregulation of *PPIL4* results in cerebrovascular simplification and hemorrhage in *Xenopus tropicalis*.

a, Lateral view of stage 39 embryos demonstrating cerebral hemorrhage upon injection of *ppil4* translational Morpholino (MO) (but not of control morpholino), or co-injection of 200 pg human mRNA harboring the $PPIL4^{G132S}$ mutation (but not $PPIL4^{WT}$) at the one-cell stage. Arrows: Hemorrhage. **b-d,** Images of embryos treated with o-dianisidine, showing hemorrhage site in *PPIL4*-MO (right) but not in control-MO (left) injected embryos; stage 46, ventral view. **e,** Western blot analysis of whole embryos injected with 9 ng or 12 ng *ppil4* MO or control, data normalized with GAPDH, n=2 sets of biological replicates. **f**, Quantification of cerebral hemorrhage after MO and/or mRNA construct injection at the one-cell stage s. n= 258, 390, 300, 432, 274, 279 embryos for 6, 9, 12 ng *ppil4* MO, 9 ng ctrl MO, 9 ng *ppil4* MO + 200 pg $PPIL4^{WT}$, 9 ng *ppil4* MO + 200pg $PPIL4^{G132S}$, respectively. **g-k**, Dorsal view of stage 46 *X. tropicalis* embryos, injected either with 9 ng control morpholino (**g** and **h)**, or with 9 ng *ppil4* MO (**i-k**) to one side of embryos at the two-cell stage. Mini-rubi (red) used as tracer and co-injected with morpholino, injected side shown with fluorescent microscopy. **k**, O-dianisidine staining of the embryo shown in (**i**). Red dashed line encircling hemorrhagic area. Statistical tests: **(f)** Pairwise Chi-Squared test with FDR correction. Scale bar: 500 μm in **a**, and 250 μm in **b,g-k**

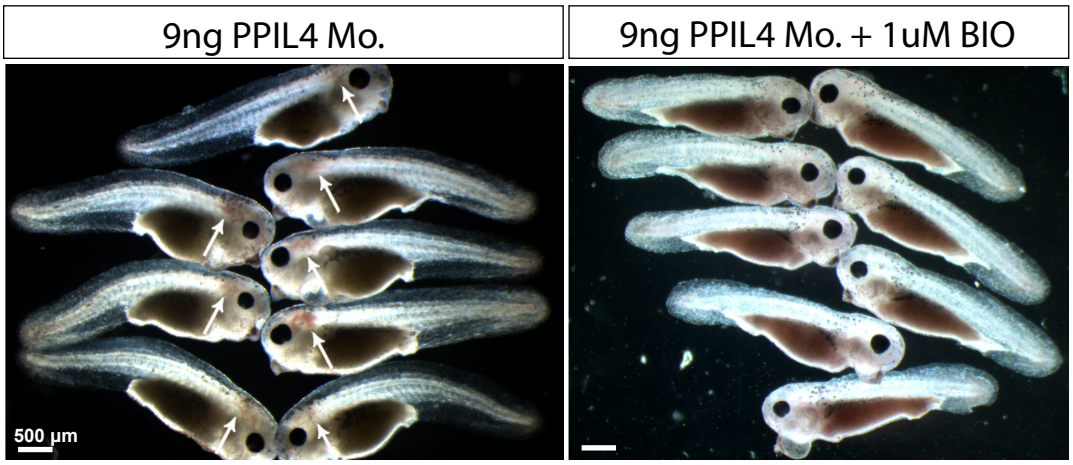
Supplementary Fig.4



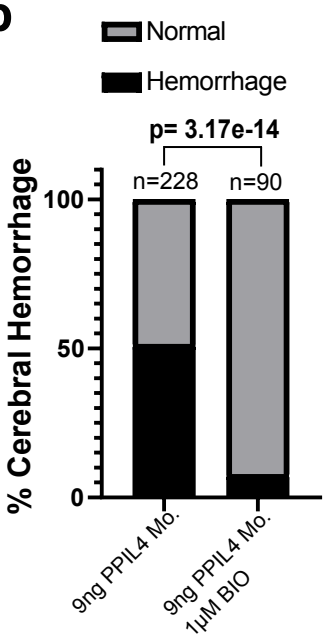
Supplementary Figure 4: *ppil4* depletion leads to impaired Wnt signaling activation in zebrafish brain parenchyma and midbrain CtAs at 60hpf.
a-c, Maximum intensity projection (MIP) of confocal z-stack images of three representative 60 hpf *ppil4*$^{-/-}$ (n=11) and **d-f,** *ppil4*$^{+/+}$ (n=10) zebrafish embryos in double transgenic *Tg(kdrl:gfp; 7xTCF-Xla.Siam:nlsmCherry)* background to visualize Wnt signaling activity (red) and endothelial cells (*green)* (dorsal-view; caudal facing up). The TCF reporter signal, quantified using the Spots application in Imaris, shows loss of TCF reporting cells in brain parenchyma and midbrain CtAs of *ppil4*$^{-/-}$ embryos. Endothelial specific Wnt-activation is calculated using Spots-Mask for the GFP channel. See methods for details of image processing and assembly.

Supplementary Fig.5

a



b



Supplementary Figure 5: Restoring Wnt signaling activation prevents cerebral hemorrhage in *X. tropicalis* embryos.
a, Lateral view of stage 39 (3 dpf) embryos demonstrating cerebral hemorrhage (arrow) in embryos injected with 9 ng *ppil4* translational MO at the one-cell stage (n=228) (left) and rescue of cerebral hemorrhage upon treatment with the WNT activator 6-bromoindirubin-3'-oxime [BIO] (1 μM) (n=90) (right). **b,** Quantification and comparison of cerebral hemorrhage ratio in **(a).** Statistical tests performed: Chi-squared test. Scale bar: 500 μm

Supplementary Fig.6

Original Gel Images for Supplementary Fig.3e

Probed for PPIL4	Reprobed for GAPDH