

Apelin as a CNS-specific Pathway for Fenestrated Capillary Formation in the Choroid Plexus

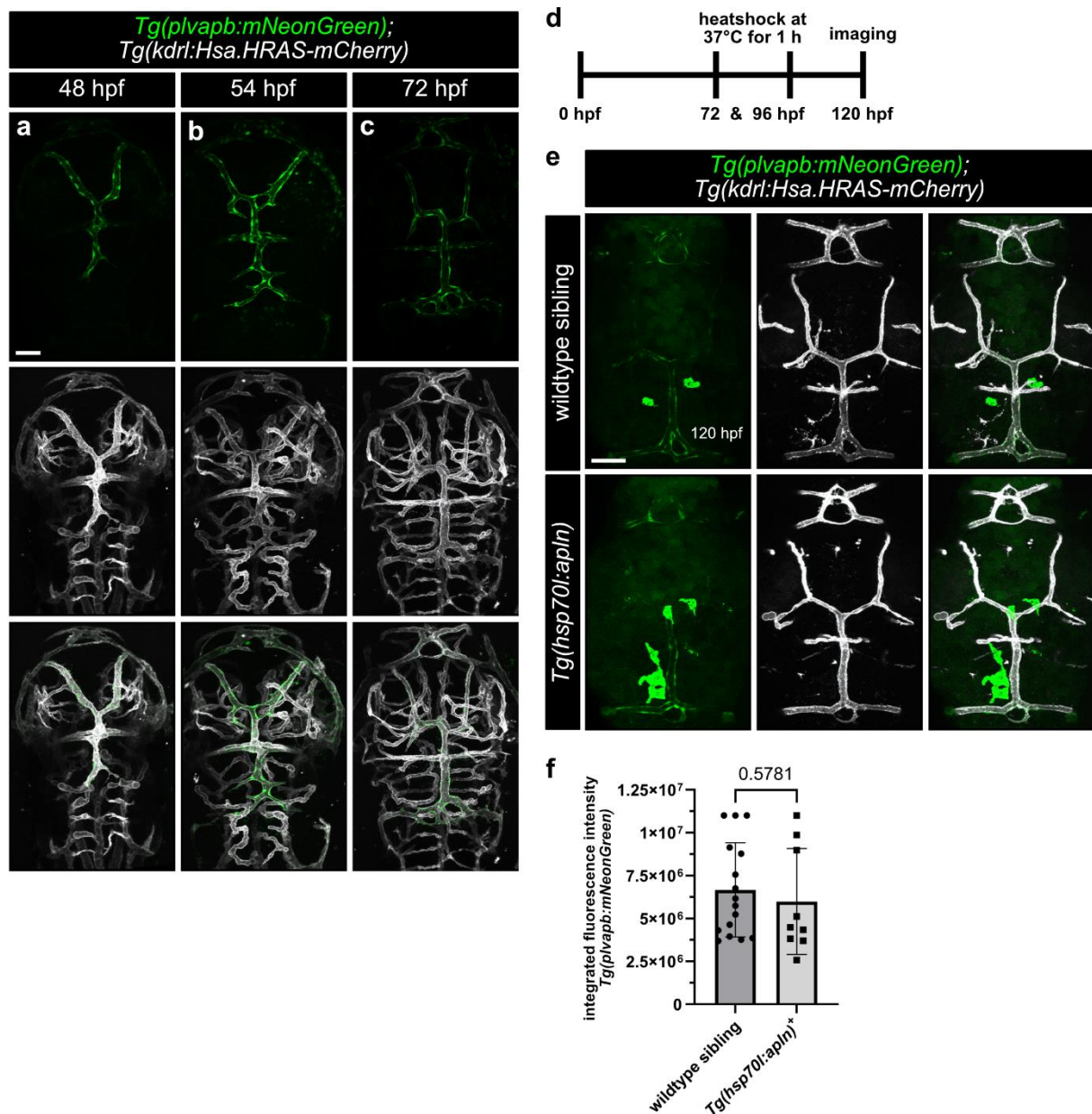
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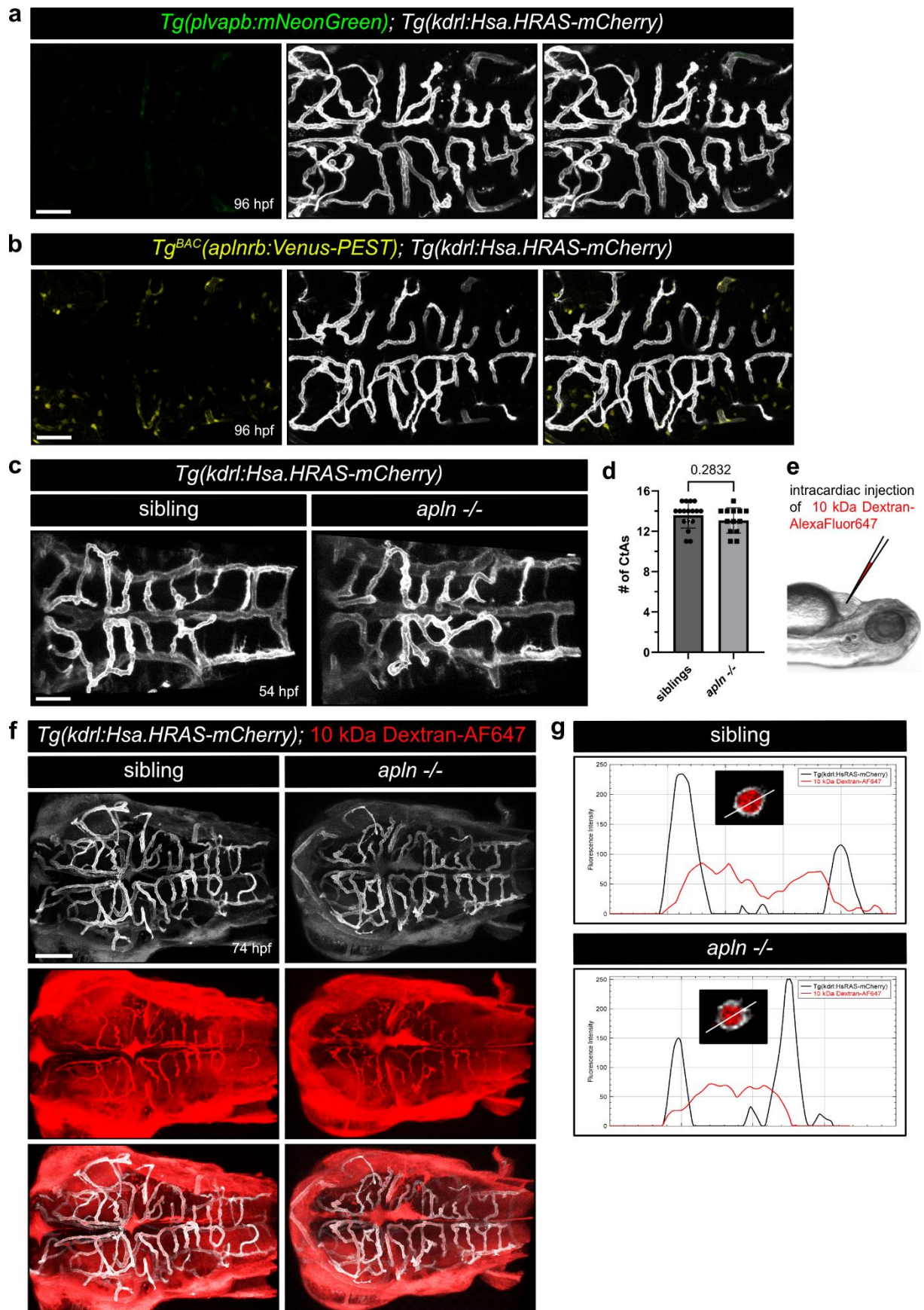
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Supplement Figure 1-10

Supplement Movie 1-6

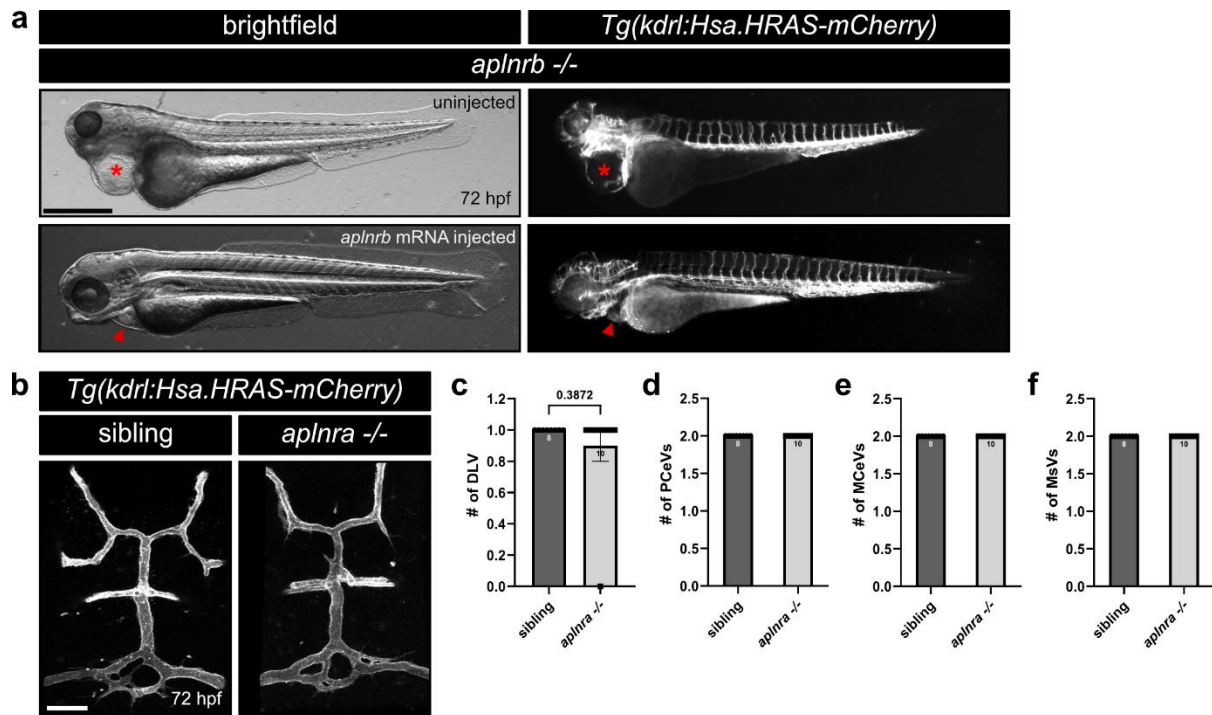


Supplement Fig. 1. Apelin signaling does not regulate *plvapb* expression in fenestrated blood vessels. (a-c) Confocal projection images of the cerebral vasculature in *Tg(plvapb:mNeonGreen); Tg(kdrl:Hsa.HRAS-mCherry)* larvae at 48 (a), 54 (b) and 72 hpf (c). (d) Schematic illustration of the experimental design. (e) Confocal projection images of the CP vasculature in *Tg(plvapb:mNeonGreen); Tg(kdrl:Hsa.HRAS-mCherry); Tg(hsp70l:apln)* and wildtype sibling larvae heat shocked for 1h at 37°C at 72 and 96 hpf and imaged at 120 hpf. (f) Quantification of integrated fluorescence intensity of *Tg(plvapb:mNeonGreen)* within the cerebral vasculature of heat shocked wildtype siblings and *Tg(hsp70l:apln)* larvae at 120 hpf. Statistical analysis was performed by using two-tailed unpaired Student's t-test with Welch's correction. Data is represented as mean $\pm$ StD. Scale bars: 50 μ m. CP – choroid plexus. Source data are provided as a Source Data file.

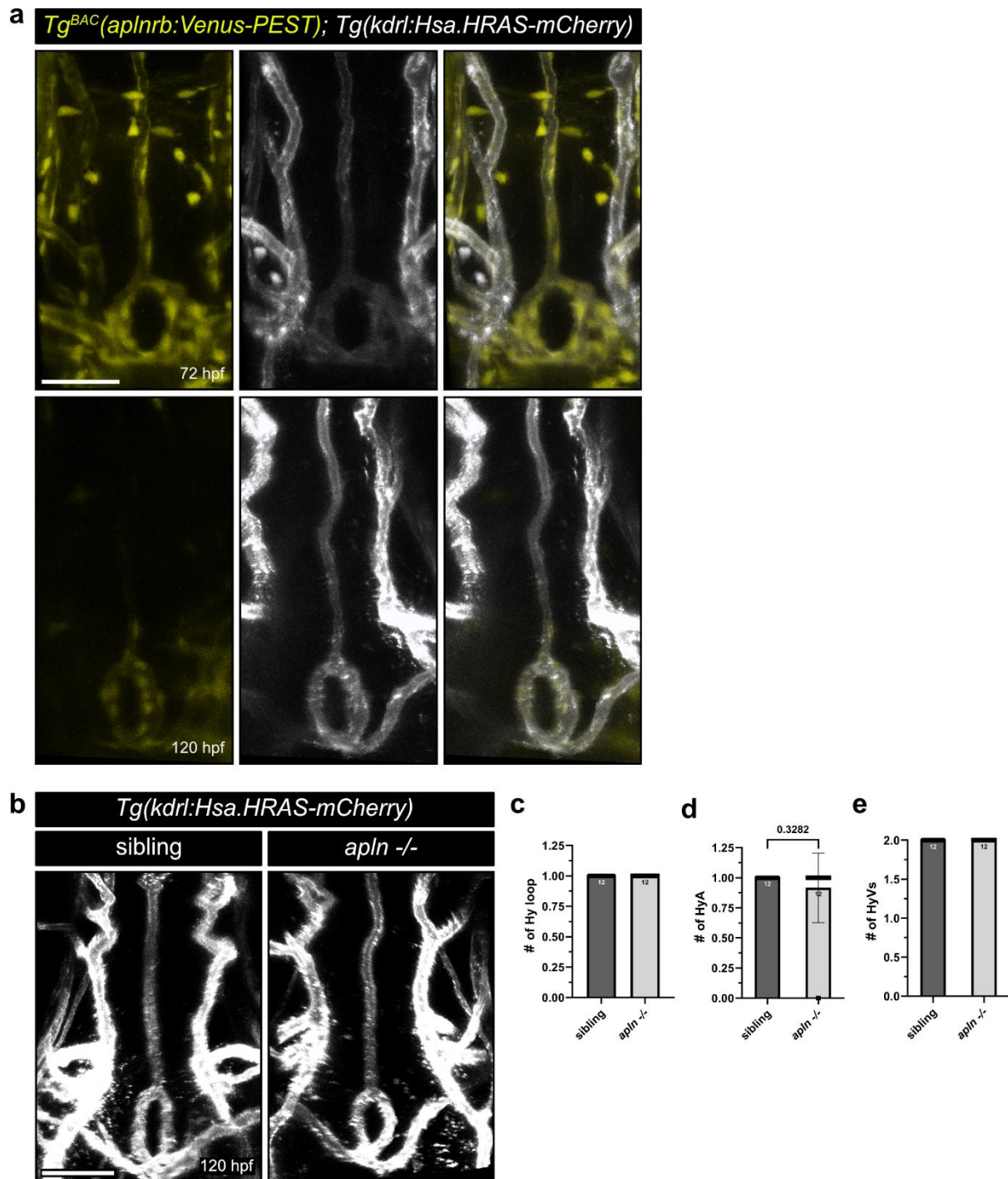


Supplement Figure 2. Apelin signaling is not required for BBB vessel formation and barrierogenesis. (a-b) Confocal projection images of the BBB-forming central arteries (CTAs) in *Tg(plvapb:mNeonGreen); Tg(kdrl:Hsa.HRAS-mCherry)* (a) and *Tg^{BAC}(aplnrb:Venus-PEST); Tg(kdrl:Hsa.HRAS-mCherry)* (b) larvae at 96 hpf. (c) Confocal projection images of hindbrain CTAs of

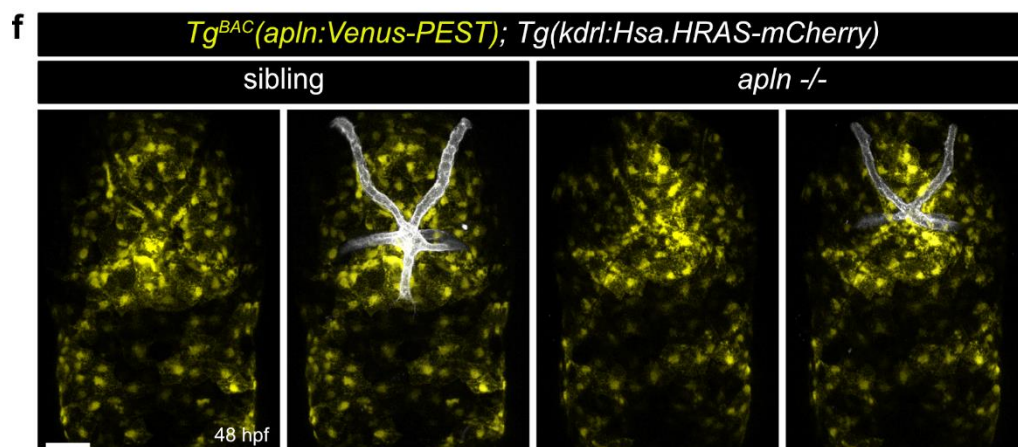
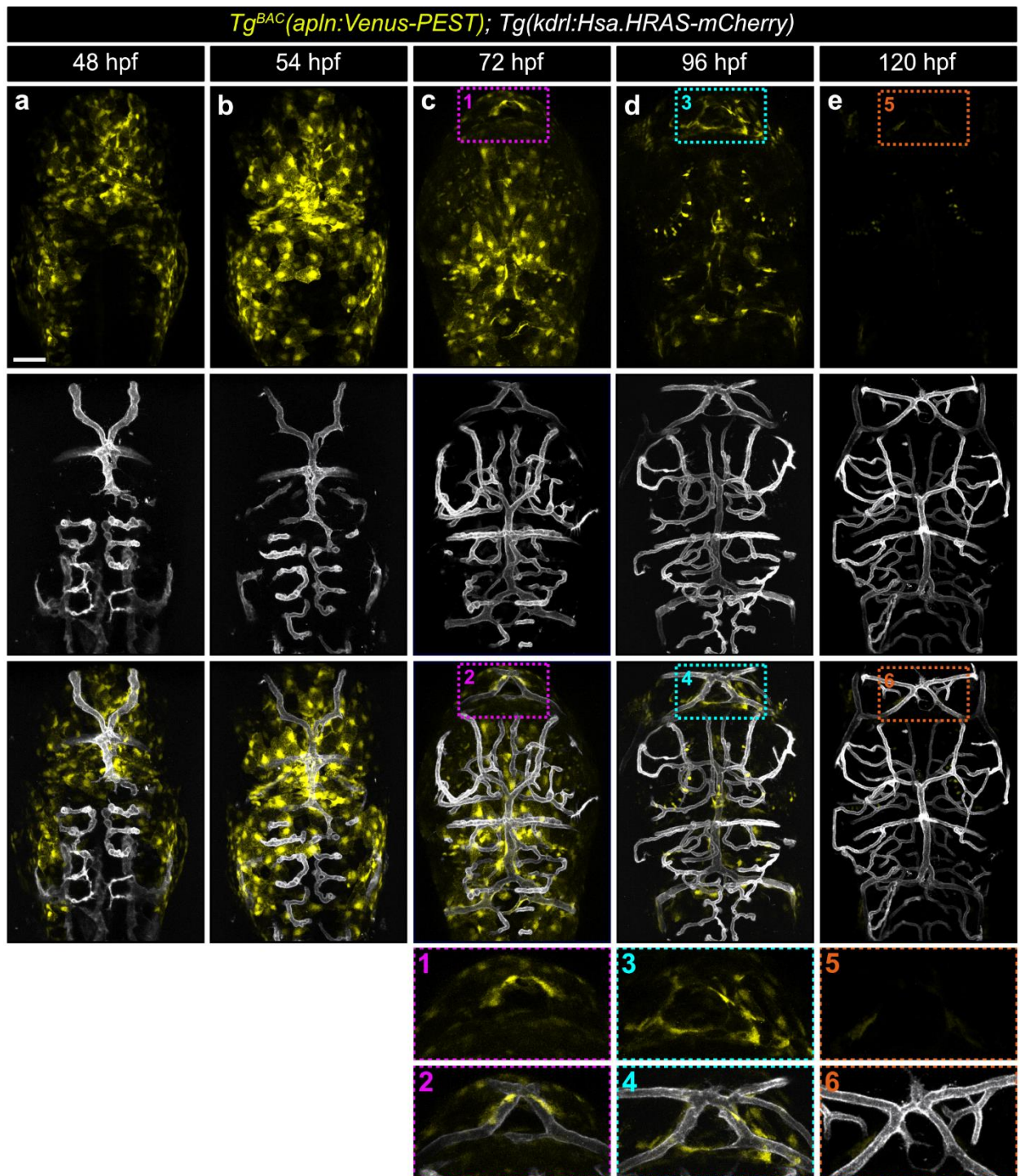
Tg(kdrl:Hsa.HRAS-mCherry) siblings and *apln* mutant larvae at 54 hpf. **(d)** Quantification of CtA numbers in siblings and *apln* mutant larvae at 54 hpf (n=17 for siblings; n=13 for *apln* ^{-/-}). **(e)** Schematic illustration of the intracardiac injection. **(f)** Confocal projection images of the cerebral vasculature of *Tg(kdrl:Hsa.HRAS-mCherry)* siblings and *apln* mutant larvae intracardially injected with a 10 kDa Dextran-AlexaFluor647 at 74 hpf. **(g)** Fluorescence intensity plots of *kdrl*:Hsa.HRAS-mCherry (black line) and of the injected Dextran (red line) in a single hindbrain CtA of a sibling and an *apln* mutant larvae. Statistical analysis was performed by using two-tailed unpaired Student's t-test with Welch's correction. Data is represented as mean $\pm$ StD. Scale bars: **(a-c)** 50 μ m; **(e)** 100 μ m. hpf – hours post fertilization. Source data are provided as a Source Data file.



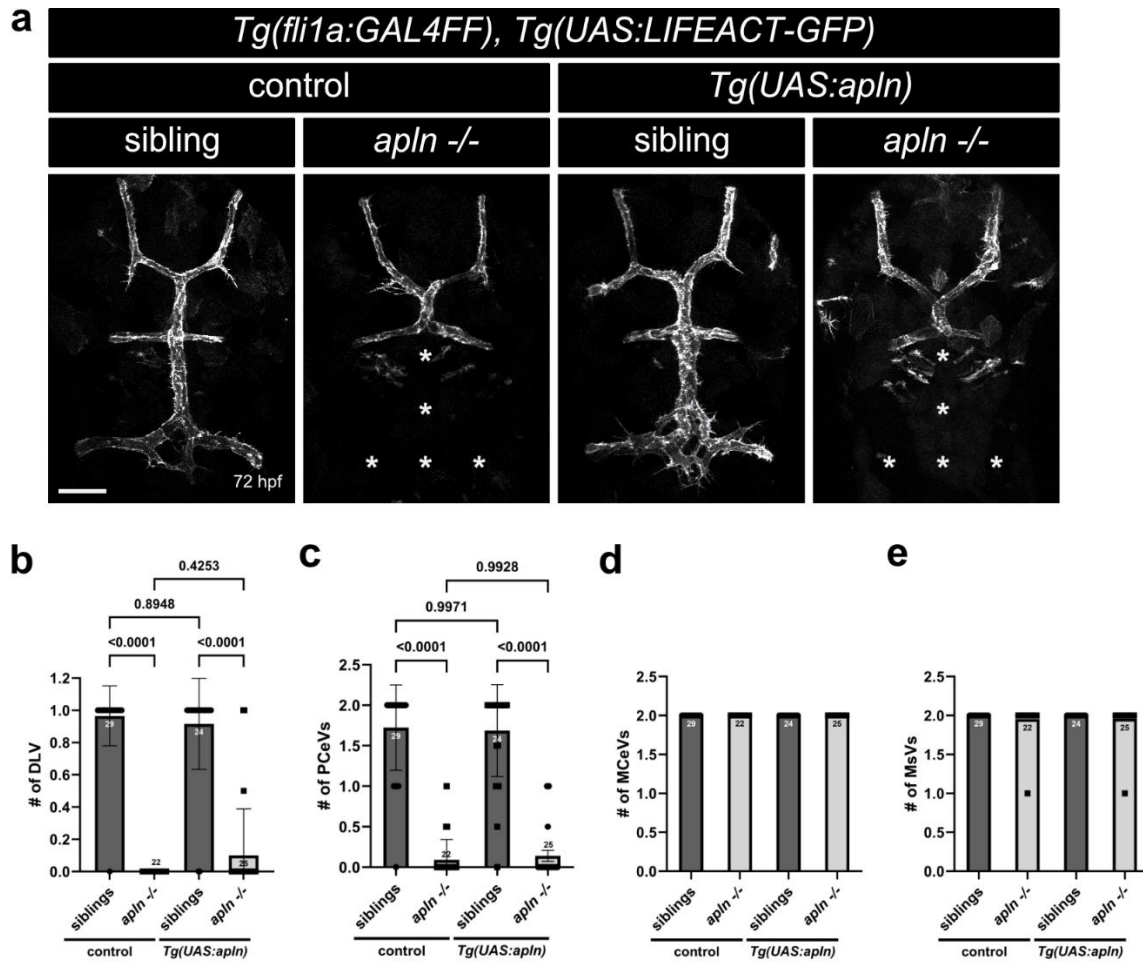
Supplement Figure 3. *Aplnr b* but not *Aplnr a* is required for fenestrated vessel formation. (a) Lateral view of *Tg(kdrl:Hsa.HRAS-mCherry)* *aplnr b* mutants with and without *aplnr b* mRNA injection at 72 hpf. *aplnr b* mRNA injection rescued the heart defects in *aplnr b* mutants (arrow head) compared to control mutants (asterisk). (b) Confocal projection images of the dorsal cerebral vasculature of *Tg(kdrl:Hsa.HRAS-mCherry)* siblings and *aplnr a* mutant larvae. (c-f) Quantification of DLV (c), PCeV (d), MCeV (e) and MsV (f) formation in siblings and *aplnr a* mutant larvae at 72 hpf (n=8 for siblings; n=10 for *aplnr a* mutant larvae). Statistical analysis was performed by using two-tailed unpaired Student's t-test with Welch's correction. Data is represented as mean $\pm$ StD. Scale bars: 500 μ m (a), 50 μ m (b). hpf – hours post fertilization; DLV – dorsal longitudinal vein; PCeV – posterior cerebral vein; MCeV – midcerebral vein; MsV – mesencephalic cerebral vein. Source data are provided as a Source Data file.



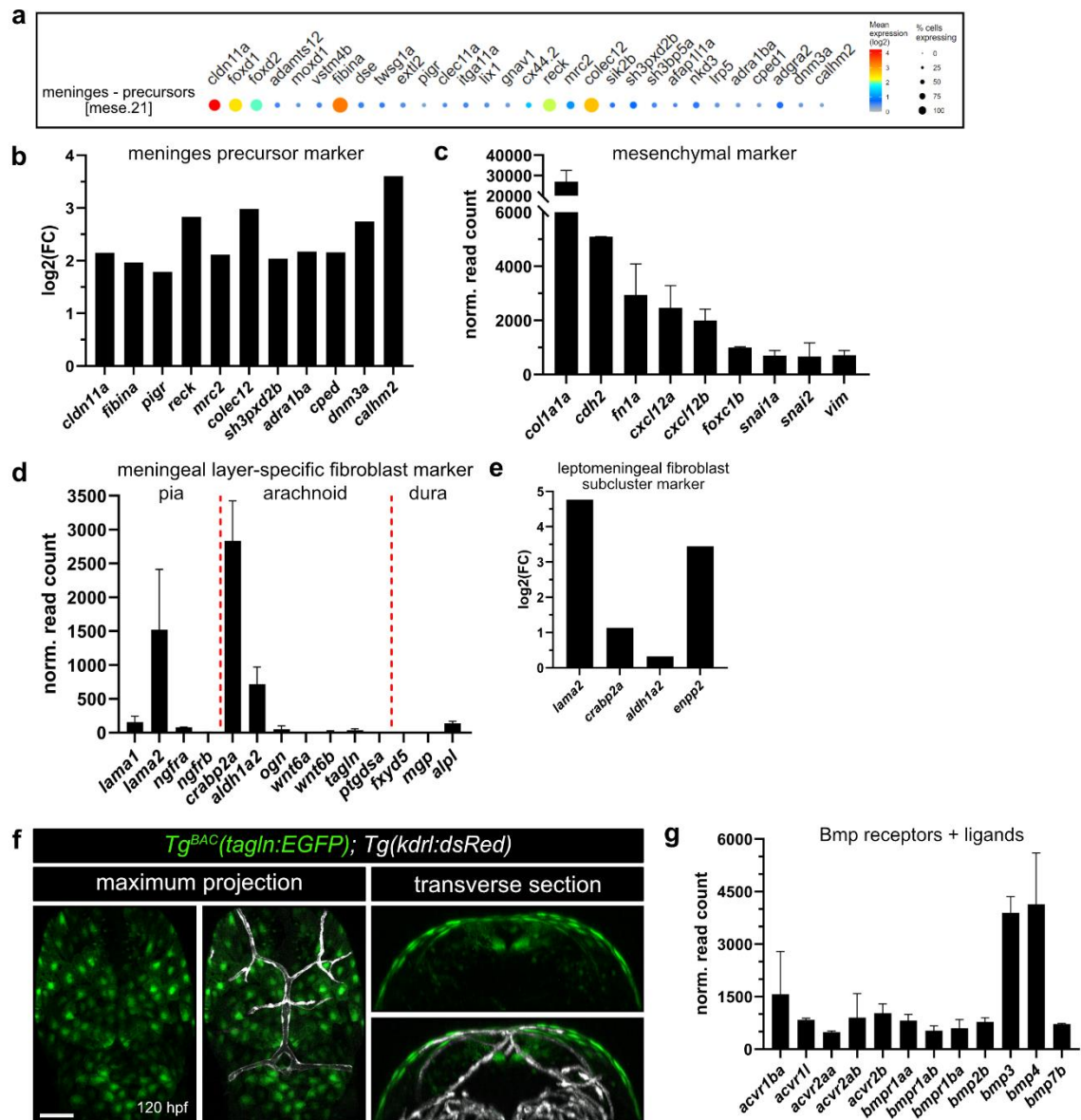
Supplement Figure 4. Apelin signaling is not required for the vascularization of the neurohypophysis. (a) Confocal projection images of the neurohypophysis vasculature of *Tg^{BAC}(aplnrb:Venus-PEST); Tg(kdrl:Hsa.HRAS-mCherry)* larvae at 72 and 120 hpf. (b) Confocal projection images of the neurohypophysis vasculature of *Tg(kdrl:Hsa.HRAS-mCherry)* siblings and *apln* mutant larvae at 120 hpf. (c-e) Quantification of Hy loop (c), HyA (d) and HyVs (e) formation in siblings and *apln* mutant larvae at 120 hpf (n=12 for siblings; n=12 for *apln*^{-/-}). Statistical analysis was performed by using two-tailed unpaired Student's t-test with Welch's correction. Data is represented as mean $\pm$ StD. Scale bars: 50 μ m. hpf – hours post fertilization; Hy – hypophyseal; HyA – hypophyseal artery; HyVs – hypophyseal veins. Source data are provided as a Source Data file.



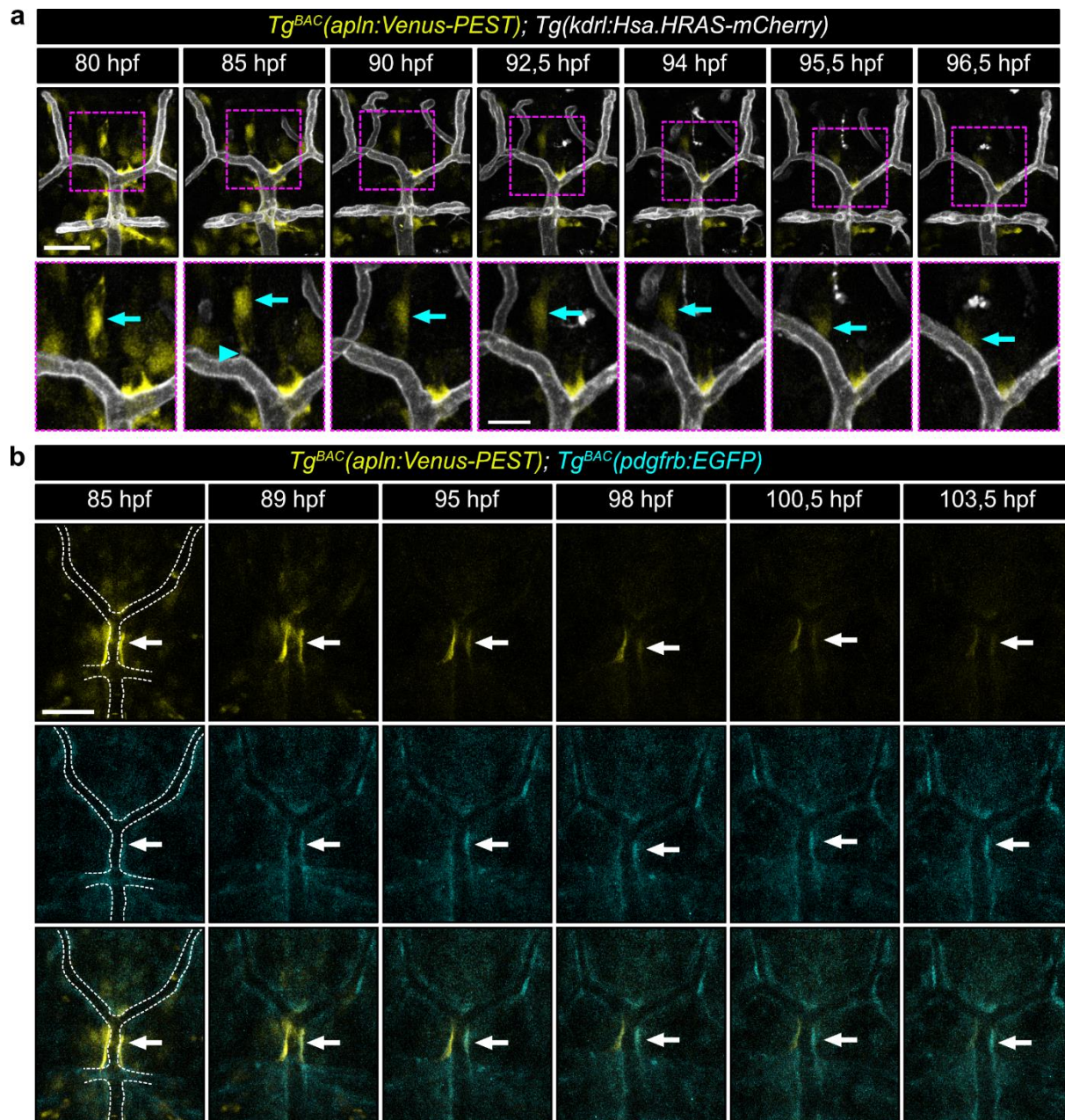
Supplement Figure 5. Apelin expression is spatiotemporally controlled during fenestrated blood vessel formation. Confocal projection images of the brain in *Tg^{BAC}(apln:Venus-PEST); Tg(kdrl:Hsa.HRAS-mCherry)* larvae at 48 (a), 54 (b), 72 (c), 96 (d) and 120 hpf. (c-e) Magnified images of the dCP vasculature are indicated by a dashed box at 72 hpf (c1-2), 96 hpf (d3-4) and 120 hpf (e5-6). (a-e) *apln:Venus-PEST* expression is observed in cells in the surrounding of the sprouting fenestrated blood vessels (a-d), but becomes downregulated after the blood vessels are formed (e). *apln:Venus-PEST* expression is also observed in perivascular cells (c-d) and in neurons of the optic tectum (d-e). (f) Confocal projection images of the dorsal cerebral vasculature of *Tg^{BAC}(apln:Venus-PEST); Tg(kdrl:Hsa.HRAS-mCherry)* siblings and *apln* mutant larvae at 48 hpf. Scale bars: 50 μ m. dCP – diencephalic choroid plexus



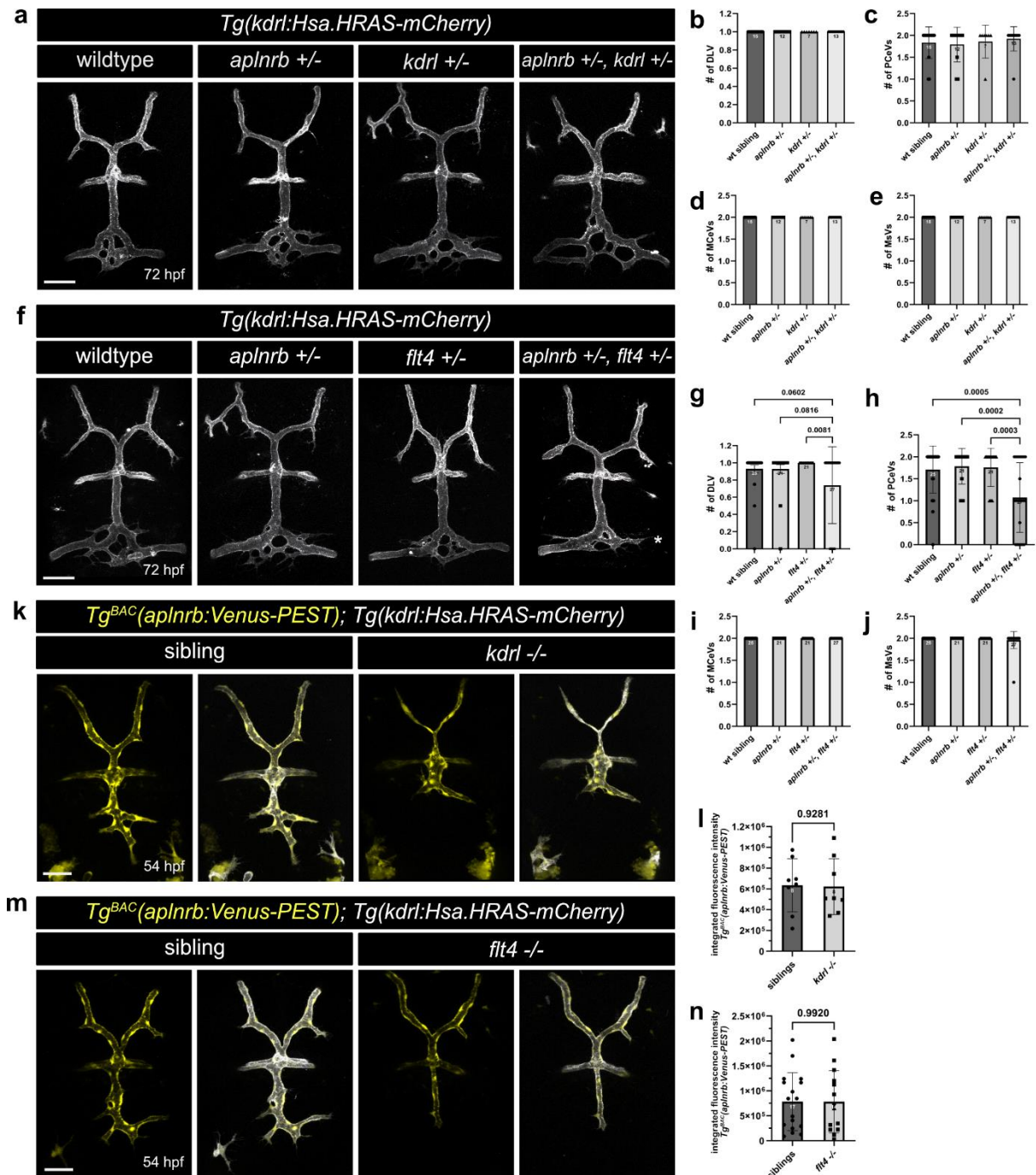
Supplement Fig. 6. Endothelium-derived autocrine Apelin is not required for fenestrated blood vessel formation in the mCP. (a) Confocal projection images of the mCP vasculature of *Tg(fli1a:GAL4FF)*; *Tg(UAS:LIFEACT-GFP)*; *Tg(UAS:apln)* siblings and *apln* mutant larvae at 72 hpf. (b-e) Quantification of DLV (b), PCeV (c), MCeV (d) and MsV (e) formation in siblings and *apln* mutant larvae with and without vascular *apln* overexpression (OE) at 72 hpf (n=29 for siblings without *apln* OE; n=22 for *apln* mutant larvae without *apln* OE; n=24 for siblings with *apln* OE; n=25 for *apln* mutant larvae with *apln* OE). Statistical analysis was performed by using ordinary One-way ANOVA with Dunnett's correction. Data is represented as mean $\pm$ StD. Scale bars: 50 μ m. mCP – myelencephalic choroid plexus; hpf – hours post fertilization; DLV – dorsal longitudinal vein; PCeV – posterior cerebral vein; MCeV – midcerebral vein; MsV – mesencephalic cerebral vein. Source data are provided as a Source Data file.



Supplement Figure 7. Marker analysis of *apln* expressing meningeal progenitor population. (a) Most specific markers for *apln* expressing meningeal precursors in zebrafish from public available DanioCell scRNA-seq database (Sur et al. 2023). (b-e) Expression analysis of meningeal precursor markers (b), mesenchymal markers (c) pia, arachnoid and dural fibroblast markers (d) and leptomeningeal fibroblast subcluster markers in *apln*:Venus-PEST expressing cells (e). (f) Confocal projection images of the brain of *Tg^{BAC}(tagln:EGFP); Tg(kdr:dsRed)* larvae at 120 hpf in a maximum projection and transverse section. Scale bar: 50 μ m. hpf – hours post fertilization. (g) Expression analysis of Bmp receptors and ligands in *apln*:Venus-PEST expressing cells. Data in (c, d, g) are presented as mean $\pm$ StD. Source data are provided as a Source Data file.

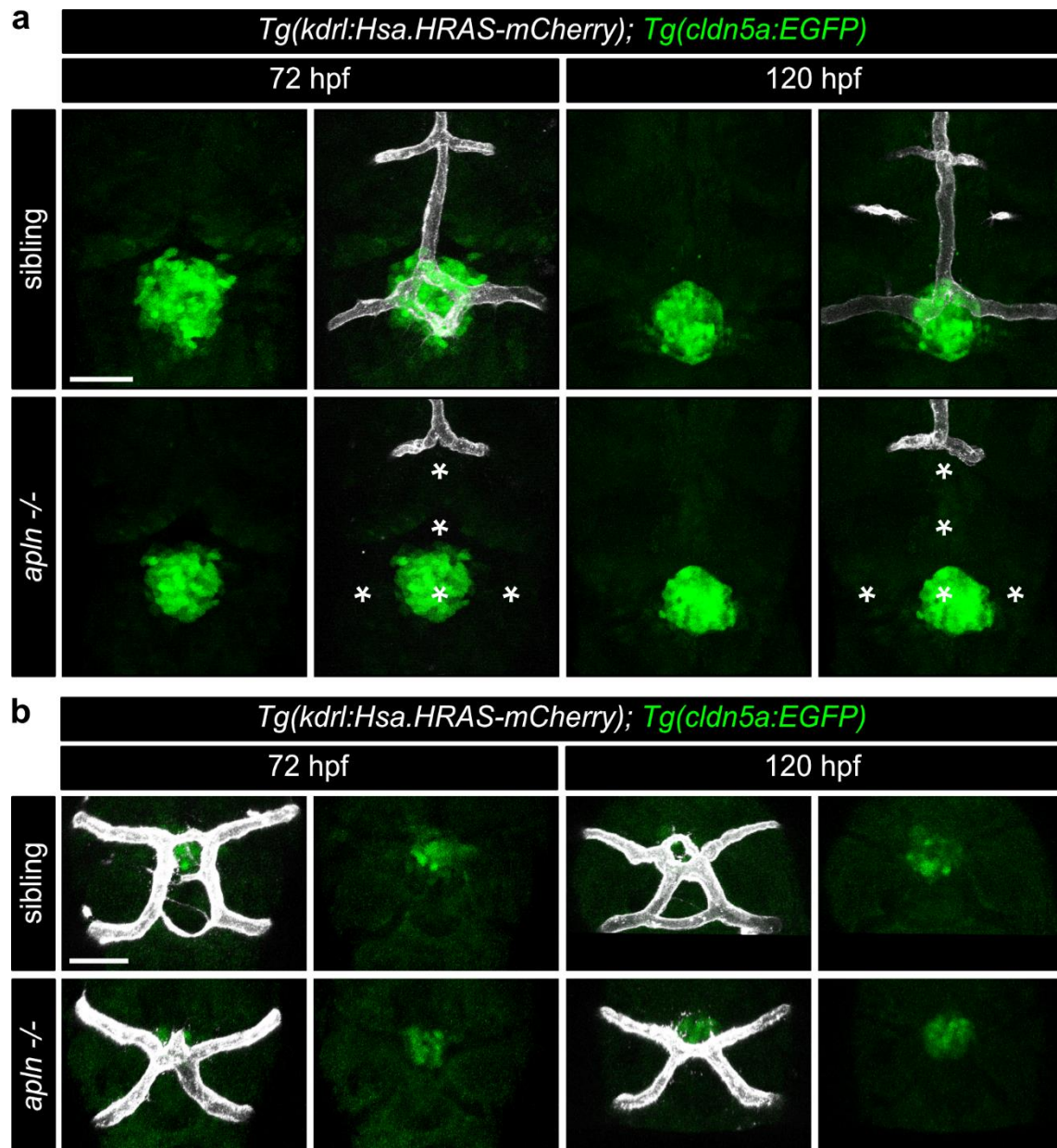


Supplement Figure 8. *apln* expressing meningeal subpopulation transition into perivascular cells. (a) Still images are taken from a time-lapse video of *Tg^{BAC}(apln:Venus-PEST); Tg(kdrl:Hsa.HRAS-mCherry)* larvae from 80-98 hpf. Arrows indicate an *apln:Venus-PEST* expressing cell migrating towards the blood vessel. Arrow head points towards the contact of the *apln:Venus-PEST* positive cell with the blood vessel. (b) Still images are taken from a time-lapse video of *Tg^{BAC}(apln:Venus-PEST); Tg^{BAC}(pdgfrb:EGFP)* larvae from 85-104 hpf. Arrow indicate a perivascular cell co-expressing *apln:Venus-PEST* and *pdgfrb:EGFP*. Scale bars: 25 μ m.



Supplement Fig. 9. Apelin receptor and Vegfr3/Flt4 signaling genetically interact during mCP vascularization. (a) Confocal projection images of the mCP vasculature of *Tg(kdrl:Hsa.HRAS-mCherry)* siblings and *aplnrb*, *kdrl* double heterozygous larvae at 72 hpf. (b-e) Quantification of DLV (b), PCeV (c), MCeV (d) and MsV (e) formation in siblings and *aplnrb*, *kdrl* double heterozygous larvae at 72 hpf (n=15 wildtype sibling; n=12 for *aplnrb* +/-; n=7 for *kdrl* +/-; n=13 for *aplnrb* +/-, *kdrl* +/-). (f) Confocal projection images of the mCP vasculature of *Tg(kdrl:Hsa.HRAS-mCherry)* siblings and *aplnrb*, *flt4* double heterozygous larvae at 72 hpf. (g-j) Quantification of DLV (g), PCeV (h), MCeV (i) and MsV (j) formation in siblings and *aplnrb*, *flt4* double heterozygous larvae at 72 hpf. (n=25 wildtype sibling; n=21 for *aplnrb* +/-; n=21 for *flt4* +/-; n=27 for *aplnrb* +/-, *flt4* +/-) (k) Confocal projection images of the mCP vasculature of *Tg^{BAC}(aplnrb:Venus-PEST); Tg(kdrl:Hsa.HRAS-mCherry)* siblings and *kdrl* mutant larvae at 54 hpf. (l) Quantification of integrated fluorescence intensity of *Tg^{BAC}(aplnrb:Venus-PEST)* within the sprouting mCP vasculature in siblings and *kdrl* mutant larvae at 54 hpf. (n=8 wildtype sibling; n=8 for *kdrl* -/-) (m) Confocal projection images of the mCP vasculature of *Tg^{BAC}(aplnrb:Venus-PEST); Tg(kdrl:Hsa.HRAS-mCherry)* siblings and *flt4* mutant larvae at 54 hpf. (n) Quantification of integrated fluorescence intensity of *Tg^{BAC}(aplnrb:Venus-PEST)* within the sprouting mCP vasculature in siblings

and *flt4* mutant larvae at 54 hpf. (n=17 wildtype sibling; n=14 for *flt4* -/-). Statistical analysis was performed by using ordinary One-way ANOVA with Dunnett's correction (**g-h**) and two-tailed unpaired Student's t-test with Welch's correction (**l, n**). Data is represented as mean $\pm$ StD. Scale bars: 50 μ m. mCP – myelencephalic choroid plexus; hpf – hours post fertilization; DLV – dorsal longitudinal vein; PCeV – posterior cerebral vein; MCeV – midcerebral vein; MsV – mesencephalic cerebral vein. Source data are provided as a Source Data file.



Supplement Figure 10. Apelin signaling is not required for larval choroid plexi morphogenesis. (a-b) Confocal projection images of the mCP (a) and dCP (b) with their associated vasculature of *Tg(kdr1:Hsa.HRAS-mCherry); Tg(cldn5a:EGFP)* siblings and *apln* mutant larvae at 72 and 120 hpf. Asterisks indicate missing blood vessels in the mCP in *apln* mutant larvae (a). Scale bars: 50 μ m. hpf – hours post fertilization; dCP – diencephalic choroid plexus; mCP – myelencephalic choroid plexus

Supplement Movie 1.

Time-lapse movie of DLV and PCeV sprouting in a *Tg(kdrl:Hsa.HRAS-mCherry); Tg(fli1a:nEGFP)* wildtype larvae from 45-67 hpf.

Supplement Movie 2.

Time-lapse movie of DLV and PCeV sprouting in a *Tg(kdrl:Hsa.HRAS-mCherry); Tg(fli1a:nEGFP) apln* mutant larvae from 45-67 hpf.

Supplement Movie 3.

Time-lapse movie of the brain in *Tg^{BAC}(apln:Venus-PEST); Tg(kdrl:Hsa.HRAS-mCherry)* larvae from 80-98 hpf.

Supplement Movie 4.

Time-lapse movie of the brain in *Tg^{BAC}(apln:Venus-PEST); Tg^{BAC}(pdgfrb:EGFP)* larvae from 85-104 hpf.

Supplement Movie 5.

Time-lapse movie of the cerebrospinal fluid flow in the hindbrain ventricle of a wildtype larvae injected with fluorescently labeled microspheres at 72 hpf. Images were captured every 200 ms and presented at 50 frames per second.

Supplement Movie 6.

Time-lapse movie of the cerebrospinal fluid flow in the hindbrain ventricle of an *apln* mutant larvae injected with fluorescently labeled microspheres at 72 hpf. Images were captured every 200 ms and presented at 50 frames per second.