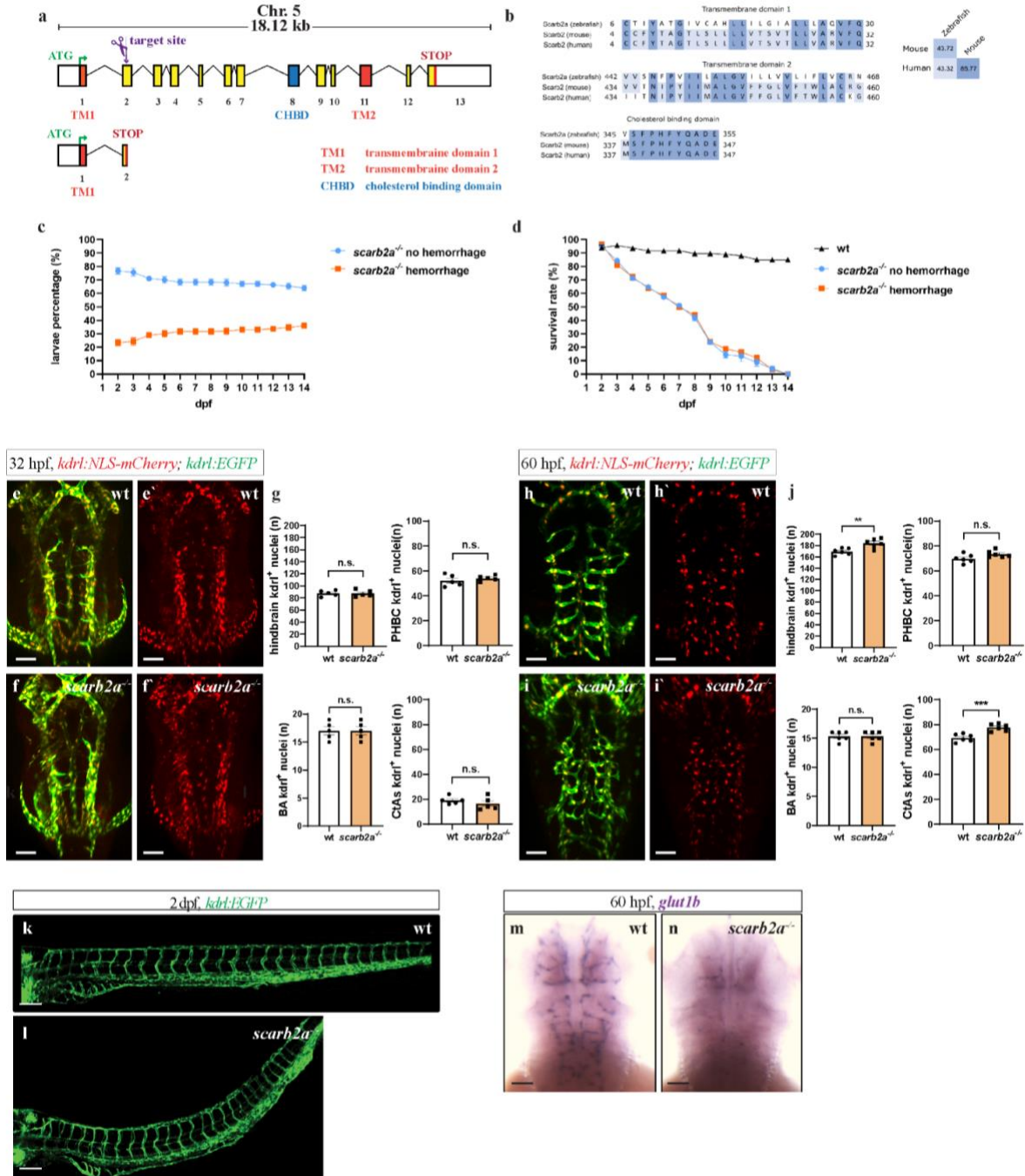
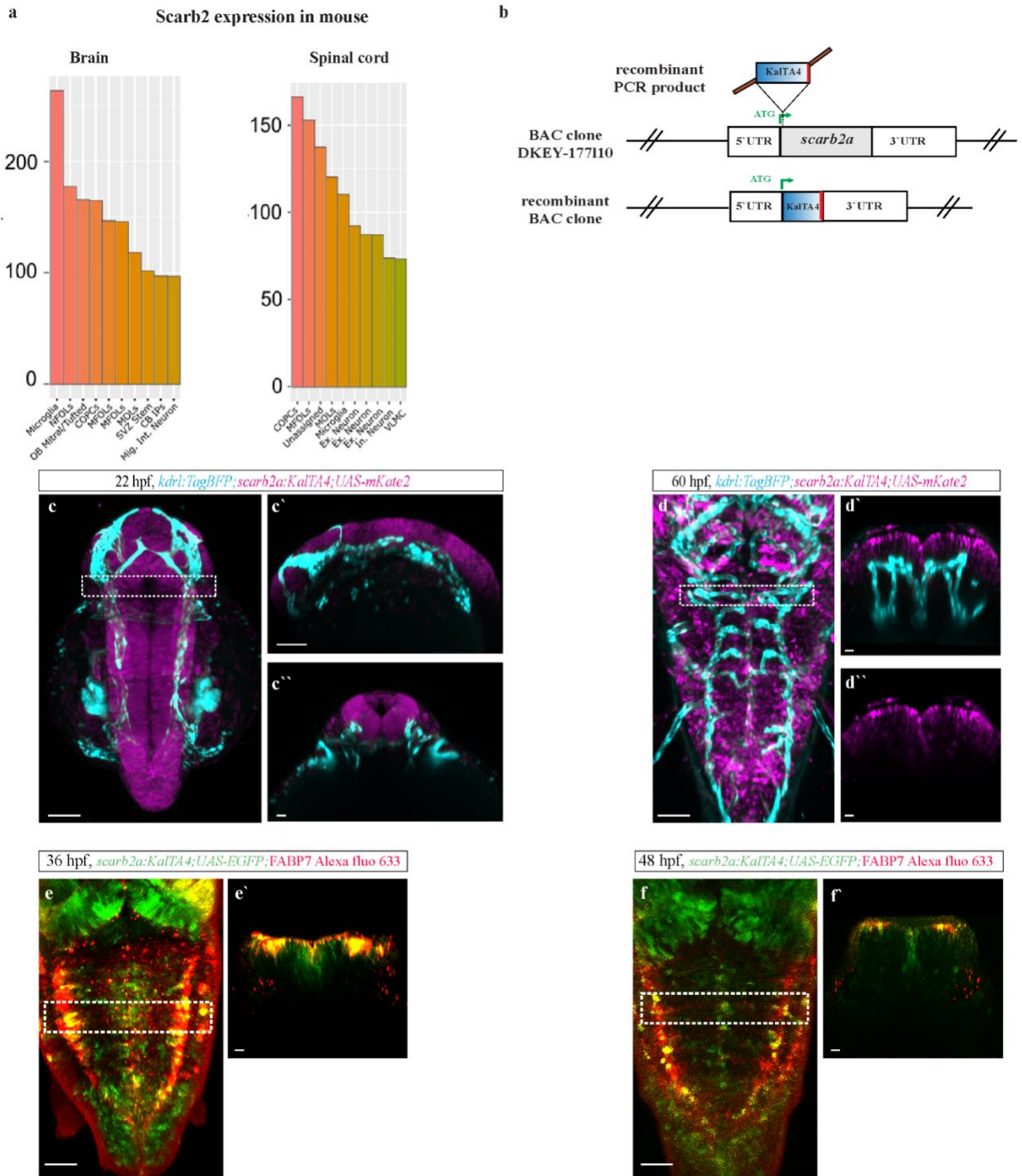


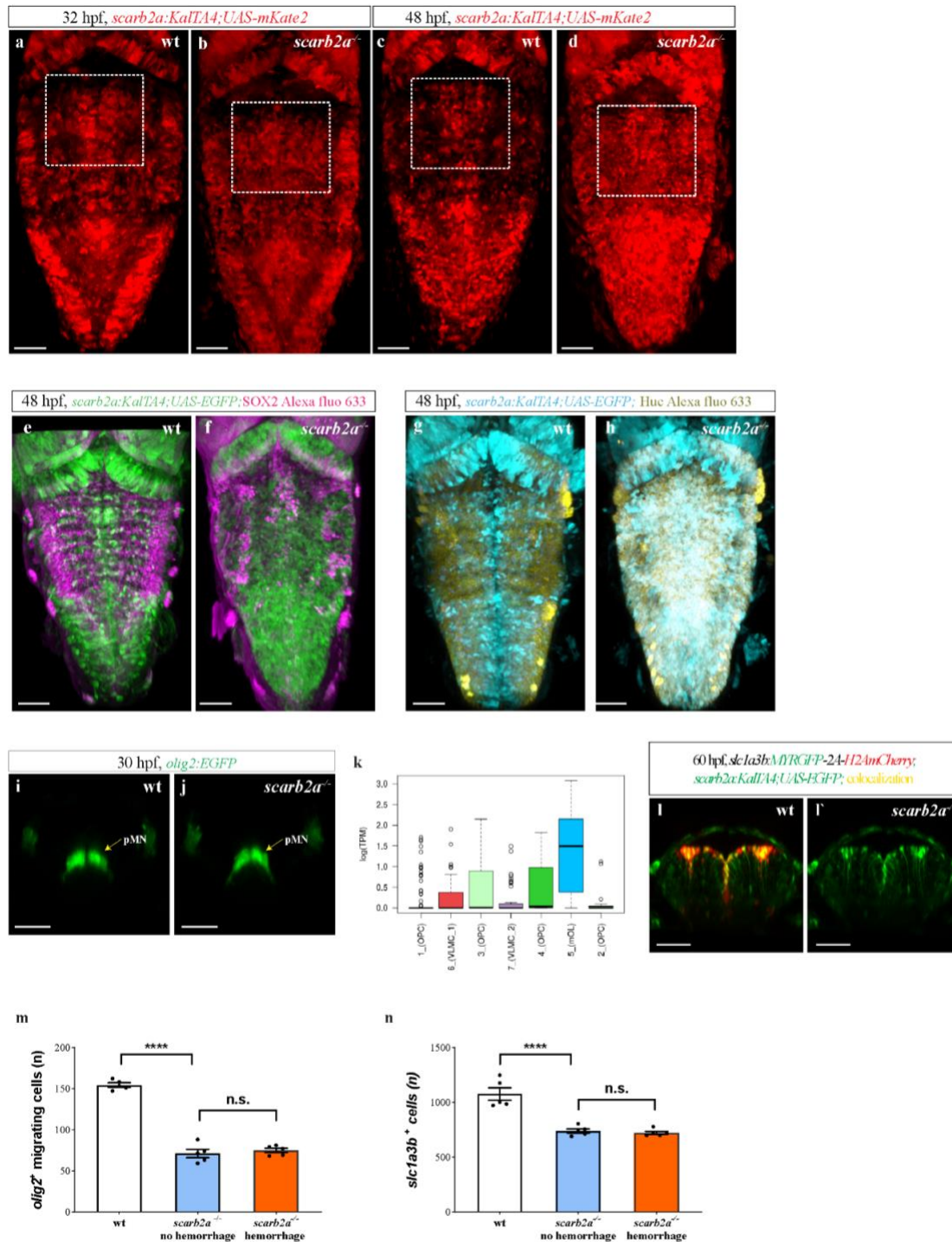
Supplementary Information



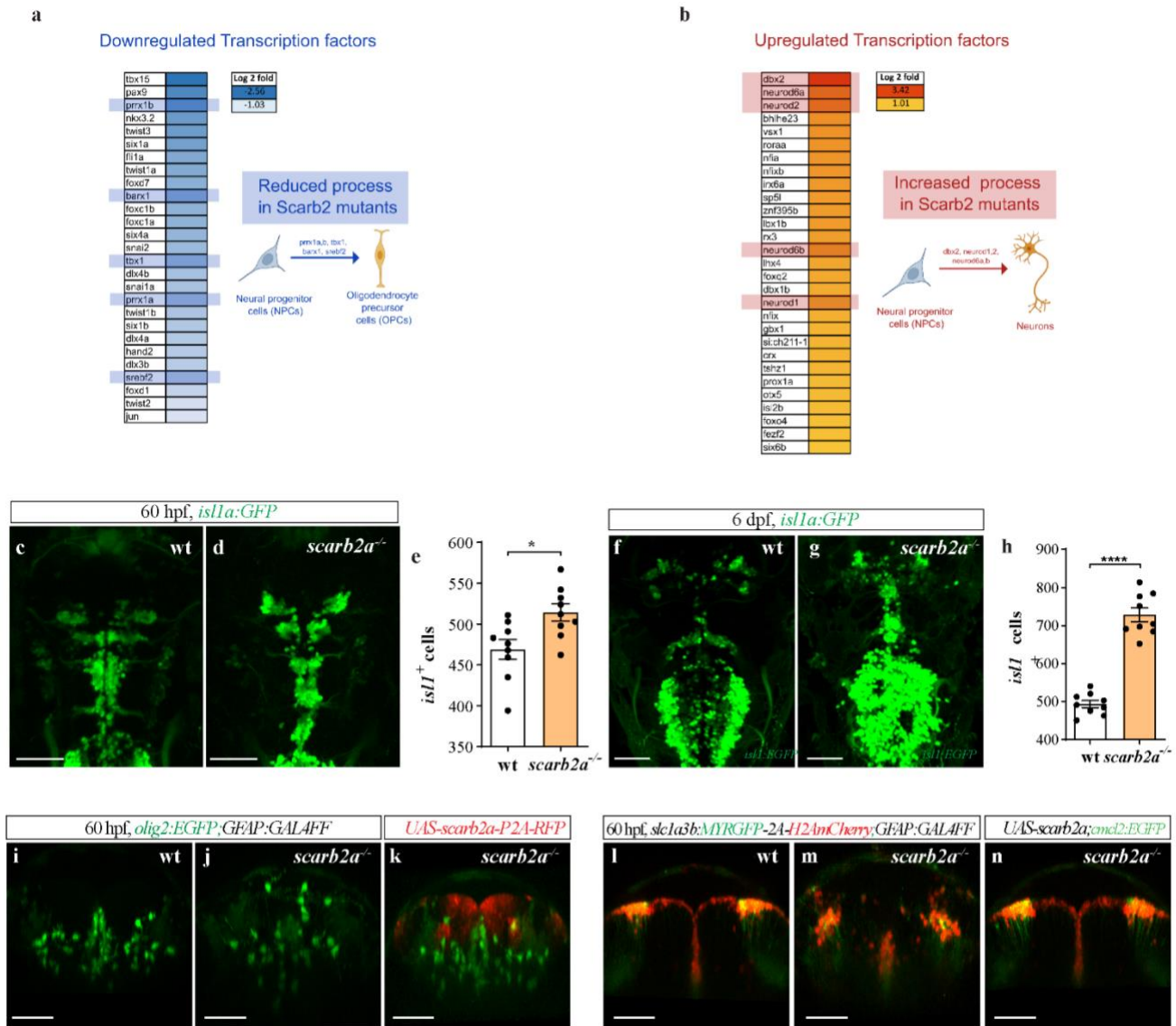
Supplementary Fig. 1: *scarb2a* mutants display specific defects in the hindbrain vasculature. **a**, Schematic illustration showing *scarb2a* wt coding sequence with target sites of guide RNAs (gRNA, purple) and resulting *scarb2a* mutant sequence. **b**, Conserved transmembrane domains and cholesterol binding domains in zebrafish (*Scarb2a*), mouse (*SCARB2*), and human (*SCARB2*). Next to the comparison is the percentage identity matrix for *SCARB2* amino acid sequences between the species. **c**, percentage of *scarb2a* mutants presenting intracranial hemorrhage over 14 dpf. **d**, survival chart of wt (black triangles dots) and *scarb2a*^{-/-} embryos with (orange square dots) or without (blue circle dots) intracranial hemorrhage over 14 dpf. **e-j**, Dorsal view of *Tg(kdr1:NLS-mCherry;kdr1:EGFP)* embryos showing EC nuclei in the PHBCs, BA and CtAs at 32 hpf (**e,f** quantified in **g**, $n=5$ embryos, two-tailed Student's t-test) and 60 hpf (**h,i**, quantified in **j**, $n=5$ embryos, two-tailed Student's t-test). **k-l**, Lateral views of *Tg(kdr1:EGFP)* wt (**k**) and *scarb2a*^{-/-} (**l**) embryos at 60 hpf showing no defects in trunk vasculature of mutant embryos. **m-n**, Whole mount ISH against *glut1b* at 60 hpf (purple, dorsal view). TM1, transmembrane domain 1; TM2, transmembrane domain 2; CHBD, cholesterol binding domain. Scale bars, **e-f**, **h-i**=60 μ m; **k-l**=200 μ m; **m-n**=50 μ m. ** $P<0.01$, *** $P<0.001$; ns, not statistically significant. Source data are provided as a Source Data file



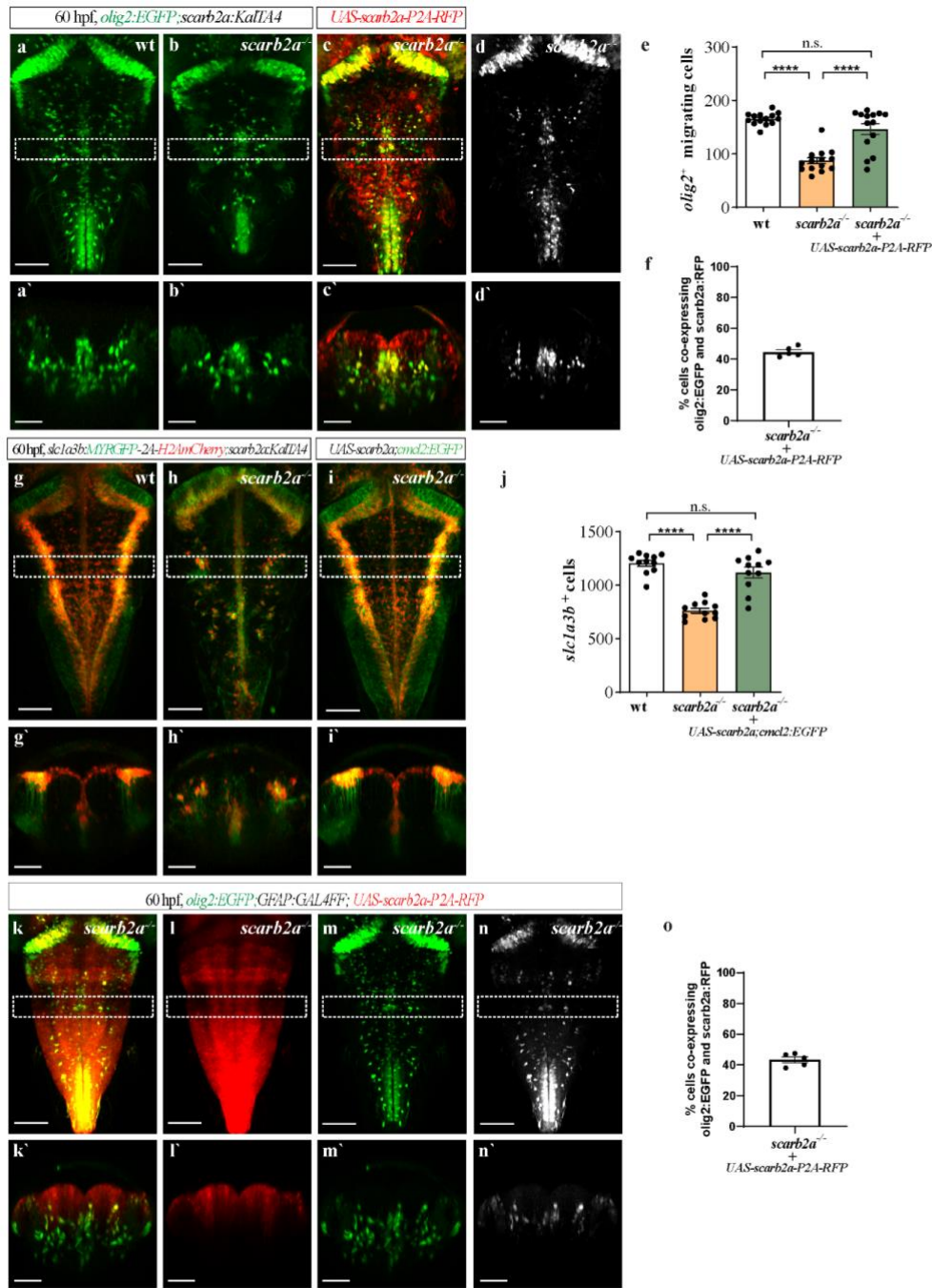
Supplementary Fig. 2: *scarb2a* is expressed in RGC progenitors. **a**, Top 10 cell types with higher expression of *Scarb2* in the developing mouse brain and spinal cord (Single-cell transcriptomics dataset re-analyzed from²⁰). **b**, Illustration of the construct used to generate *TgBAC(scarb2a:KalTA4)* reporter. **c-d''**, Dorsal (c,d), lateral (c') and transverse (c'',d',d'') views of *Tg(kdr1:TagBFP;scarb2a:KalTA4;UAS-mKate2)* (*scarb2a* in magenta) in wt embryos at 22 hpf (dashed square in c and d marks the region shown in c'' and d',d'' respectively) showing no colocalization of *scarb2a* and *kdr1* (blue). **e-f**, *fabp7a* immunostaining on *Tg(scarb2a:KalTA4;UAS-EGFP)* embryos at 36 (e,e') and 48 hpf (f,f') showing *fabp7a*/*scarb2a* co-localization in a subset of RGCs in the ventricular zone of wt embryos (co-localization channel depicted in yellow; dashed square in e and f marks the region shown in e' and f' respectively). Scale bars, c''=70 μ m; c'',d'',e',f'=30 μ m; c,d,e,f= 60 μ m.



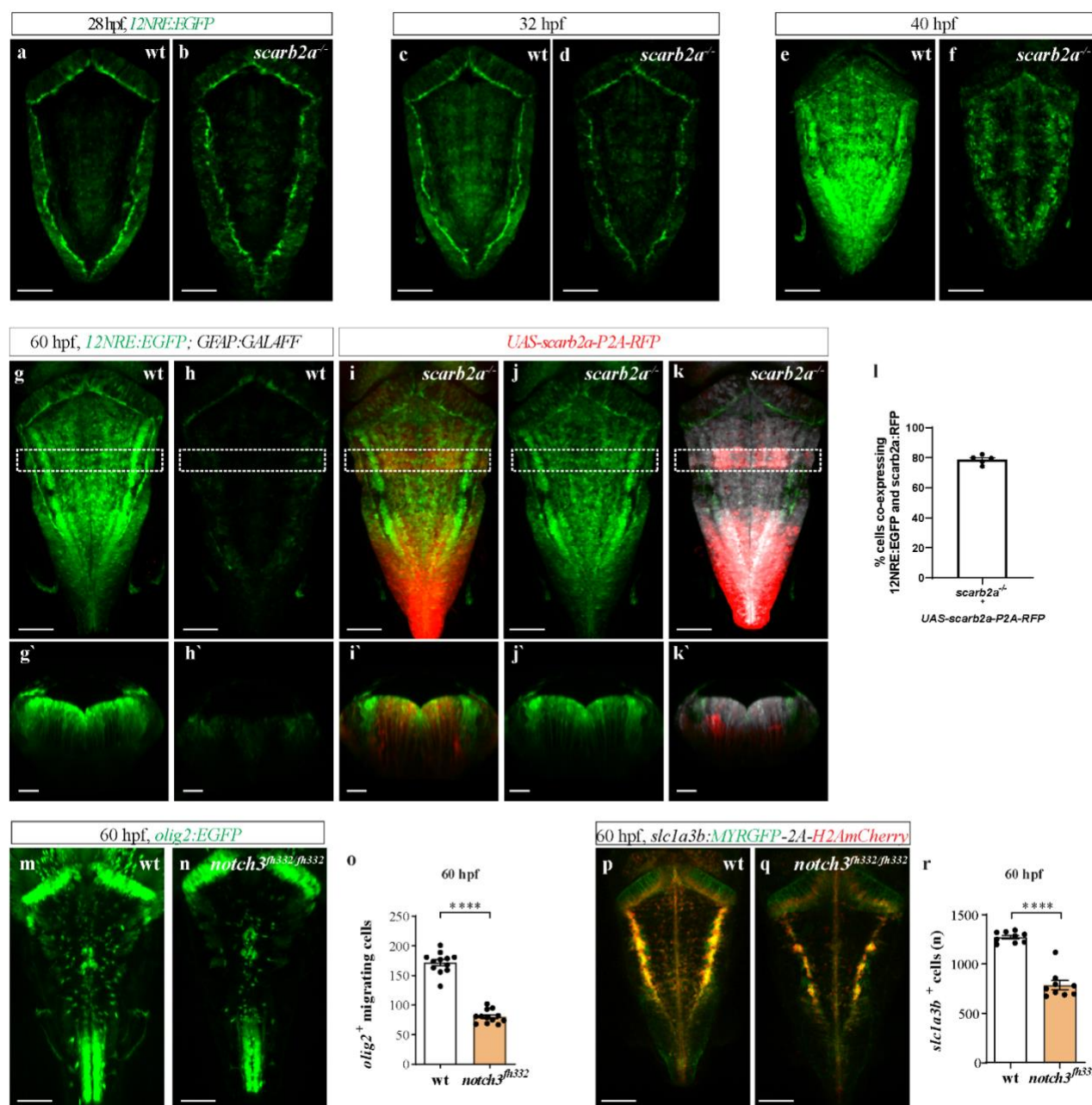
Supplementary Fig. 3: characterization of *scarb2a* expression in wt and mutant hindbrains. **a-d**, Selected confocal images from *Tg(scarb2a:KalTA4;UAS-mKate2)* (Supplementary movie 2) at 32 (a,b) and 48 hpf (c,d). **e-f**, Dorsal views of *Tg(scarb2a:KalTA4;UAS-EGFP)* at 48 hpf immunostained with SOX2 antibody (magenta). **g-h**, HuC immunostaining (yellow) on *Tg(scarb2a:KalTA4;UAS-EGFP)* (blue) embryos at 48 hpf showing HuC/*scarb2* colocalization (white) in mutant hindbrains. **i-j**, Transverse optical section at the level of the pMN in *Tg(olig2:EGFP)* wt (i) and *scarb2a* mutant (j). **k**, Analysis of single-cell transcriptomic data obtained from³⁶ (Oligodendrocyte precursors (OPC)) = 1_OPC, 2_OPC, 3_OPC, 4_OPC; mature oligodendrocytes (mOLs) = 5_mOL, vascular and leptomeningeal cells (VLMCs) = 6_VLMC_1, 7_VLMC_2. **l-l'**, Transverse optical section of *Tg(slc1a3b:MYRGFP-2A-H2AmCherry;scarb2a:KalTA4;UAS-EGFP)* double transgenic reporter. **m-n**, Quantification of *olig2*⁺ migrating cells (m) and *slc1a3b*⁺ nuclei (n) in wt and *scarb2a* mutants with and without hemorrhage (m,n, n=5 embryos, One-way ANOVA, multiple comparisons with Tukey post-hoc test). pMN= primary motor neuron; Scale bars: a-d, e-h=60 μ m; i-j, l-l'=30 μ m. Error bars are mean $\pm$ s.e.m. ****P<0.0001; ns, not statistically significant. Source data are provided as a Source Data file.



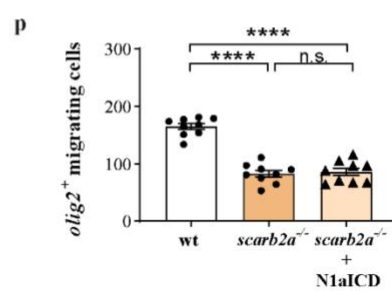
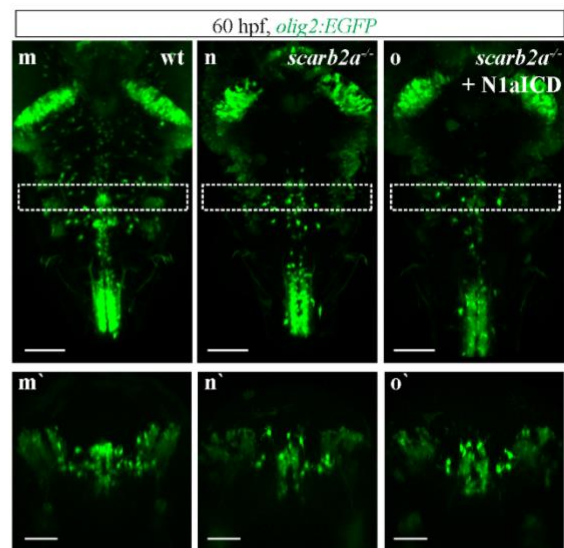
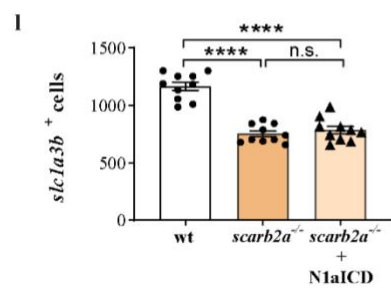
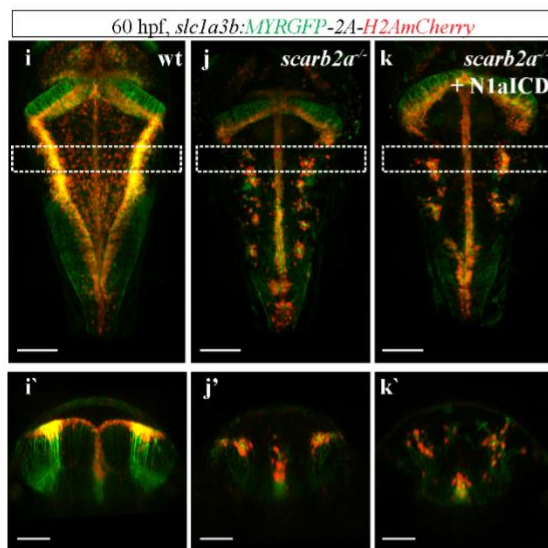
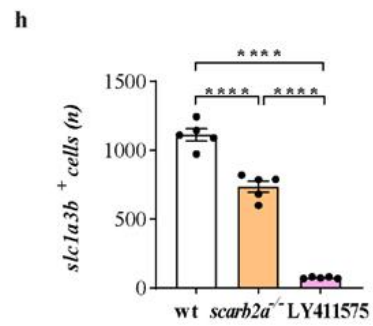
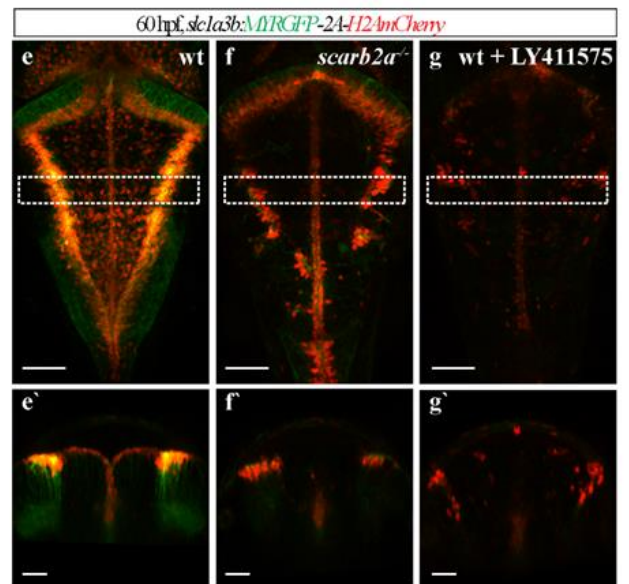
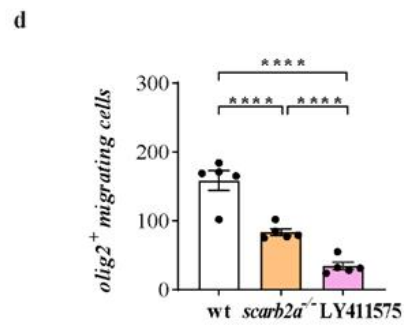
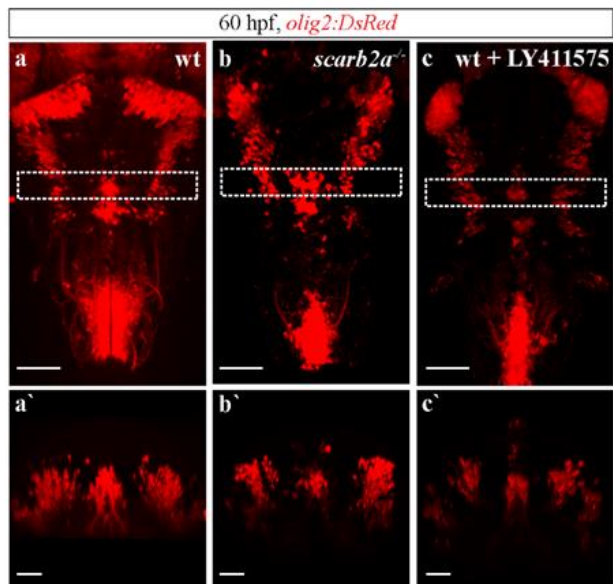
Supplementary Fig. 4: *Scarb2a* depletion leads to excess neurogenesis and reduced gliogenesis. **a,b**, RNA-seq data analysis showing down (a) and up-regulated (b) TF expression in *scarb2a* mutant embryos, identified using Uniprot gene ontology. Created in BioRender.com <http://BioRender.com>. **c-h**, Dorsal views of *Tg(isl1:EGFP)* reporter showing increased numbers of *isl1*⁺ neurons in mutant hindbrains at 60 hpf (c-e, n=9 embryos, two-tailed Student's t-test) and 6 dpf (f-h, n=9 embryos, two-tailed Student's t-test). **i-k**, Transverse optical sections of *Tg(olig2:EGFP;GFAP:Gal4FF)* in wt (i), *scarb2a*^{-/-} (j), and *scarb2a*^{-/-} following injection of UAS-*scarb2a*-P2A-RFP (k, red). **l-n**, Transverse optical sections of *Tg(slc1a3b:MYRGFP-2A-H2AmCherry;GFAP:Gal4FF)* in wt (l), *scarb2a*^{-/-} (m) and *scarb2a*^{-/-} injected with UAS-*scarb2a;cmcl2:EGFP* construct (n). Scale bars, i-n=30 μ m; c-d,f-g=50 μ m. Error bars are mean $\pm$ s.e.m. *P<0.05, ****P<0.0001; ns, not statistically significant. Panels **a** and **b** were created with BioRender.com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license. Source data are provided as a Source Data file



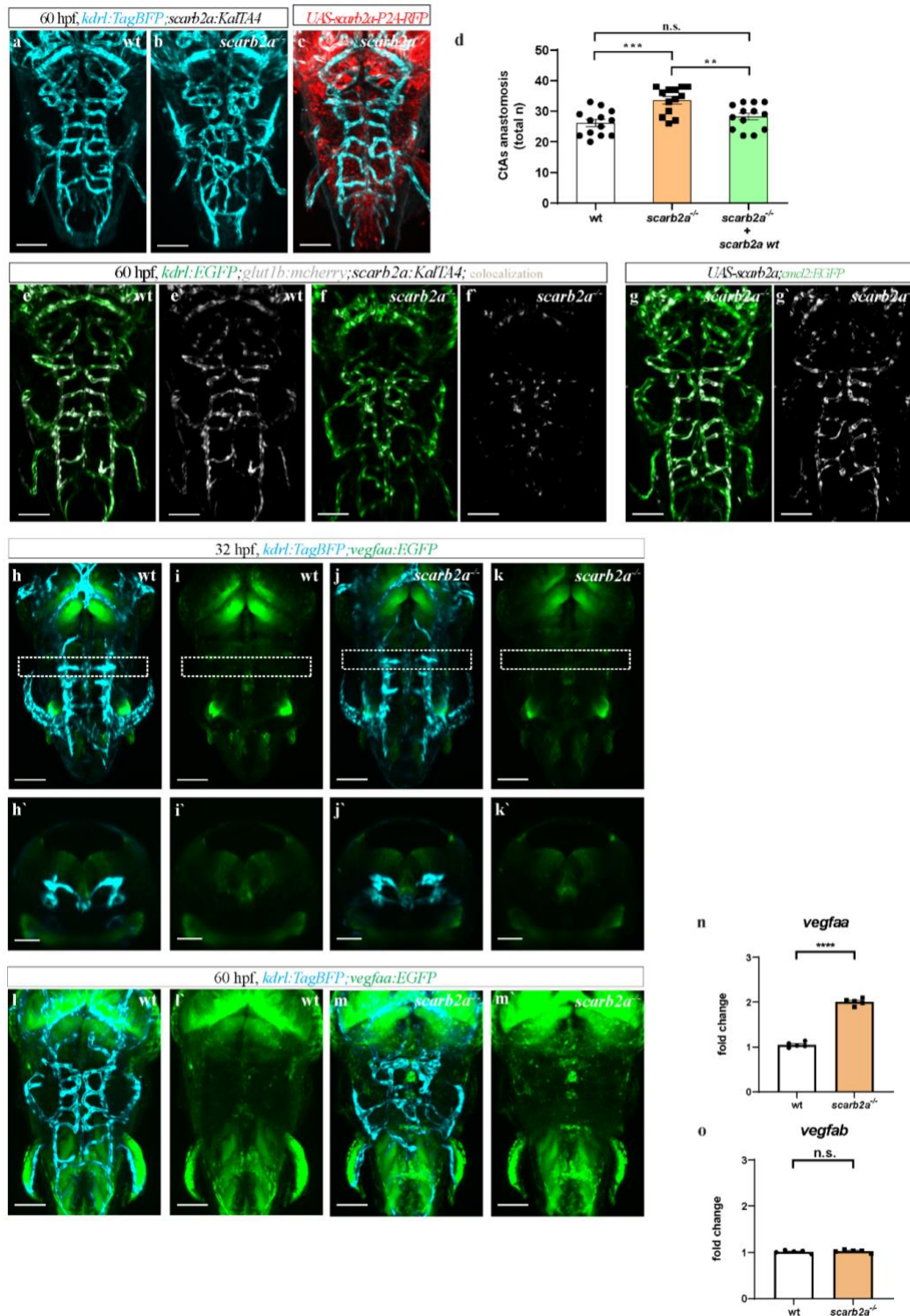
Supplementary Fig. 5: Restoring Scarb2a expression in *scarb2a*⁺ cells rescues the glial phenotype a-c, Dorsal views (a-c), and transverse optical sections (a'-c') of *Tg(olig2:EGFP;scarb2a:KalTA4)* at 60 hpf. Injection of *UAS-scarb2a-P2A-RFP* rescues OPC numbers in *scarb2a*^{-/-} embryos (c-c'). **d-d'**, Dorsal view and transverse optical section of *scarb2a* mutant hindbrain showing colocalization (white) of *olig2:EGFP* and *scarb2a* (red) reporters. **e**, Quantification of migrating *olig2*⁺ cells in a-c (n=14 embryos; One-way ANOVA, multiple comparisons with Tukey posthoc test). **f**, Percentage of cells with *olig2* and *scarb2a* colocalization as shown in d-d' (n=5 embryos). **g-i'**, Injection of *UAS-scarb2a-cmcl2:EGFP* construct into *Tg(slc1a3b:MYRGFP-2A-H2AmCherry;scarb2a:KalTA4);scarb2a*^{-/-} results in full recovery of the astrocyte population (i-i') (dashed square in g-i, marks the region shown in g'-i'). **j**, Quantification of *slc1a3b*⁺ red nuclei in g-i (n= 11 embryos; One-way ANOVA, multiple comparisons with Tukey post-hoc test). **k-m'**, Dorsal views (k-m) and transverse optical sections (k'-m') of *Tg(olig2:EGFP;GFAP:Gal4FF);scarb2a*^{-/-} at 60 hpf after injection of *UAS-scarb2a-P2A-RFP* (dashed square in k-m, marks the region shown in k'-m'). **n-n'** Dorsal view and transverse optical section of *scarb2a* mutant hindbrain showing colocalization (white) of *olig2:EGFP* and *scarb2a* (red) reporters). **o**, Percentage of cells with *olig2* and *scarb2a* colocalization as shown in n-n' (n=5 embryos). Scale bars, a'-d', g'-i', k'-n'=30 μm; a-d, g-i, k-m=50 μm. Error bars are mean ± s.e.m. ****P<0.0001; ns, not statistically significant. Source data are provided as a Source Data file.



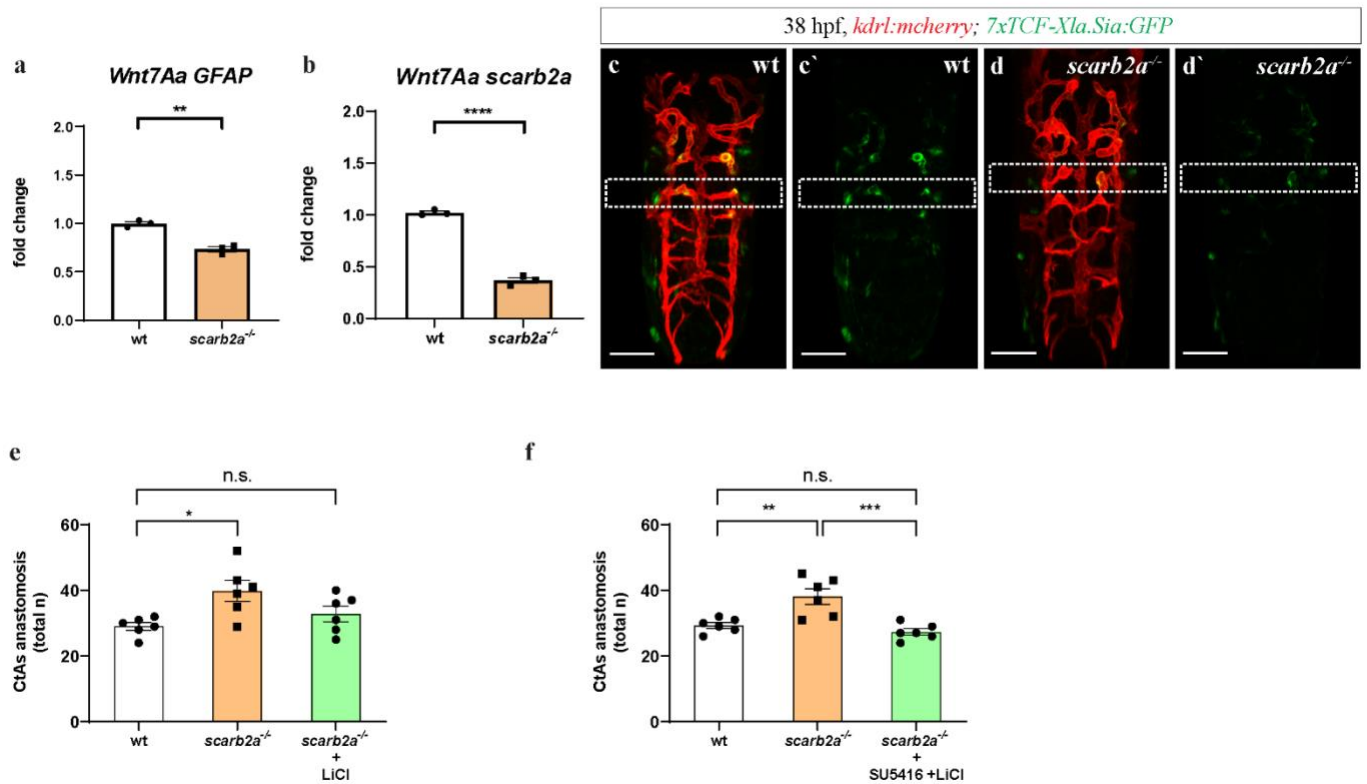
Supplementary Fig. 6: Notch signaling is downregulated in *scarb2a* mutants. **a-f**, Dorsal views of *Tg(12NRE:EGFP)* reporter, at 28 (a-b), 32 (c,d) and 40 (e,f) hpf depicting significant reduction of Notch signaling activation in mutant hindbrains starting at 32 hpf (d,f). **g-k'**, Dorsal views (g-k) and transverse optical sections (g'-k') of *Tg(12NRE:EGFP;GFAP:Gal4FF)* embryos showing complete restoration of Notch signaling in *scarb2a*^{-/-} embryos after injection of *UAS-scarb2a-P2A-RFP* (dashed square in g-k, marks the region shown in g'-k'). **l**, Percentage of cells with *olig2* and *scarb2a* colocalization as depicted in k-k' (n=5 embryos). **m-o**, Confocal images of *Tg(olig2:EGFP);notch3^{fh332/fh332}* hindbrains showing significant decrease of OPC numbers (n) as compared to wt siblings (m), (quantified in (o) (n=12 embryos, two-tailed Student's *t*-test, *P*<0.0001). **p-r**, Confocal images of *Tg(slc1a3b:MYRGFP-2A-H2AmCherry);notch3^{fh332/fh332}* revealed an absence of *slc1a3b*⁺ cells in the vz of *notch3* mutant (q) quantified in (r) (n=9 embryos, two-tailed Student's *t*-test, *P*<0.0001). Scale bars: a-f= 50 μ m; g-k, m-n, p-q=60 μ m; g'-k'=30 μ m. Error bars are mean $\pm$ s.e.m. *****P*<0.0001; ns, not statistically significant. Source data are provided as a Source Data file



Supplementary Fig. 7: Dissection of Notch signaling components affected in *scarb2a* mutants a-d, Dorsal (a-c) and transverse (a'-c') views of *Tg(olig2:DsRed)* embryos at 60 hpf showing recapitulation of *scarb2a* mutant phenotype following treatment of wt embryos with the Notch signaling inhibitor LY411575, quantified in (d) (n=5 embryos, One-way ANOVA, multiple comparisons with Tukey post-hoc test). **e-h**, Dorsal (e-g) and transverse (e'-g') views of *Tg(slc1a3b:MYRGFP-2A-H2AmCherry)* embryos showing reduced *slc1a3b*⁺ cells numbers in *scarb2a* mutants and following LY411575 treatment of wt embryos, quantified in (h), (n=5 embryos, One-way ANOVA, multiple comparisons with Tukey posthoc test). **i-k**, Confocal images of heat-shock induced overexpression of *notch1* intra-cellular domain (N1aECD) in *Tg(slc1a3b:MYRGFP-2A-H2AmCherry;hsp70l:MYC-notch1a-cryaa:Cerulean)* mutant embryos (dashed square in i-k, marks the region shown i'-k'). **l**, Quantification of *slc1a3b*⁺ astrocytes in the hindbrain of i-k (n=10 embryos, One-way ANOVA, multiple comparisons with Tukey posthoc test). **m-o**, Dorsal (m-o) and transversal (m'-o') views of *Tg(olig2:EGFP;hsp70l:MYC-notch1a,cryaa:Cerulean);scarb2a* embryos with or without heat-shock induced overexpression of N1ICD. **p**, Quantification of *olig2*⁺ migrating OPCs (n=9 embryos, One-way ANOVA, multiple comparisons with Tukey post-hoc test). Scale bars: dorsal views a-c, e-g, i-k, m-o= 60 µm; a'-c', e'-g', i'-k', m'-o'=30 µm. Error bars are mean ± s.e.m. ****P<0.0001; ns, not statistically significant. Source data are provided as a Source Data file



Supplementary Fig. 8: The vascular phenotypes in *scarb2a* mutants are caused by defective specification of RGCs. a-c, Dorsal views of *Tg(kdrl:TagBFP;scarb2a:KalTA4)* hindbrains in wt (a), *scarb2a*^{-/-} (b) and *scarb2a*^{-/-} following *UAS-scarb2a-P2A-RFP* injection (c) embryos. d, Quantification of CtA interconnections in a-c (n= 13 embryos; One-way ANOVA, multiple comparisons with Tukey posthoc test). e-g, Dorsal views of *Tg(kdrl:EGFP;glut1b:mCherry;GFAP:Gal4FF)* depicting complete recovery of *glut1b* expression (white) following injection of *UAS-scarb2a,cmcl2:EGFP* in mutant embryos (g-g'). h-m, Confocal images of *Tg(kdrl:TagBFP;vegfaa:EGFP)* at 32 hpf (h-k) and 60 hpf (l-m) showing increased *vegfaa* expression in *scarb2a* mutant hindbrains at 60 hpf (m-m', dashed square in h-k, marks the region shown in h'-k'). n-o, qTR-PCR at 60 hpf showing increased levels of *vegfaa* in *scarb2a*^{-/-} embryos (N=5, n= 20 heads/sample, two-tailed Student's *t*-test). Scale bars, h'-k'=30 μ m; a-c, e-g, h-k, l-m'=50 μ m. Error bars are mean $\pm$ s.e.m. ** *P*<0.01, ****P*<0.001; *****P*<0.0001; ns, not statistically significant. Source data are provided as a Source Data file.



Supplementary Fig. 9: Reduced Wnt signaling in *scarb2a* mutants leads to aberrant CNS angiogenesis. **a-b, h** qRT-PCR analyses showing mRNA levels of *wnt7aa* in *mKate2*⁺ cells sorted from *Tg(GFAP:Gal4FF;UAS-mkate2)* (a) and *Tg(scarb2a:KaITa4;UAS-mkate2)* (b) heads (N=3, n=20 heads/sample, two-tailed Student's t-test). **c-d'**, Dorsal views of *Tg(kdrl:mcherry; 7xTCF-Xla.Sia:GFP)* showing reduced Wnt/ β -catenin activation in the CtAs of *scarb2a*^{-/-} (d-d'). **e-f**, Quantification of CtA interconnections in *scarb2a*^{-/-} after administration of LiCL (e) and LiCL+SU5416 (f) (for both experiments n=6 embryos; One-way ANOVA, multiple comparisons with Tukey posthoc test). Scale bars: c-d' =60 μ m; Error bars are mean $\pm$ s.e.m. *P<0.05, ** P<0.01, ***P<0.001; ****P<0.0001; ns, not statistically significant Source data are provided as a Source Data file