

Supplemental Figure Legends

Supplemental Figure 1. Reactive gliosis in the hemorrhagic neonatal mouse brain. (A);

Sagittal sections through P5 WT (left) and KO (right) cerebral cortices were immunohistochemically labeled with anti-Iba1 (upper panels) or anti-GFAP (lower panels). Note the increased numbers of reactive Iba1⁺ microglia/macrophages and GFAP⁺ astroglial cells in the hemorrhagic cortex (arrows). **(B);** Sagittal sections through the P10 WT (left) and KO (right) cerebral cortices were immunostained with anti-Iba1 (upper panels) or anti-GFAP (lower panels). Note the reactive Iba1⁺ microglia/macrophages and GFAP⁺ astroglial cells in the KO sample (arrows).

Supplemental Figure 2. scRNAseq experimental workflow and sample analysis. (A);

Experimental workflow for scRNAseq experiments to identify genes and pathways linked to hemorrhage pathophysiology in the neonatal cerebral cortex. **(B);** Uniform manifold approximation and projection (UMAP) visualization of integrated WT and KO cells in P0 mice split by samples (top). Shown are violin plots of number of nFeatures (middle) per sample, and a dot plot of marker genes used for cluster annotation (bottom). **(C);** Uniform manifold approximation and projection (UMAP) visualization of integrated WT and KO cells in P5 mice split by samples (top). Shown are violin plots of number of nFeatures (middle) per sample, and a dot plot of marker genes used for cluster annotation (bottom). **(D);** Uniform manifold approximation and projection (UMAP) visualization of integrated WT and KO cells in P10 mice split by samples (top). Shown are violin plots of number of nFeatures (middle) per sample, and a dot plot of marker genes used for cluster annotation (bottom).

Supplemental Figure 3. Analysis of Itgb8 mRNA expression levels. (A); Dot plot of Itgb8 expression in astrocytes for each individual P0 KO and WT sample (top left). Feature plot of Itgb8 expression in astrocyte clusters for P0 KO and WT samples (top right). Dot plot of Itgb8 expression in all cell types for P0 KO and WT samples (bottom). **(B);** Dot plot of Itgb8 expression in astrocytes for each individual P5 KO and WT sample (top left). Feature Plot of Itgb8 expression in astrocyte clusters for P5 KO and WT samples (top right). Dot Plot of Itgb8 expression in all cell types for P5 KO and WT samples (bottom). **(C);** Dot plot of Itgb8 expression in astrocytes for each individual P10 KO and WT sample (top left). Feature Plot of Itgb8 expression in astrocyte clusters for P10 KO and WT samples (top right). Dot plot of Itgb8 expression in all cell types for P10 KO and WT samples (bottom).

Supplemental Figure 4. GO enrichment analysis of DEGs in endothelial cells and microglia/macrophages from scRNAseq. (A); TreePlot of top 15 gene ontology (GO) enrichment pathways expressed at lower levels in endothelial cells from KO compared to WT mice at P0 (top), P5 (middle) and P10 (bottom). **(B);** TreePlot of top 15 gene ontology (GO) enrichment pathways activated at lower levels in microglia/macrophages from KO compared to WT mice at P0 (top), P5 (middle) and P10 (bottom).

Supplemental Figure 5. Xenium experimental workflow and sample analysis. (A); Experimental workflow for Xenium-based analysis of gene expression. One mouse was used per age, per each condition. **(B);** UMAP of P0 integrated WT and KO samples (left). Stacked bar plots of P0 cell type proportion per condition (top right). Total cell numbers per sample are

listed (WT: n = 32,866, KO: n = 33,221). Individual cell numbers split by cell type used for downstream analyses can be found in Supp. Table 3. Dot plot of P0 marker genes used for cluster annotation (bottom left). **(C)**; UMAP of P5 integrated WT and KO samples (left). Stacked bar plots of P5 cell type proportion per condition (top right). Total cell numbers per sample are listed (WT: n = 41,078, KO: n = 30,619). Individual cell numbers split by cell type used for downstream analyses can be found in Supp. Table 3. Dot plot of P5 marker genes used for cluster annotation (bottom left). **(D)**; UMAP of P10 integrated WT and KO samples (left). Stacked bar plots of P10 cell type proportion per condition (top right). Total cell numbers per sample are listed (WT: n = 39,856, KO: n = 37,212). Individual cell numbers split by cell type used for downstream analyses can be found in Supp. Table 3. Dot plot of P10 marker genes used for cluster annotation (bottom left).

Supplemental Figure 6. Spatial organization of Xenium annotated cell types. (A); Spatial positions of cells in KO (top left) and WT (top right) in P0 mouse cortex. H&E images of KO (bottom left) and WT (bottom right) P0 brains with overlaid pseudocolored cells from Xenium Explorer software. **(B)**; Spatial positions of cells in KO (top left) and WT (top right) in P5 mouse cortex. H&E images of KO (bottom left) and WT (bottom right) P5 brains with overlaid pseudocolored cells from Xenium Explorer software. **(C)**; Spatial positions of cells in KO (top left) and WT (top right) in P10 mouse cortex. H&E images of KO (bottom left) and WT (bottom right) P10 brains with overlaid pseudocolored cells from Xenium Explorer software. **(D)**; Representative image of spatial organization of cells around blood vessels in P5 control and KO samples. Images shown from left to right are H&E, H&E with overlaid pseudocolored cells, pseudocolored cells plotted by coordinates, and pseudocolored cells split by cell type.

Supplemental Figure 7. Immunofluorescence analysis of Hmox1 expression. (A, B);

Sagittal sections through the cerebral cortices of P0 and P5 WT (A) and KO mice (B) were stained with H&E (upper panels) or immunofluorescently labeled with anti-CD31 (red) and anti-Hmox1 (green) antibodies. Note the upregulation of Hmox1 protein expression in perivascular microglia/macrophages in control and hemorrhagic cortices at P0 and P5 (arrows). Scale bars, 100 μ m.

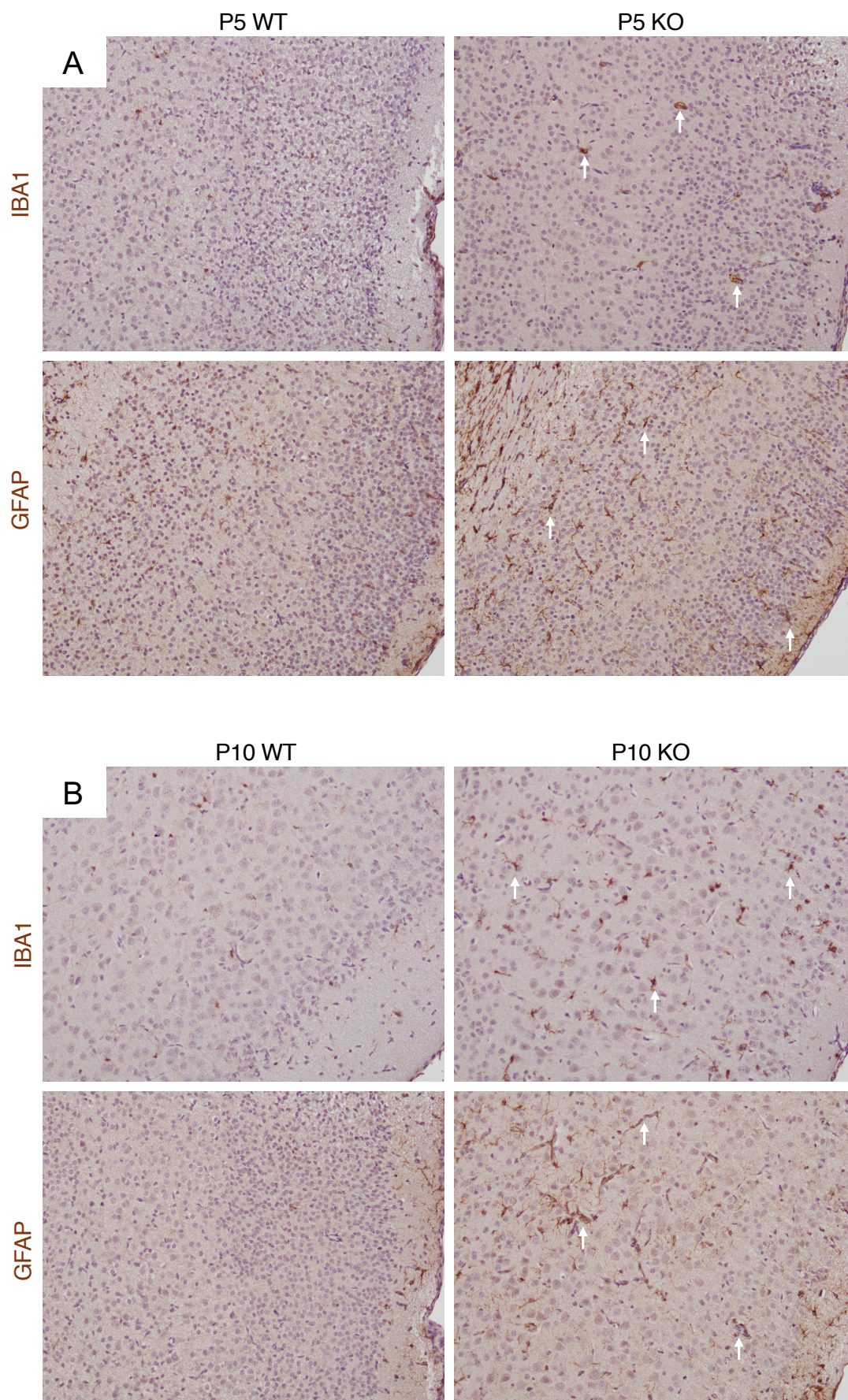
Supplemental Figure 8. CellChat analysis of cortical cell-cell communication pathways

from Xenium spatial profiling. Heatmap of cell–cell communication probabilities between sender and receiver cells (y-axis) across all signaling pathways for all cell types (x-axis). Rows split by age and condition.

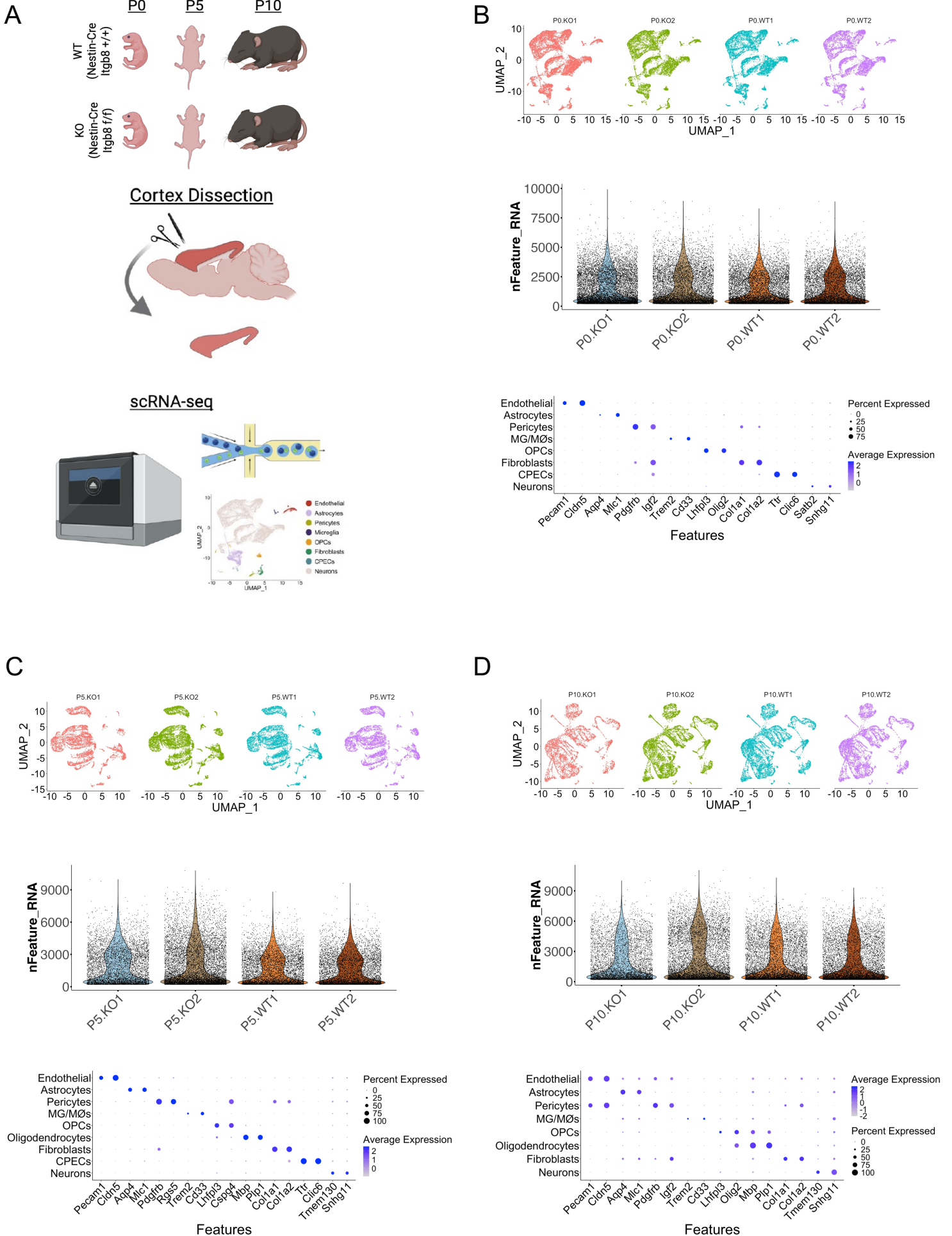
Supplemental Figure 9. A model for pathological cell adhesion and signaling pathways at the NVU that drive neonatal brain hemorrhage development and resolution. (A);

Diagram depicting cell types comprising the neurovascular unit (NVU) (left). NVU crossed section (right) shows key components surrounding the blood vessel. **(B);** Graphical representation of a healthy (top) and hemorrhagic (bottom) NVU showing vessel wall rupture and red blood cell extravasation. **(C);** Summary diagram of key genes and pathways at the NVU involved in hemorrhage. **(D);** Summary diagram of key genes and pathways involved in hemorrhage repair. Images were created with BioRender.

Supp. Figure 1

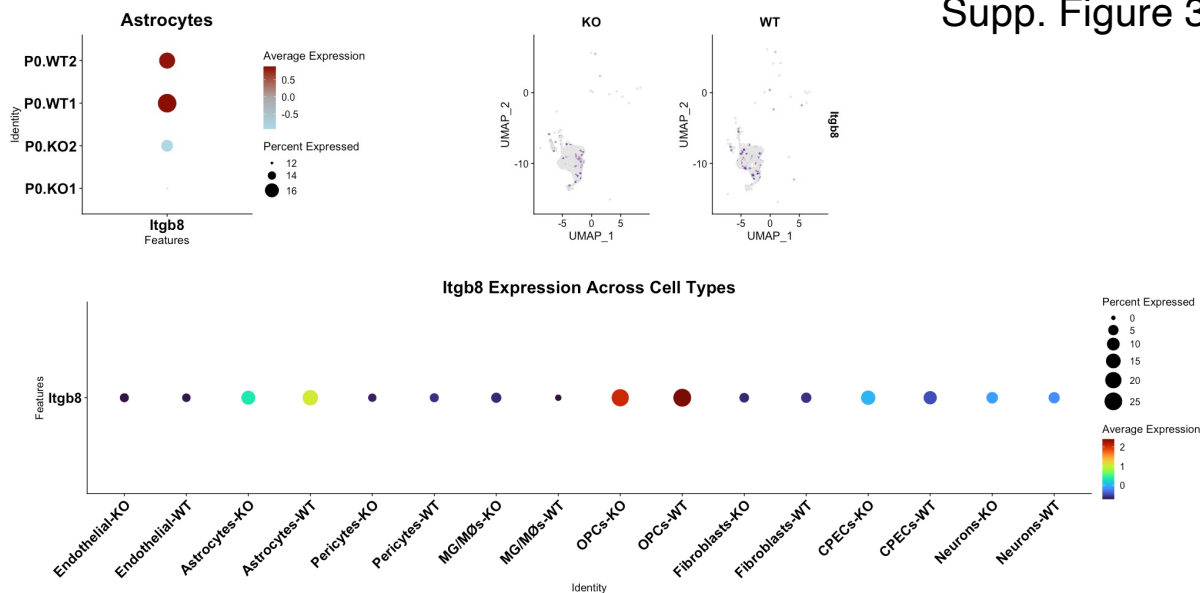


Supp. Figure 2

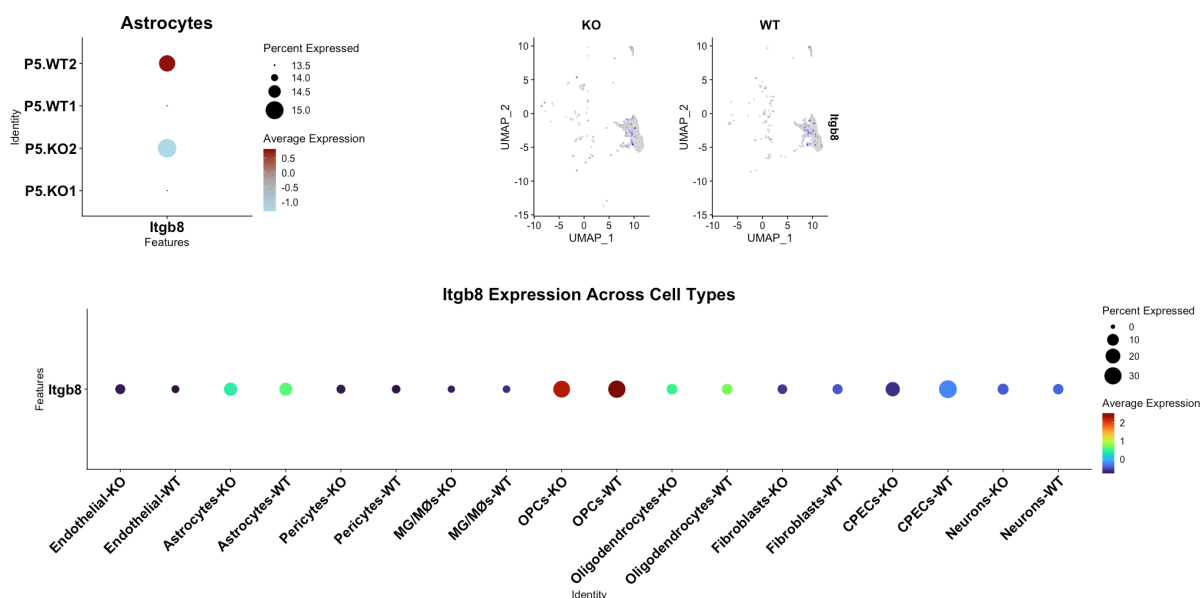


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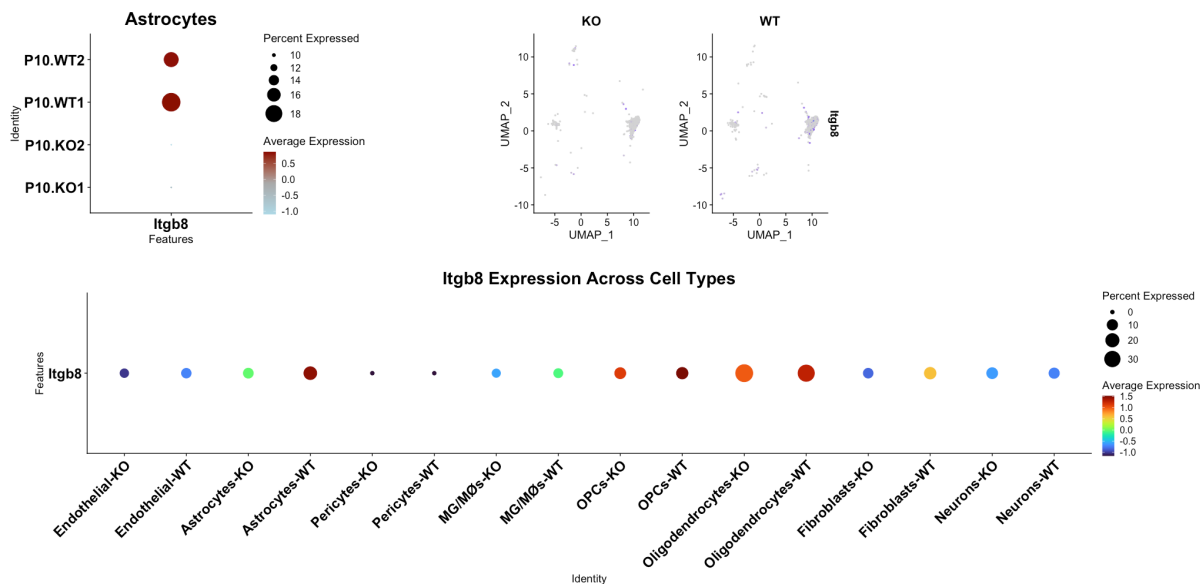
A



B

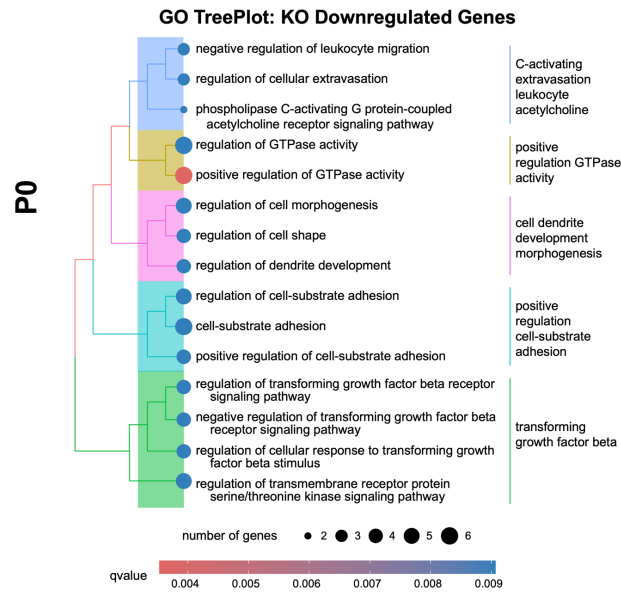


C



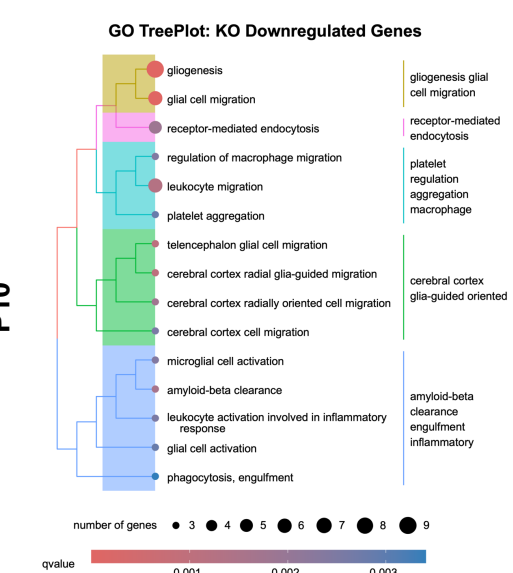
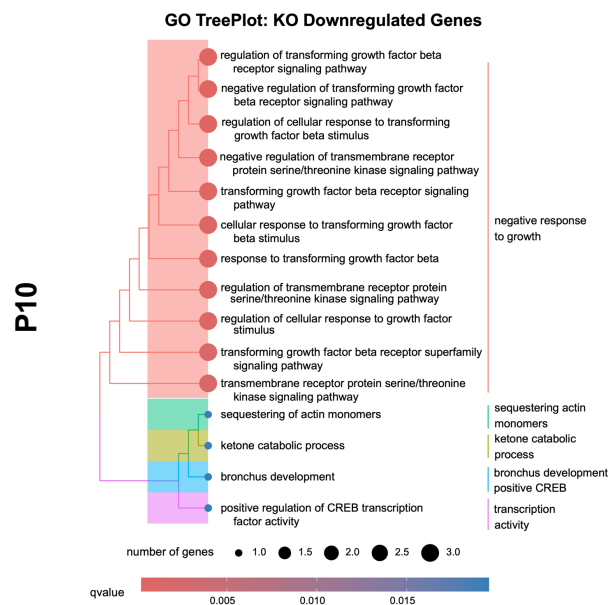
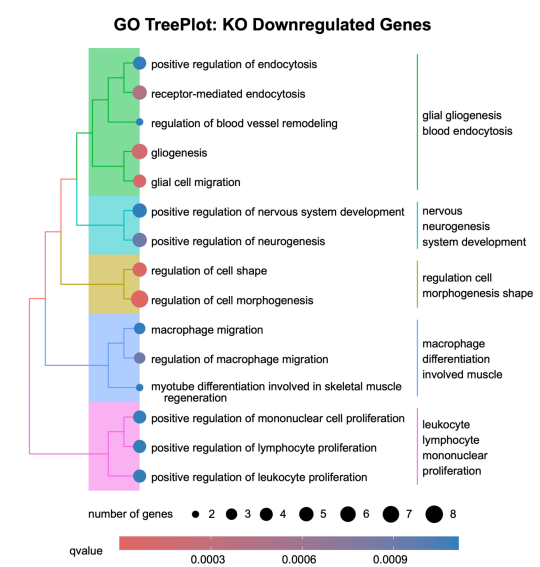
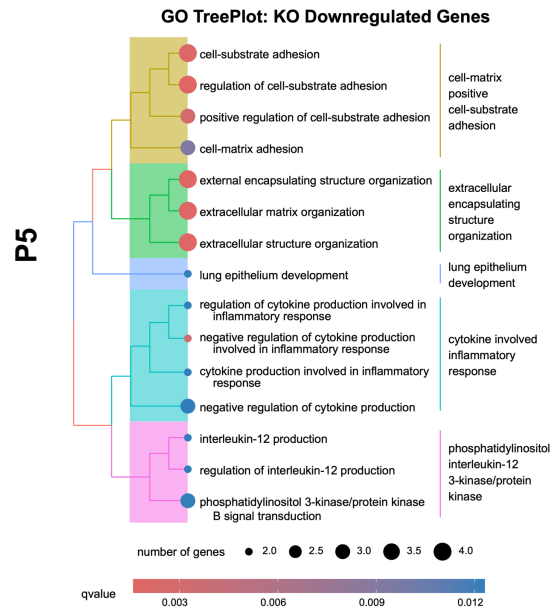
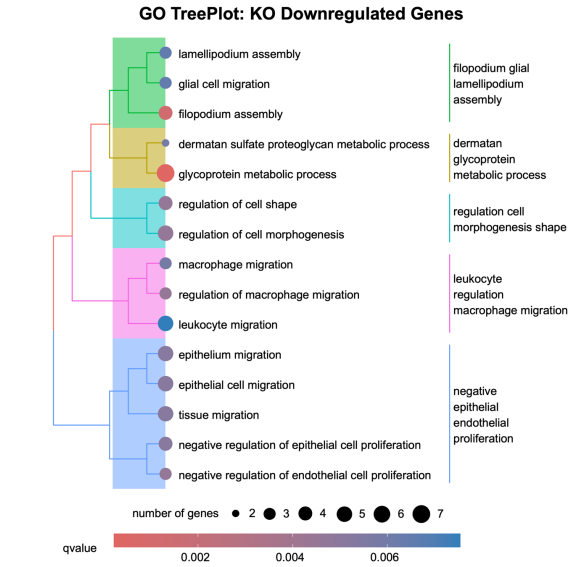
A

Endothelial

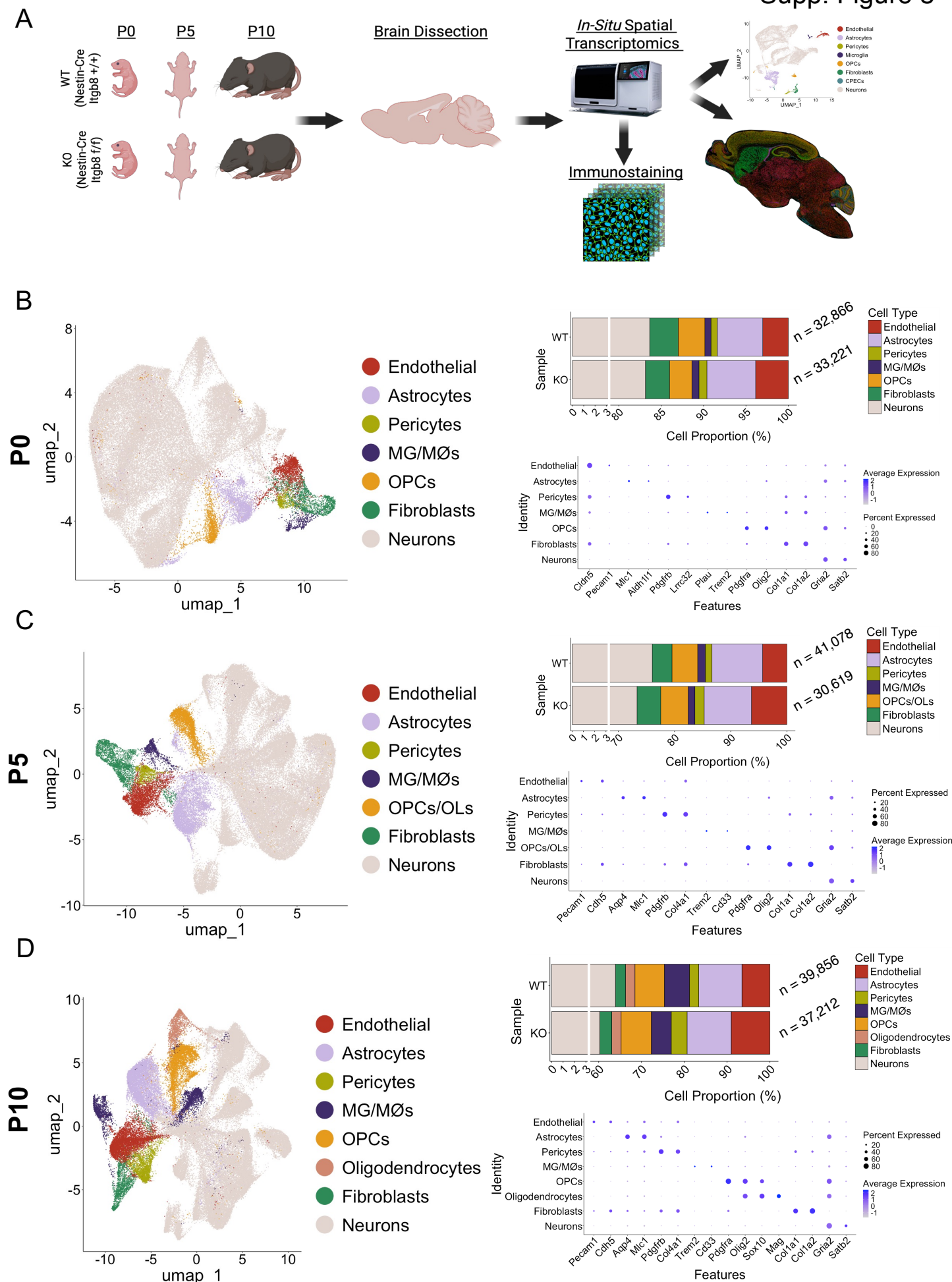


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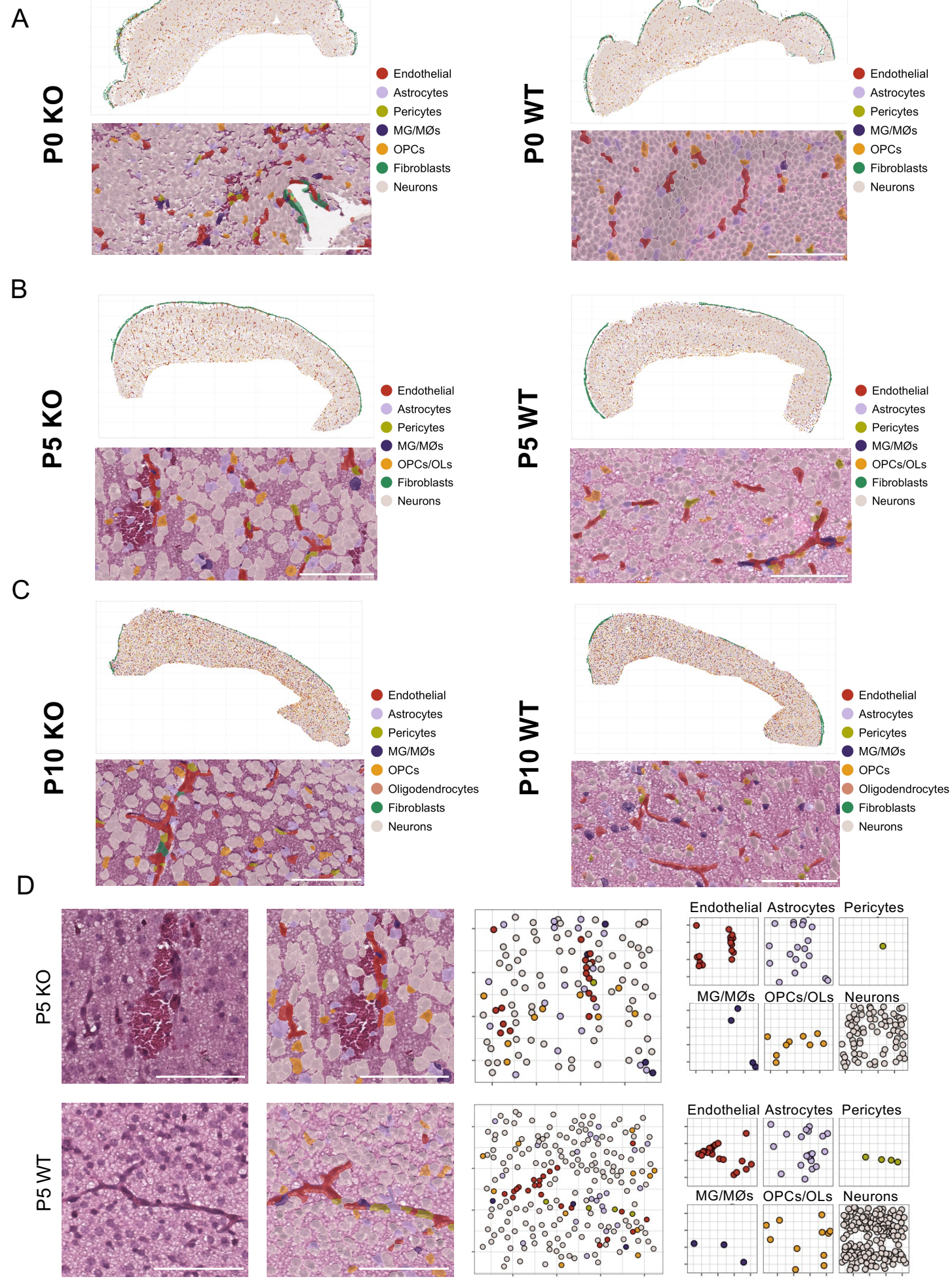
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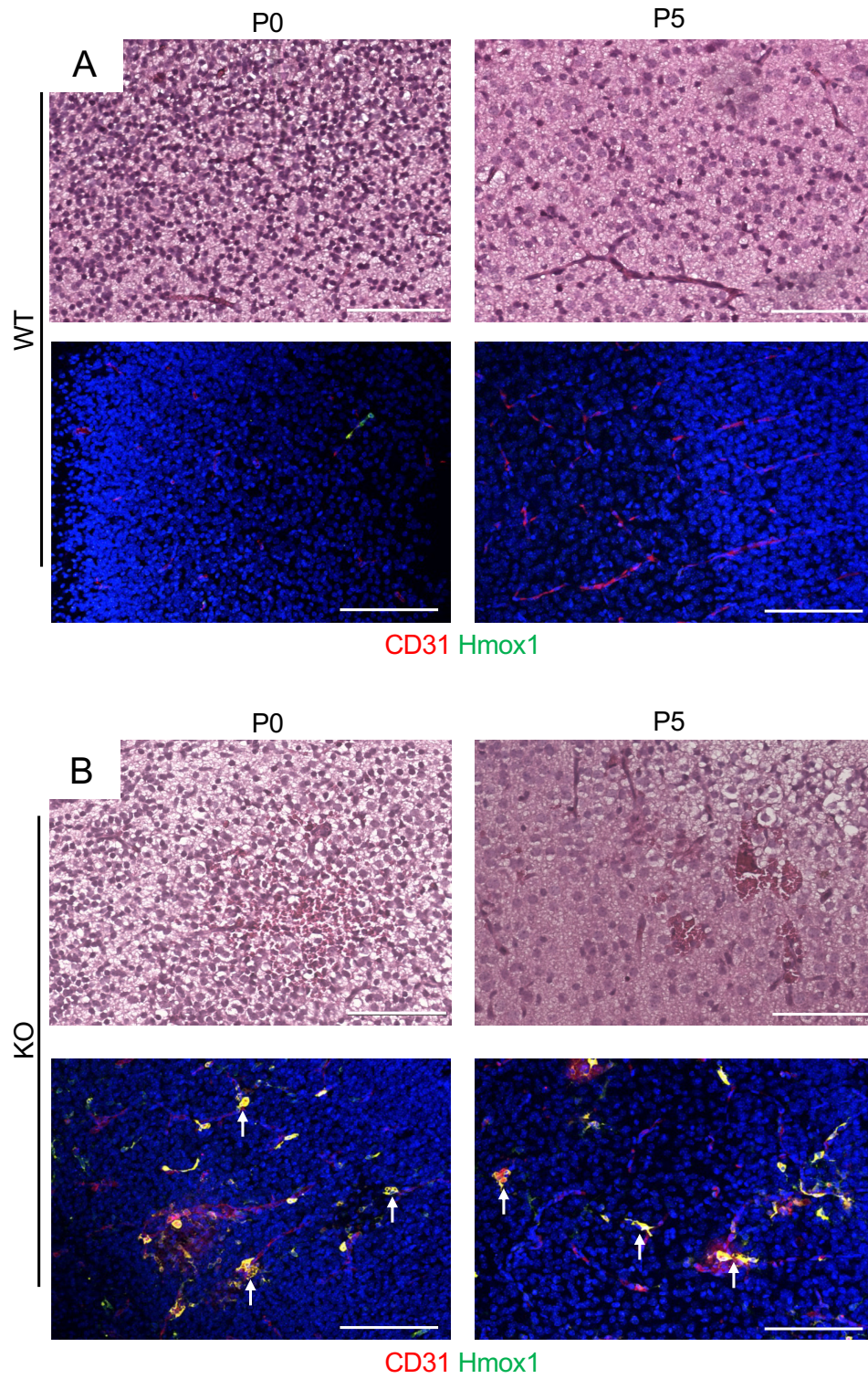
Supp. Figure 5



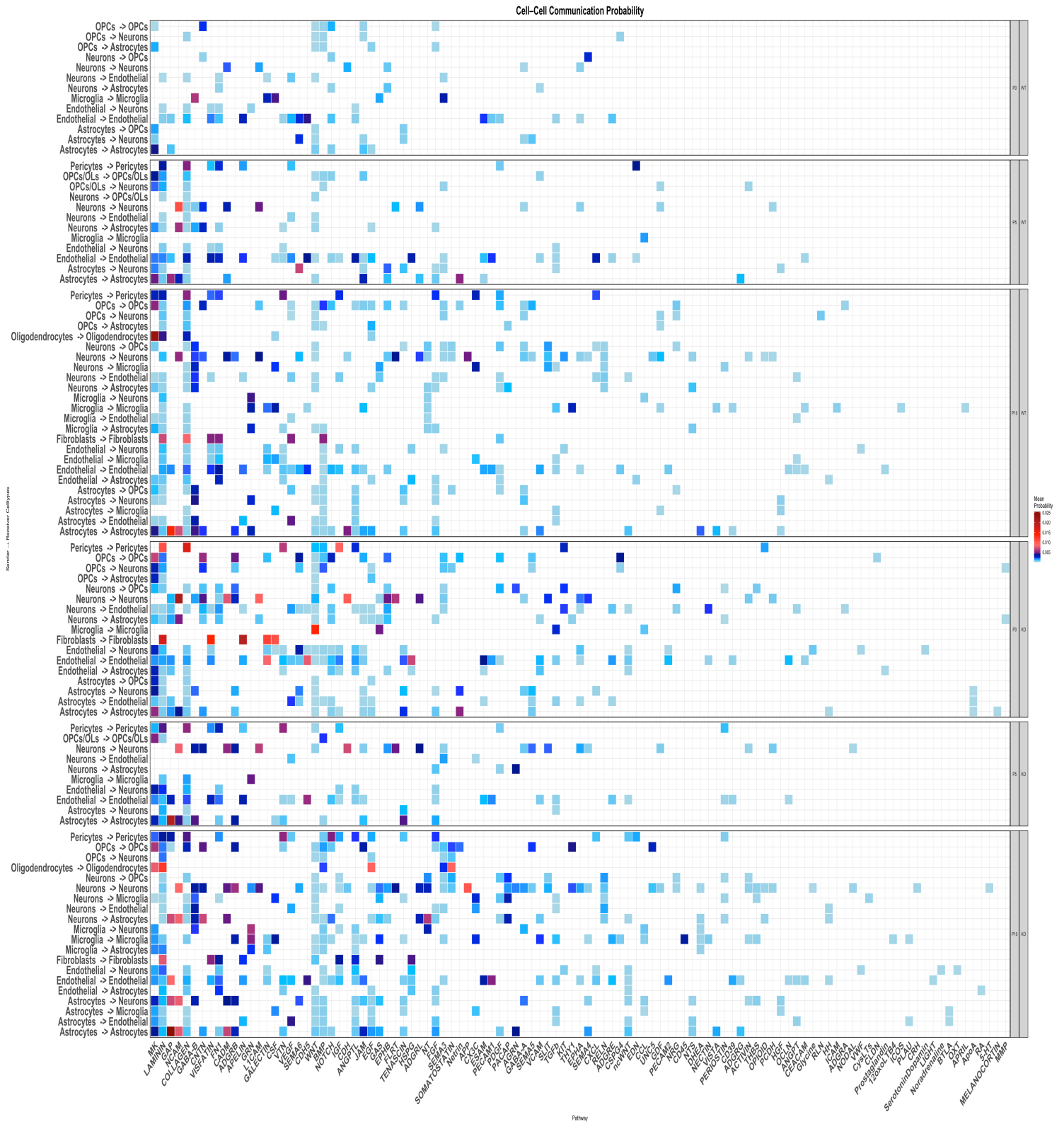
Supp. Figure 6



Supp. Figure 7



Supp. Figure 8



Supp. Figure 9

