

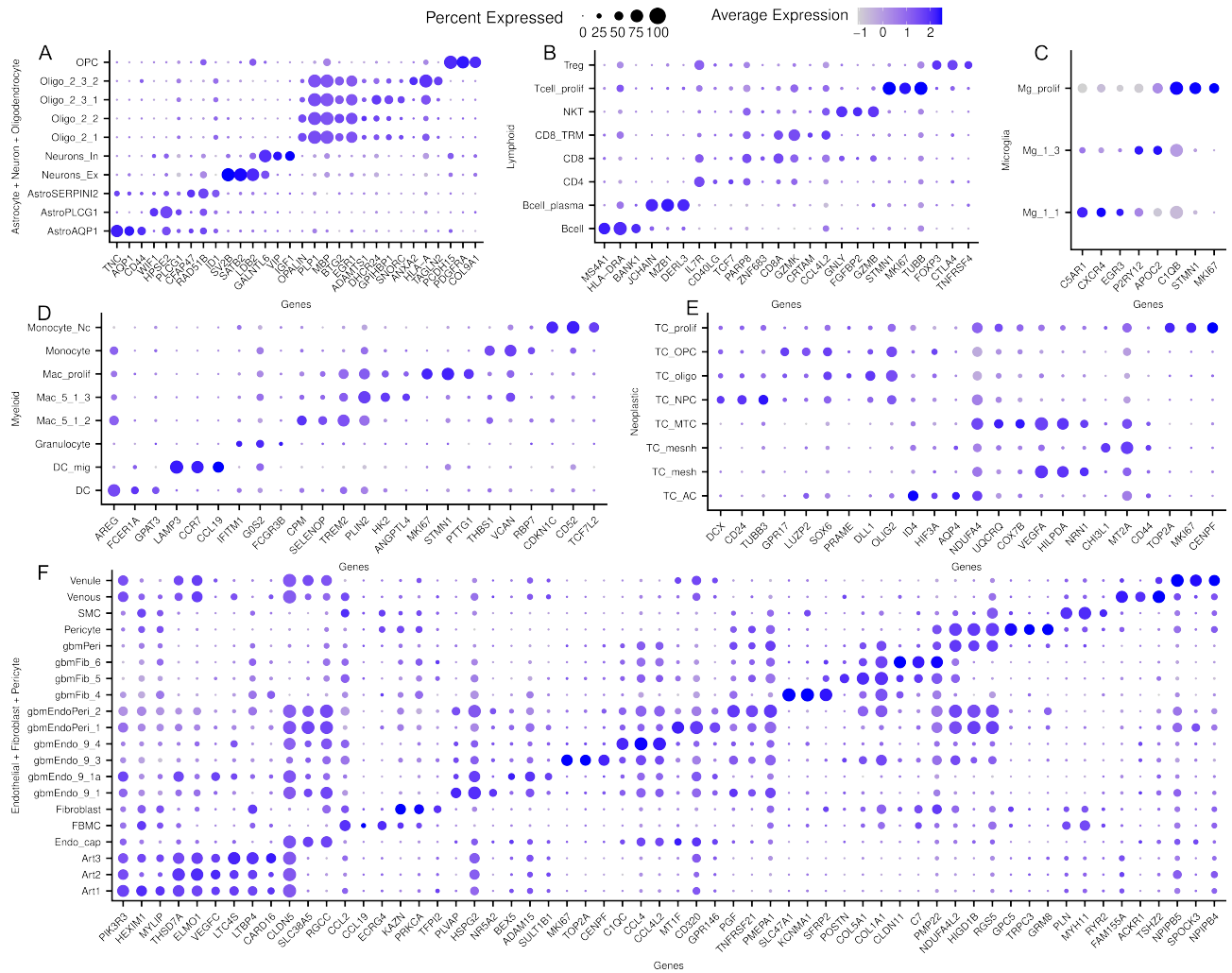


Figure S1:

Canonical markers for cell subtypes. Cell subtype marker genes for (A) astrocytes, neurons, and oligodendrocytes, (B) lymphoid, (C) microglia, (D) myeloid, (E) neoplastic, and (F) endothelial and stromal cells. The rows indicate genes and the columns indicate minor cell type assignments.

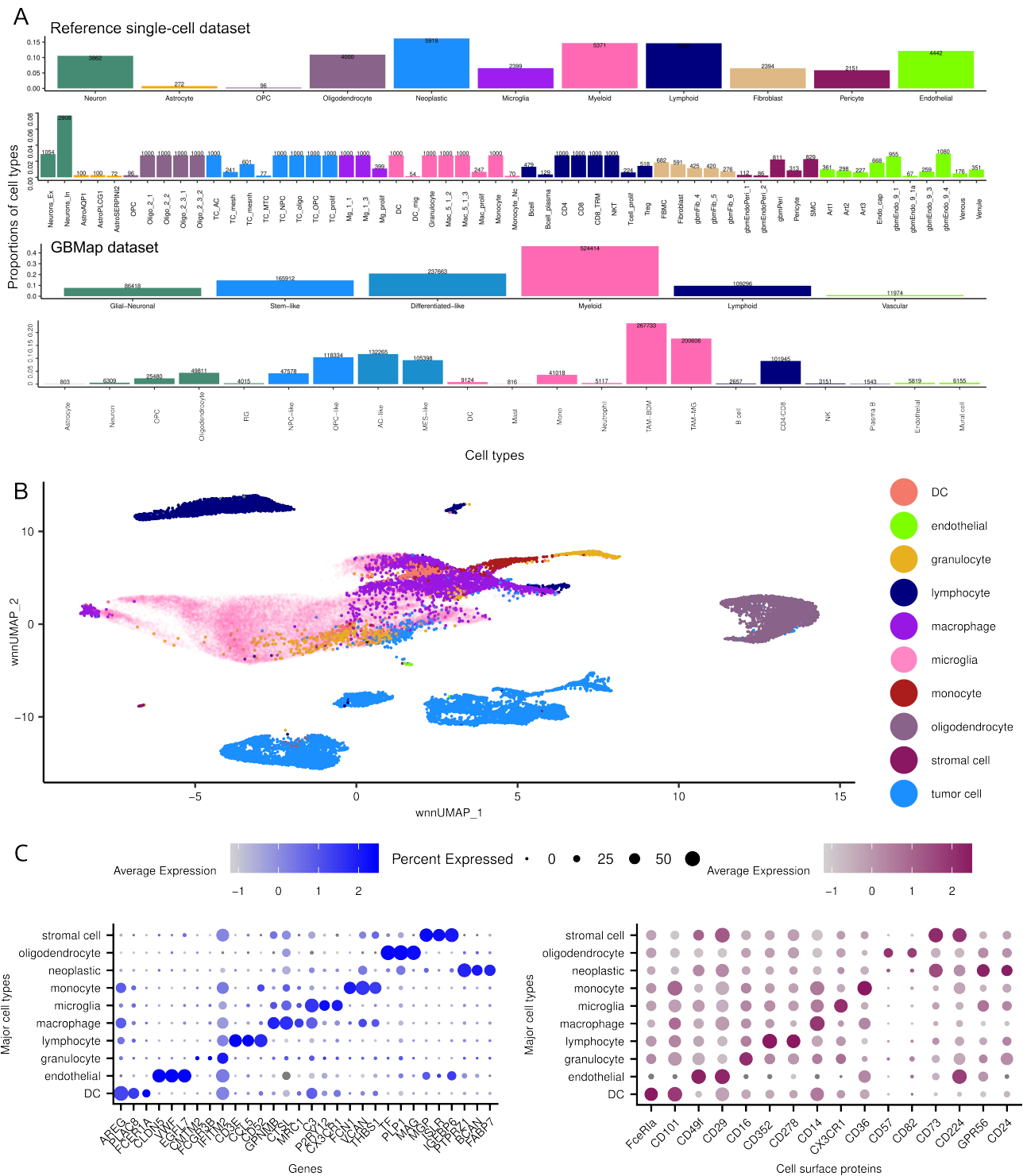


Figure S2:

(A) Major and minor cell type proportions in reference single-cell dataset and GBMap¹⁶. Bar plots show aggregated proportions of cell types across samples. Values represent descriptive summaries of the datasets; no statistical testing was performed. (B) UMAP representation single-cell profiled using CITE-seq overlaid with major cell type assignments. (C) Canonical marker genes (left) and cell surface protein markers (right) for major cell types.

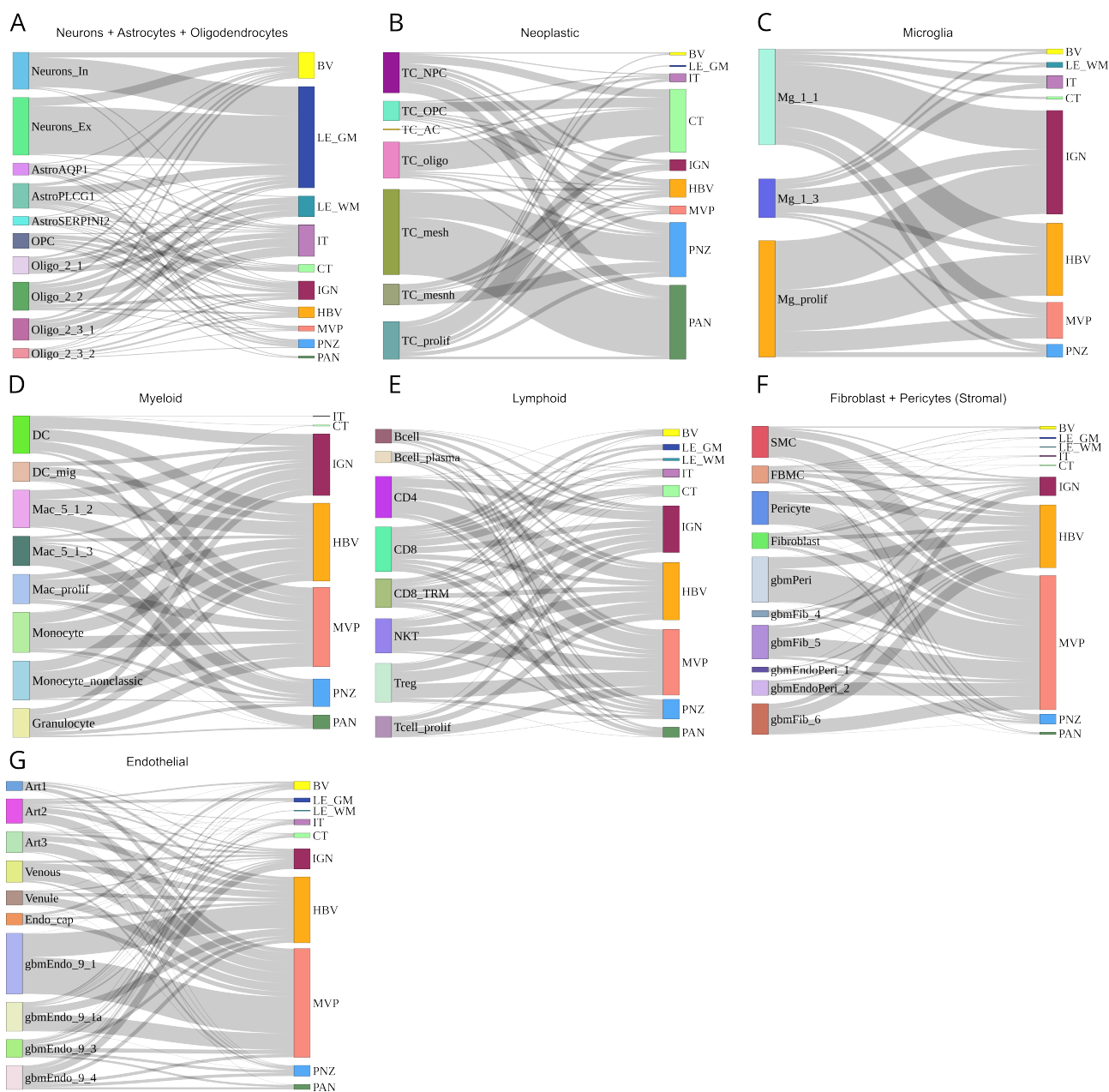
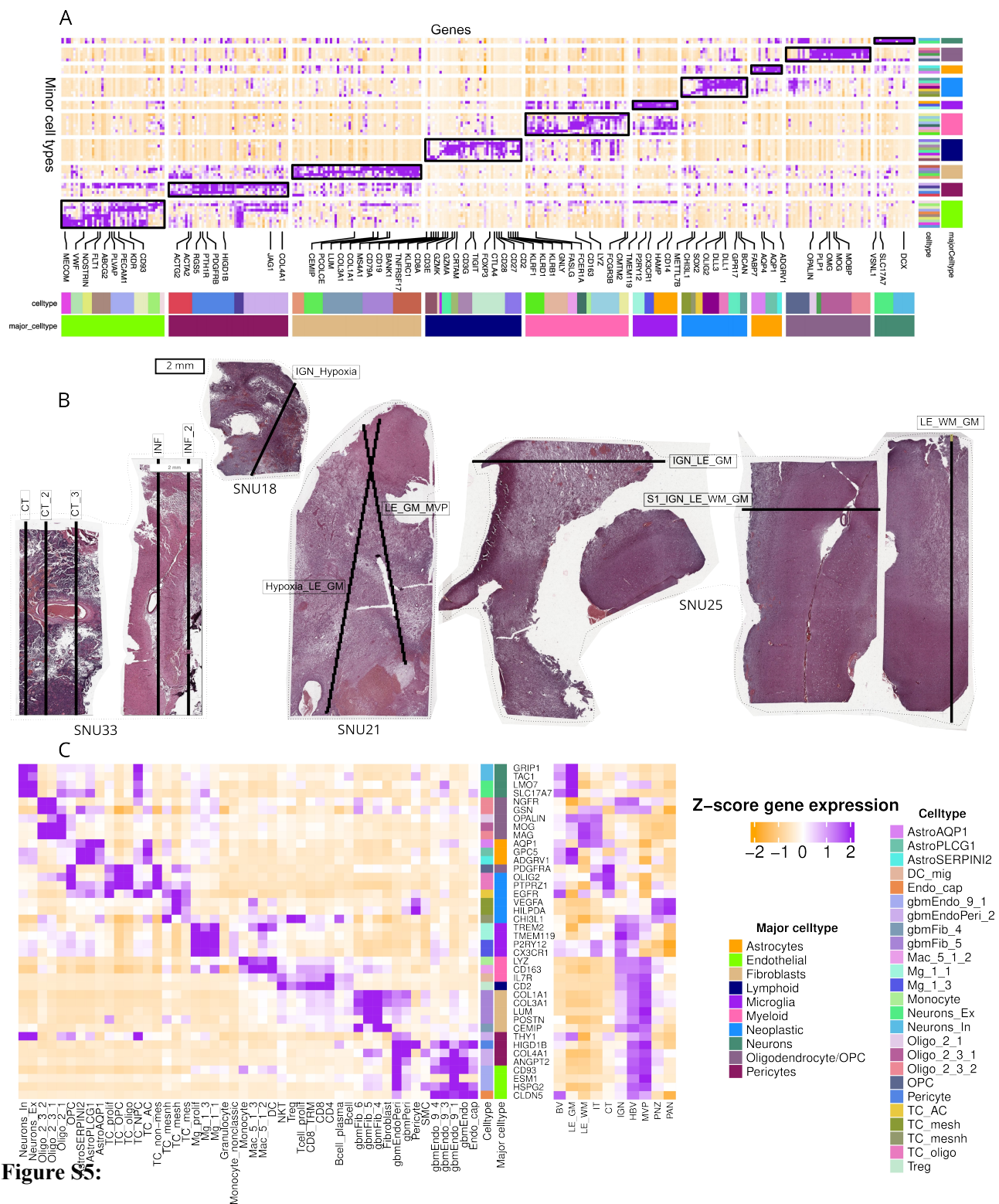


Figure S4:

River plot depicting (A) brain-specific (neurons + astrocytes + oligodendrocytes/OPCs), (B) neoplastic, (C) microglia, (D) myeloid, (E) lymphoid, (F) stromal, and (G) endothelial cell subtype contribution across AFs. (H) Heatmap representing average gene set expression of cellular states across all the AFs.



(A) Custom gene marker panel used for Xenium analysis. Genes used for major cell type assignment are highlighted. (B) H&E image with spatial trajectories selected for AF annotation. (C) Heatmap showing the average expression of 40 genes—used for anatomical feature (AF) assignment—across minor cell types from the reference single-cell RNA-seq dataset and anatomical features derived from the Visium spatial transcriptomics dataset. Scale bars: 2 mm.

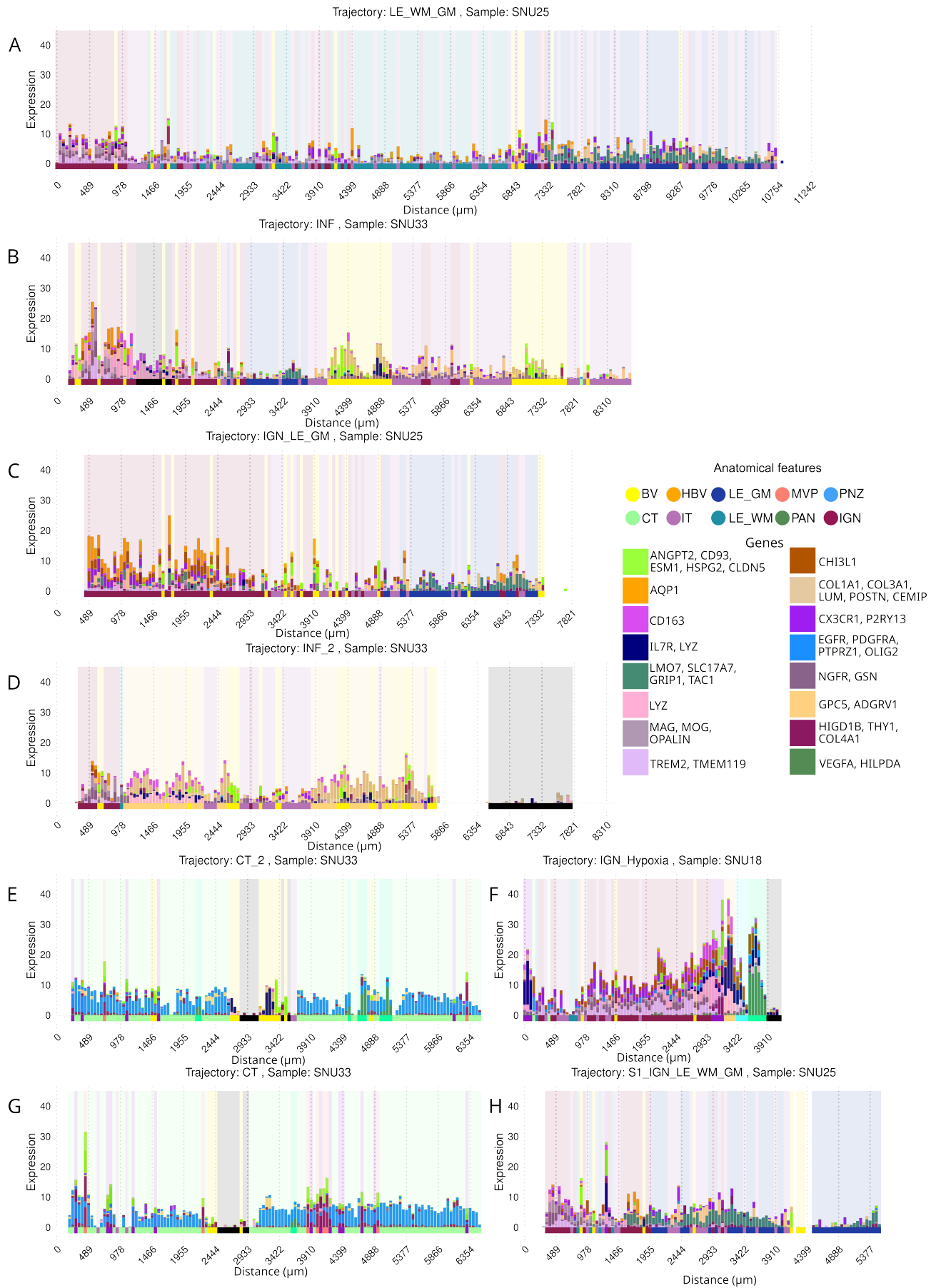


Figure S6:

Snapshots of trajectories selected for AF assignments (A-H). (A) LE_GM_WM, (B) INF, (C) IGN_LE_GM, (D) INF_2, (E) CT_2, (F) IGN_Hypoxia, (G) CT, and (H) S1_IGN_LE_WM_GM. The x-axis is the distance in micrometers, and the y-axis is the transcript expression of 40 genes selected for assignment.

recurrent GBM cases. Each bar represents a descriptive summary across samples; individual data points are not shown and no statistical testing was performed. (B) Differential gene expression analysis was performed between all oligodendrocyte subtypes and the Oligo_2_3_2 subtype in the single-cell transcriptomic dataset using pseudo-bulk patient-level data. DESeq2³⁹ package was used to identify differentially expressed genes. Genes with an adjusted p-value < 0.01 and $|\log_2FC| > 0.8$ were considered significant, with $\log_2FC > 0.8$ classified as up-regulated and $\log_2FC < -0.8$ as down-regulated. (C) SSGSEA Score Analysis Based on Oligo_2_3_2 Gene Expression. Single-sample Gene Set Enrichment Analysis (SSGSEA) scores were computed using genes differentially expressed in the Oligo_2_3_2 cell type. Genes downregulated in Oligo_2_3_2 exhibited higher expression scores in IDH-mutant, 1p/19q-codeleted gliomas, whereas upregulated genes showed increased expression in IDH-wildtype tumors. This pattern was consistently observed across both the TCGA (IDH-mutant/1p19q-codeleted, $n = 151$; IDH-mutant/1p19q non-codeleted, $n = 227$; IDH-wildtype, $n = 225$) and CGGA (IDH-mutant/1p19q-codeleted, $n = 182$; IDH-mutant/1p19q non-codeleted, $n = 315$; IDH-wildtype, $n = 435$) datasets. Differential expression profiles were analysed using non-directional one-way ANOVA. (D) Spatial Distribution of Oligo_2_1 and Oligo_2_3_2 in the 10x Xenium Dataset. The spatial proximity of Oligo_2_1 and Oligo_2_3_2 to other cell types was assessed using the 10x Xenium dataset. Oligo_2_1 exhibited closer spatial association with normal brain cell types, Mg_1_3 (inactive microglia), pericytes, and Endo_cap (normal endothelial cells). In contrast, Oligo_2_3_2 was more proximal to neoplastic mesenchymal non-hypoxic cells, Mg_1_1 (active microglia), Mac_5_1_2, and GBM-associated stromal/endothelial cell types. (E) Mutual Exclusivity of Oligo_2_3_2 and TC_mesh Gene Sets. Mutual exclusivity of the Oligo_2_3_2 and TC_mesh gene sets was assessed using the 10x Visium dataset. In the corresponding figure, white regions denote the absence of both gene sets, red regions indicate the presence of Oligo_2_3_2 genes, and green regions represent the presence of TC_mesh genes. Overlapping occurrences of both gene sets were rare, further supporting the hypothesis of mutual exclusivity. The mutual exclusivity was tested using the 'getMutexGroup' function in R. (F) Negative Correlation Between Mesenchymal Hypoxic and Oligodendrocyte Cell Types in the Greenwald Codex Dataset¹⁵. Cell type proportions were calculated based on author-provided annotations in the Greenwald Codex dataset. Oligodendrocyte cells exhibited a positive correlation with reactive astrocytes but a negative correlation with mesenchymal hypoxic cells. The '*' indicates significant pearson correlation between celltypes.

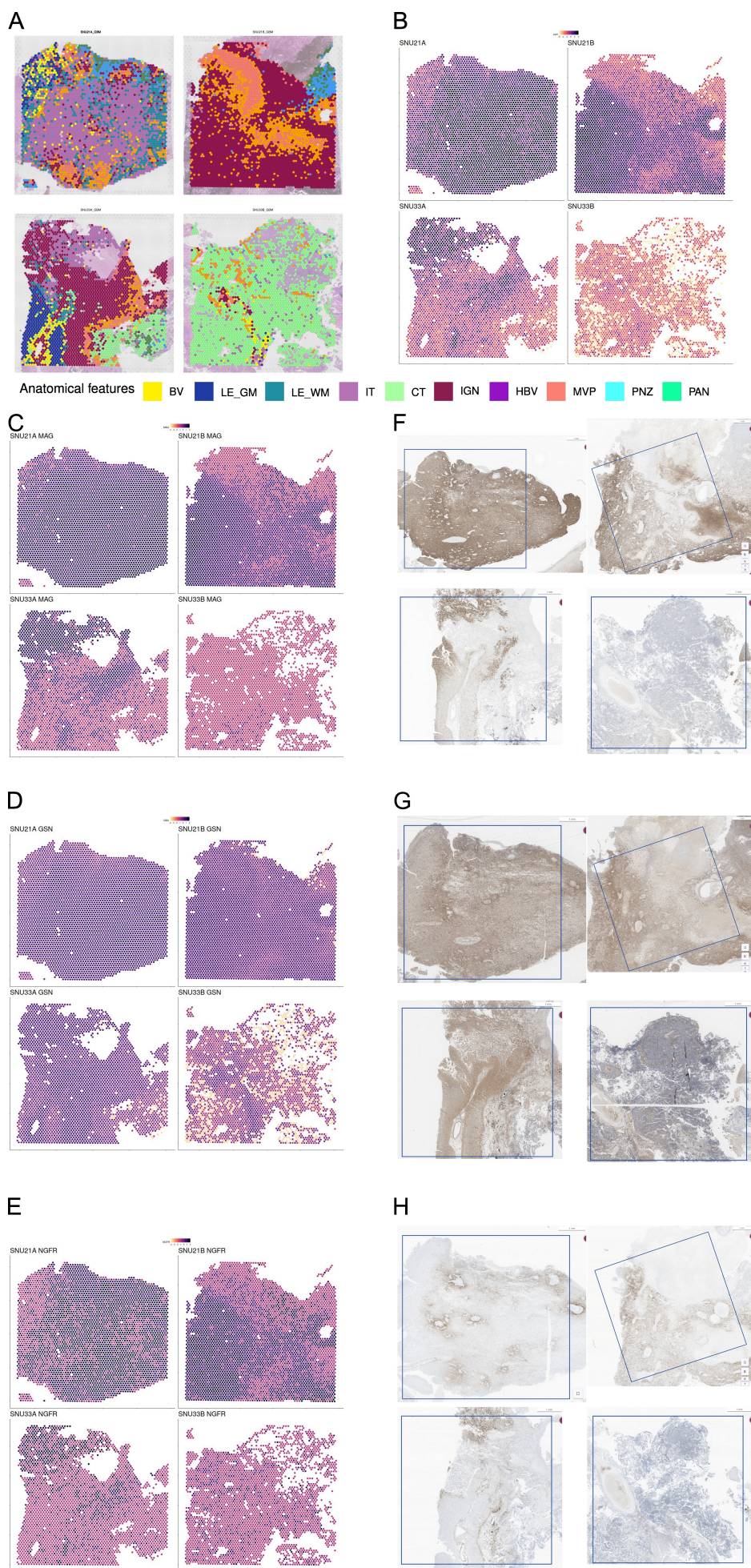


Figure S8:

IHC validation of GSN and NGFR markers upregulated in GBM-associated oligodendrocytes.

(A) Anatomic features of SNU21 (FFPE) and SNU33 visium slides. (B) Combined expression of MAG, GSN, and NGFR genes on SNU21 and SNU33 Visium slides. (C-E) Expression of MAG, GSN, and NGFR genes. (F-H) IHC results of corresponding proteins on the same blocks with blue squares highlighting the region equivalent to the regions of Visium slides. Scale bars: 1 mm.

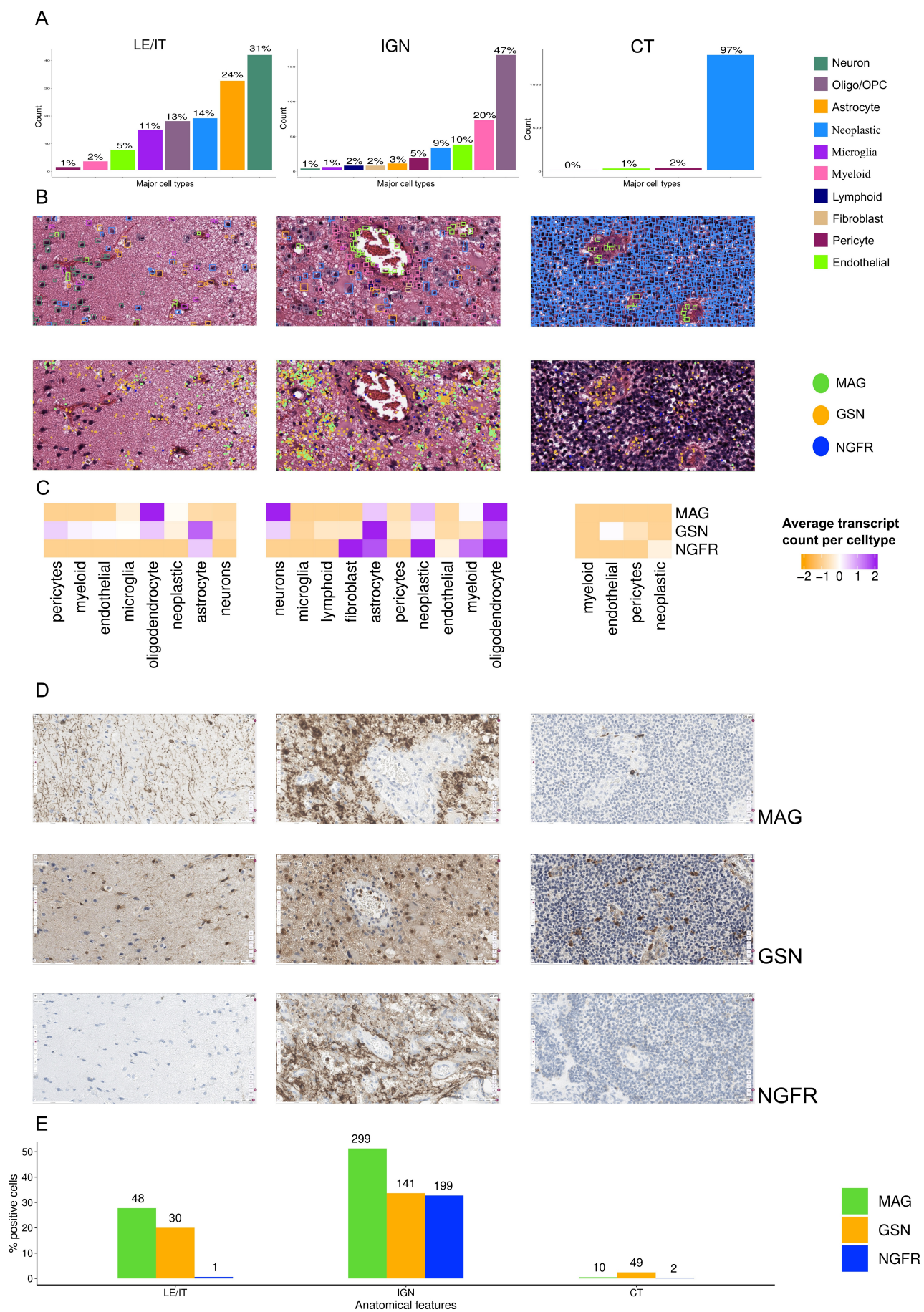


Figure S9:

Xenium and IHC validation of Oligo_2_3_2 cell type markers MAG, GSN, and NGFR

Three regions (LE/IT, IGN, and CT) from the SNU33 sample were selected for further validation.

(A) Barplots showing major cell type counts in all three regions. Neurons were highly enriched in LE/IT region, oligodendrocytes were enriched in the IGN region, and neoplastic cells were enriched in the CT region. The percentages indicate the proportion of each cell type within the respective region. (B) Overlaid major cell type assignment and presence of MAG, GSN and NGFR in all three regions. (C) Heatmaps showing the averaged transcript counts of MAG, GSN and NGFR across all the cell types in the LE/IT, IGN, and CT regions. (D) IHC validation of MAG, GSN, and NGFR markers in SNU33 sample showing their protein expression patterns in tissue sections. (E) Quantitative expression of MAG, GSN and NGFR in marked regions. The bar plots show the percentage of positive cells in each regions, whereas the values displayed are the absolute cell counts. Data represent aggregated cell counts across regions and are shown for descriptive purposes; individual data points are not shown and no statistical testing was performed. Scale bars: 20 μm .

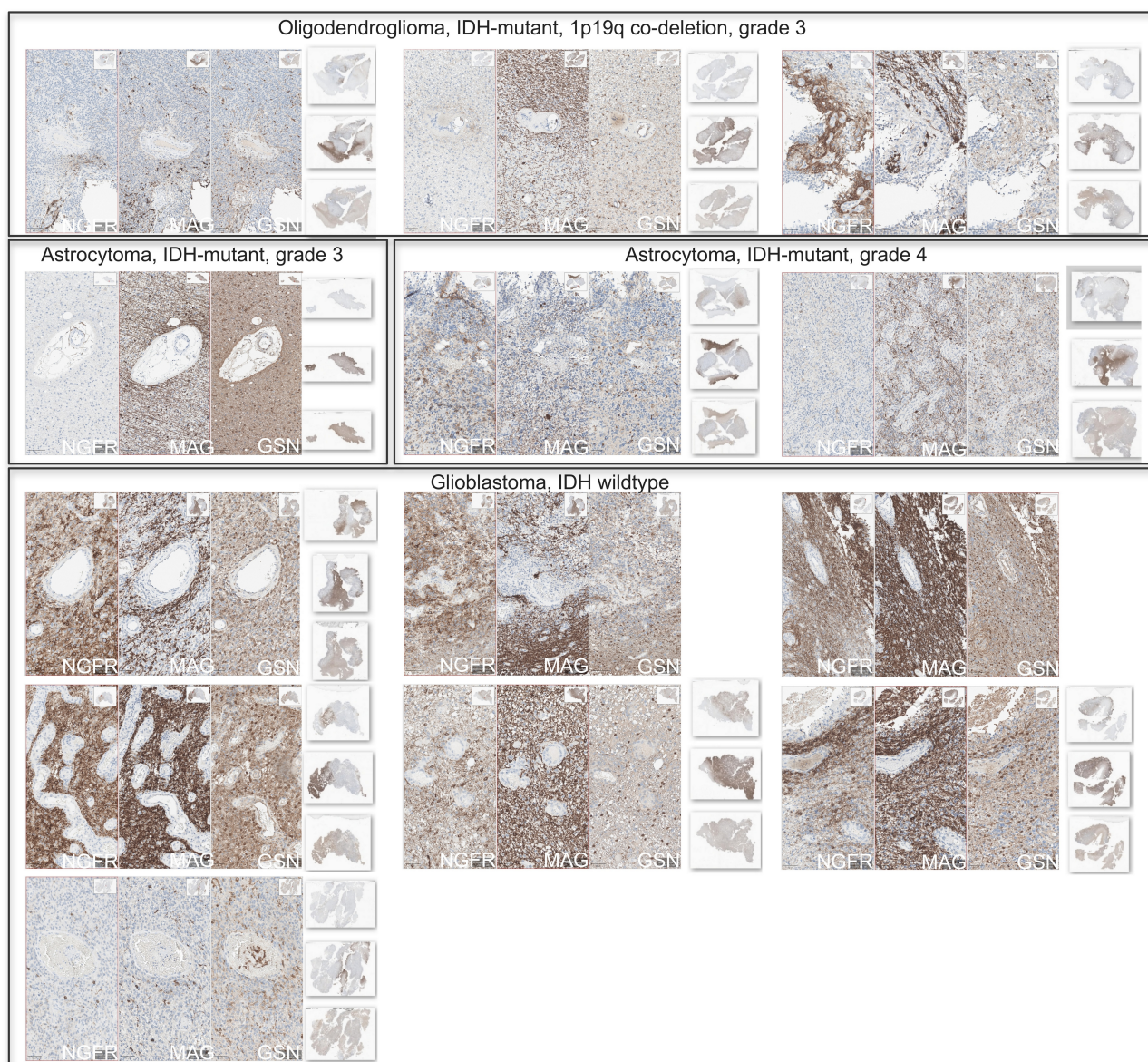


Figure S10:

Immunohistochemical analysis of NGFR, MAG, and GSN expression in representative Oligodendroglioma, IDH-mutant and 1p/19q-codeleted (n = 3), Astrocytoma, IDH-mutant (n = 3), and Glioblastoma, IDH-wildtype (n = 5) samples.

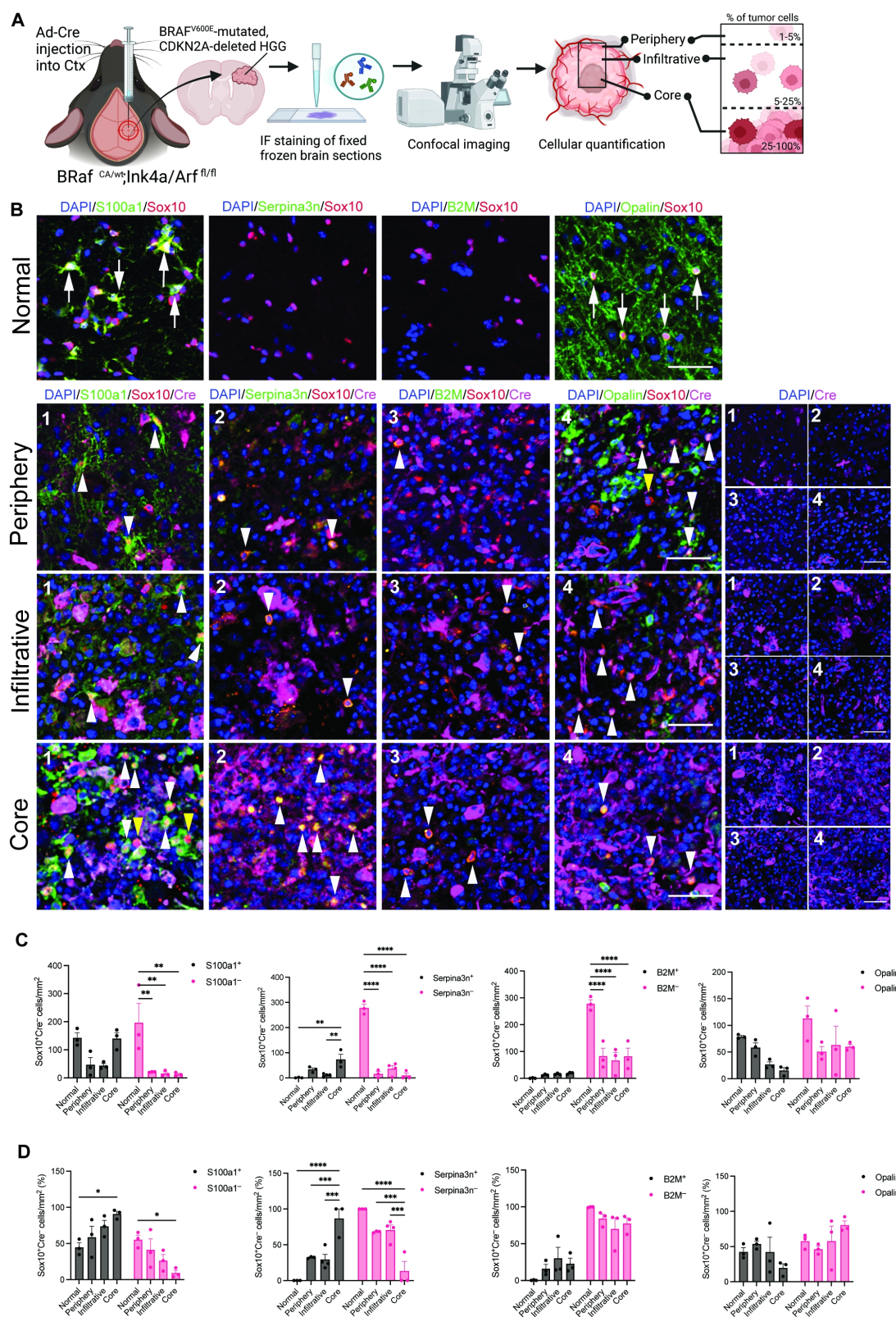


Figure S11:

In vivo validation of Oligo_2_3_2 markers in glioma-associated oligodendrocytes

(A) Representative IF images of normal and tumor-bearing mouse brains with BRAFV600E-mutated, CDKN2A-deleted HGG. Mouse tissue sections were stained for Oligo_2_3_2 markers including S100a1, Serpina3n, B2M and Opalin, as well as Sox10 and Cre to detect glioma-associated oligodendrocytes and

tumor cells, respectively. DAPI is used to stain nuclei. In the normal brain sections, arrows indicate triple-positive cells. *Serpina3n* and *B2M* are undetectable under normal condition. In the tumor-bearing brain sections, white triangles indicate triple-positive glioma-associated oligodendrocytes that are negative for Cre tumor cell marker. Yellow triangles indicate Cre-negative, *S100a1*/*Opalin*-negative oligodendrocytes. Separate DAPI/Cre-stained images are shown on the right to distinguish Cre from *Sox10* in the corresponding numbered images. (B-C) Cellular quantification of (B) showing densities (C) or percentages of *Sox10*+Cre⁻ glioma-associated oligodendrocytes positive or negative for *S100a1*, *Serpina3n*, *B2M* or *Opalin* in the normal and tumor-containing regions. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (2-way ANOVA with Sidak's multiple comparisons test). N = 3 mice per group, data represented as mean $\pm$ SEM. Scale bars: 50 μ m.