

Supplementary Information

A blueprint for local and distal invasion programs in glioblastoma

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Figure S1

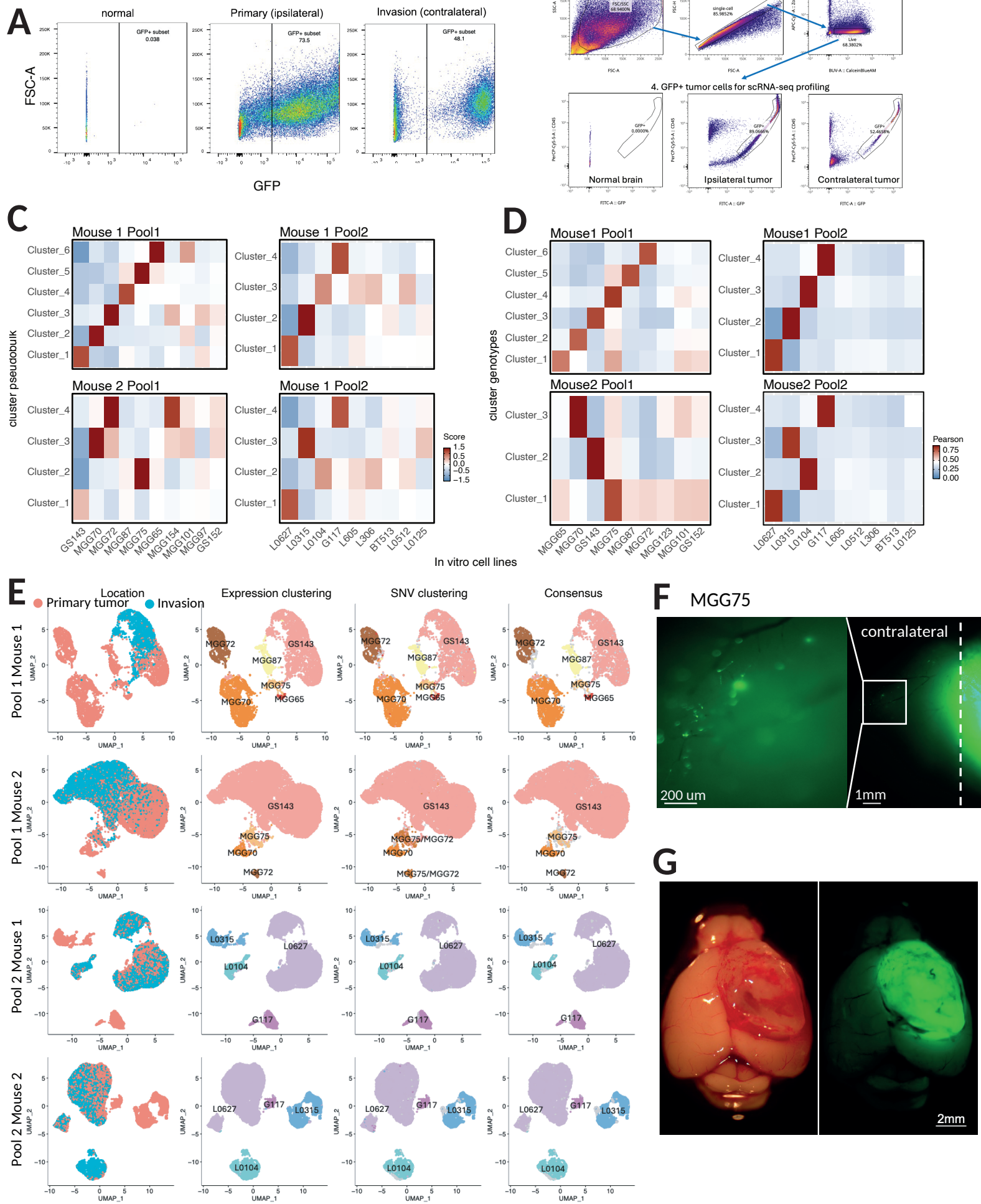
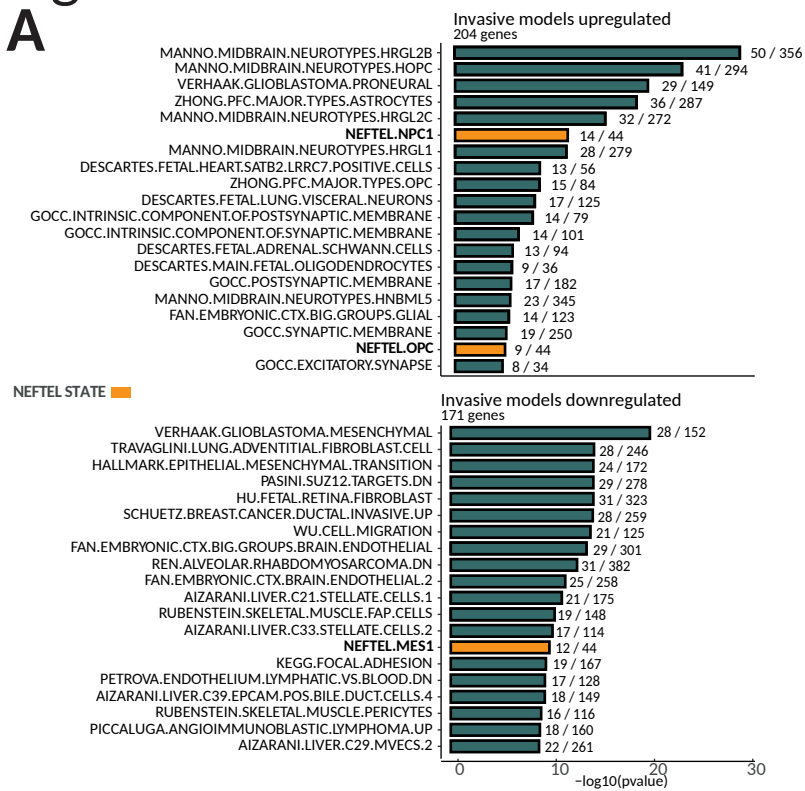


Figure S1: Multiplex system. (A) FACS plots of normal brain (left), ipsilateral hemisphere (middle) and contralateral hemisphere (right) for GFP to quantify GFP+ malignant cells at each location. (B) FACS gating strategy for S1A. (C) Heatmaps show the scores of each Louvain cluster (y axis) from each mouse (2 pools, 2 mice) for each in vitro cell line signature (x axis). (D) Heatmaps show the correlation of the genotypes for each souporecell cluster (y axis) from each mouse with the genotypes of the in vitro cell lines (x axis). (E) UMAPs in each row of panels show one mouse, where cells are colored by (left to right): location isolated, expression-based classification, SNV-based classification, or consensus classification. (F) GFP fluorescence images of brain with GFP-labelled MGG75 model, showing GFP+ malignant single-cells in the contralateral hemisphere. (G) GFP fluorescence images of whole brain with GFP-labelled non-infiltrative D270 model, showing GFP signal observed only in the ipsilateral hemisphere.

Figure S2

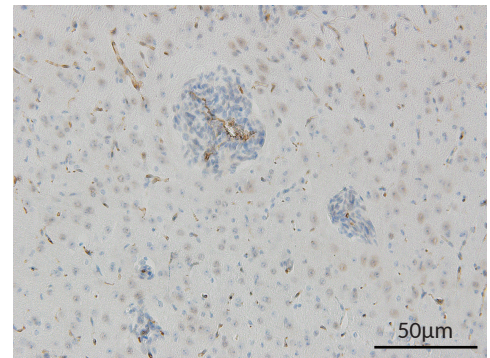
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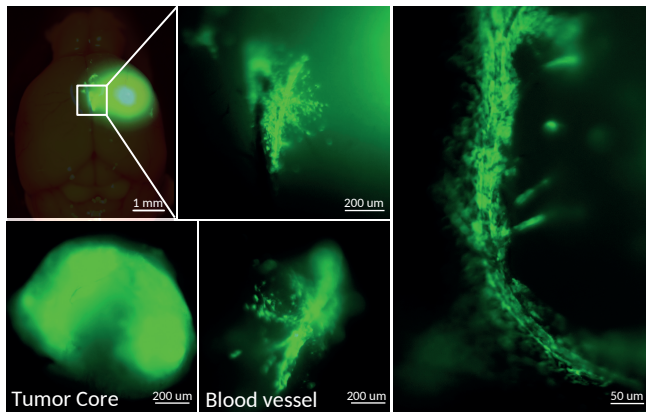
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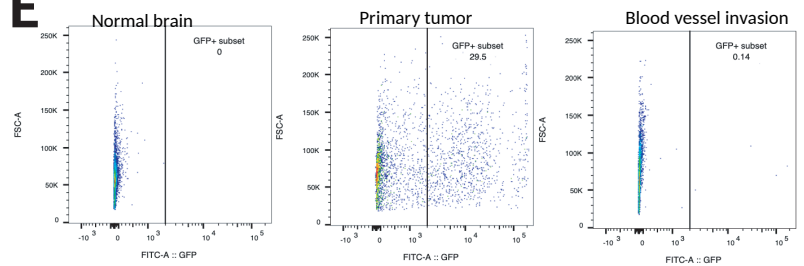


Figure S2: Comparing distant and local invasion. (A) Gene pathway enrichment plots for differentially expressed genes from (Fig 2A), showing pathways upregulated in invasive (top) and in noninvasive (bottom) models. (B) Barplots show the proportion of each metaprogram from Neftel et al in each model. Models ordered by invasive (left), noninvasive (middle) and right two bars represent the averages of invasive and noninvasive models. (C) Immunohistochemistry analysis detecting CD31 (brown color) and nuclei (blue) in MGG123 model, showing perivascular invasion patterns. (D) GFP fluorescence images of brain with GFP-labelled MGG123 model. GFP signal from the tumor periphery showing vascular structural patterns. (E) FACS plots of GFP intensity in a normal mouse brain (left), tumor core (middle), or cells collected along blood vessels (right). Source data are provided as a Source Data file.

Figure S3

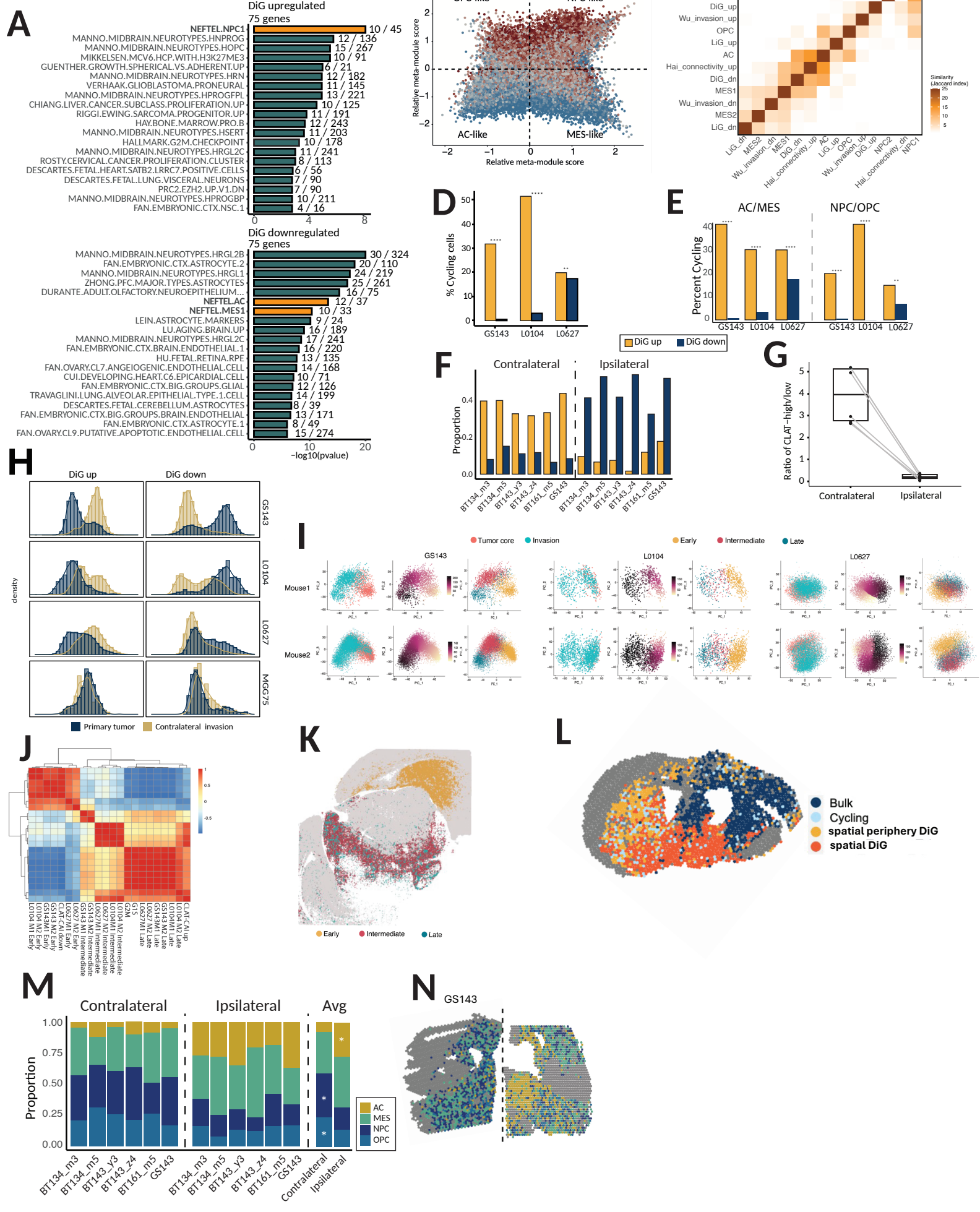
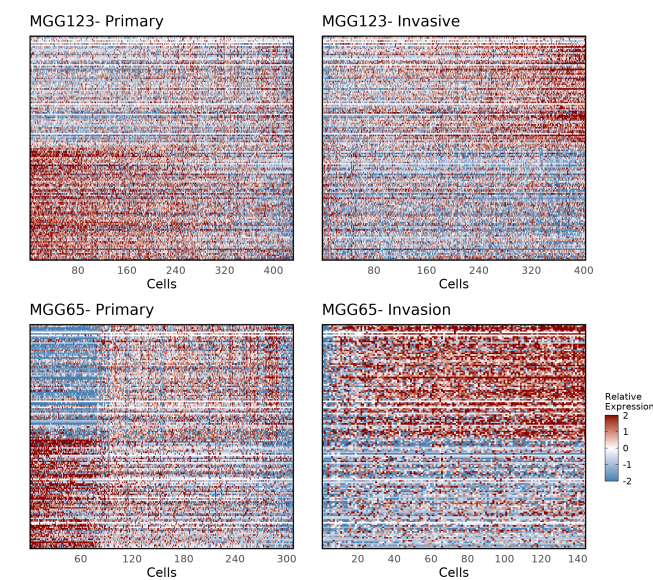


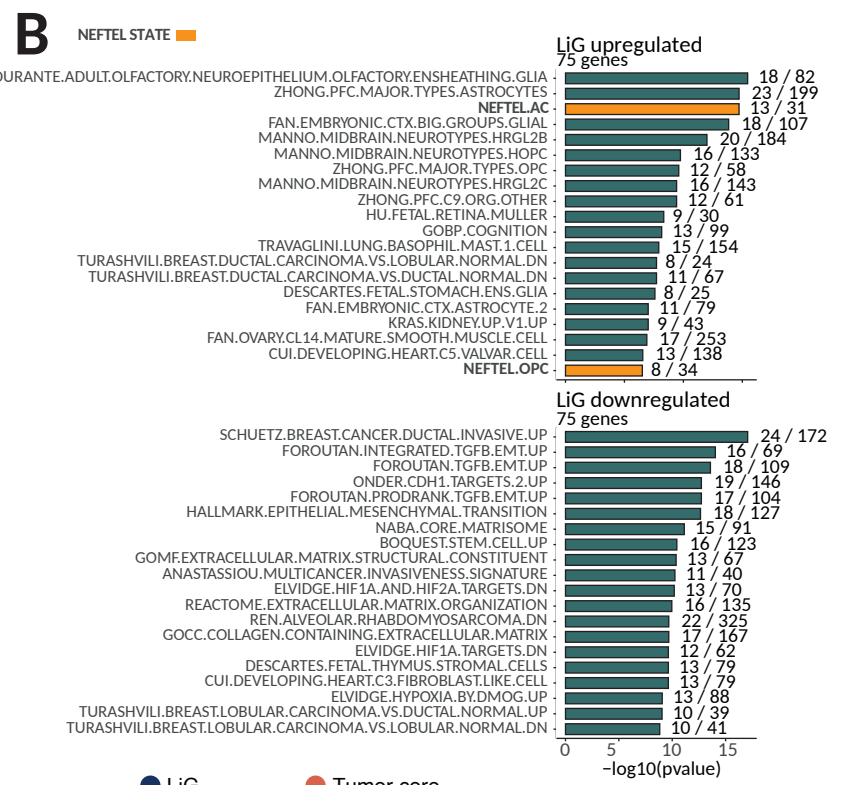
Figure S3: Profiling distally invading cells. (A) Gene pathway enrichment plots, showing pathways upregulated (top) or downregulated (bottom) in DiG. (B) Scatterplot ("star plot") displays the distribution of malignant cells across the GBM metaprogram space, colored by their DiG score. (C) Heatmap illustrating Jaccard similarity (gene overlap percentage) between invasion signatures from this study (LiG/DiG), canonical GBM metaprograms (Neftel et al.), and published invasion programs (Venkataramani et al./Wu et al.). (D-E) Barplots showing the percent of cells in the cell cycle states G1S and G2M across three invasive models (GS143, L0104, L0627). Cells are grouped by location (core: navy; invasive: yellow) in (D), and subset into AC/MES-like (left) and NPC/OPC-like (right) states in (E). Standard error bars and paired t-test significance ($p < 0.05$) are shown. (F) Barplots quantifying proportions of malignant spatial spots assigned as DiG high (yellow) or DiG low (navy) in the contralateral (left) versus ipsilateral (right) hemispheres across six samples. (G) Boxplot comparing the ratio of average DiG upregulated to DiG downregulated scores between the contralateral and ipsilateral hemispheres. Paired dots (same sample) are connected by lines; significance is by Wilcoxon rank test ($p < 0.05$). (H) Histograms showing the scores for the spatial DiG upregulated (left) and downregulated (right) programs, colored by location (core: navy; invasive: yellow). (I) Scatterplots (PC plots) of the three invasive models (GS143, L0104, L0627), separated by mouse (two rows). Plots are colored by Location, Pseudotime, and assignment to a Time-associated program (early, intermediate, late). (J) Correlation plot showing the correlation between time-associated program expression, invasion programs, and cell cycle programs. (K) Spatial map of GS143 (Visium HD), where malignant cells are colored by their assignment to the time-associated programs. (L) Spatial map of BT134, where malignant spots are colored by assignment to the spatial periphery DiG (yellow), spatial DiG (orange), tumor core (dark blue), or cycling (blue). (M) Boxplot comparing the proportion of cells in each canonical Neftel state in contralateral versus ipsilateral malignant spots, with paired lines connecting same-sample data ($p < 0.05$, Wilcoxon rank test). (N) Spatial map of GS143 where malignant spots are colored by canonical Neftel states. Source data are provided as a Source Data file.

Figure S4

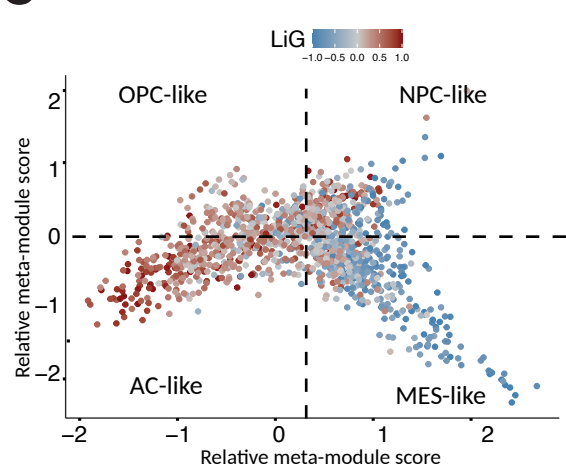
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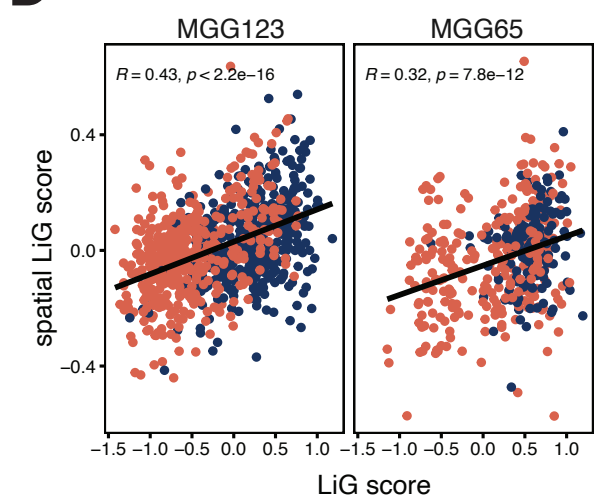
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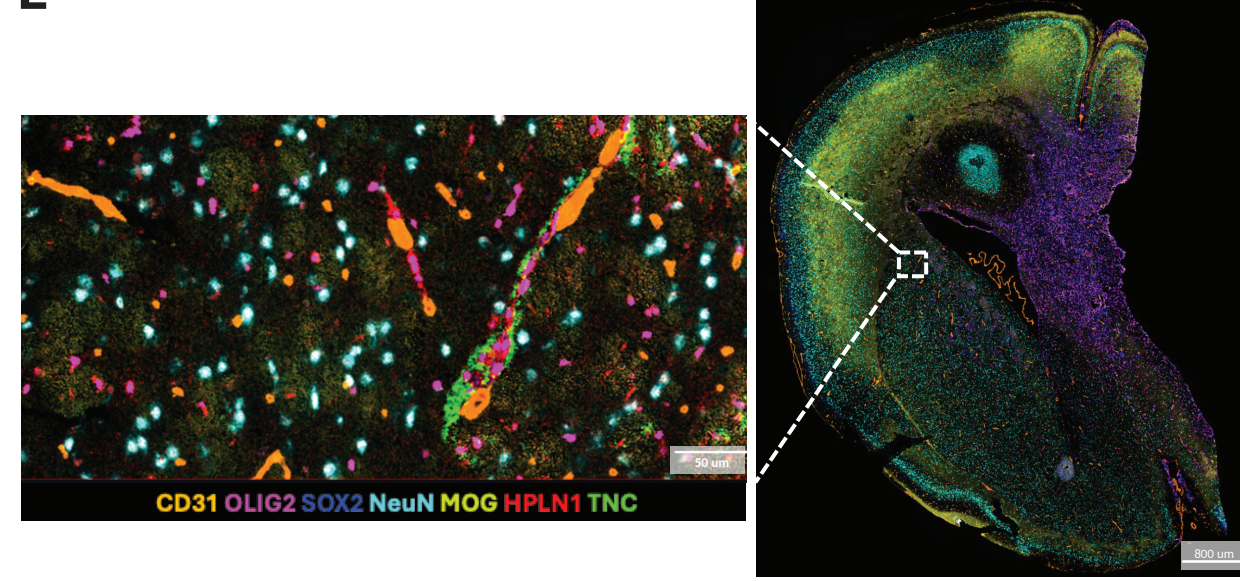


Figure S4: Profiling locally invading cells. (A) Heatmaps show the expression of LiG program genes (bottom: downregulated, top: upregulated) in the tumor core cells (left) and blood vessel invading cells (right) in either MGG23 (top) or MGG65 (bottom) (B) Gene pathway enrichment plots, showing pathways upregulated in LiG upregulated (top) and in downregulated genes (bottom). (C) Scatterplot shows the “star plot” showing the distribution of metaprogram cell states from the cells of the blood-vessel invading models, as presented in Neftel et al. Cells are colored by their score for LiG. (D) Scatterplot shows the correlation between LiG score (X-axis) and the spatially defined LiG score (Y-axis) for single cells in either MGG123 or MGG65. Cells are colored by their location (tumor core in red, BV invading cells in blue). (E) Representative CODEX image of the contralateral hemisphere showing OPC glioma cells (OLIG2+) with invasion along blood vessels (CD31+) and deposition of the ECM proteins (TNC+, HPLN1+).

Figure S5

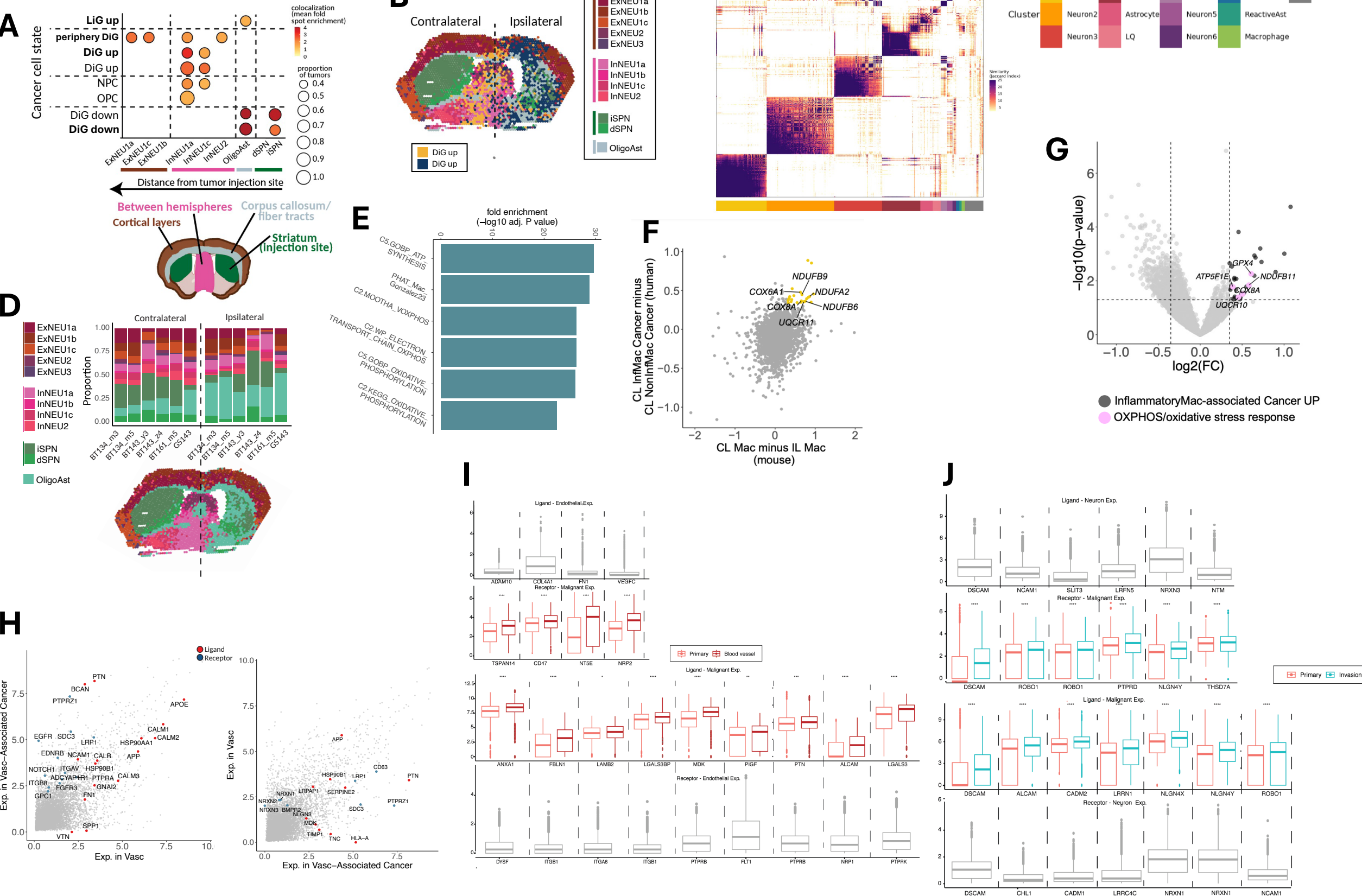
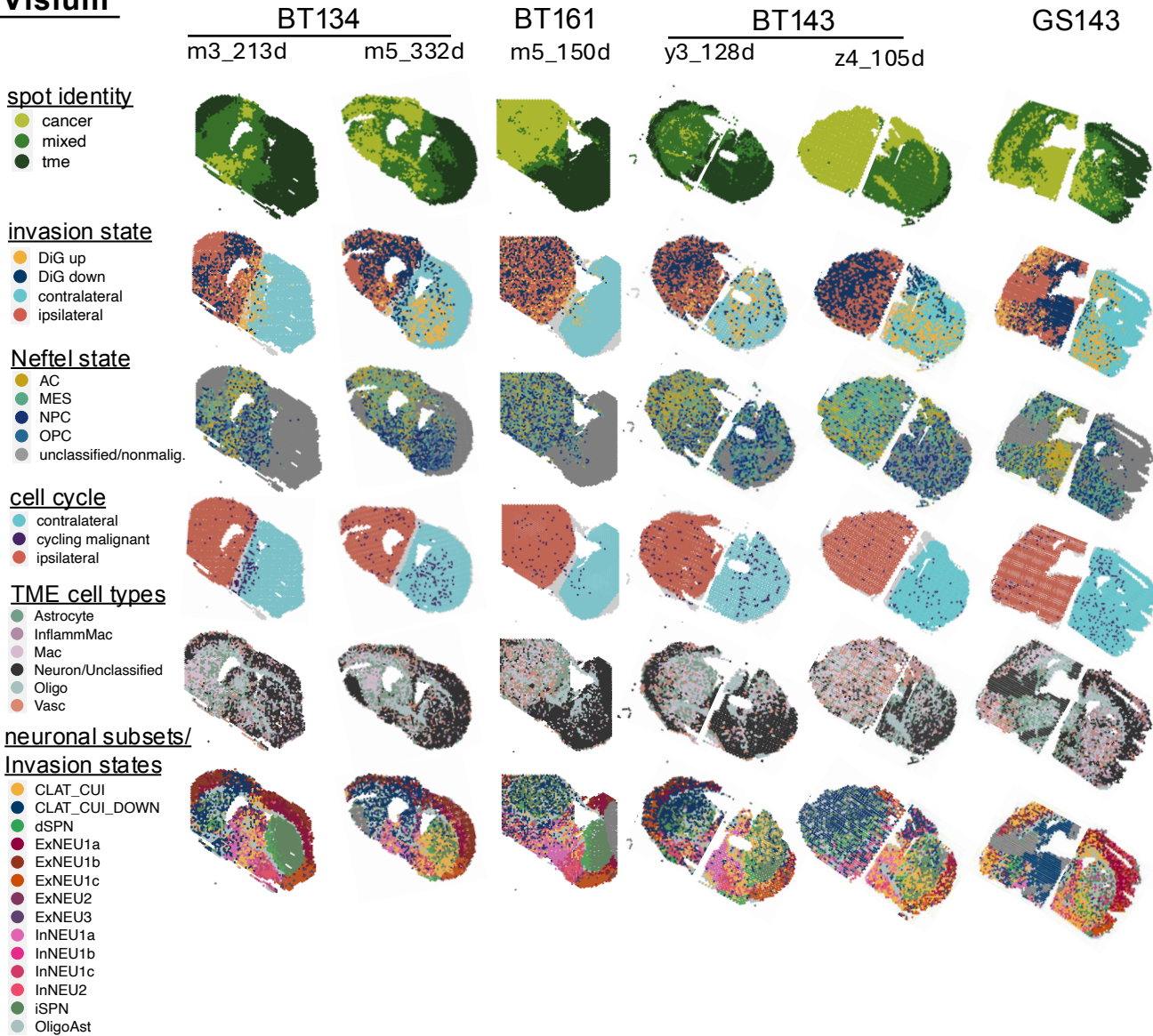


Figure S5: Spatially dissolved invasion programs and their associations with TME. (A) Heatmap shows spatial associations between spatial neuronal programs (X-axis) and different cancer cell states (Y axis), where the color of the circle is the mean fold spot enrichment and the size of the circle represents the proportion of tumors in which the association is found. A scheme of the anatomical structure of the neuronal subsets is drawn below. (B) Spatial map of BT134, where nonmalignant spots are colored by the neuronal spatial programs and the malignant spots are colored by DiG programs. (C) Heatmap shows the Jaccard similarity of the nonmalignant cell type spatial metaprograms. (D) Spatial map of BT134, where nonmalignant spots are colored by the neuronal spatial programs (E) Barplot shows the enrichment of different programs (Y-axis) in DE expressed genes by contralateral compared to ipsilateral macrophages. (F) Scatterplot shows differential expression of contralateral Mac – ipsilateral Mac spots (mouse) (X-axis) vs contralateral InfMac-associated cancer vs. – contralateral non-InfMac-associated cancer (human) (Y-axis). OXPHOS genes are colored in yellow. (G) Volcano plot shows log2FC (X-axis) vs -log10pvalue for the comparison of cancer cells in the contralateral hemisphere that were coupled to Inf-Mac vs not. OXPHOS genes are labeled in pink and InfMac program genes in dark grey. (I-K) Ligand-receptor analysis: (H) Left: dot plots show average expression of genes in vascular-containing Visium spots (x-axis) and in spots with malignant cells associated with vascular (y-axis). Ligands in red are highly expressed in vascular with corresponding receptors (blue) expressed highly in vascular-associated malignant cells. Right: Same as let, but in the reverse direction. (I) Top: Ligands expressed in endothelial cells with corresponding receptors in blood-vessel invading cells. Bottom: Ligands expressed in blood-vessel invading cells with receptors expressed in endothelial cells from. (J) Top: Ligands expressed in neurons from with corresponding receptors in contralateral invading cells. Bottom: Ligands expressed in contralateral invading cells with receptors expressed in neurons.

Figure S6

Visium



Visium HD

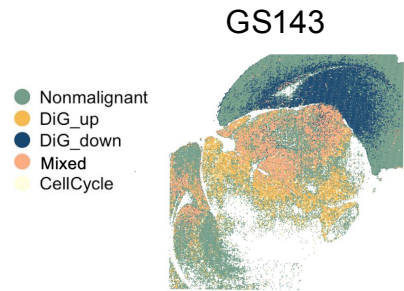


Figure S6: Spatial maps of the 6 samples. Top: (each column) profiled by Visium ST. GS143 is from this study and the others were profiled by Manoharan et al. Each row is colored by a different spot identity. Row 1: Colored by spot origin (cancer, mixed, or TME). Row 2: Colored by DiG. Spots that scored highly for DiG up are colored in yellow and spots that scored highly for DiG downregulated genes are colored in blue. Spots that did not have enough human reads (i.e., nonmalignant spots) or did not score highly for either program are colored by hemisphere (ipsilateral: red, contralateral: blue). Row 3: Spots are annotated by Neftel cell states. Nonmalignant spots are colored in grey. Row 5: Spots are annotated by cell cycle status. Cycling malignant spots are colored purple. All other spots are colored by hemisphere. Row 6: Non-malignant spots are colored by TME cell types. Neurons and malignant spots are colored grey. Row 7: Nonmalignant spots are colored by neuronal subsets, and malignant spots are colored by DiG states as in Row 2. Bottom: Visium HD spatial map of GS143. Spots that scored highly for DiG up are colored in yellow and spots that scored highly for DiG downregulated genes are colored in blue. Neuronal subtypes are colored by shades of green and light blue. Cycling malignant spots are colored in yellow.