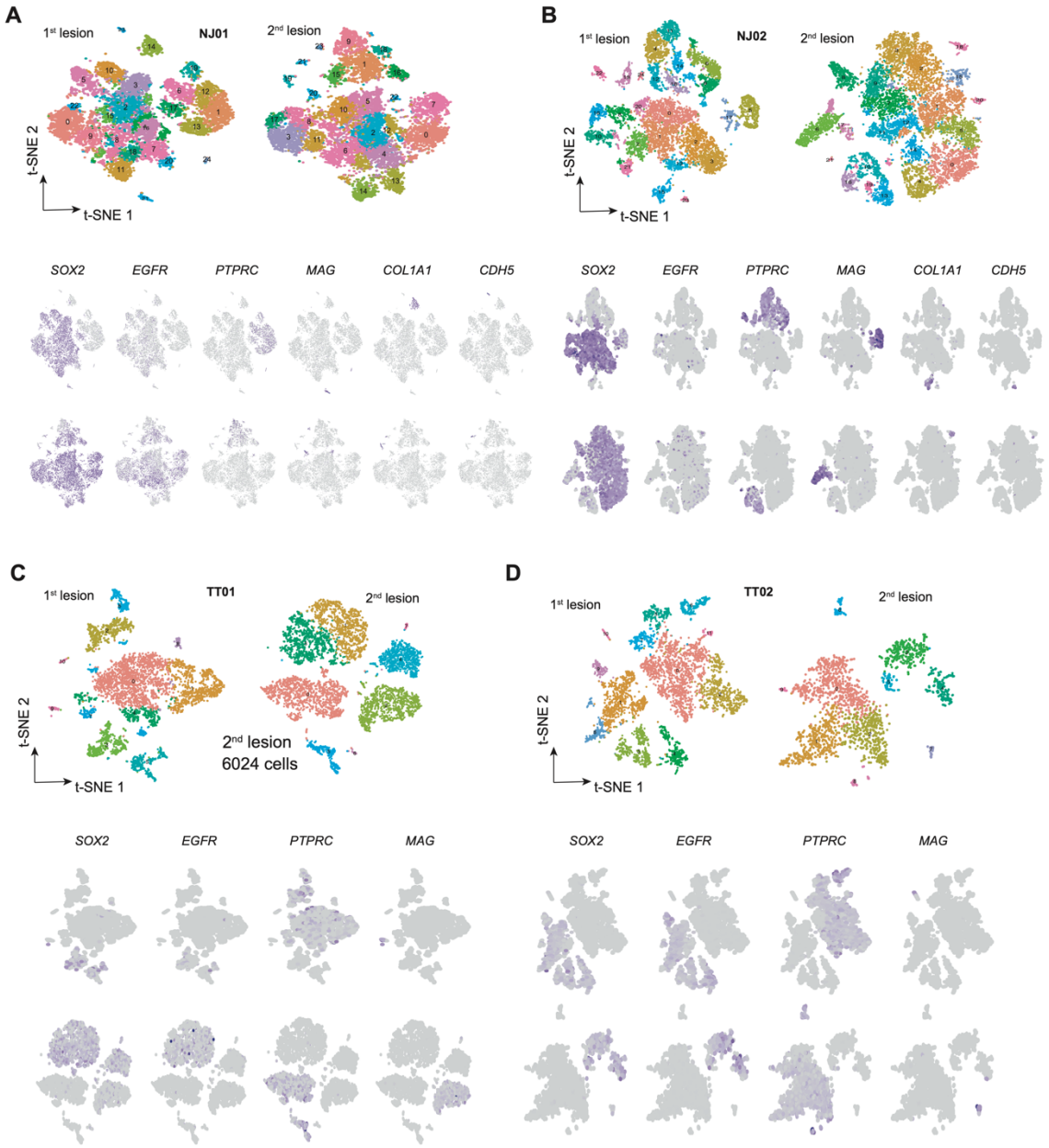
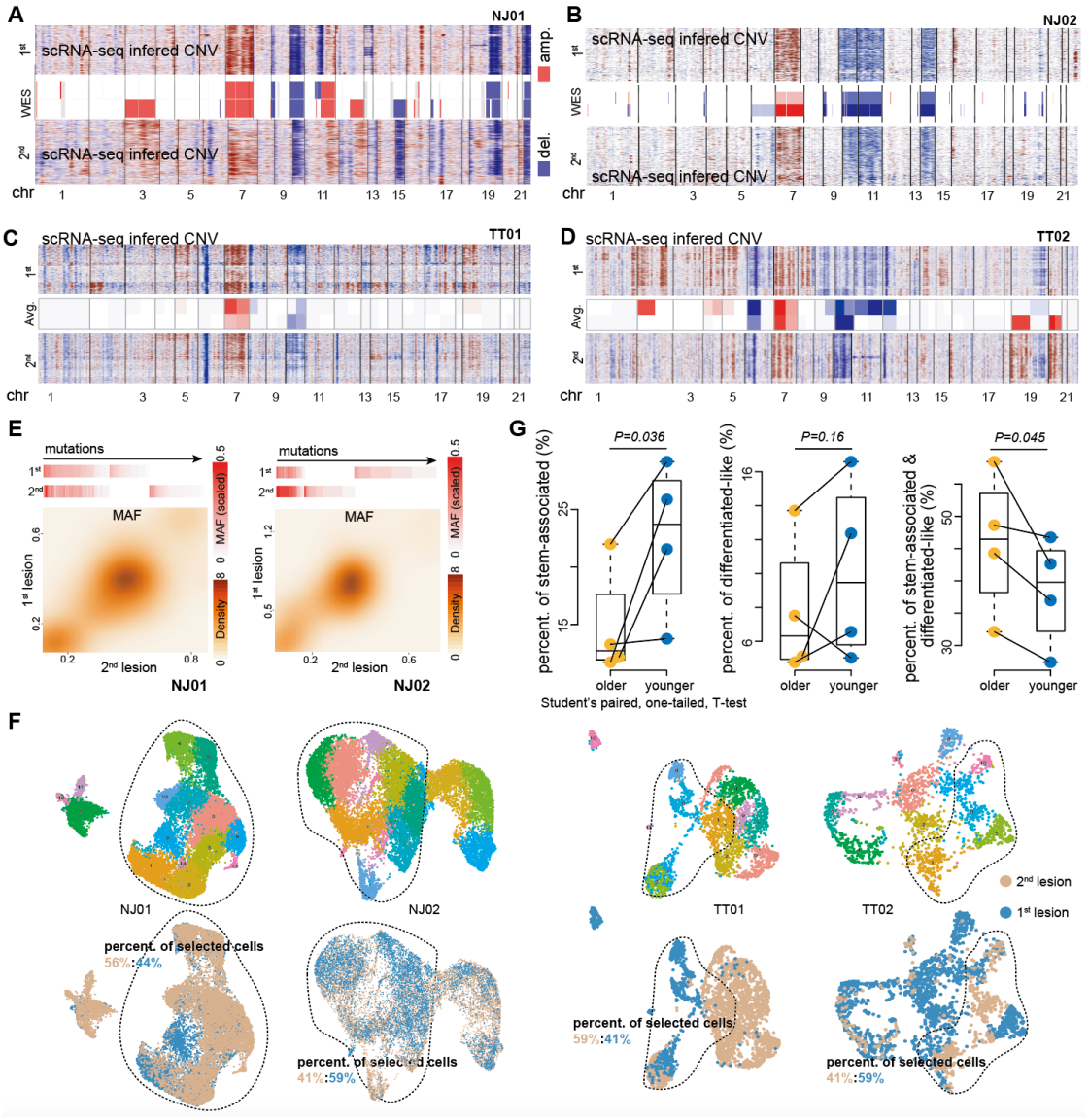
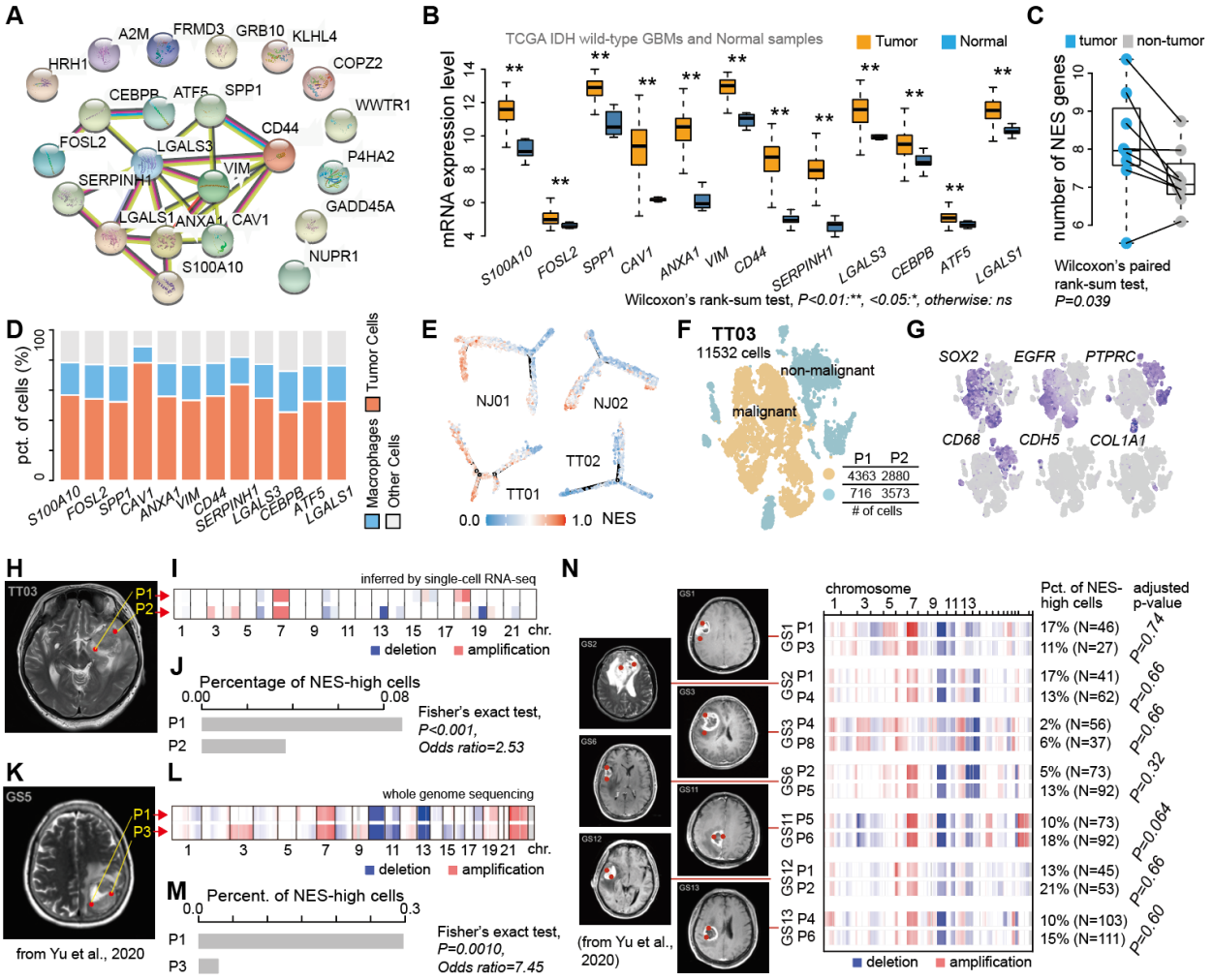
Supplementary Figures



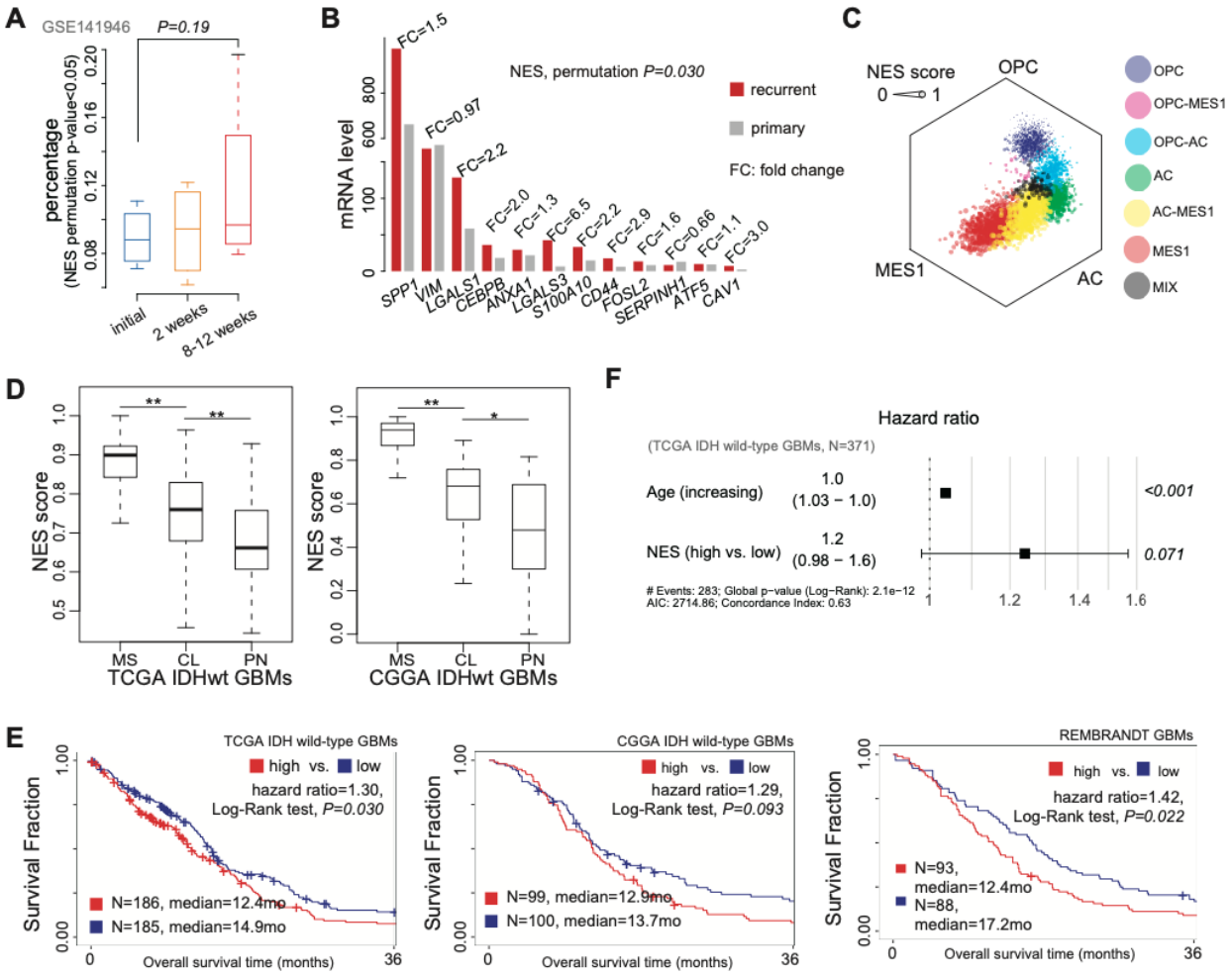
Supplementary Figure S1, related to Figure 1. A-D, Distribution of the indicated cell marker genes overlaid on the 2D-tSNE plot of the 1st and 2nd lesion across the indicated cases.



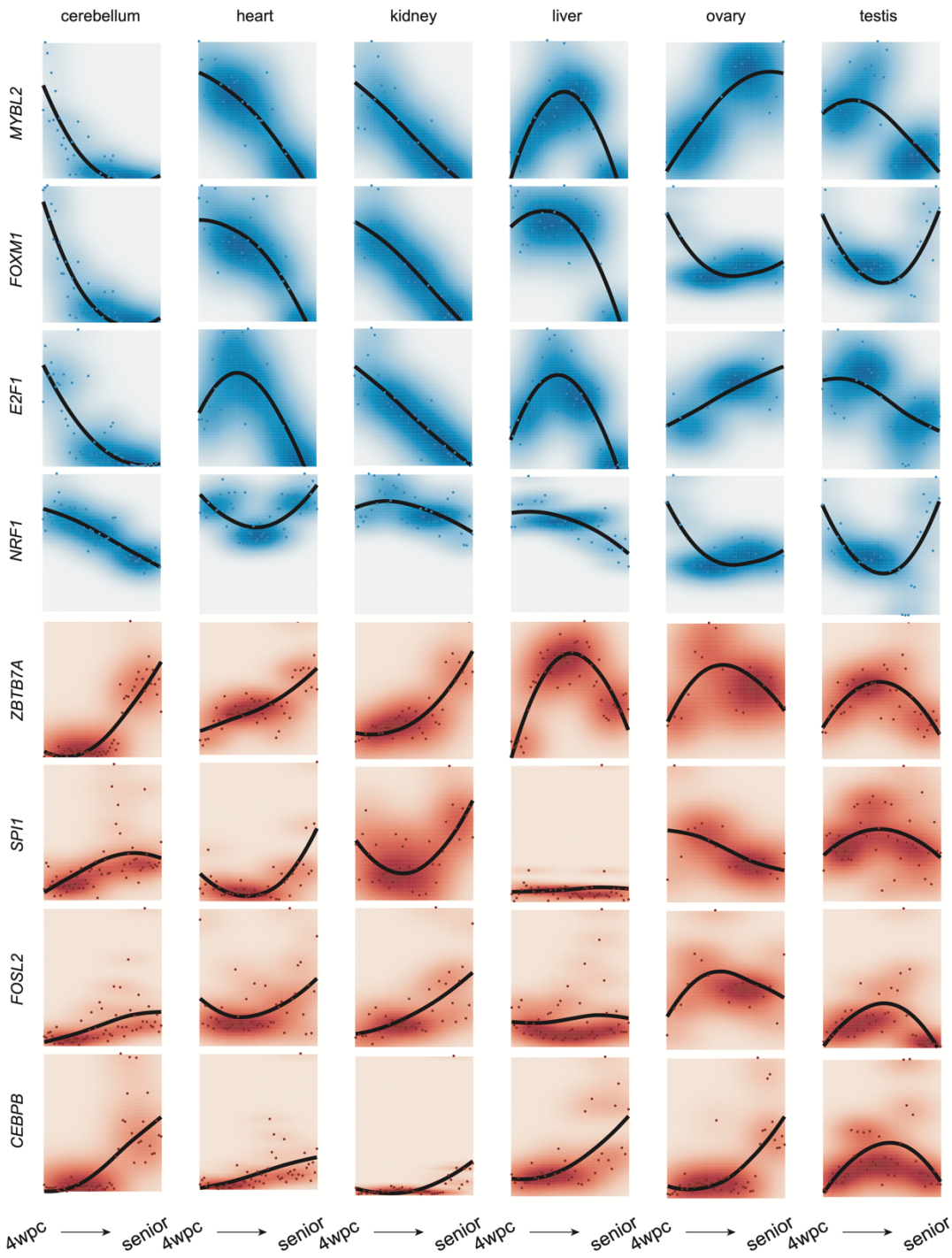
Supplementary Figure S2, related to Figure 1. A-B, Comparison of absolute copy number scores among different clusters across the 1st and 2nd lesion across indicated cases. **Top and bottom panel:** Representative inferred copy number variation of the first and second lesion using scRNA-seq. **Middle panel**: Representative inferred copy number variation of the first (**top**) and second (**bottom**) lesions using whole-exome sequencing. **C-D**, Comparison of absolute copy number scores among different clusters across the 1st and 2nd lesion across indicated cases. **Top and bottom panel:** Representative inferred copy number variation of the first and second lesion using scRNA-seq. **Middle panel**: Representative inferred average copy number variation of the first (**top**) and second (**bottom**) lesions using scRNA-seq. **E**, **Top:** Comparison of shared and private mutations between the 1st and 2nd lesions. The color from white to red represents the scaled mutation allele frequency from low to high. **Bottom:** Scatter plot of MAFs between the first (y-axis) and second (x-axis) lesions. **F**, Distribution of tumor cells overlaid on the 2D-tSNE plot of the integration of the 1st and 2nd lesion across the indicated cases. Cell clusters enclosed by a dashed line were subjected to transition state analysis (**see Supplementary Methods**). **G**, Comparison of the percentage of the indicated tumor cells (e.g., differentiated-like tumor cells, etc.) between the older and younger lesions across NJ01, NJ02, TT01, and TT02 cases.



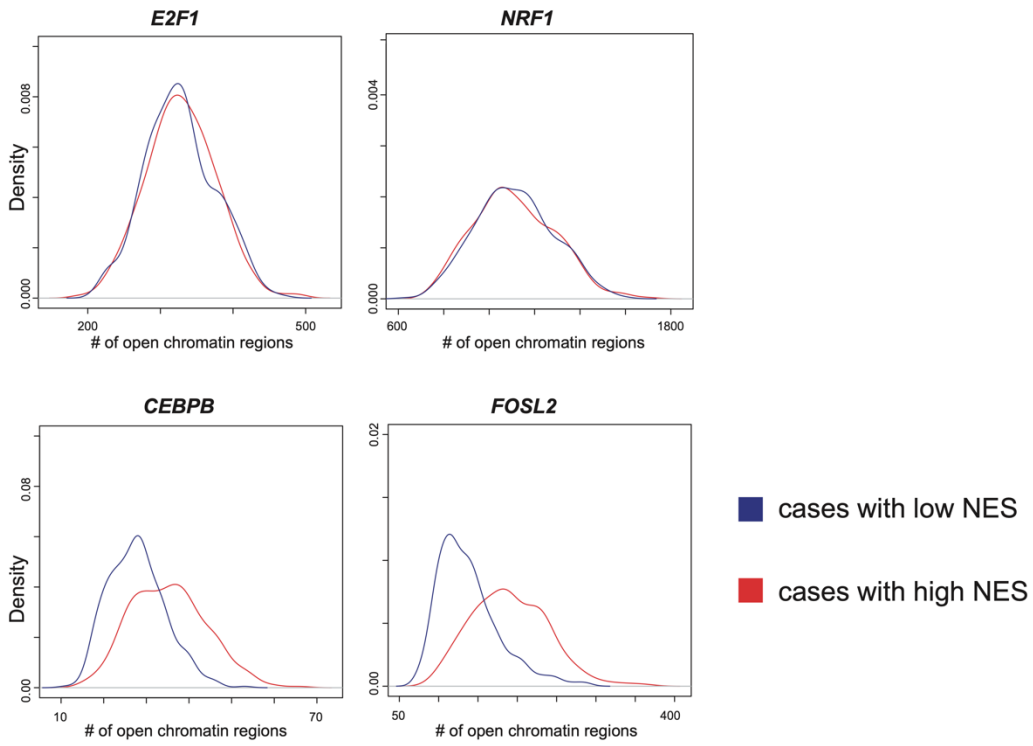
Supplementary Figure S3, related to Figure 2. A, Protein-protein interaction network based on 22 genes (generated by STRING)**. B,** heat map of average mRNA expression of the 12 NES genes in tumor cell clusters from the 1st and 2nd lesions. **C**, Comparison of the number of expressed NES genes between the indicated groups. **D**, The proportion of the indicated cell types expressing the indicated genes across GBMs. **E**, Trajectory analysis of GBMs based on monocle2 across the indicated cases. **F**, t-SNE plot of malignant (orange dots) and non-malignant (blue dots) cells of the two sectors derived from TT03. The table shows the number of the indicated cells among two sectors. **G**, Distribution of the indicated cell marker genes overlaid on the 2D-tSNE plot of all cells derived from TT03. Representative MRI (**H**) and the copy number variations (**I**) (inferred by scRNA-seq data) for the indicated sectors derived from TT03. **J**, Comparison of the percentage of NES-like tumor cells between the indicated sectors derived from TT03. Representative MRI (**K**) (from doi: 10.1093/n- sr/nwaa099) and the copy number variations (**L**) (inferred by whole-genome sequencing data) for the indicated sectors derived from GS5. **M**, Comparison of the percentage of NES-like tumor cells between the indicated sectors derived from GS5. **N**, Comparison of the percentage of NES-high tumor cells between the indicated sectors across other seven cases provided by Yu et al. The MRI images are cited from doi: 10.1093/nsr/nwaa099.



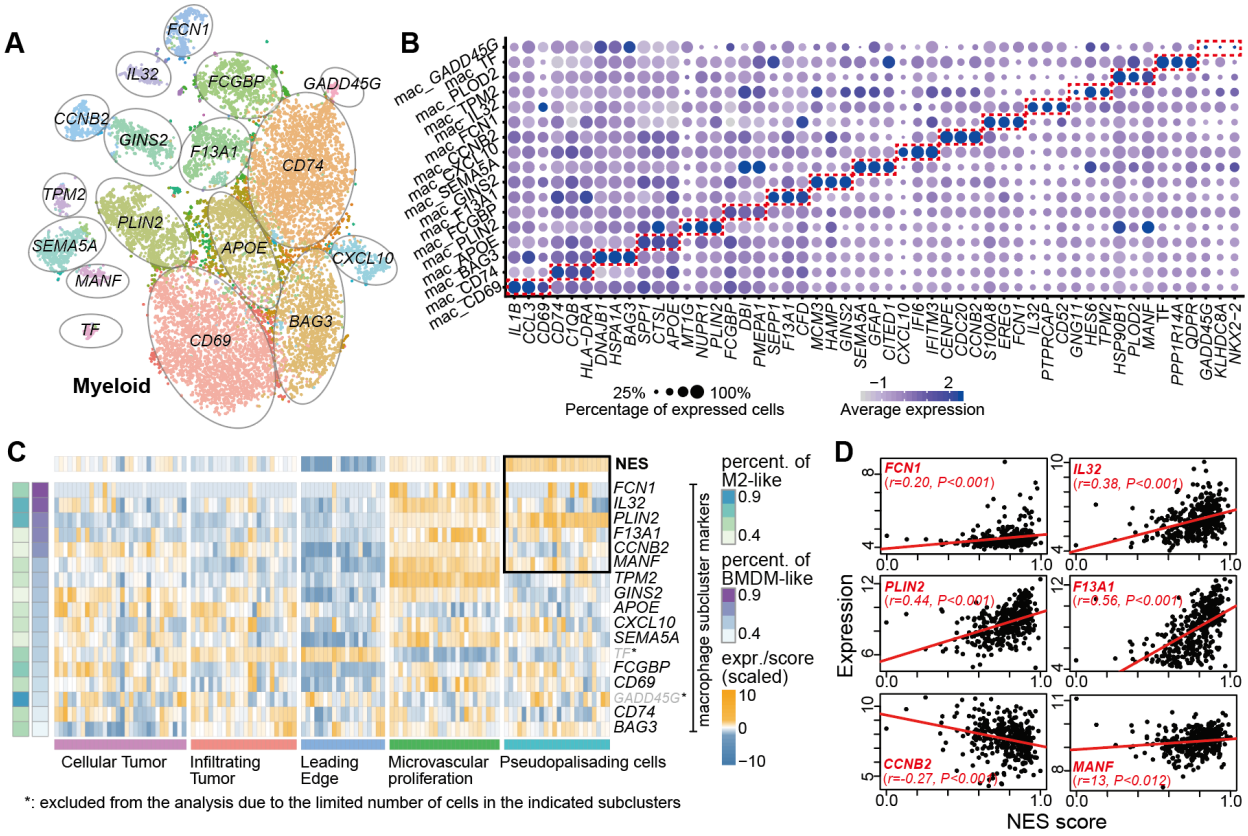
Supplementary Figure S4, related to Figure 2. A, Comparison of the percentage of NES-like tumor cells between the indicated groups. **B**, Comparison of mRNA levels of the NES-related genes between the primary (gray) and matched recurrent (red) samples. The fold change (recurrent vs. primary) for the indicated gene were presented above the corresponding bar. **C**, Hexagonal plots depict different cellular state or subtype signature scores for malignant cells in the 1st and 2nd lesions. Each data point corre- sponds to a single cell and is positioned along three axes according to its relative scores for the indicated cellular states. The size of the data point reflects the NES score of the cell. **D**, Comparison of NES among GBMs with different subtypes. Wilcoxon's rank-sum test, P<0.01:**, <0.05:*. **E**, Survival analysis of and comparison for IDH wild-type GBMs between indicated groups (hNES versus lNES). Datasets used for analyses were indicated on the top of each KM plot. **F**, Multivariable Cox's regression analysis of overall survival of TCGA IDH wild-type GBMs.



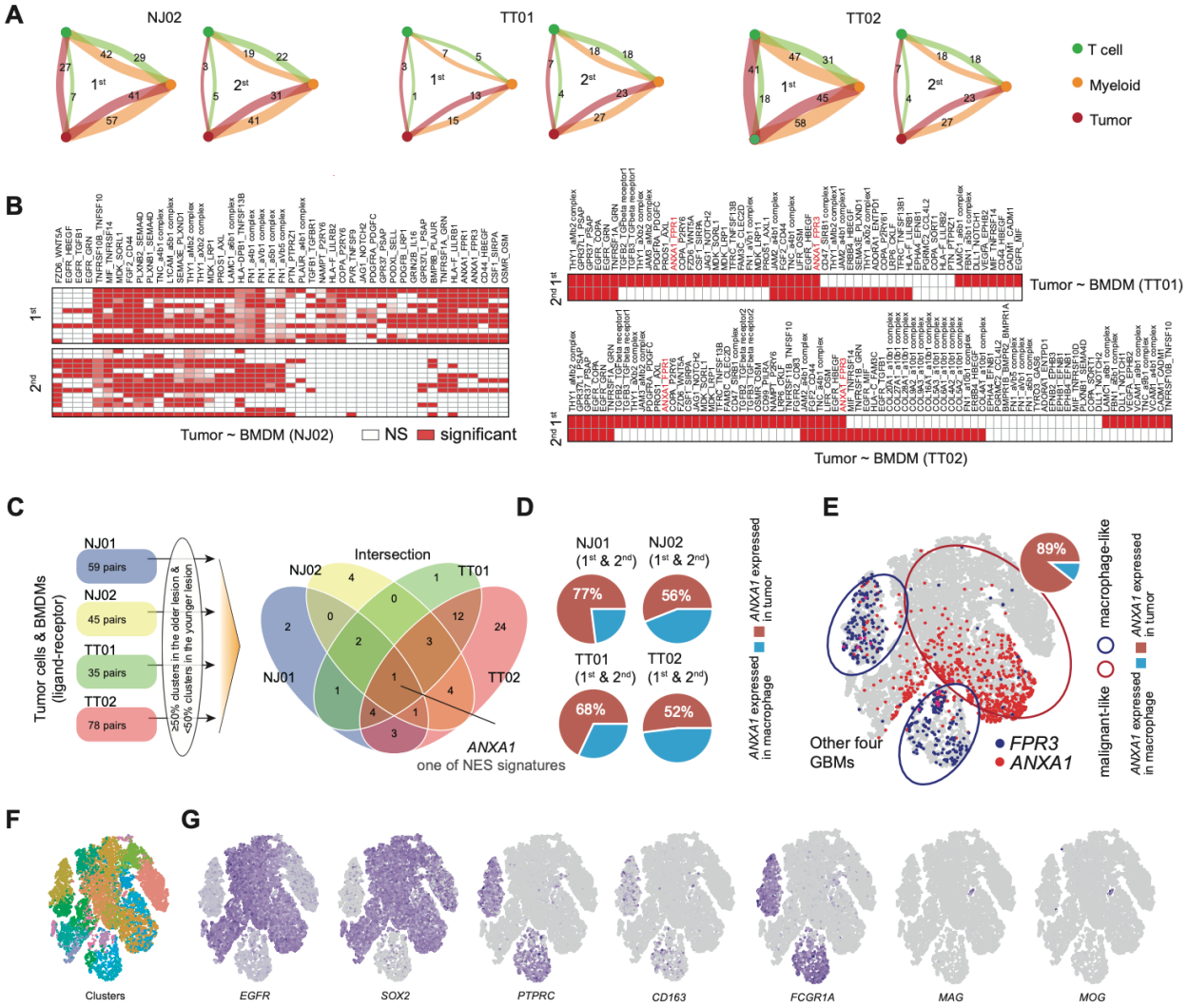
Supplementary Figure S5, related to Figure 3. Distribution of indicated genes among different stages of indicated organ development.



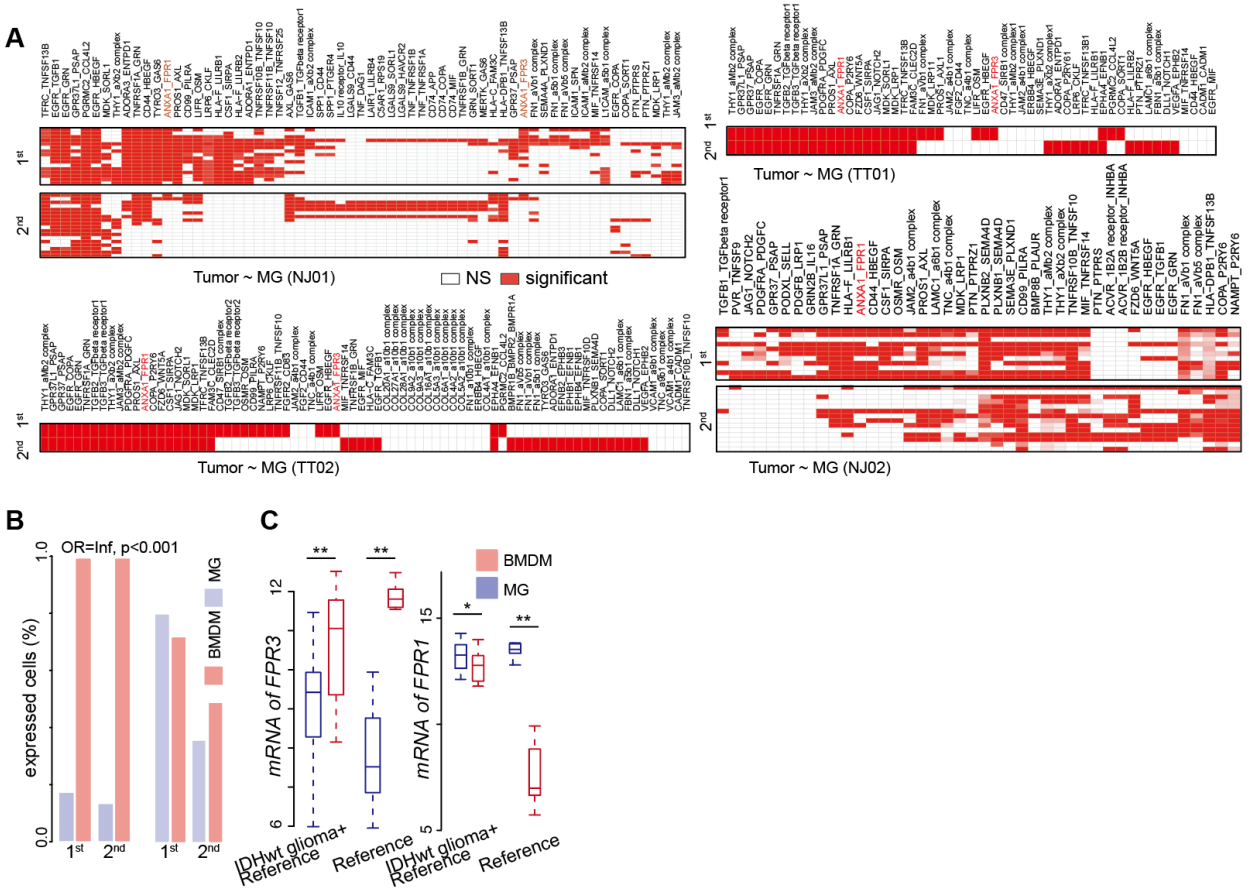
Supplementary Figure S6, related to Figure 3. Density plot depicts the number of open chromatin regions for the indicated TFs.



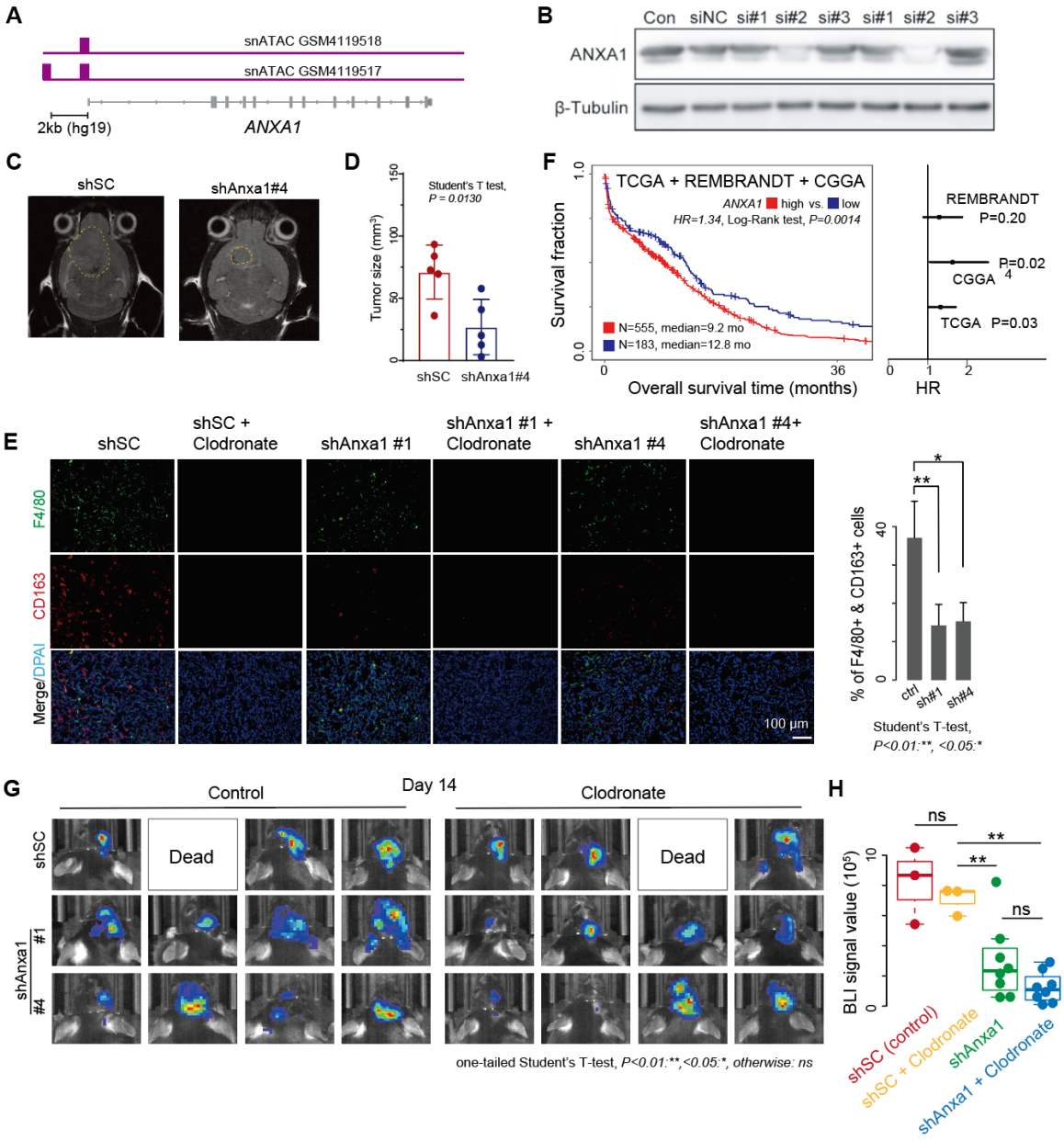
Supplementary Figure S7, related to Figure 4. A, t-SNE plot of myeloid cells derived from four pairs of multifo- cal GBMs. **B**, A dot plot for expression of macrophage-associated markers across different subclusters. The size of dot point denotes the percentage of expressed cells. **C**, A heat map for expression of indicated genes and signature scores among different regions. The color bars on left panel represent the percentage of M2-like and BMDM-like cells, respectively. **D**, Correlation between the mRNA level of the indicated genes and NES score across TCGA IDH wild-type GBMs.



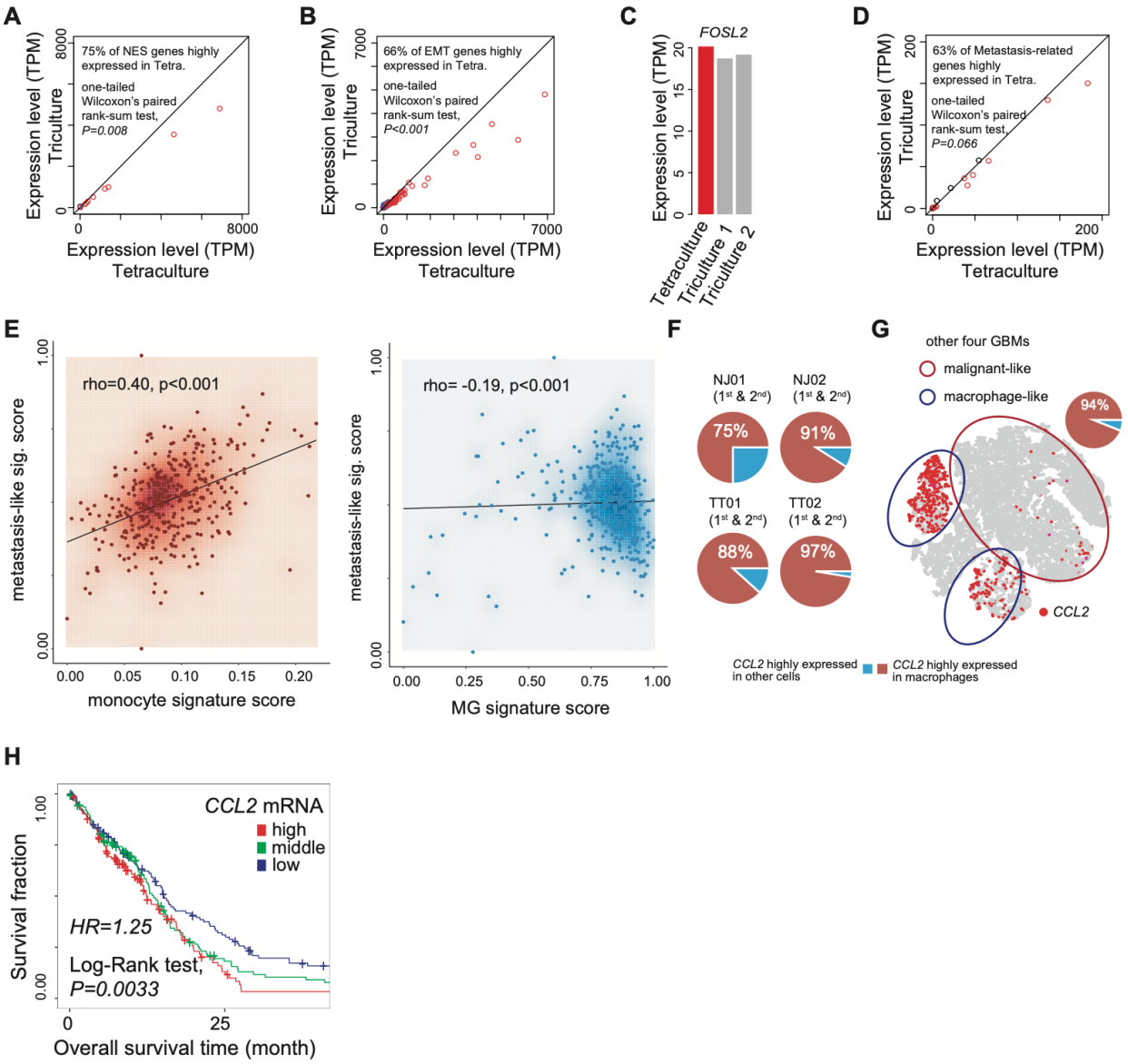
Supplementary Figure S8, related to Figure 4. **A**, Interaction among different cell types. The width of links represents the number of significant ligand-receptor interactions between the indicated cell types. **B**, heat map for ligand-receptor interactions between indicated cell types in the two lesions (NJ02, TT01 and TT02). **C**, Schematic workflow for the identification of the ligands associated with the older tumors across four multifocal GBMs. **D**, Percentage of cells expressing *ANXA1* in the indicated cell types. **E**, Distribution of the indicated genes (*ANXA1* and *FPR3*) overlaid on the 2D-tSNE plot of the cells derived from four GBMs. Pie plot demonstrates the percentage of cells expressing *ANXA1*. **F**, t-SNE plot for all cells in the sample consisting of four GBMs. **G**, Distribution of indicated cell marker genes overlaid on the 2D-tSNE plot of the sample consisting of four GBMs.



Supplementary Figure S9, related to Figure 4. **A**, heat map for ligand-receptor interactions between indicated cell types in the two lesions (NJ01, NJ02, TT01, and TT02). **B**, Comparison of percentage of expressed *FPR1* and *FPR3* between the BMDM and MG across the 1st and 2nd lesion (NJ01). OR indicates odds ratio. **C**, Comparison of mRNA of FPR1/3 between the indicated groups. Wilcoxon's rank-sum test, P<0.01:**, <0.05:*.



Supplementary Figure S10, related to Figure 5. A, snATAC-seq analysis of GBM cells. The representative IGV tracks at the *ANXA1* locus show the location with significant peaks upstream of the TSS (<5KB). **B**, Western Blot analysis of *ANXA1*. **C**, Representative MRI from mice after intracranial injection of GL261 with lentiviral vectors carrying scambled shRNA or shAnxa1#4. T2 sequences demonstrate infiltrative tumors in the mouse brain (yellow line). **D**, Tumor volume was measured by the T2 MRI scan. **E, Left:** representative immunofluorescence (IF) staining of macrophages in a M2-like, defined as CD163+, tumor field, stained for markers for macrophages (F4/80, green), and nuclei (DAPI, blue). Scale bars, 100 μm. **Right:** quantification of the fraction of CD163+ & F4/80+ macrophages across the indicated groups. **F, Left:** Survival analysis of GBM patients. **Right:** Comparison of hazard ratios among indicated data sets. **G,** Tumor growth was measured by bioluminescence imaging on an IVIS Spectrum In Vivo Imaging System. **H,** Comparison of bioluminescence imaging (BLI) signal value of each tumor between the indicated groups. One-tailed Student's T-test, P<0.01:**, <0.05:*, otherwise: ns.



Supplementary Figure S11, related to Figure 6. A, Comparison of the mRNA expression level (quantified with Transcripts per Million, TPM) of NES genes in GSCs between the 3D tr-culture and tetra-culture model. **B,** Comparison of the mRNA level of the indicated genes between the indicated groups. **C-D**, Comparison of the mRNA level of EMT-related genes **(C)** and Metastasis-related genes **(D)** in GSCs between the 3D tr-culture and tetra-culture model. **E,** Correlation between metastasis-like signature and indicated immune cell signatures across TCGA IDH wild-type GBMs. **F,** Percentage of cells expressing *CCL2* in the indicated cell types. **G,** Distribution of the indicated gene (*CCL2*) overlaid on the 2D-tSNE plot of the indicated lesions. Pie plot demonstrates the percentage of macropages (red) highly expressing *CCL2*. **H,** Survival analysis of TCGA IDH wild-type GBMs among indicated groups.