

Supplementary figure legends

Supplementary Figure 1. **A)** UMAP visualization of the 58 clusters identified through Seurat FindClusters. **B)** Heatmap of enrichment of cell type specific pathways for each nuclei. Tracking bar indicates cell cluster identity from the UMAP in **1A**.

Supplementary Figure 2. Expression levels of commonly used markers to isolate cancer stem cells and stemness score calculated from set of stem cell associated genes identified in Tirosh et al for each major cell type⁴⁶⁸.

Supplementary Figure 3. Boxplot of the stemness scores of all nuclei for each sample.

Supplementary Figure 4. **A)** Median stemness score distribution by tumor types. Horizontal line for each tumor type indicates median stemness score per tumor type. Comparisons between embryonal tumors and other tumor types were tested with Wilcoxon rank-sum test. **B)** Median stemness score distribution by grade. Comparison between median stemness scores of low and high grade conducted using Wilcoxon rank-sum test. **C)** Correlation between age at diagnosis and median stemness score of tumors. Correlation calculated using the Spearman rank method. Linear regression line and 95% confidence interval indicated by the blue line and gray band, respectively. **D)** Median stemness score distribution by tumor location class. Comparison between median stemness scores of subtentorial and supratentorial regions conducted using Wilcoxon rank-sum test.

Supplementary Figure 5. Distribution of cell types present per sample, categorized by tumor types. **A:** Astrocyte; **EMB:** Embryonal tumor cells; **EN:** Endothelial cells; **MC:** Macrophage; **MG:** Microglia; **NEU:** Neuron; **NEU_EX:** Excitatory neuron; **NEU_GN:** Granular neuron; **NEU_INH:** Inhibitory neuron; **NEU_INT:** Interneuron; **NSC:** Neural stem cell; **OLIG:** Oligodendrocyte; **OPC:** Oligodendrocyte precursor cell; **RGC:** Radial glial cell; **ST:** Stromal cell; **TC:** T cell; **UBC:** Unipolar brush cell.

Supplementary Figure 6. **A)** Proportion (out of 196 PID pathways tested) of pathways specific to cell types compared to all other nuclei. **B)** Hierarchical clustering of all 196 PID pathways

tested for each cell type. Dark green indicates pathways relatively specific/important to the cell type.

Supplementary Figure 7. A) Volcano plot of differentially expressed genes for each tumor type compared to non-tumor tissue, *not* adjusted for cell type. Number of genes on the left of the volcano plot indicate genes that are downregulated compared to non-tumor tissue. Number of genes on the right of the plot indicate genes that are upregulated compared to non-tumor tissue. **B)** Heatmap of differential expression direction and significance in all 4000 genes tested in the cell type unadjusted differential expression analyses. Red indicates significantly upregulated in the tumor type compared to non-tumor tissue. Blue indicates significantly downregulated in the tumor type compared to non-tumor tissue. Gray indicates the gene is not significantly differentially expressed. Tracking bar indicate the gene type.

Supplementary Figure 8. Top 10 Reactome pathways associated with differentially expressed genes in **A)** astrocytoma, **B)** embryonal tumors, **C)** ependymoma, **D)** glioblastoma, **E)** glioneuronal/neuronal tumors, and **F)** Schwannoma.

Table legends

Table 1. Subject demographics.

Table 2. Classic markers for cell types in the brain.

Table 3. Number of significantly differentially expressed genes shared among all or subsets of tumor types

Supplementary table legends

Supplementary Table 1. Detailed sample information.

Supplementary Table 2. Enriched pathways per cell type.

Supplementary Table 3. Differentially expression analysis results from cell type-adjusted model and cell type-unadjusted model.

Supplementary Table 4 – 9. Full list of pathways associated with differentially expressed genes in the cell type-adjusted model.