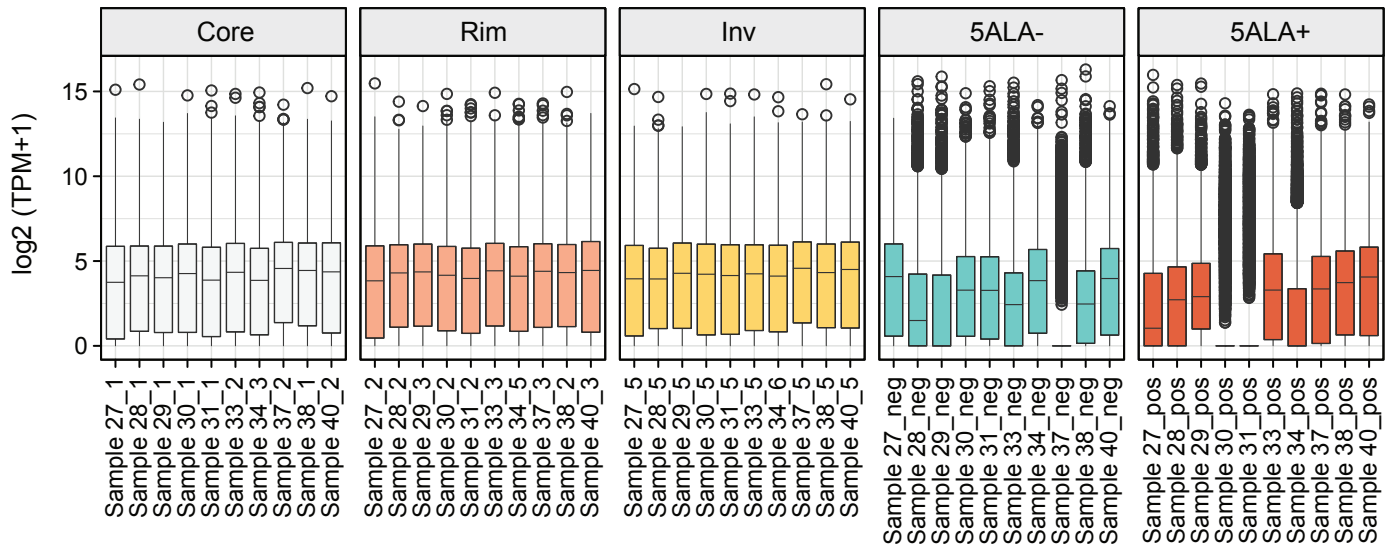
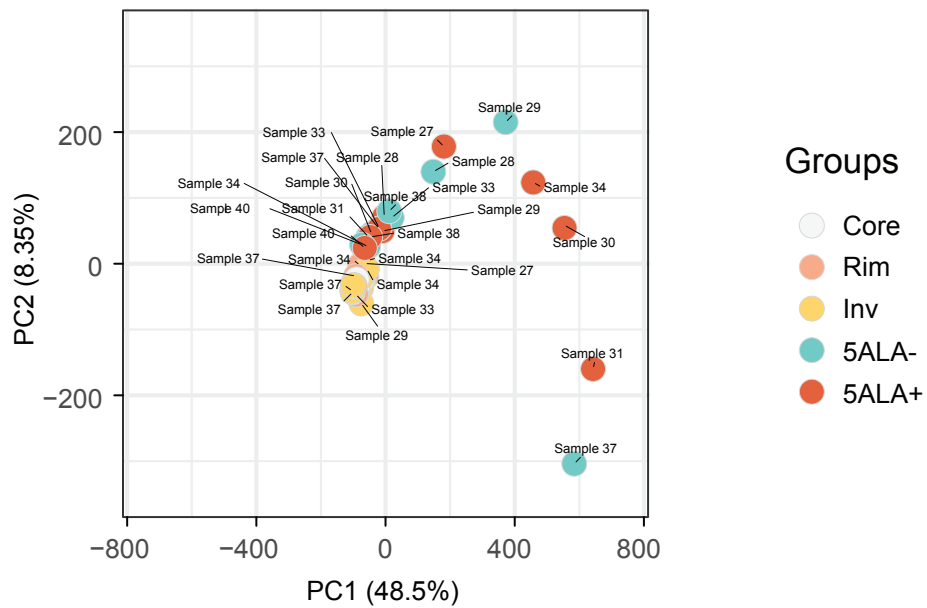


Fig S1

A



B



C

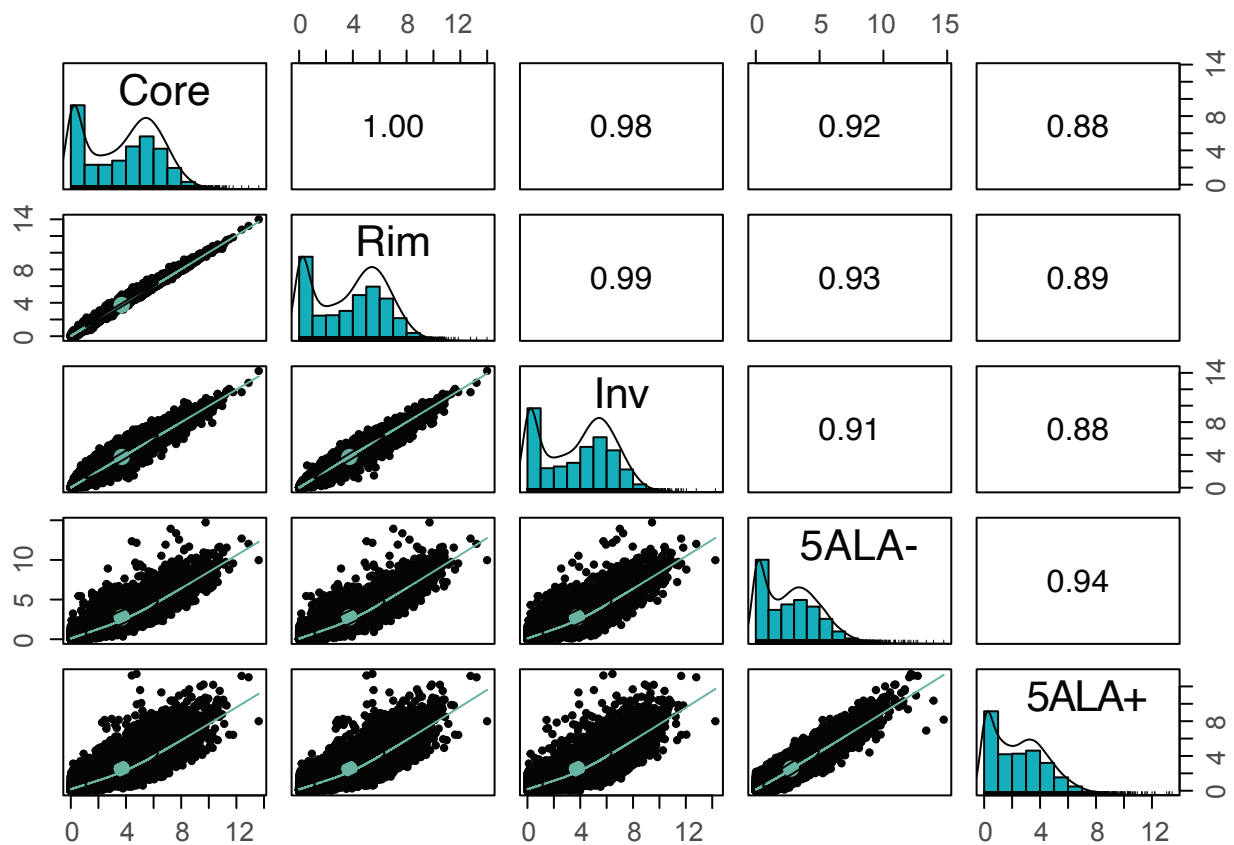
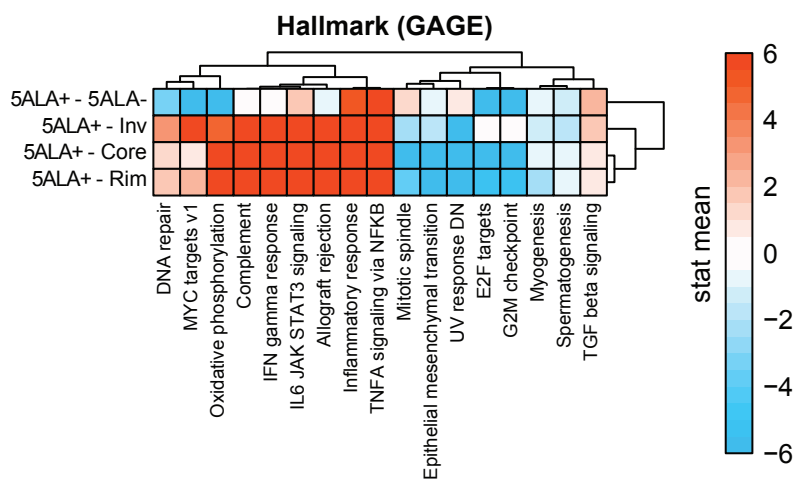


Fig. S1: Quality assessment of spatial-resolved bulk RNA profiling (SPRP) dataset

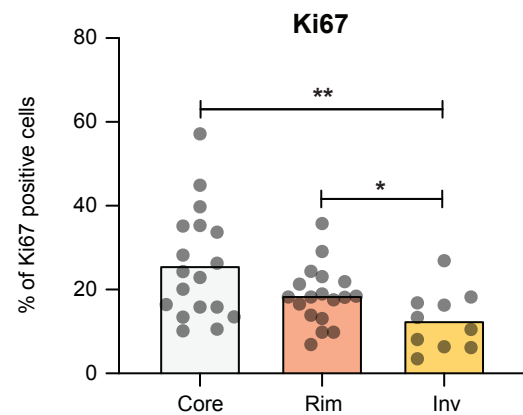
The boxplot of log normalized TPM expression values of 50 samples from 10 patients divided into five brain regions (Core, Rim, Invasive-margin, 5ALA-, 5ALA+) (A). The PCA plot is based on spatially-resolved RNA profiles (SPRP) across 10 GBM patients (B). The scatter plot matrix shows the histogram and correlation coefficients of the global transcriptome without housekeeping genes across brain regions and FACS-sorted cells (C). The lower triangle boxes represent the pairwise scatter plots, the diagonal boxes show the brain regions with the distribution of the values for those regions, and the correlation coefficients are presented in the upper triangle boxes (C).

Fig S2

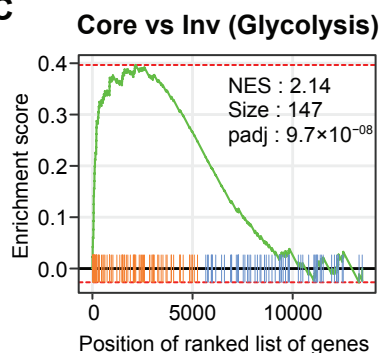
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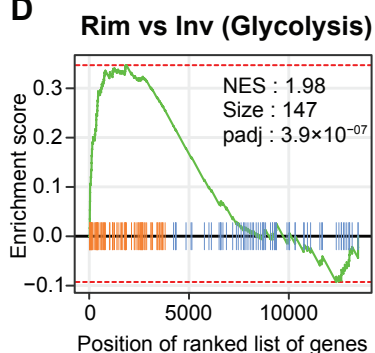
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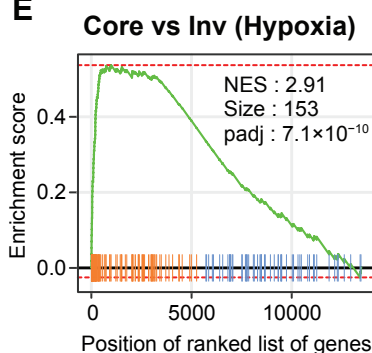
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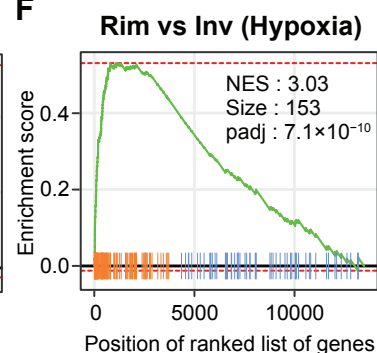
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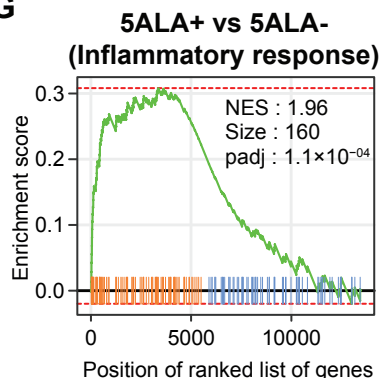
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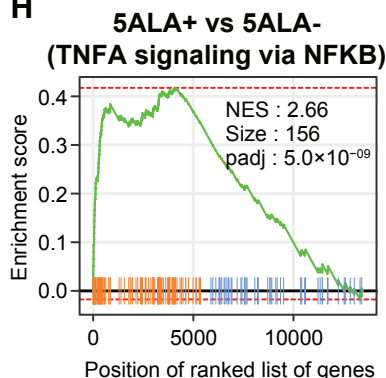
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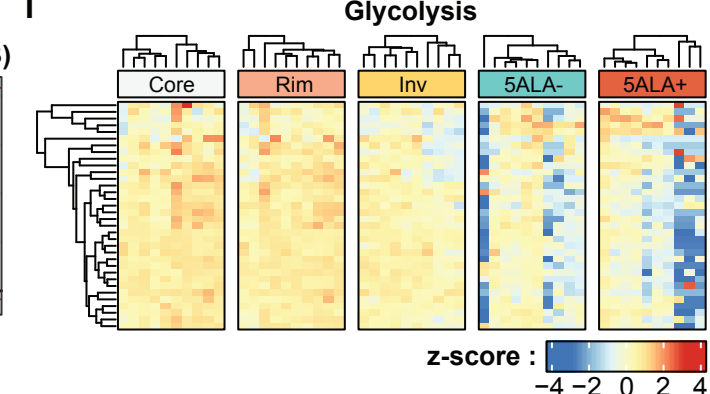
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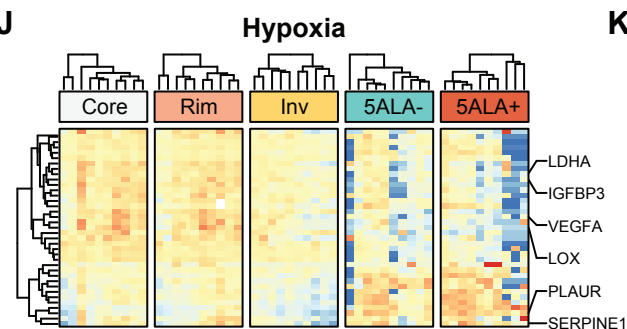
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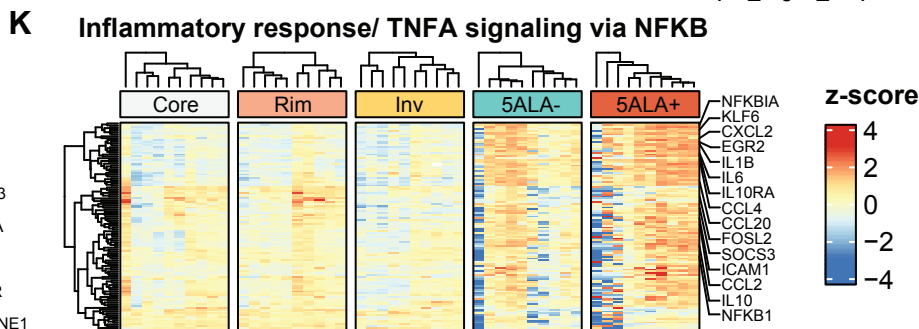
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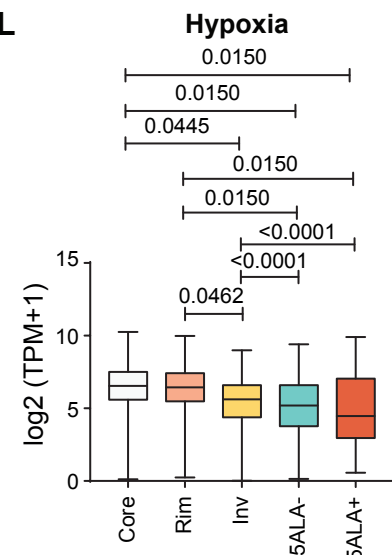
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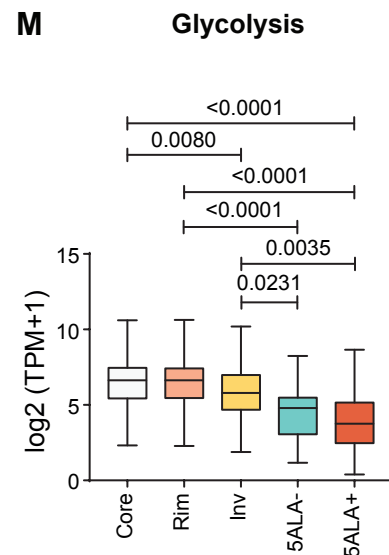
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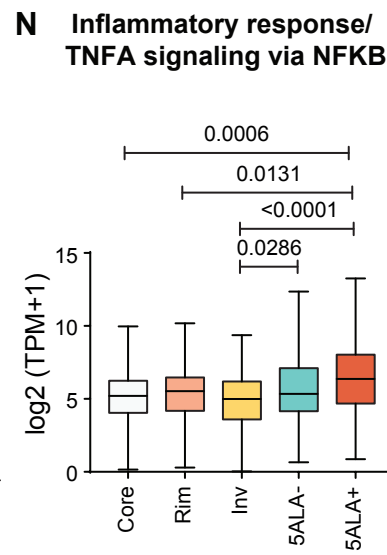
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M



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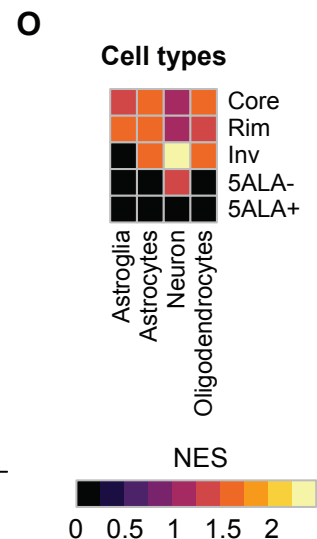


Fig. S2: Hallmark gene-set enrichment analysis of SPRP profile

Hallmark enrichment heatmap showing the enriched cancer hallmark pathways based on the significantly differentially expressed genes as identified by Limma ($\text{padj} < 0.05$) between 5ALA+ cells and Core, Rim, Invasive-margin, and 5ALA- cells (A). Color code indicates enrichment score where red and blue colors represent positive and negative enrichment. Percentage of Ki67 positive cells across Core, Rim, and Inv regions (B). GSEA enrichment plots of representative gene sets from Glycolysis (Core vs. Invasive-margin) (C), Glycolysis (Rim vs. Invasive-margin) (D), Hypoxia (Core vs. Invasive-margin) (E), Hypoxia (Rim vs. Invasive-margin) (F), Inflammatory response (5ALA+ vs. 5ALA-) (G), and TNF- α signaling via NFkB (5ALA+ vs. 5ALA-) (H). The heatmaps show hierarchical clustering based on z-scored expression ($\text{Log}_2 \text{TPM} + 1$) of the leading edge genes of Glycolysis (I), hypoxia (J), and inflammatory response/ TNF- α signaling via NFkB (K). Some rows are labeled with the symbols of selected genes of interest. Boxplots illustrating significant expression (Benjamini hochberg < 0.05) changes between brain regions for differentially regulated leading-edge genes (Limma; $\text{padj} < 0.05$) from- Hypoxia (L), Glycolysis (M), and Inflammatory response/ TNF-A signaling via NFkB (N). Hierarchical clustering heatmap of brain cell type (oligodendrocytes, neurons, astrocytes, and cultured astroglia) specific enrichment analysis (O). The color intensities depict the normalized enrichment scores (NES) of each cell type on different brain regions (Core, Rim, Invasive-margin, 5ALA-, 5ALA+).

Fig S3

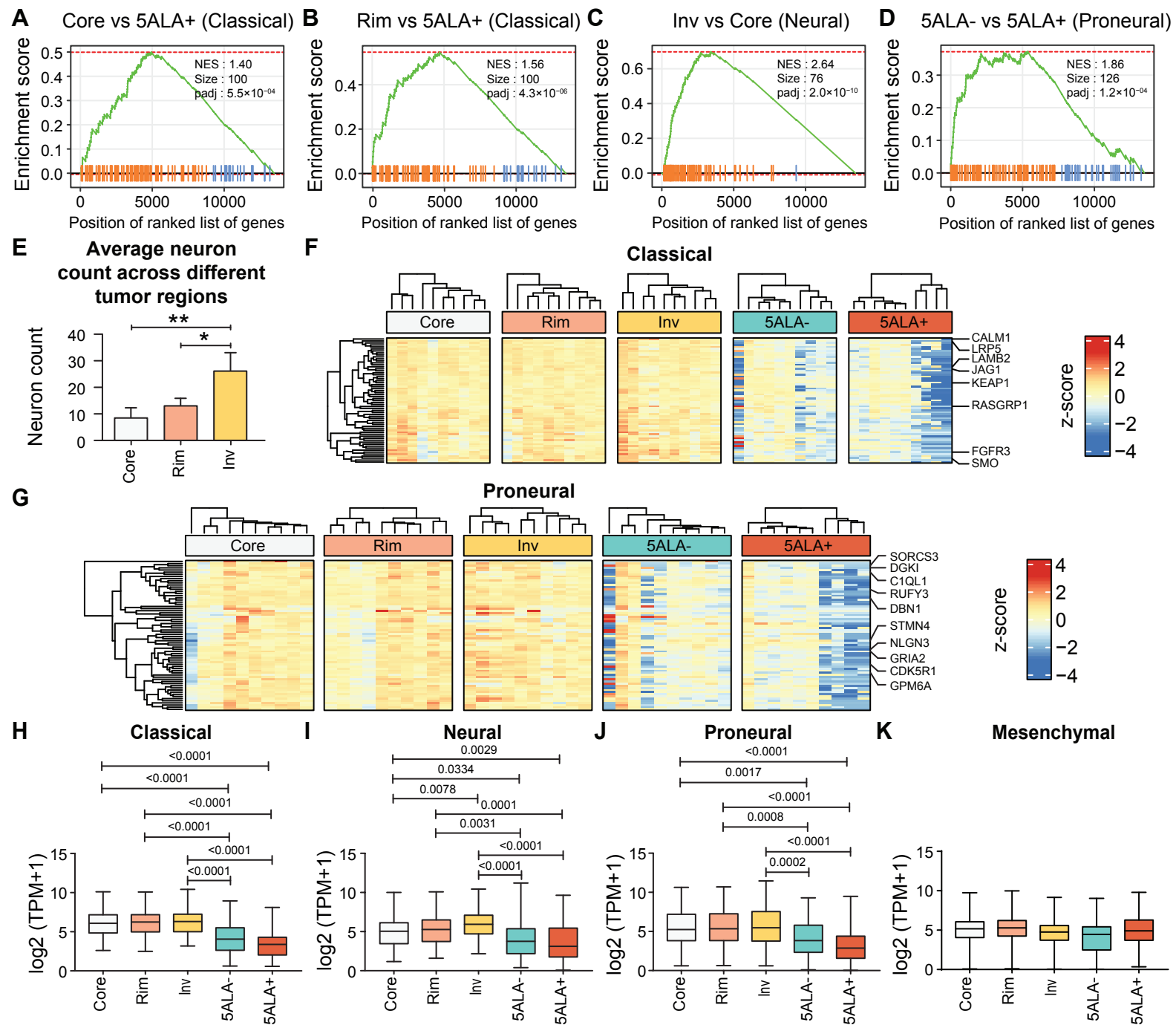


Fig. S3: GBM subtype gene-set enrichment analysis of SPRP profile

GSEA plots of gene clusters from GBM subtypes – Classical (Core vs. 5ALA+) (A), Classical (Rim vs. 5ALA+) (B), Neural (Invasive-margin vs. Core) (C), and Proneural (5ALA- vs. 5ALA+) (D). Bar plot representing the number of NeuN positive cells in patient-matched spatially distinct GBM regions (Core, Rim, and Invasive-margin) across three GBM patients (E). The star (*) symbol indicates the p-value as estimated by the patient-matched paired T-test. Error bar shows standard deviation. Differential z-scored normalized expression $\text{Log}_2(\text{TPM}+1)$ of the leading-edge genes of GBM subtypes (from Verhaak *et al.*) – Classical (F) and Proneural (G) - are shown as a heatmap. Column (samples) and row (genes) are clustered by the Euclidian distance method. The color code represents the differential expression of genes (Yellow: higher expression; Blue = lower expression). The boxplots represent the \log_2 (TPM+1) expression levels for each brain region (Core, Rim, Invasive-margin) and 5ALA sorted cells (5ALA-, 5ALA+), with significant expression differences (Limma, $\text{padj} < 0.05$) denoted among GBM regions and 5ALA sorted cells for GBM subtypes – Classical (H), Neural (I), Proneural (J) and Mesenchymal (K).

Fig S4

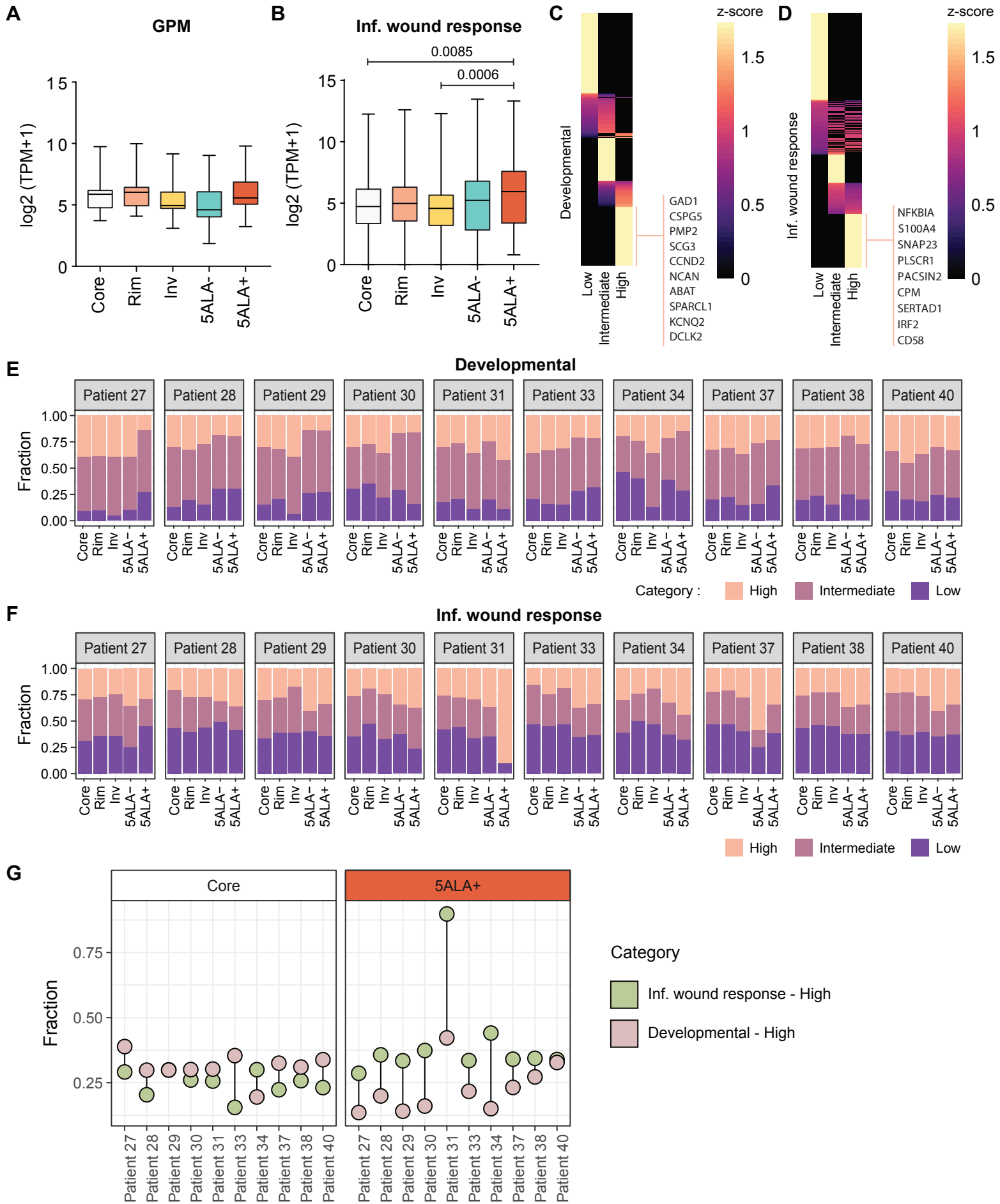


Fig. S4: Metabolic and transcriptional signature analysis and deconvolution of SPRP

Box plots showing the relative \log_2 (TPM+1) expression levels of differentially regulated (Limma; $p_{adj} < 0.05$) cellular and metabolic state gene sets – GPM (A), Inf. wound response (B), for Core, Rim, Invasive-margin, 5ALA- and 5ALA+ cells. Respective p-values (if < 0.05) are also shown. The signature-matrix generated from the single-cell RNA-seq data (Richards *et al.*) by CIBERSORTx deconvolution algorithm is shown. The matrix represents three categories (High, Intermediate, and Low) based on the expression of two transcriptional programs – Development (C) and Inf. wound response (D). Highly expressed canonical markers genes for Developmental-High and Inf. wound response-High are shown. Color code represents the differential expression (yellow: High, red: Low, Black: No expression). Patient-wise stacked bar plot representing the estimated fraction of cells with High, Intermediate, and Low transcriptional programs - Development (E) and Inf. wound response (F) across 10 GBM samples. The color indicates three different categories (High, Intermediate, and Low) of the transcriptional programs as reported by Neftel *et al.* (Development and Inf. wound response). The patient-wise estimated fractions of cells with High transcriptional programs (Development and Inf. wound response) in Core (left) and 5ALA+ cell (right) populations (G).

Fig S5

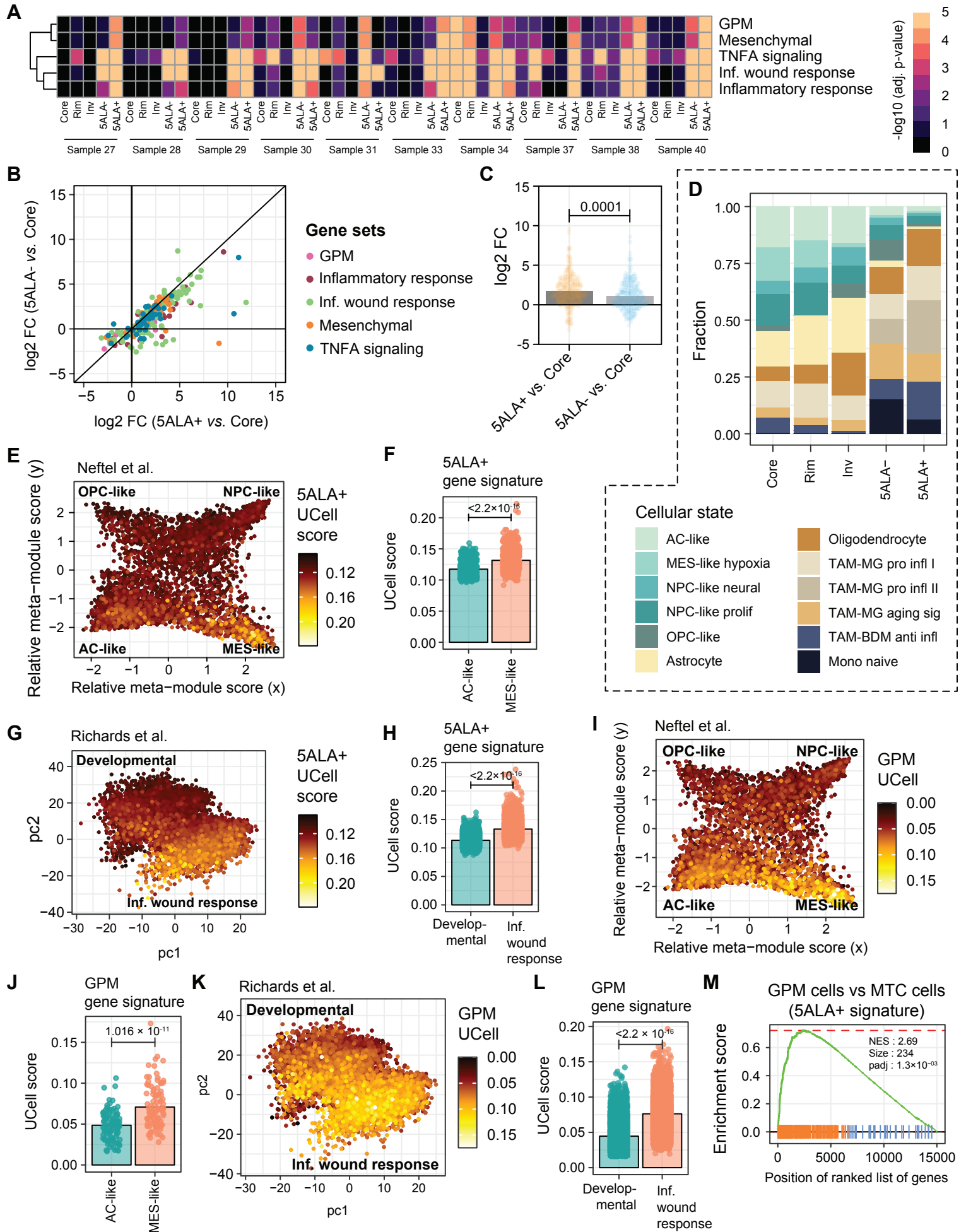


Fig. S5: Single-cell analysis from independent cohorts

Single sample GSEA (ssGSEA) was performed on distinct GBM regions (Core, Rim, Invasive-margin, 5ALA+, and 5ALA-) across ten GBM patients separately for five unique gene-signatures in 5ALA+ cells – GPM, MES, TNF-A signaling, Inflammatory wound response, and Inflammatory response (A). Log2 fold change (FC) of 5ALA+ genes between 5ALA+ vs. Core (x-axis) and 5ALA- vs. Core (y-axis) (B). Bar jitter plot showing the Log2 FCs (5ALA+ vs. Core and 5ALA- vs. Core) (C). The p-value was calculated by T-test between the FCs. The stacked bar plot represents the average cell-type estimates based on a GBmap data across 10 GBM samples; the color indicates different cell types (D). Two-dimensional scatter plot of cellular states based on RNA-seq dataset (Nettel *et al.*) where each quadrant corresponds to one distinct cellular state. The dots represent the positions of GBM cells in a two-dimensional plane reflecting their relative scores for the meta-modules - OPC-like, NPC-like, AC-like, and MES-like (E). The color code is based on the single-cell wise UCell scores of 5ALA+ gene signature across the GBM cells where yellow and black indicate high and low UCell scores, respectively. 5ALA+ scores were compared between MES-like and AC-like cells (F). The p-value (Wilcox test) is shown. Principle component analysis (PCA) plot of GSCs (N= 65,655 cells) based on RNA-seq dataset (Richards *et al.*) (G). Each dot represents a single GSC positioned by expression of Developmental (PC1-low) and Injury Response (PC1-high) programs. The color code is based on the single-cell wise UCell scores of 5ALA+ gene signature across the GBM cells where yellow and black indicate high and low UCell scores. 5ALA+ scores were compared between Developmental (N= 10,000) and Inf. wound response/Injury (N= 10,000) cells. The p-value = 2.2×10^{-16} (H). Mapping GPM UCell scores on the single cells from Nettel (I) and Richards *et al.* datasets (K). GPM scores were compared

between MES-like and AC-like cells. The p-value for this particular comparison was 1.016×10^{-11} (J). GPM scores were compared between Developmental (N= 10,000) and Inf. wound response/Injury (N= 10,000) cells. The p-value = 2.2×10^{-16} (L). Enrichment of 5ALA+ gene signature in GPM PDCs compared to MTC PDCs (M).

Fig S6

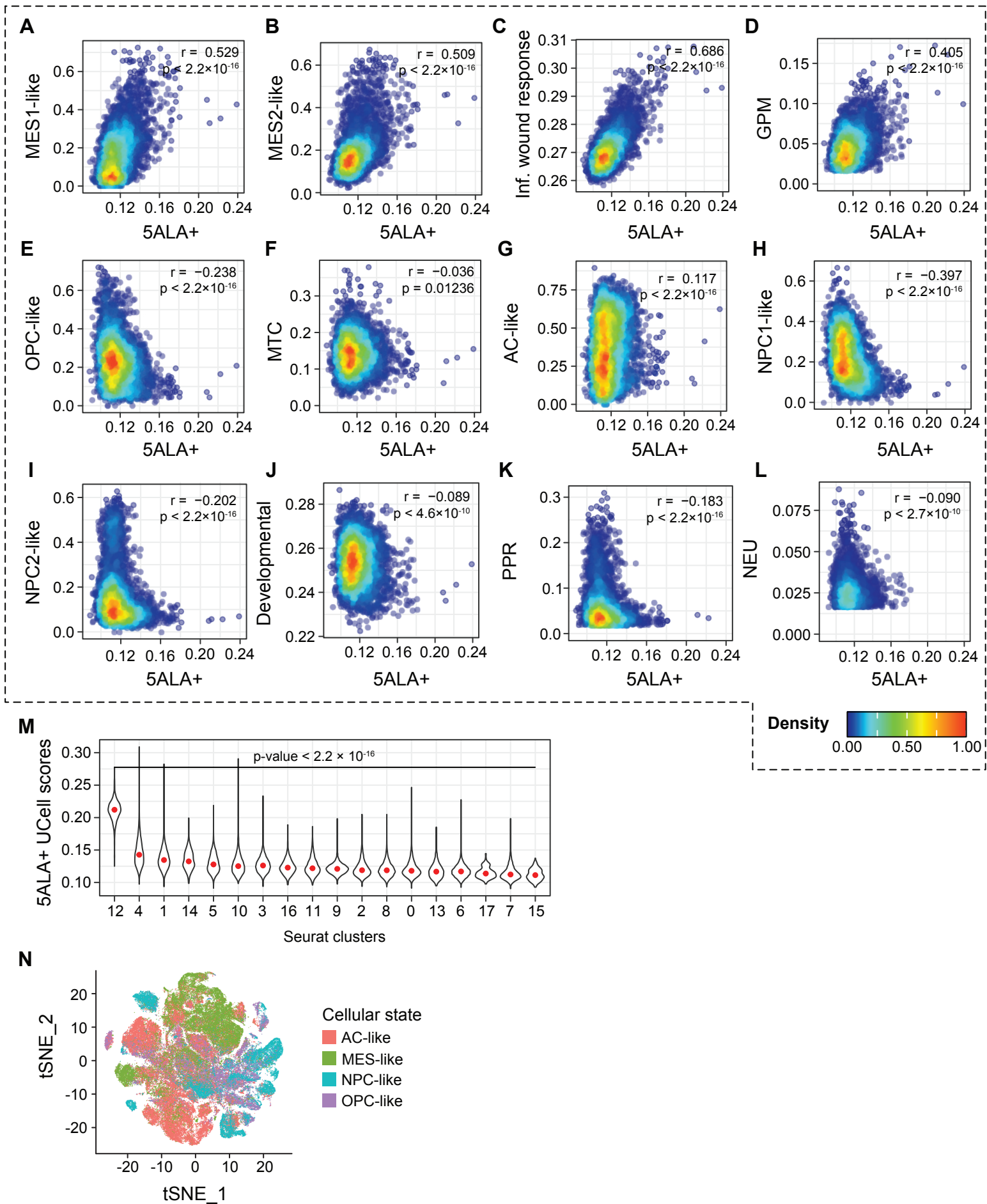


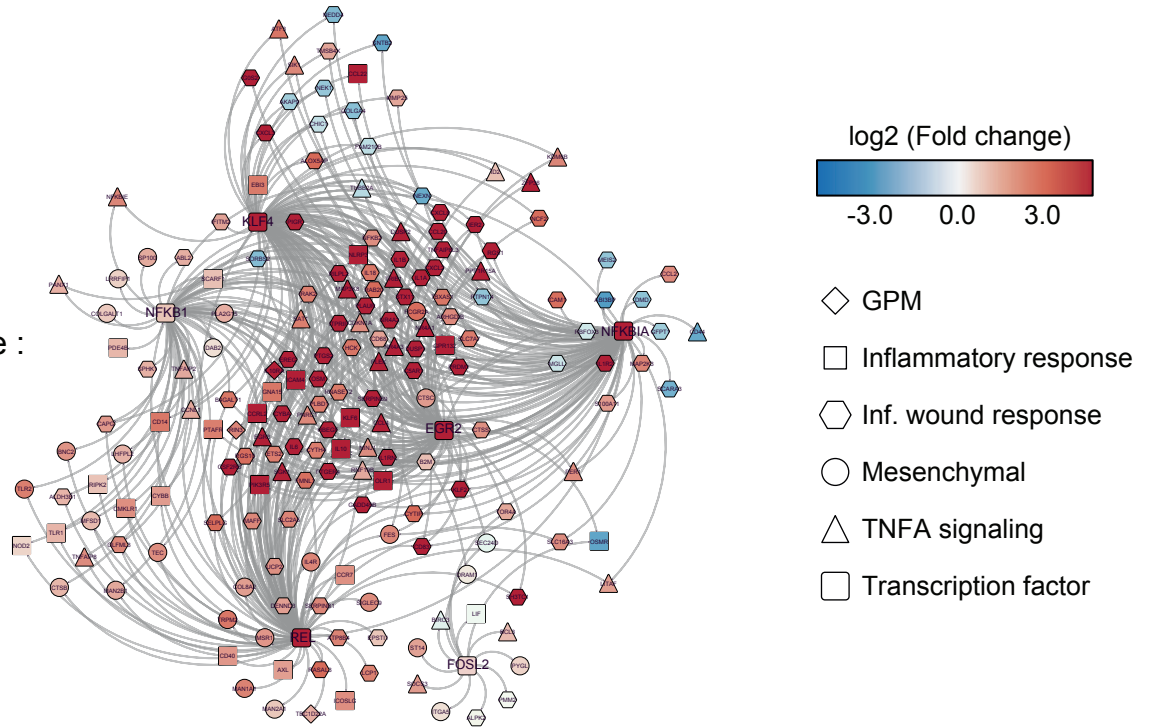
Fig. S6: Single-cell wise UCell enrichment analysis

Scatter plots representing the UCell score correlation between 5ALA+ gene signature and MES1-like (A), MES2-like (B), Inf. wound response (C), GPM (D), OPC-like (E), and MTC (F), AC-like (G), NPC1-like (H), NPC2-like (I), Developmental (J), PPR (K), NEU (L). and. The Pearson correlation coefficient (r) and p-values are given for each scatter plot. P-values of correlation are given. Average 5ALA+ UCell scores across different Louvain clusters (M). We performed bootstrap analysis where 100 cells were selected randomly 1000 times for the comparison followed by the Wilcox test. The median p-value between cluster-12 and cluster-4 was 2.2×10^{-16} . All the other comparisons between Cluster-2 and the rest of the clusters resulted in p-value below 2.2×10^{-16} . Cell state annotations of the tSNE plot based on neoplastic cells from the GBmap dataset (N).

Fig S7

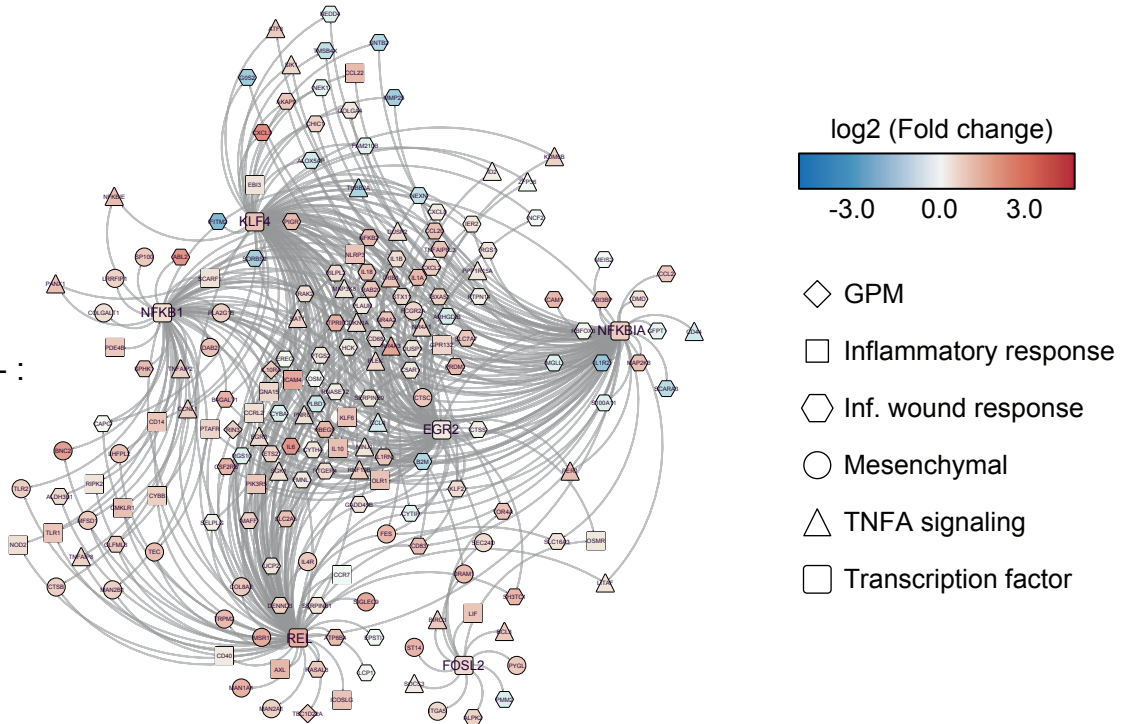
A

5ALA+ vs. Core :



B

5ALA+ vs. 5ALA- :



C

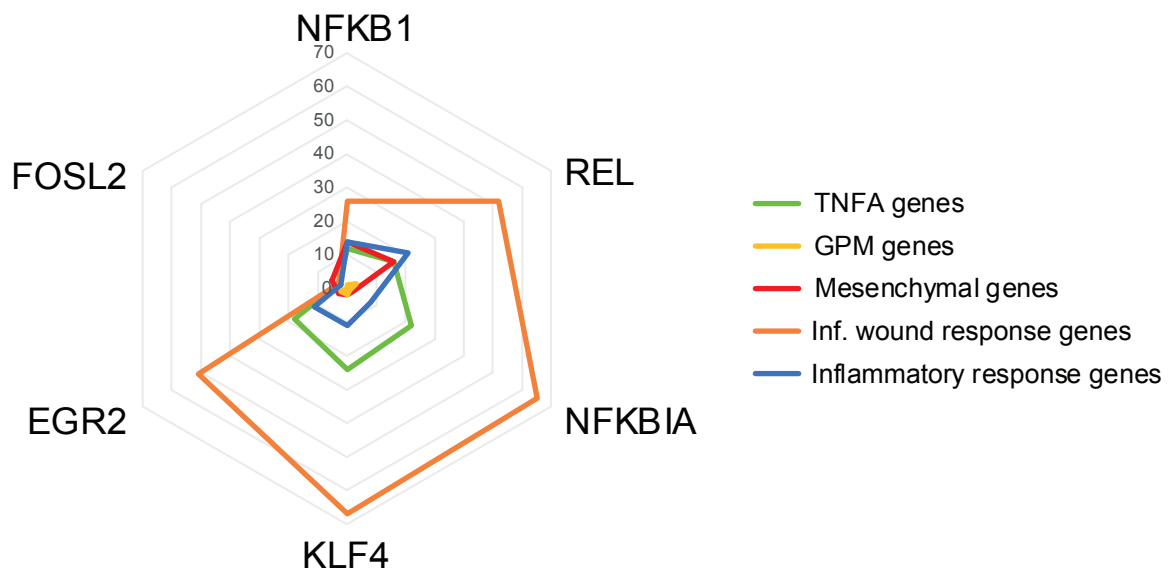


Fig. S7: Transcription factor – Target gene network analysis

Transcription networks upregulated in 5ALA+ cells in comparison to Core (A) and 5ALA- cells (B) are shown. Transcription factors (TF) were identified in the differentially regulated leading-edge genes that were enriched in 5ALA+ cells by using ENCODE TF-annotation. The association between TF and corresponding target genes (TG) was retrieved from the ENCODE database. A transcription network is constructed representing TF-TG association by using the ARACNE algorithm. The diagram illustrates that TGs (Nodes) associated with enriched gene sets (Inflammatory response, Inflammatory wound response, MTC, Mesenchymal subtype, GPM, and TNF- α signaling), are mutually controlled by different TFs (Hubs). The association between a node (TG) and a hub (TF) is shown as edges (grey curved lines) and nodes/hubs are color-coded according to the Log₂ fold change (FC) values between 5ALA+ and Core (A) and 5ALA- cells (B), where red and blue indicate higher and lower FCs respectively. A radar plot representing TG (of different enriched subtypes) counts for the TFs (C). The colored dots represent the TG count for a specific TF.

Fig S8

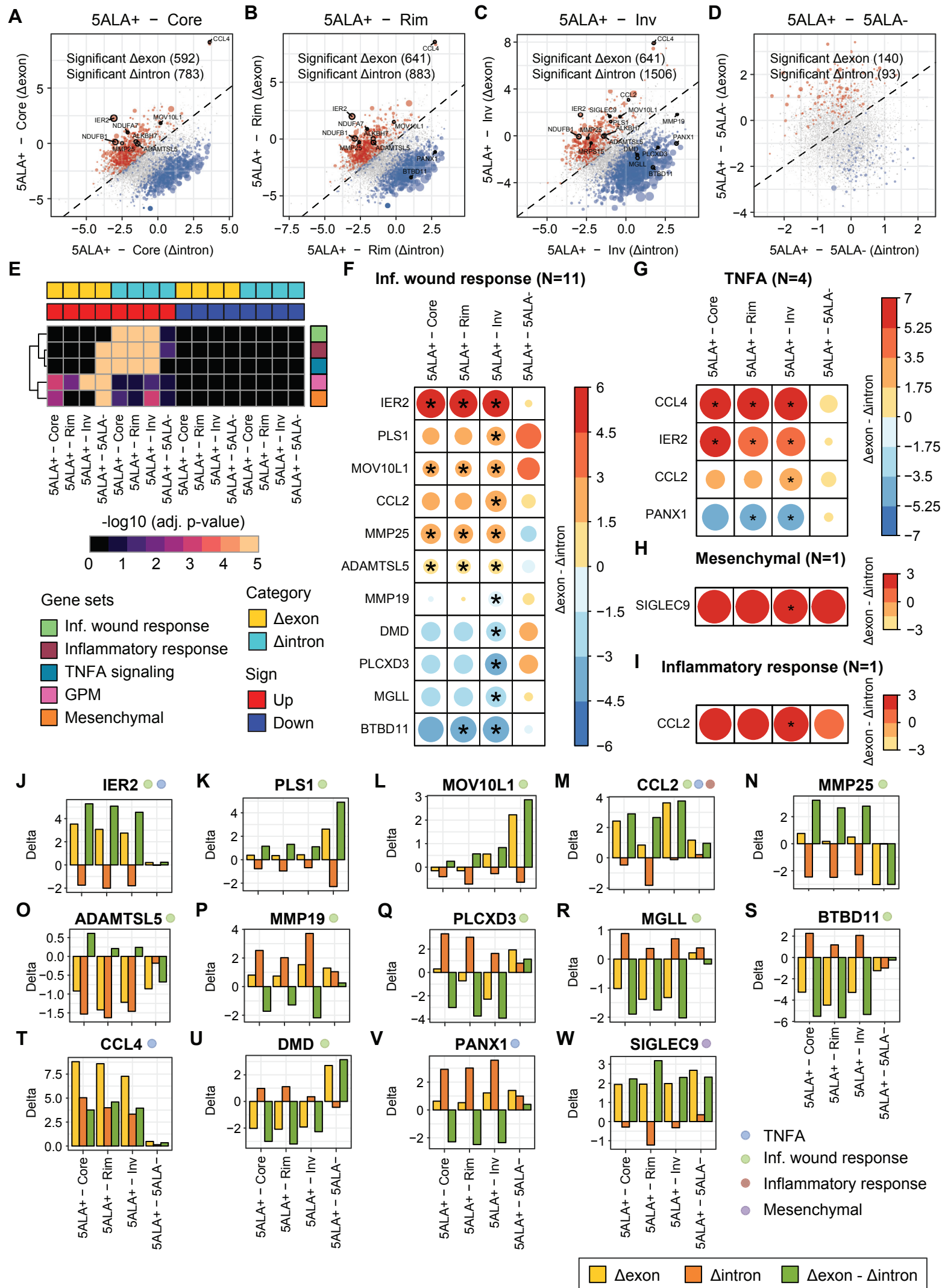


Fig. S8: Exon-intron split analysis

Exon-intron split analysis (EISA) of enriched cellular and metabolic subtypes in 5ALA+ cells. Scatter plot showing the gene-specific changes in exon (delta-exon) and intron (delta-intron) counts between 5ALA+ cells and tumor Core (A), Rim (B), Invasive-margin (C) and 5ALA- cells (D). X- and Y-axis represent the delta-intron and delta-exon, respectively. Color code represents the genes that showed significant regulation with adjusted p-value<0.05 (Blue: a significant change in delta intron and Red: a significant change in delta exon) between 5ALA+ cells and intra-tumor regions or 5ALA- cells. Heatmap showing the $-\log_{10}$ adjusted p-value of the hallmark enriched pathways (E). The samples representing the comparisons of 5ALA+ with other regions are given in columns (5ALA+ vs. tumor Core; 5ALA+ vs. Rim; 5ALA+ vs. Invasive-margin; and 5ALA+ vs. 5ALA). Each row represents the different cellular and metabolic subtypes. Columns are divided into upregulated (Red) or downregulated (Blue) segments based on the exon and intron regulation of genes in 5ALA+ cells. Finally, delta-exon (yellow) and delta-intron (Light blue) are annotated for each column. The color code in the heatmap is representative of $-\log_{10}$ p-values (Orange is highly significant and Black is not significant). Regulated DEGs with a significant change in exon and intron counts are shown as heatmaps for Inflammatory wound response (F), TNF- α signaling (G), Mesenchymal subtype (H), and Inflammatory response (I). The color code represents the difference between delta exon and delta intron (Δ exon- Δ intron). The significant changes of Δ exon- Δ intron are shown as (*). The size of the circle is according to the adjusted $-\log_{10}$ p-value. Bar plots represent delta exon (Δ exon), delta intron (Δ intron), and the difference between Δ exon and Δ intron (Δ exon - Δ intron) for the significant DEGs (J-W). The circles adjacent to the gene symbol represent the gene-signature and the colors of the circle indicate different gene-signatures.

Fig S9

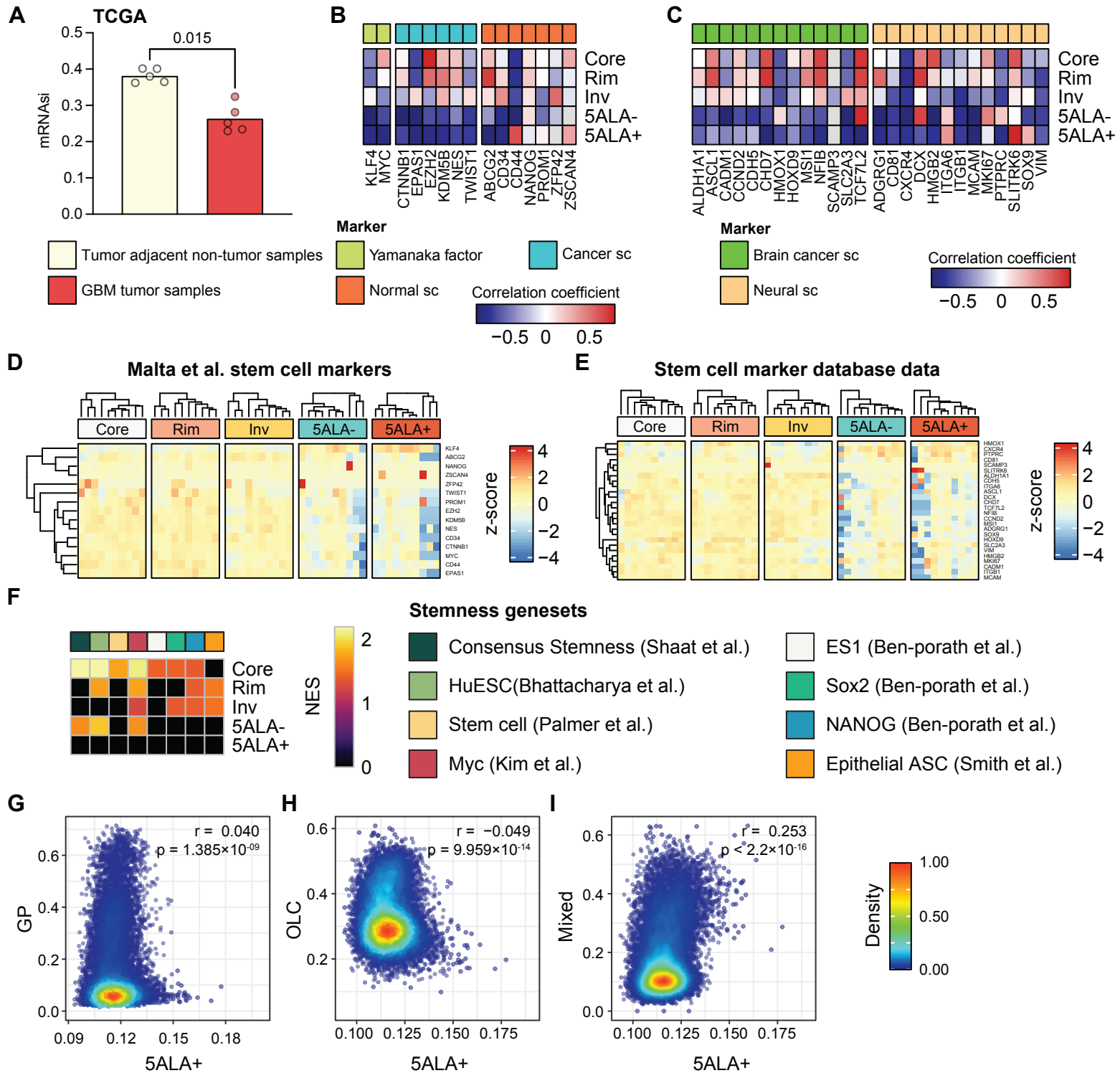


Fig. S9: Stemness signature analysis

Comparison of mRNAsi of adjacent non-tumor tissue (NAT) and GBM tumor tissue (A). P-value is calculated by the Wilcox test following a bootstrap analysis where an equal number of samples from GBM tissue were drawn 100 times to compare with NAT. The median p-value of 100 comparisons is shown. Correlation between mRNAsi values and mRNA expression for available Malta *et al.* stem cell markers (B). Correlation between mRNAsi and mRNA expression for markers from stem cell marker database (C). Heatmap of RNA-seq expression z-scores for the Malta *et al.* stem cell marker signatures (D) and markers from stem cell marker database (E). Heatmap illustrating normalized enrichment scores (NES) of GSEA with $\text{padj} < 0.05$ for selected stemness gene-sets from different studies (F). Scatter plots representing the UCell score correlation between gene signature of 5ALA+ cells and GP (G), OLC (H), and Others mixed population (I). The Pearson correlation coefficient (r) is given for each scatter plot. P-values of correlation are given.

Fig S10

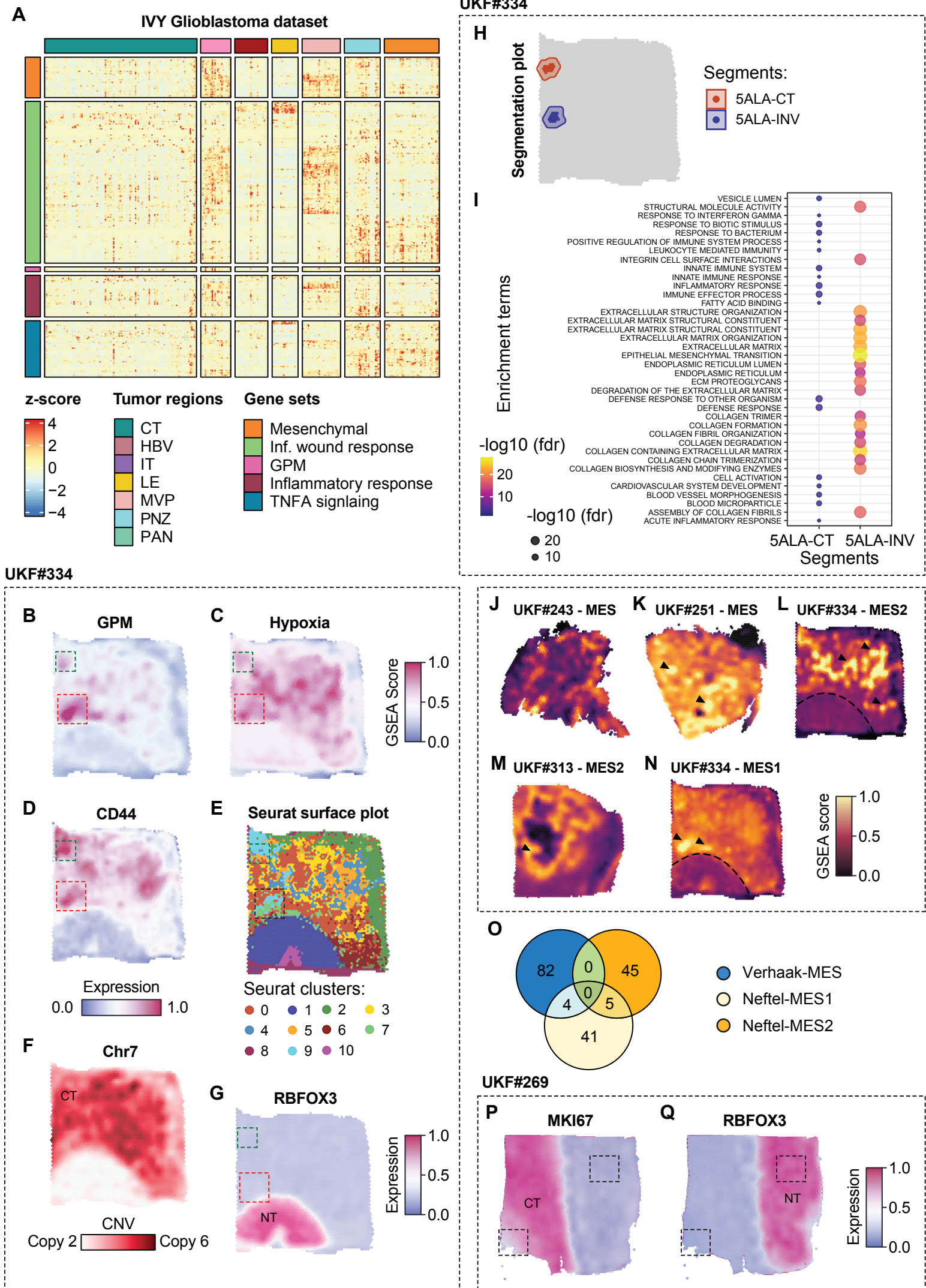
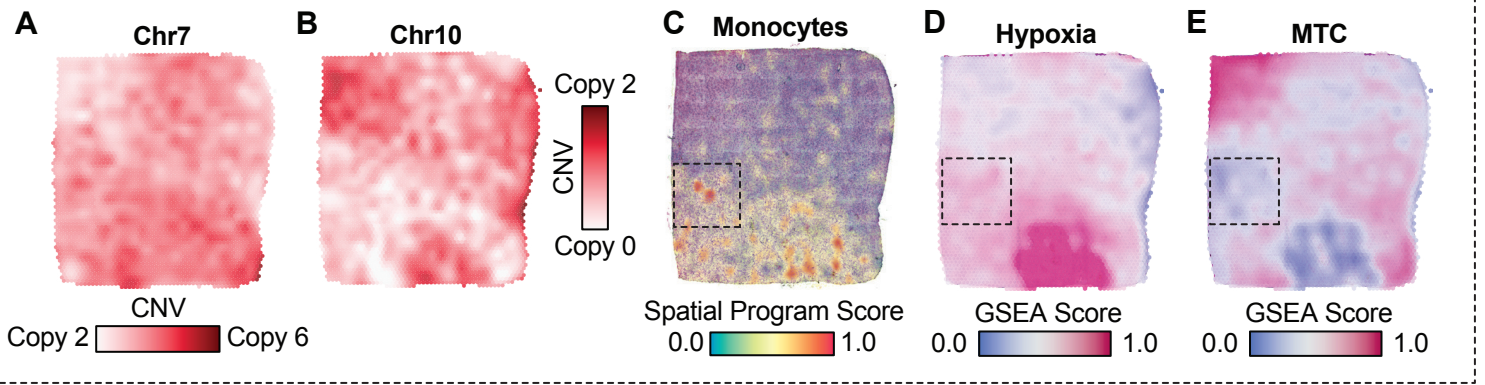


Fig. S10: Spatial segmentation and differential gene expression analysis

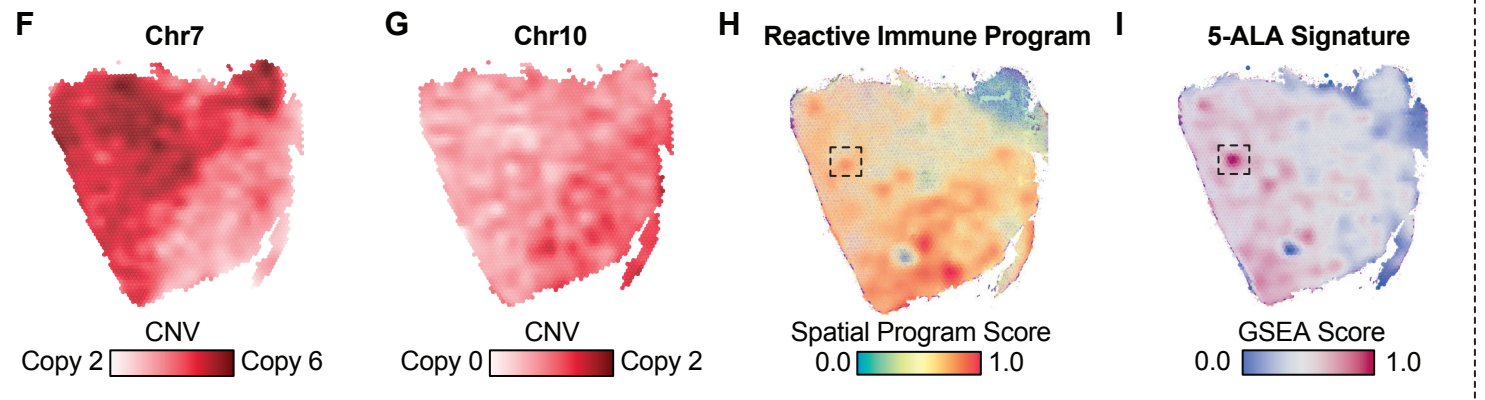
Heatmap representing the enrichment of the 5ALA+ specific genes across different anatomical regions (CT: Cellular Tumor; HBV: Hyperplastic blood vessels; IT: Infiltrative tumor; LE: Leading-edge; MVP: Microvascular proliferation; PNZ: Perinecrotic zone, and PAN: Pseudopalisading cells around necrosis) based on the IVY Glioblastoma dataset (A). GSEA score of GPM (B), Hypoxia (C), and single gene score (z-scored) for *CD44* (D) are shown for sample UKF#334, where the enriched and random spots are shown in red and blue, respectively. The spatial localization of the enriched spots is marked by a dashed box where the black and green box represents the 5ALA+ spots within the invasive margin (5ALA-INV) and inside the cellular tumor (5ALA-CT), respectively. Seurat Clusters representing the transcriptional diversities are mapped in the tissue section based on Spatial Transcriptomics data (E). Color codes are indicative of different clusters. The Inferred CNV analysis of Chromosome-7 (F) is presented as a spatial surface plot where the gain of Chromosome-7 is color-coded. The z-scored gene-expression value for *RBFOX3* (G) is mapped and represented as a surface plot. The spatial locations of the segmented 5ALA-INV and 5ALA-CT spots are shown (H) where 5ALA-INV and 5ALA-CT segments are represented by blue and red colors. GSEA scores of the enriched pathways based on the top-20 differentially expressed genes between the segmented 5ALA-INV and 5ALA-CT regions are shown as a heatmap (I). The color code is based on enrichment score: $-\log_{10}(\text{FDR})$ values. Spatial Gene-set enrichment analysis of MES, MES1, and MES2 genes across GBM tissues (J-N). Venn Diagram showing minimal or no overlap among MES, MES1, and MES2 gene sets (O). Spatial expressions (z-scored) of *MIK67* (Ki67) (P) and *RBFOX3* (NeuN) (Q) genes in sample UKF#269 are shown as surface plots.

Fig S11

UKF#275



UKF#251



UKF#243

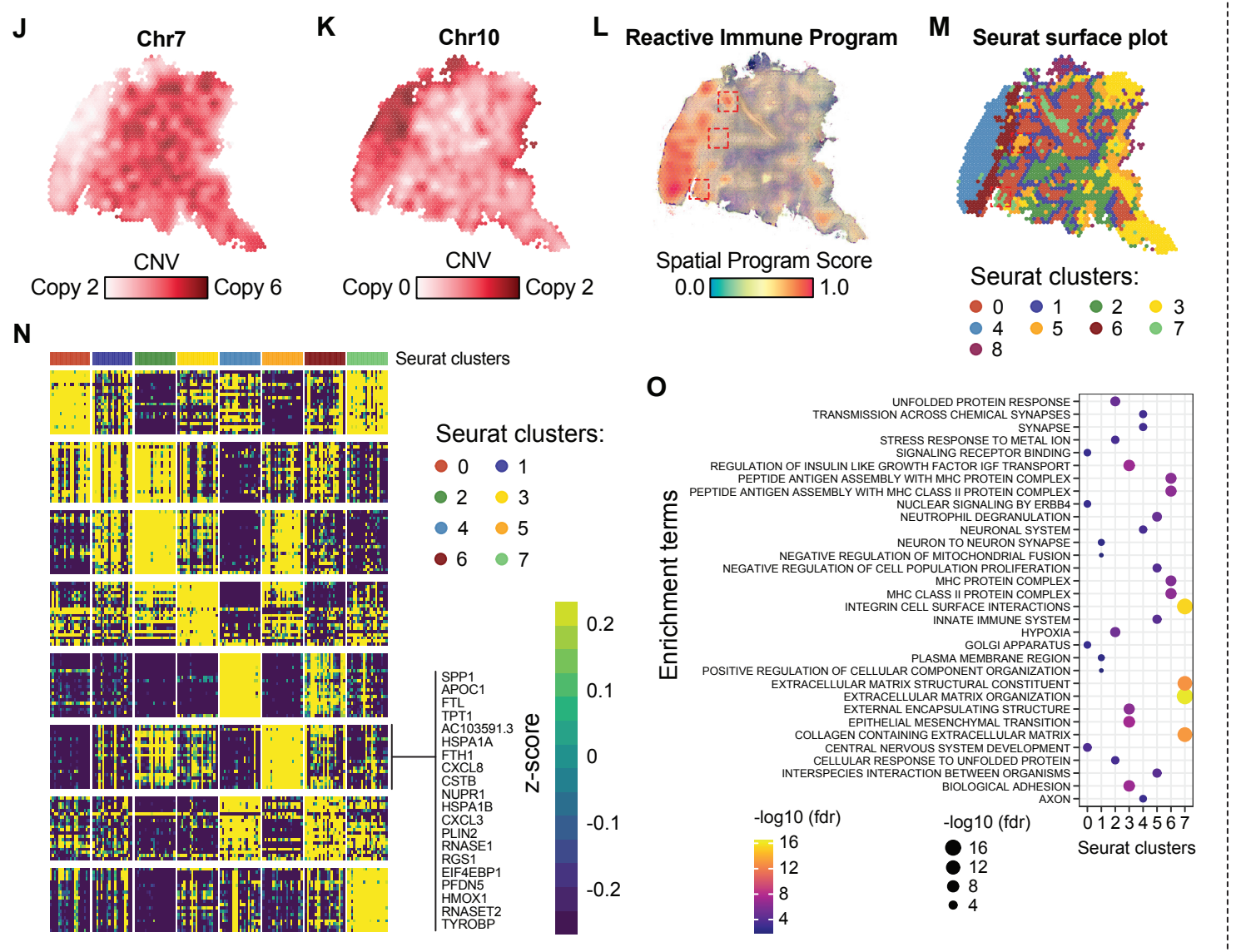


Fig. S11: Spatial copy number variation and enrichment analysis

Inferred CNV analysis of Chromosome-7 (A) and -10 (B) are presented as spatial surface plots where the gain and loss of Chromosome-7 and -10, respectively are color-coded for sample UKF#275. GSEA scores of monocyte (C), Hypoxia (D), and MTC (E) are represented as surface plots by showing the enriched and random spots in red and blue, respectively. For sample UKF#251 the inferred CNVs of Chromosome-7 (F) and -10 (G) are shown. The spatial location of the enrichment of the Reactive immune program (RIP) (H) and 5ALA+ gene-signature are shown (I). For sample UKF#243, the inferred CNVs of Chromosome-7 (J) and -10 (K) are shown. The spatial localization of the spots enriched with RIP (Red color) is presented as a surface plot (L). Seurat Clusters representing the transcriptional diversities are mapped in the tissue section based on Spatial Transcriptomics data (M). Color codes are indicative of different clusters. Heatmap represents the top20 differential genes of each Seurat cluster (N). The genes belonging to Cluster-5 are shown. The color code indicates the differential expression of the genes. GSEA scores of the enriched pathways based on the top-20 differentially expressed genes across the Seurat clusters are shown as a heatmap (O). The color code is based on enrichment score: $-\log_{10}(\text{FDR})$ values.

FigS12

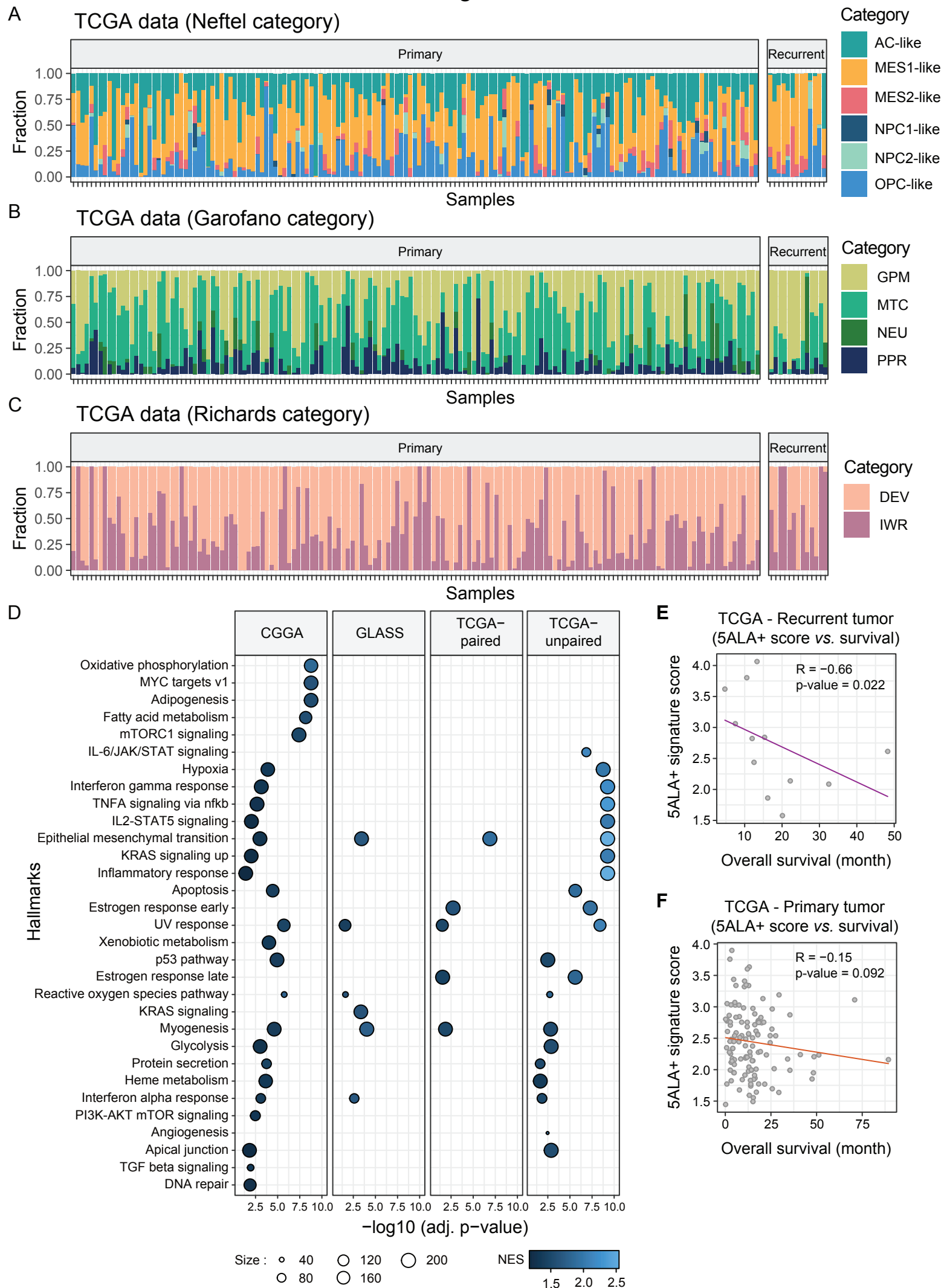


Fig. S12: CIBERSORTx derived Cell-type fraction estimation across primary and recurrent GBM patients

CIBERSORTx derived Cell-type fraction estimation across primary and recurrent GBM patients based on signature matrices from Netfel (A), Garofano (B), and Richards (C) *et al.* Hallmark enrichment analysis of DEGs between recurrent and primary GBM tumors. The hallmarks that are enriched in recurrent tumors in comparison to primary tumors from TCGA, CCGA, and GLASS cohorts are illustrated (D). Each bubble indicates one particular hallmark (y-axis). The x-axis indicates a $-\log_{10}$ adjusted p-value. The color gradient of the bubble indicates the normalized enrichment score (NES) whereas the size of the bubble depends on the gene-count for each hallmark. Paired and unpaired analysis was performed for the TCGA cohort. Scatter plots representing the correlation between 5ALA+ gene-signature scores and overall survival (months) in recurrent and primary patients from TCGA (E and F) cohort. Spearman correlation coefficient (R) and p-values are shown.