Supplementary Information

Tumor microenvironment is critical for the maintenance of cellular states found in primary glioblastomas.

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Patient	WCM1	WCM2	WCM3	WCM4	WCM5
Cell-line	GSC-728	GSC-320	GSC-810	GSC-517	GSC-1206
Clinical Diagnosis	Glioblastoma	Glioblastoma	Glioblastoma	Glioblastoma	Glioblastoma
Surgery	Primary	Primary	Primary	Primary	Primary
IDH	wt	wt	wt	wt	wt
Molecular Subtype	Mesenchymal	Proneural; mesenchymal	Classical; mesenchymal	N/A	Classical
EGFR Amplification	Exon 20 insertion	No	Focal amplification	No	G598V
MET	wt	wt	5323G	F1269fs*17	wt
TP53 mutation	No	H179Y (Missense)	No	No	D281E
PTEN	W111 (Nonsense)	R130 (Nonsense)	wt	Loss	wt
Age	80	70	69	48	51
Sex	M	M	M	M	F

Table S1. Clinical Cohort of Glioblastomas.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit Monoclonal DLL3 Antibody (7H24L4), ABfinity™	Thermo Fisher Scientific	703623
Rabbit Antibody SOX4	SIGMA	HPA029901
Mouse polyclonal BCAN	Novus Biologicals	H00063827-B01P
Chicken polyclonal anti-GFP	Abcam	ab13970
Goat anti-chicken Alexa Fluor 488	Thermo Fisher Scientific	A11039
Goat anti-rabbit Alexa Fluor 568	Thermo Fisher Scientific	A11036
Chemicals, Peptides, and Recombinant Proteins		
ROCK inhibitor (Y-27632 2HCl)	Selleck Chemicals	Cat# S1049
Matrigel	Corning	#354277
Critical Commercial Assays		
Papain Dissociation System	Worthington Biochemical	LK003150
Deposited Data		
Single-cell RNA-sequencing data	NCBI Sequence Read Archive	PRJNA595375
Experimental Models: Cell Lines		
Patient-Derived Glioma Stem Cells: WCM1 (0728), WCM2 (0320), WCM3 (0810), WCM4 (0517), WCM5 (1206)	National Institutes of Health and Weill Cornell Medicine/NYU Presbyterian Hospital	N/A
NIH-registered human H1 (WA01) embryonic stem cells	WiCell Research Institute	Cat# WA01; RRID: CVCL_9771
NIH-registered human H9 (WA09) embryonic stem cells	WiCell Research Institute	Cat# WA09; RRID: CVCL_9773
Recombinant DNA		
pLenti PGK GFP Blast (w510-5)	Campeau et al., 2009	Addgene Plasmid# 19069

Table S2. Detailed Resource Information.

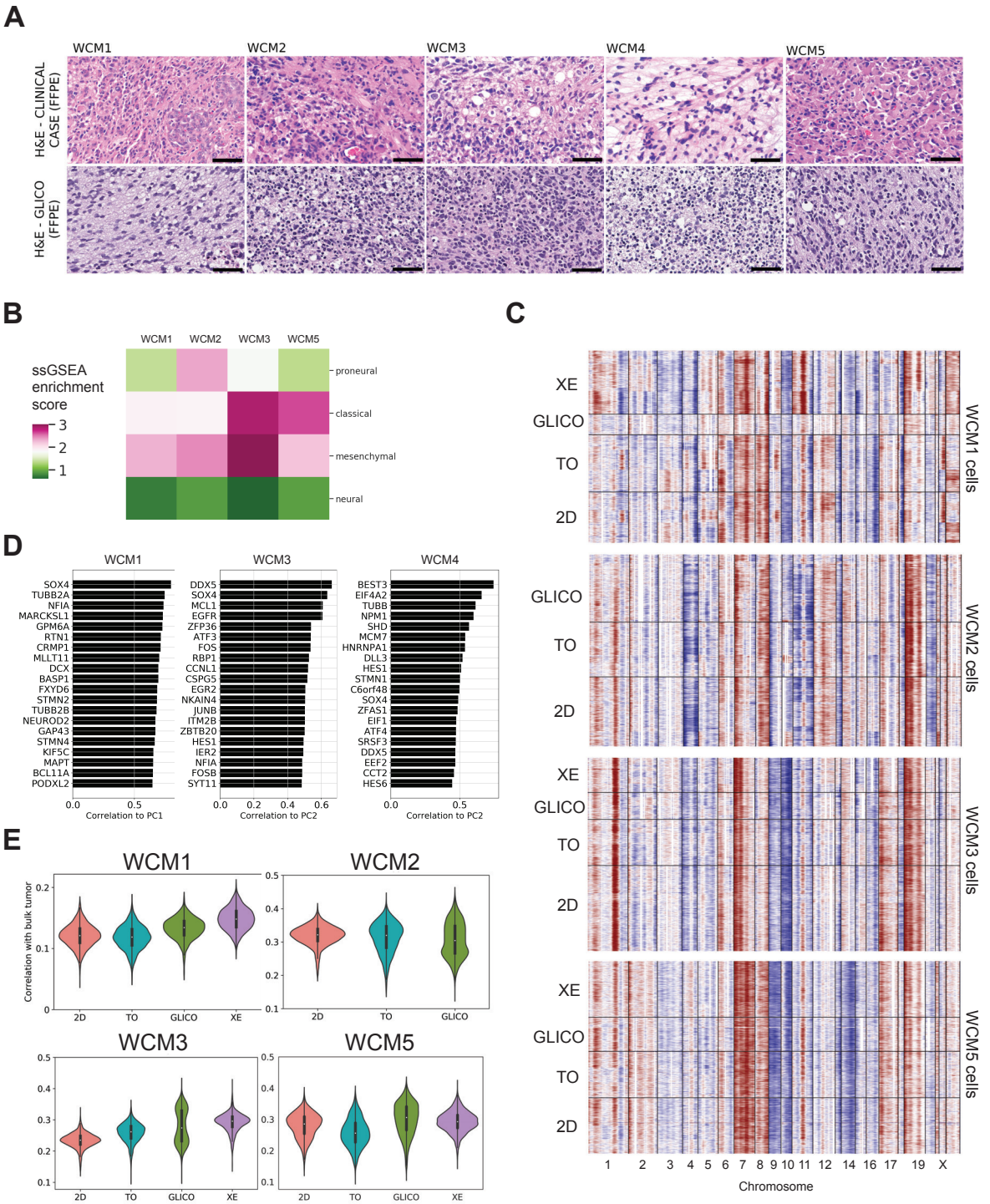

Figure S1. (A) H&E staining for all patient tumors and their respective GLICOs, FFPE. Scale bar: 50um. (B) ssGSEA of the available bulk RNA-seq patients' tumor profiles into GBM transcriptional subtypes. (C) Inferred copy number variation profiles calculated from single-cell RNA-seq data for WCM1, WCM2, WCM3, WCM5 (see Methods). (D) Bar plot of the top genes with the highest correlation to the representative principal component in WCM1, WCM3 and WCM4. (E) Distributions of the Spearman correlation of each model type with the bulk patient tumor using the expression signatures of genes highly variable in the bulk sample, grouped by patient.

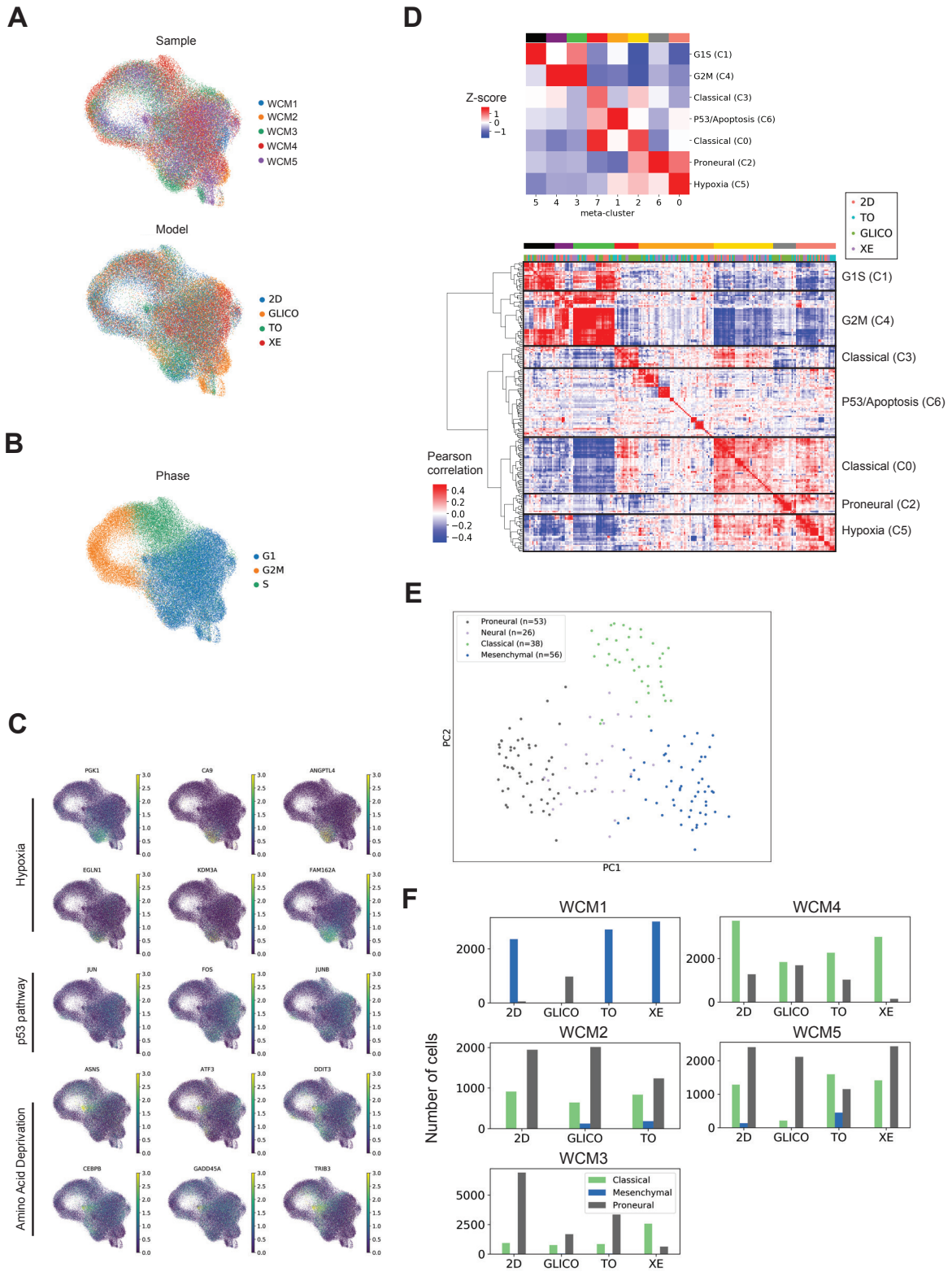

Figure S2. (A) UMAP projection colored by sample and condition (B) by cell cycle phase score (C) by gene expression of marker genes for clusters enriched for hypoxia, p53 pathway, and amino acid deprivation signatures. (D) (Top panel) Gene set scores for each meta-cluster using differentially expressed genes from main clusters found in Fig 2A. (Bottom panel) Hierarchical clustering (one minus Pearson correlation) showing meta-clusters of aggregated transcriptomes from Louvain/NMF on each of the 19 samples (N=220). Heatmap colored by meta-cluster identity and model type, labeled by highest scoring reference cluster. (E) Principal component analysis of the reference dataset used for TCGA subtype analysis. (F) Number of cells assigned to each subtype per patient and model type.

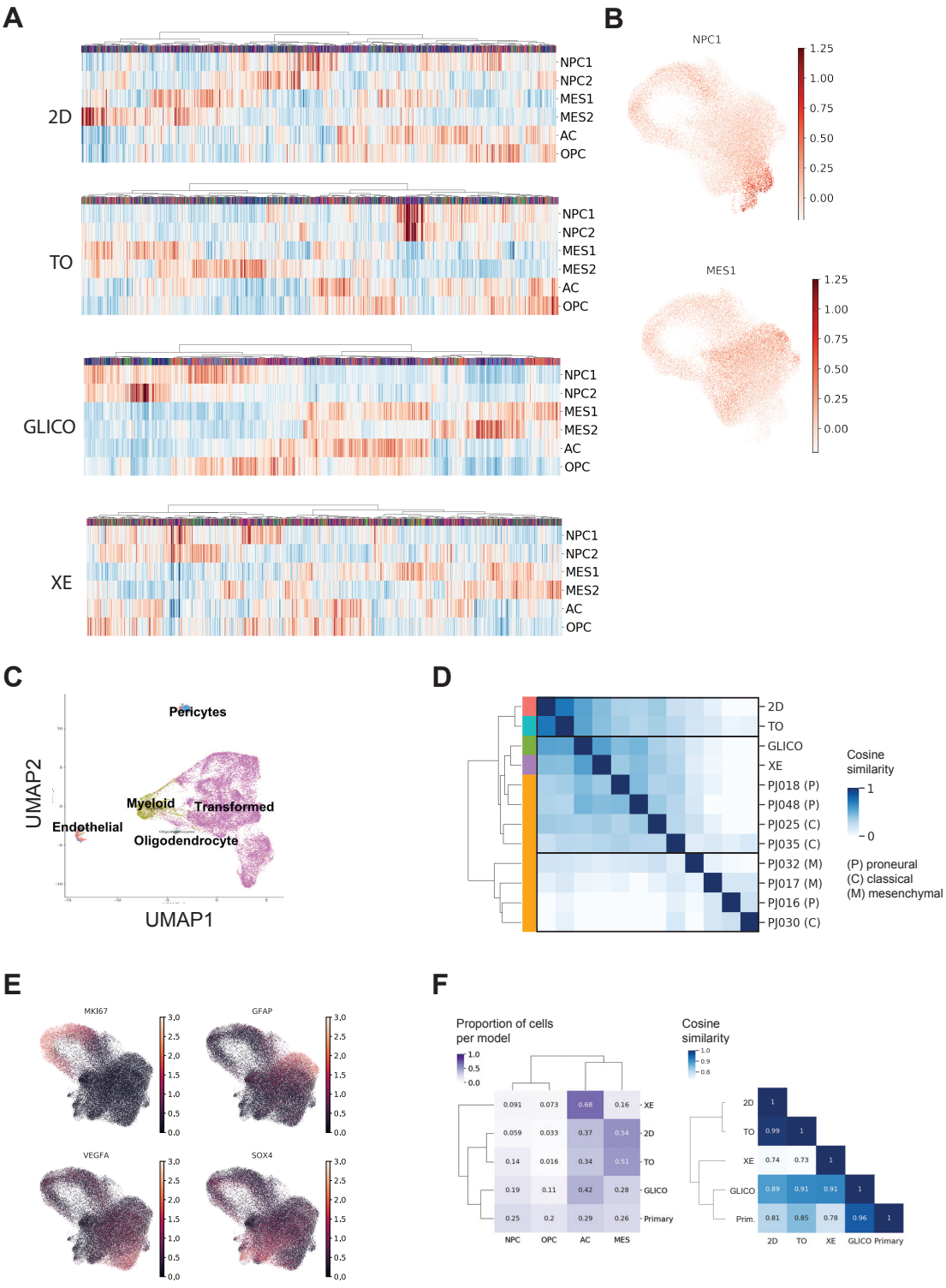

Figure S3. (A) Heatmap of glioblastoma meta-modules for each model. Cells are along the y-axis and the columns are colored by patient. (B) UMAP projection of NPC1 and MES1 gene set scores. (C) UMAP projection of non-malignant cell type labeling. Analysis was completed with only malignant cell types as done previously. (D) Hierarchical clustering (Ward's method, Cosine distance) of the averaged transcriptomes of each model type and the eight primary scRNA-seq published samples (see Methods). (E) UMAP projection with primary GBM samples colored by marker gene expression. (F) Proportion of NPC, OPC, AC, and MES cells across models and aggregated primary samples (left). Clustering of the models and primary samples (Cosine distance), visualized with cosine similarity between each model and primary samples (right).

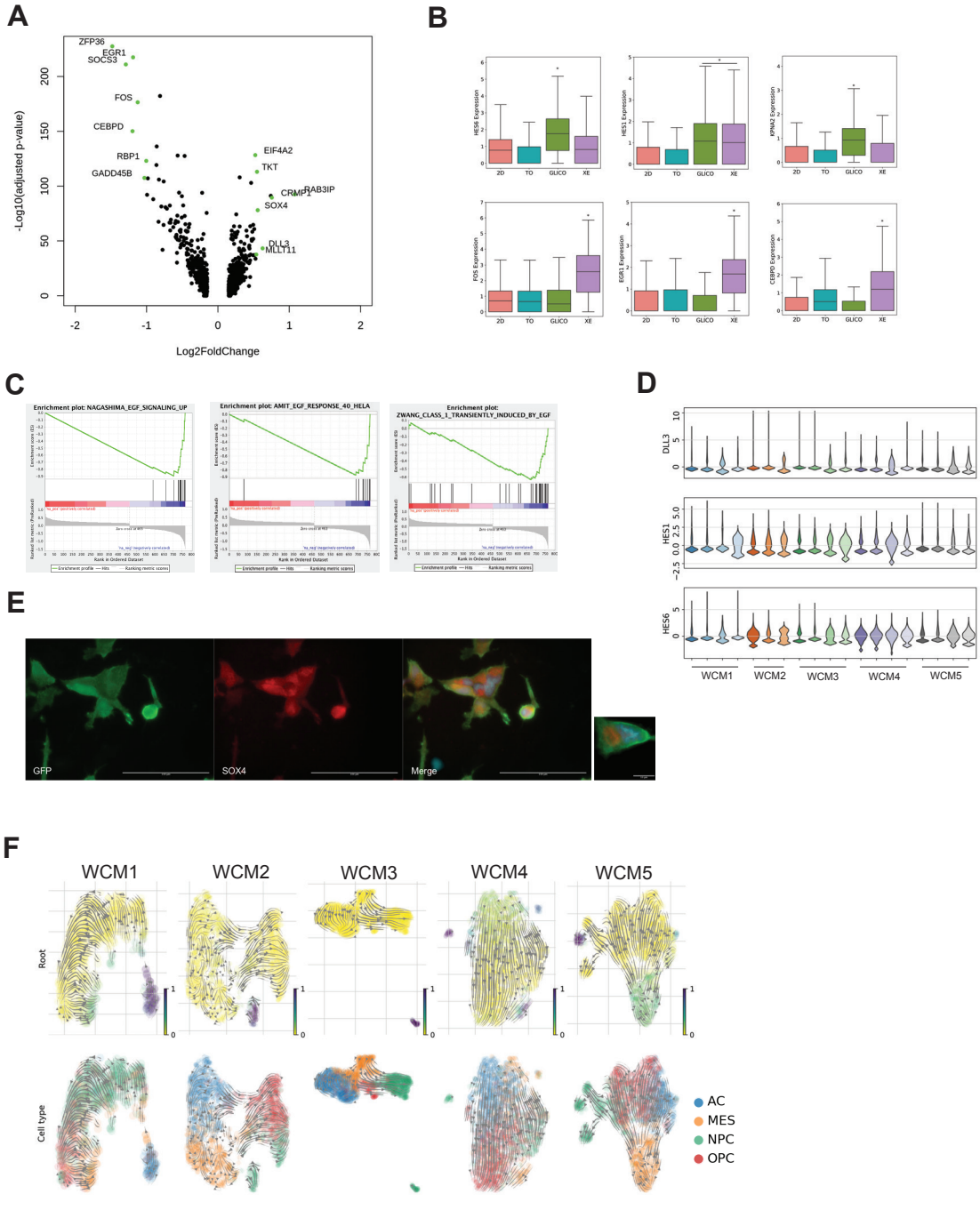

Figure S4. (A) Volcano plot of GLICO model vs. XE model. Genes with an absolute log fold change > 0.6 are labeled. (B) Expression of GLICO-enriched genes *HES1, HES6*, and *KPNA2* and XE-enriched genes *FOS, EGR1,* and *CEBPD* across models (* indicate p < 0.05 compared to all other models). (C) Top gene set enrichment results for XE model vs. GLICO model. (D) Violin plots of scaled expression values across all patients and models for Notch pathway members *DLL3, HES1,* and *HES6.* (E) Immunofluorescence staining of *SOX4* in GSCs isolated from the 2D model (40X and 100X respectively). (F) UMAP projection of RNA velocity stream plots and cell type assignments for GLICO across all patients.

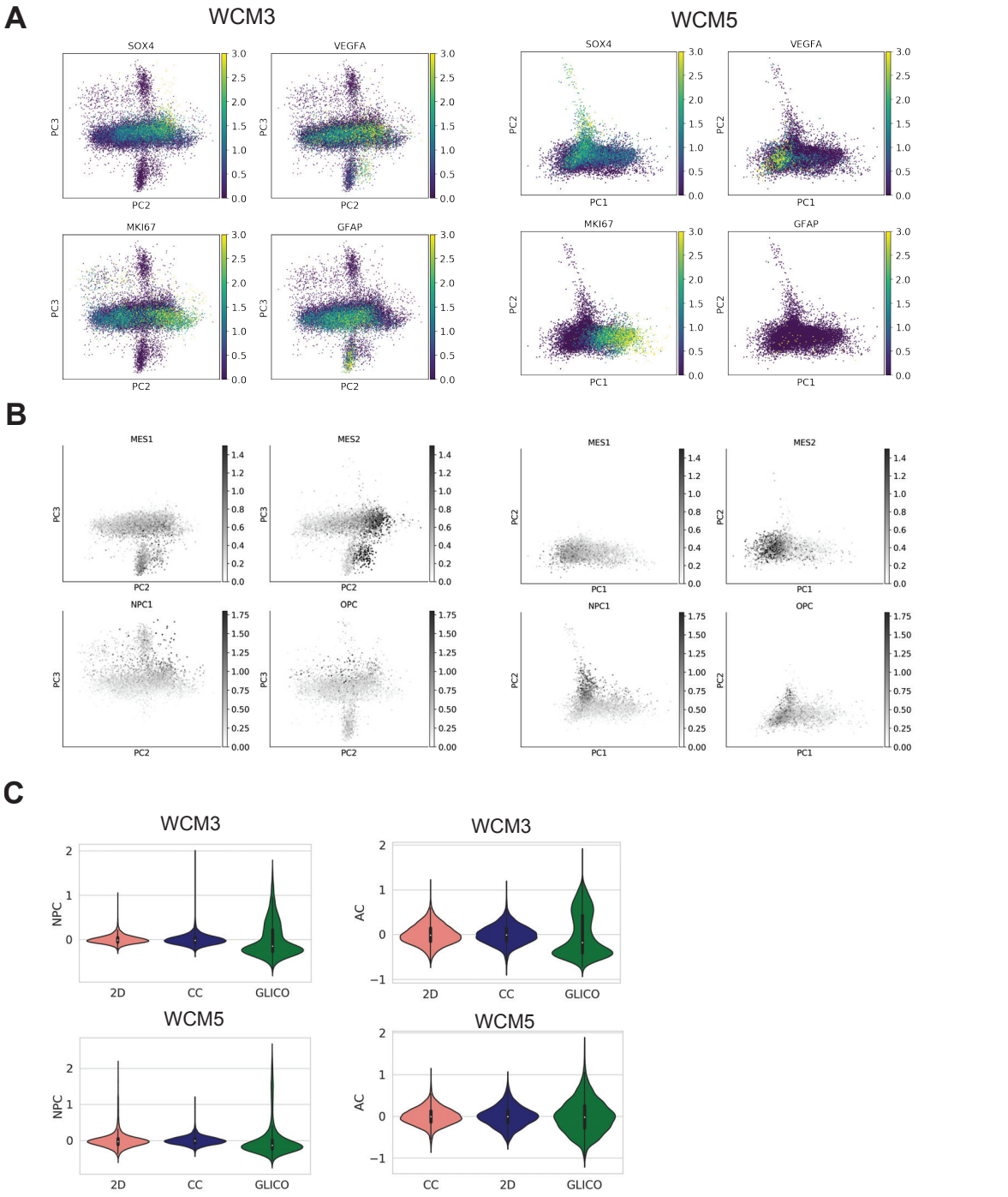

Figure S5. (A) PCA projections colored by cluster marker genes from Figure 2A for patient samples cultured in GLICO and then re-plated in 2D for 30 days, GLICO and 2D samples. (B) PCA projections colored by additional cellular state gene set scores for patient samples cultured in GLICO and then re-plated in 2D for 30 days, GLICO and 2D samples. (C) Violin plots of NPC and AC-like gene set scores across 2D, GLICO, and re-plating (CC) in WCM3 and WCM5.