

Expanded View Figures

Figure EV1. *In vitro* culture of patient-derived organoids (PDOs).

- A Schematic representation of primary tumor samples management for generation of PDOs as tumor single cells spheroid.
- B–D Brightfield images of tumor single cells spheroids EPN- (B), MB- (C), and LGG- (D) derived PDOs at different timepoints.
- E, F Confocal images of DAPI staining and immunofluorescence of human nuclear antigen and Ki67 of sagittal brain sections of immunodeficient mice engrafted with MB-derived PDOs.
- G, H Copy number variation profiles comparison between primary parental tumor and 3 different MB-PDOs (H) and EPN-PDOs (G).

Data information: X axis: chromosomes; Y axis: Log_2 copy number ratio. Scale bar 200 μm (B–D), 100 μm (E, F). DNA methylation (CNV) experiments (G, H) were performed once per primary tumor/matching PDOs.

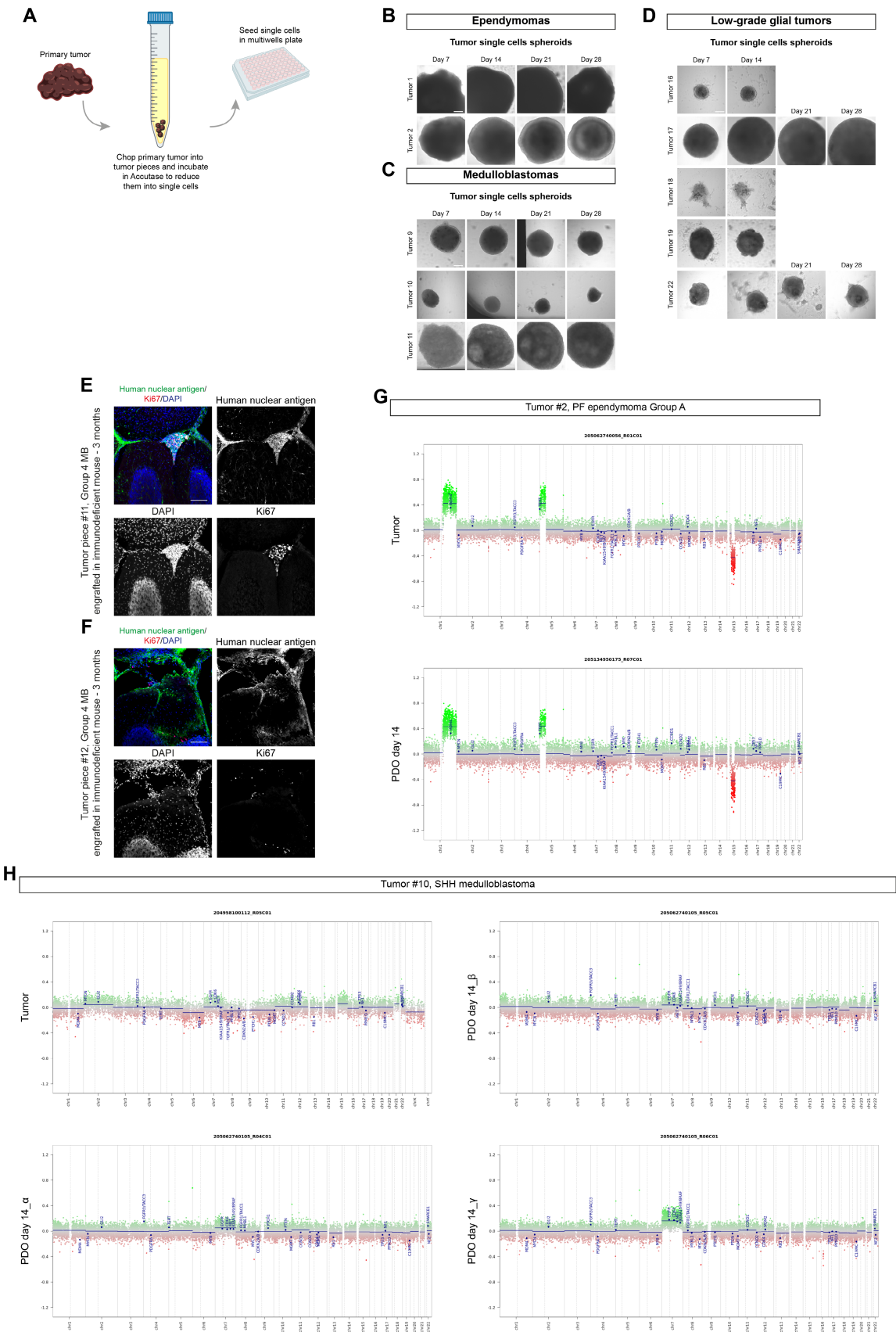


Figure EV1.

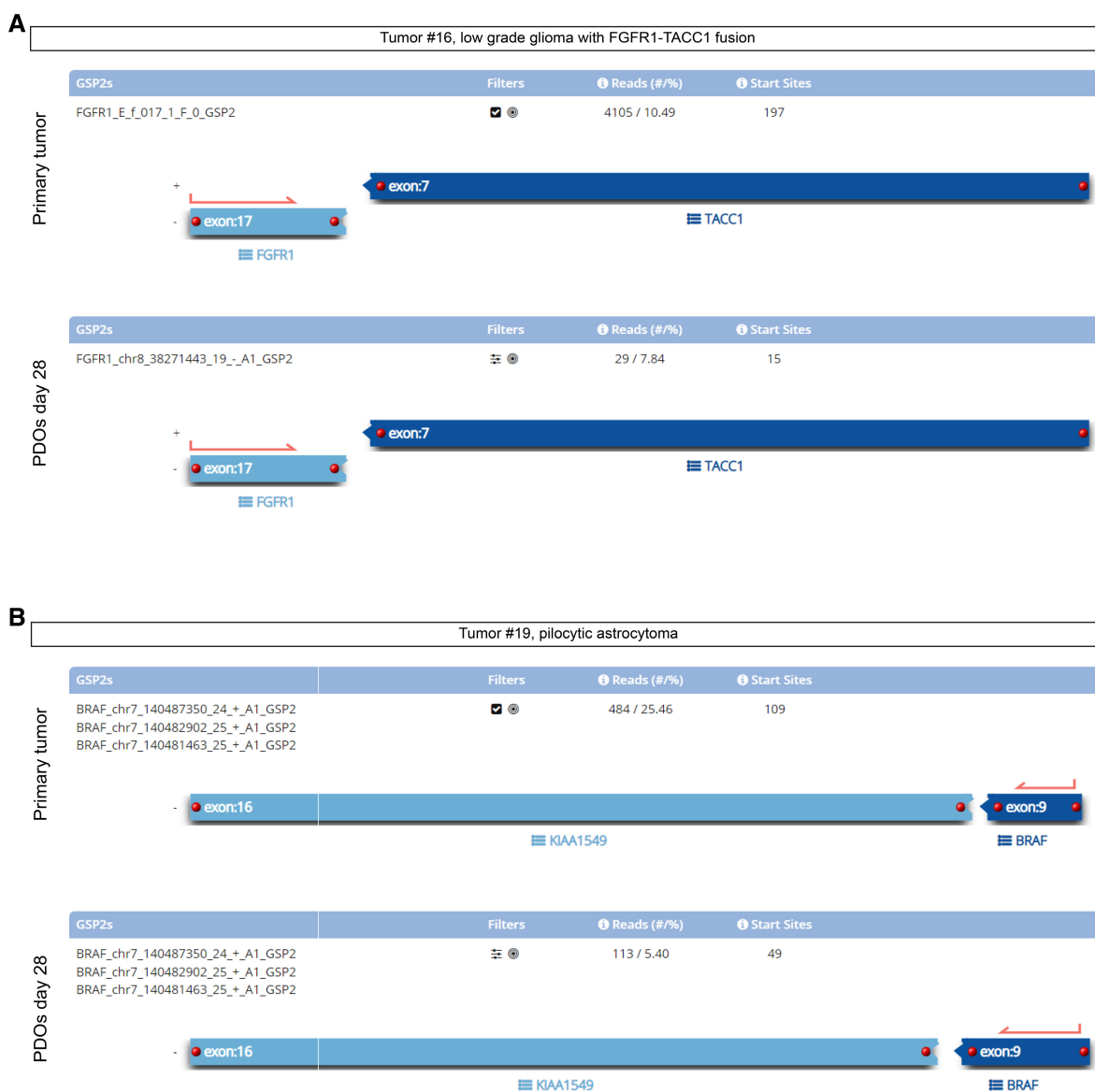


Figure EV2. Data from RNA-based assay showed maintenance of genomic features of fusion-positive tumors and their corresponding PDOs.

A Tumor #16, LGG with *FGFR1-TACC1* fusion compared to derived PDOs at day 28: the assay detects the same fusion.

B Tumor #19, *KIAA1549-BRAF* fusion was detected in pilocytic astrocytoma and in derived PDOs at day 28. Both PDOs shared the same breakpoints compared to primary parental tumors.

Data information: Experiments were performed once per primary tumor/matching PDOs.

Figure EV3. Maintenance of cellular heterogeneity in PDOs and comparison with already published medium.

- A–C Confocal images of immunofluorescence of Ki67, SOX2, OLIG2, Nestin, IBA1, CD3, GFAP, B3-tubulin, CD34 of EPN- (A'–A''), MB- (B) and LGG- (C) derived PDOs.
- D Brightfield images of LGG-derived PDOs as tumor pieces at different timepoints in PDOs medium and cultured according to (Abdullah et al, 2022) (D'), confocal images of immunofluorescence of Ki67, SOX2, OLIG2, Nestin, IBA1, CD3, GFAP, B3-tubulin (D'') and quantification in PDOs of Ki67⁺, SOX2⁺, OLIG2⁺ and IBA1⁺ cells (D''').
- E, F Confocal images of immunofluorescence of YAP1 (E', F') and p75 NGFR (E'', F'') of MB-derived PDOs.
- G, H Confocal images of immunofluorescence of synaptophysin of LGG-derived PDOs. Quantifications are shown as percentage of specific marker⁺ cells/DAPI (D''').

Data information: Data are presented as mean $\pm$ s.e.m.; each dot represents a ROI/image. For each marker, $n = 5–7$ ROI/image of primary tumor was considered. For each marker, $n = 2–3$ PDOs (biological replicates) were considered; for each PDO, $n = 3–4$ ROI/image was used. Quantification experiments were performed once per primary tumor/matching PDOs. Kruskal–Wallis test with Dunn's *post hoc* correction; ** $P \leq 0.01$, * $P \leq 0.05$. Adjusted and exact P values are reported in figure. The white arrows highlight specific cells. Scale bar 50 μm (A–C, D'', E–H), 200 μm (D').

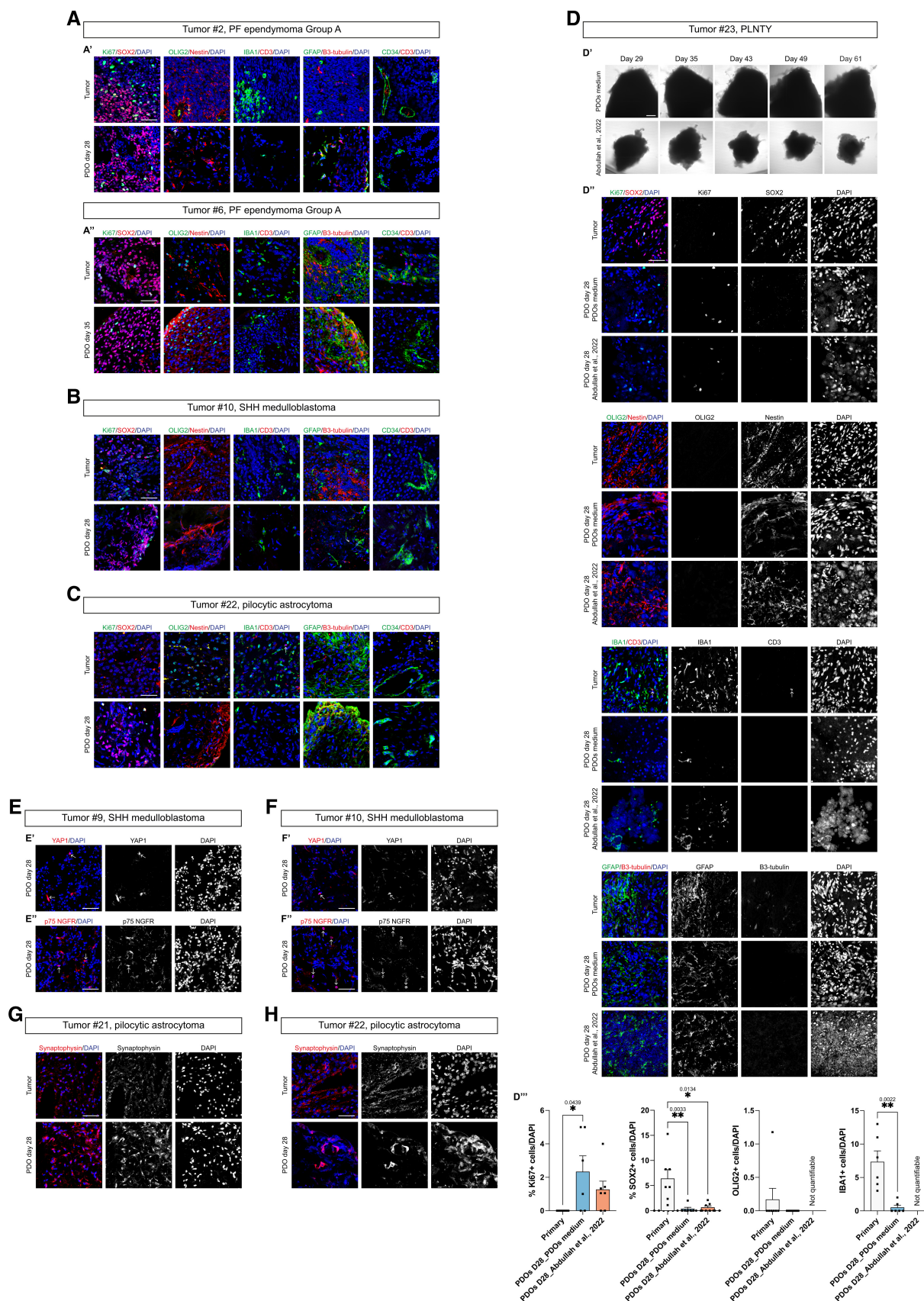


Figure EV3.

Figure EV4. Maintenance of morphological features and cellular heterogeneity in PDOs and PDOs-derived tumors.

A–C Morphological features (A', B', C') and immunohistochemical expression of lineage markers GFAP (A''), H3K27me3 (A''), synaptophysin (B''), and OLIG2 (C'') of 2 EPN, 2 MB and 2 LGG paired parental tumors/PDOs samples.

D, F Confocal images of immunofluorescence of human nuclear antigen and GFAP of sagittal brain sections of immunodeficient mice engrafted with EPN- (D) and MB- (F) derived PDOs.

E, G Confocal images of immunofluorescence of human nuclear antigen and OLIG2 of sagittal brain sections of immunodeficient mice engrafted with EPN- (E) and MB- (G) derived PDOs.

H Confocal images of immunofluorescence of human nuclear antigen and SOX9 of sagittal brain sections of immunodeficient mice engrafted with MB-derived PDOs.

Data information: Scale bar 100 μm (A, C, D–H), 50 μm (B).

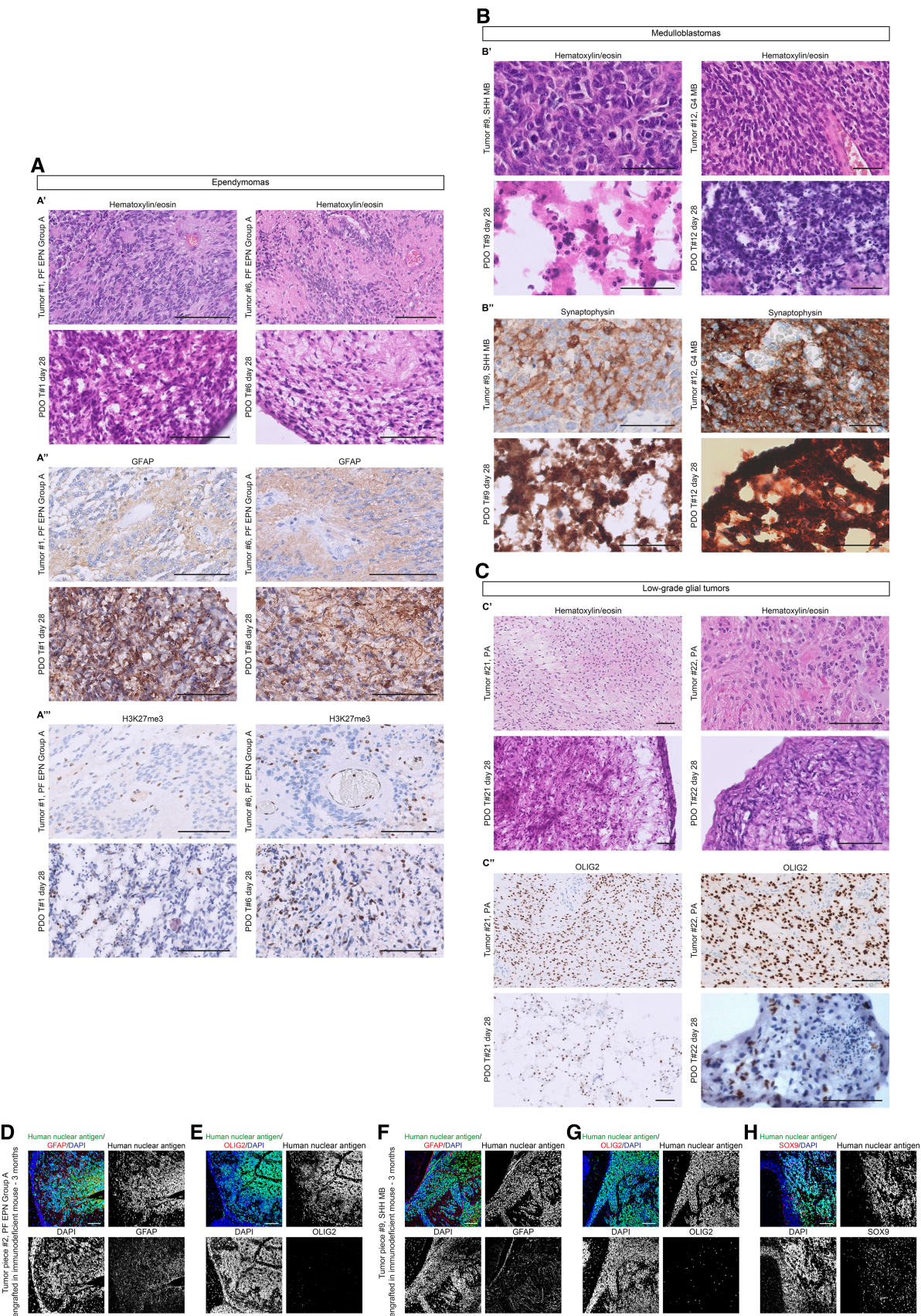


Figure EV4.

Figure EV5. scRNA-seq data analysis of primary tumor #2 and matching PDOs samples describes EPN PFA-specific intratumoral heterogeneity, recapitulated in the PDOs model.

- A UMAP dimensionality reduction plot showing the cluster distribution of cells obtained from tumor and PDO samples.
- B UMAP plot showing the different independent clusters obtained by integrating the malignant cells from “Primary tumor #2,” “PDO Day 14,” and “PDO Day 28” datasets.
- C Expression dotplot representing the key markers identified for each cluster and belonging to cellular and/or functional categories.
- D FeaturePlot showing the expression levels of key markers in each cell.
- E Stacked barplot representing the relative proportion (expressed in %) of the tumor and PDO cells across the different subclusters.

Data information: scRNA-seq experiment was performed once per primary tumor/matching PDOs.

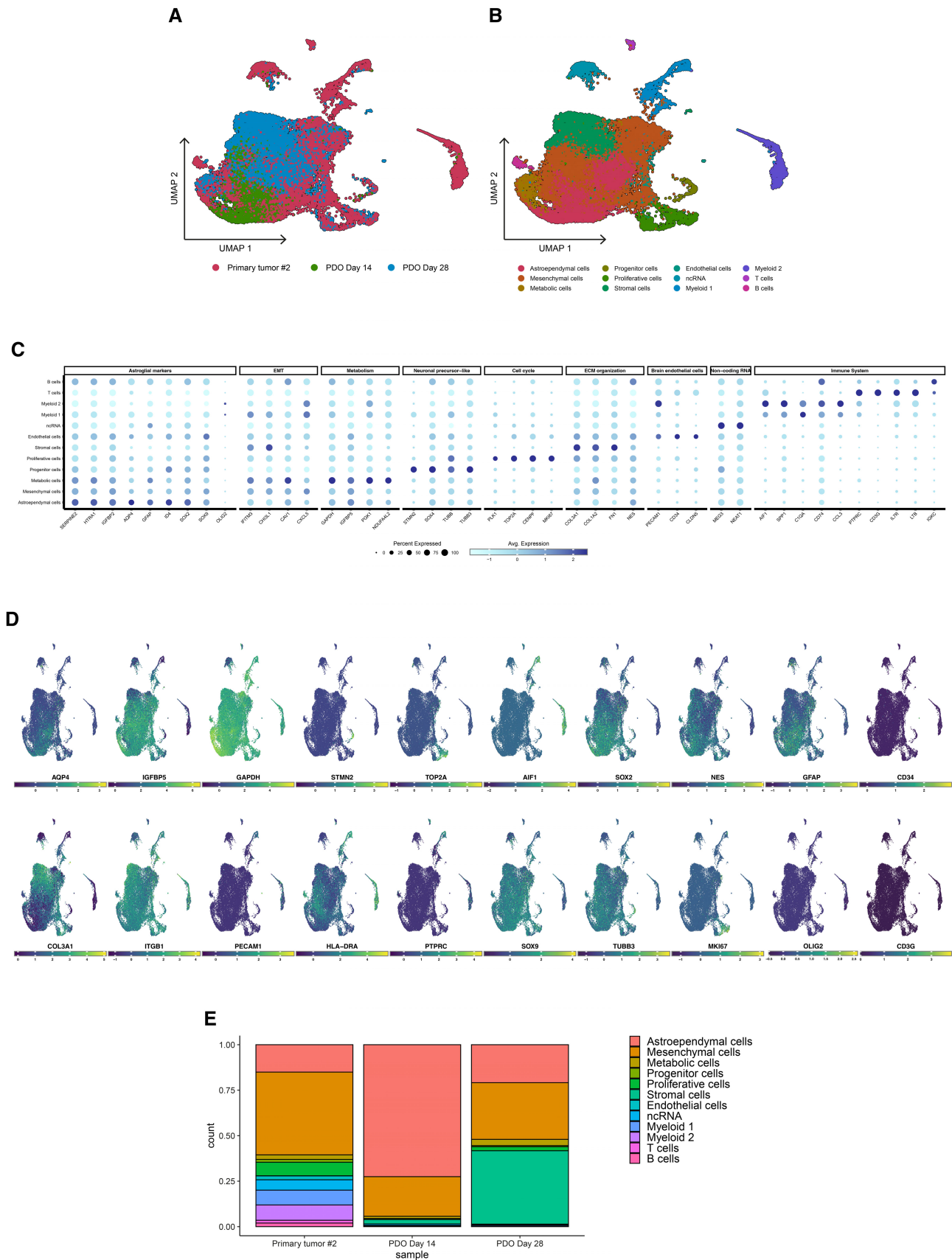


Figure EV5.