

Spatially Resolved Multiomics Profiling of Glioblastoma Reveals Immune-Tumor Microenvironment Architecture

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Introduction

Glioblastoma (GBM) is a highly heterogeneous and aggressive brain tumor with a dismal prognosis, necessitating advanced spatial multiomics approaches to decode its complex biology. Here, we employed the CosMx[®] Spatial Molecular Imager to perform high-resolution, same-cell spatial multiomics profiling on serial sections from a GBM patient tissue block, integrating two complementary high-plex protein panels with the whole-transcriptome RNA panel (~19,000 gene transcripts, covering >99% of protein-coding genes). The neuro-pathology panel enables integrated analysis of up to 76 proteins linked to tissue morphology and neuropathologic pathways, revealing distinct spatial distributions of phosphorylated Tau variants associated with GBM cell subtypes across tumor and peri-tumoral regions. In parallel, the immuno-oncology panel highlighted region-specific expression of immune checkpoint proteins, underscoring spatially heterogeneous tumor-immune interactions. Collectively, these findings demonstrate the complementary strengths of protein and RNA data in spatial profiling, positioning the CosMx multiomic assay as a powerful tool for translational neuroscience research.

CosMx Multiomics Assay Workflow

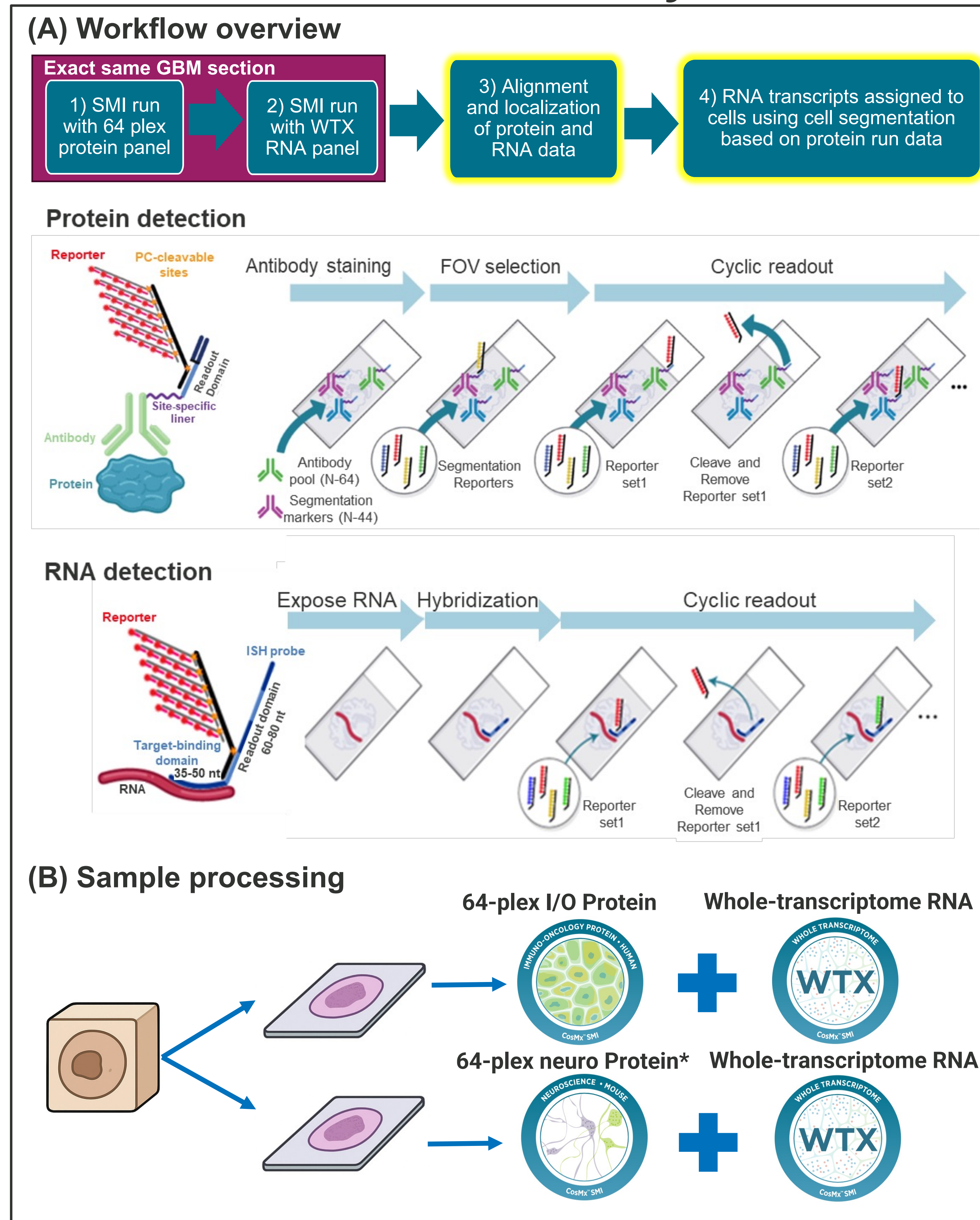


Fig 1. (A) The CosMx SMI multiomics assay sequentially detects protein and RNA targets with oligonucleotide-conjugated antibodies and barcoded RNA probes via sequential rounds of reporter binding and fluorescence imaging. (B) Serial GBM sections were subjected to WTX multiomic profiling with neuro-pathology and immuno-oncology protein panels.

CosMx SMI Multiomics for Spatial Neuro-Pathology Profiling

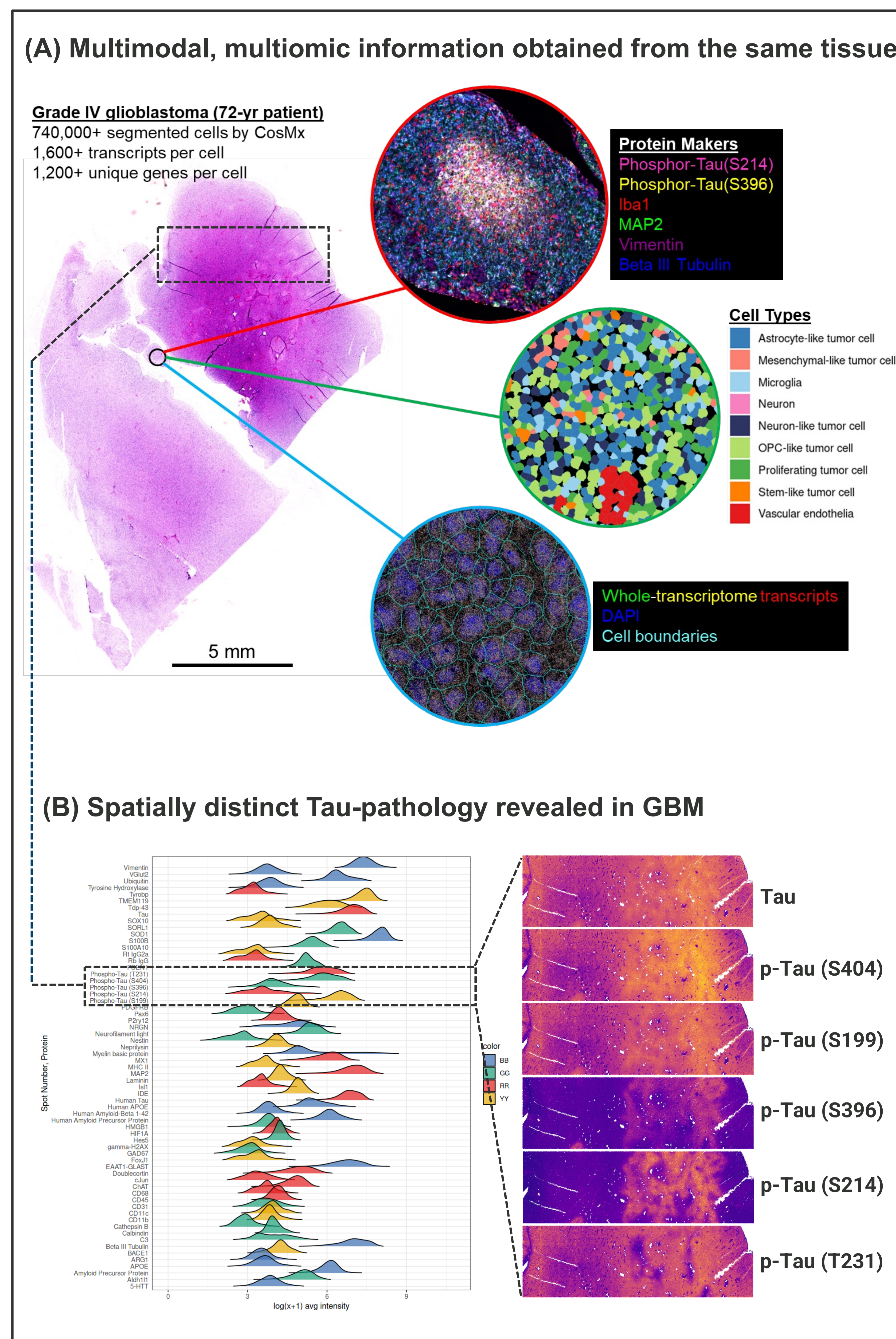


Fig 2. (A) CosMx same-cell multiomics assay provides histology, morphology, tissue composition, and ultrahigh-plex protein-RNA information from a single tissue sample. (B) The CosMx neuro-pathology protein panel revealed heterogeneous quantity and distinct localization of phospho-Tau variants in this GBM patient.

High RNA quality in Multiomics

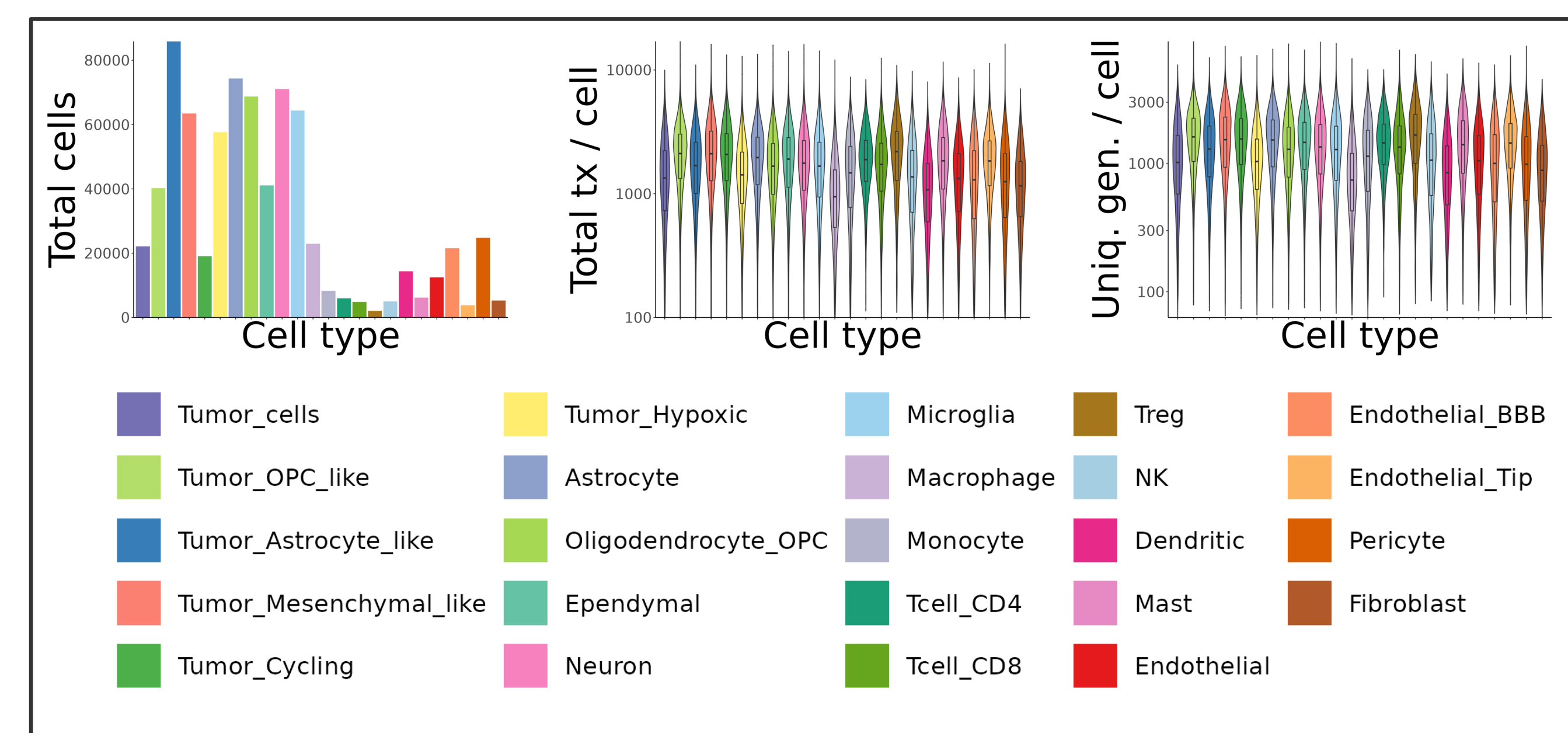


Fig 3. Quality control statistics indicate high quality RNA persistence after protein assay. After filtering low quality cells, there were 745,481 cells, with a median of 1683 transcripts per cell and 1284 unique genes per cell.

RNA and Protein Complement in Cell Type Identification

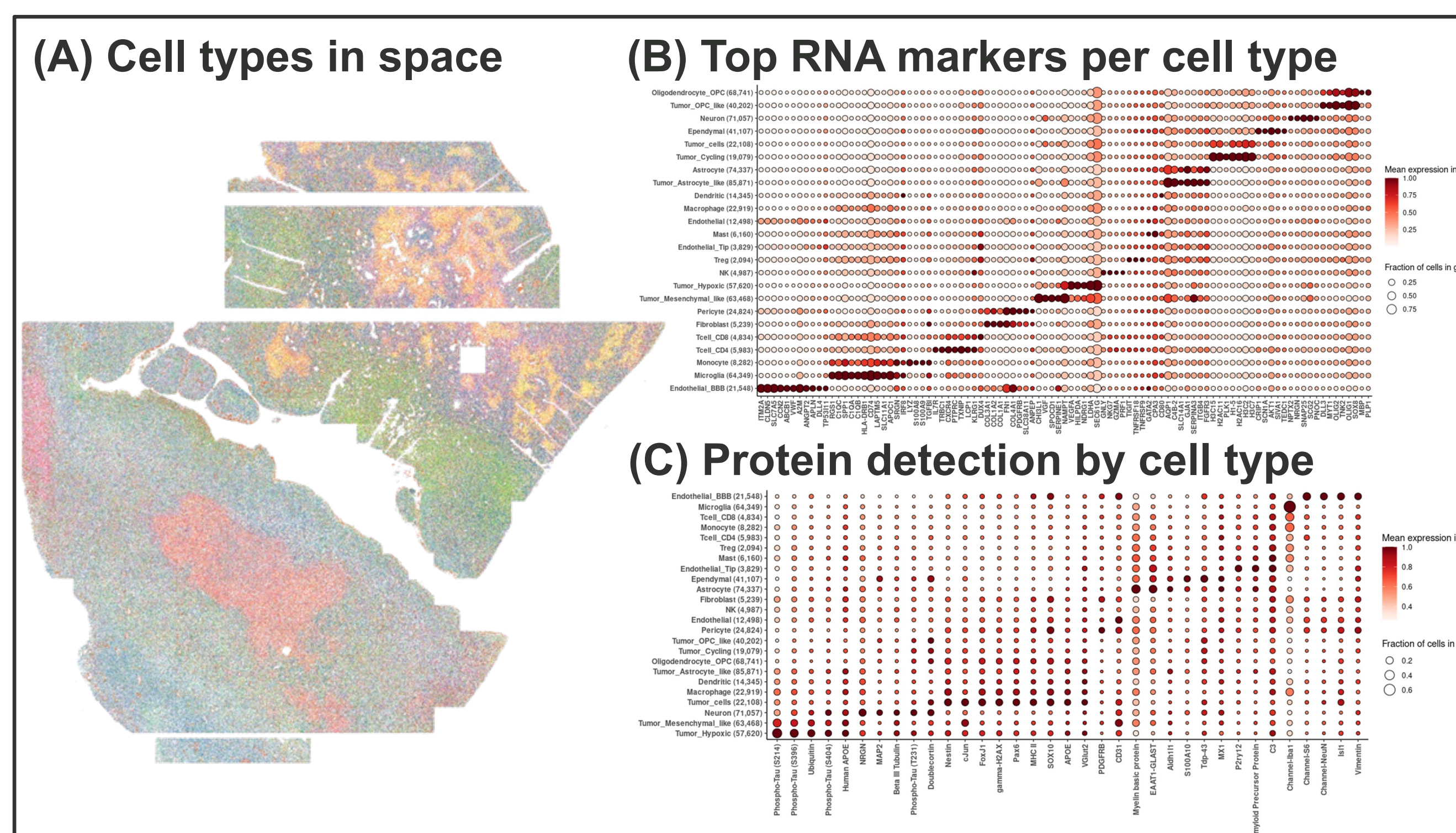


Fig 4. Cell type assignments derived from multiomic marker-based algorithm "Hieratype". Cell type color key in Fig 3.

Specific Phospho-Tau-Enriched Neighborhood Correlates with Elevated Hypoxia and EMT

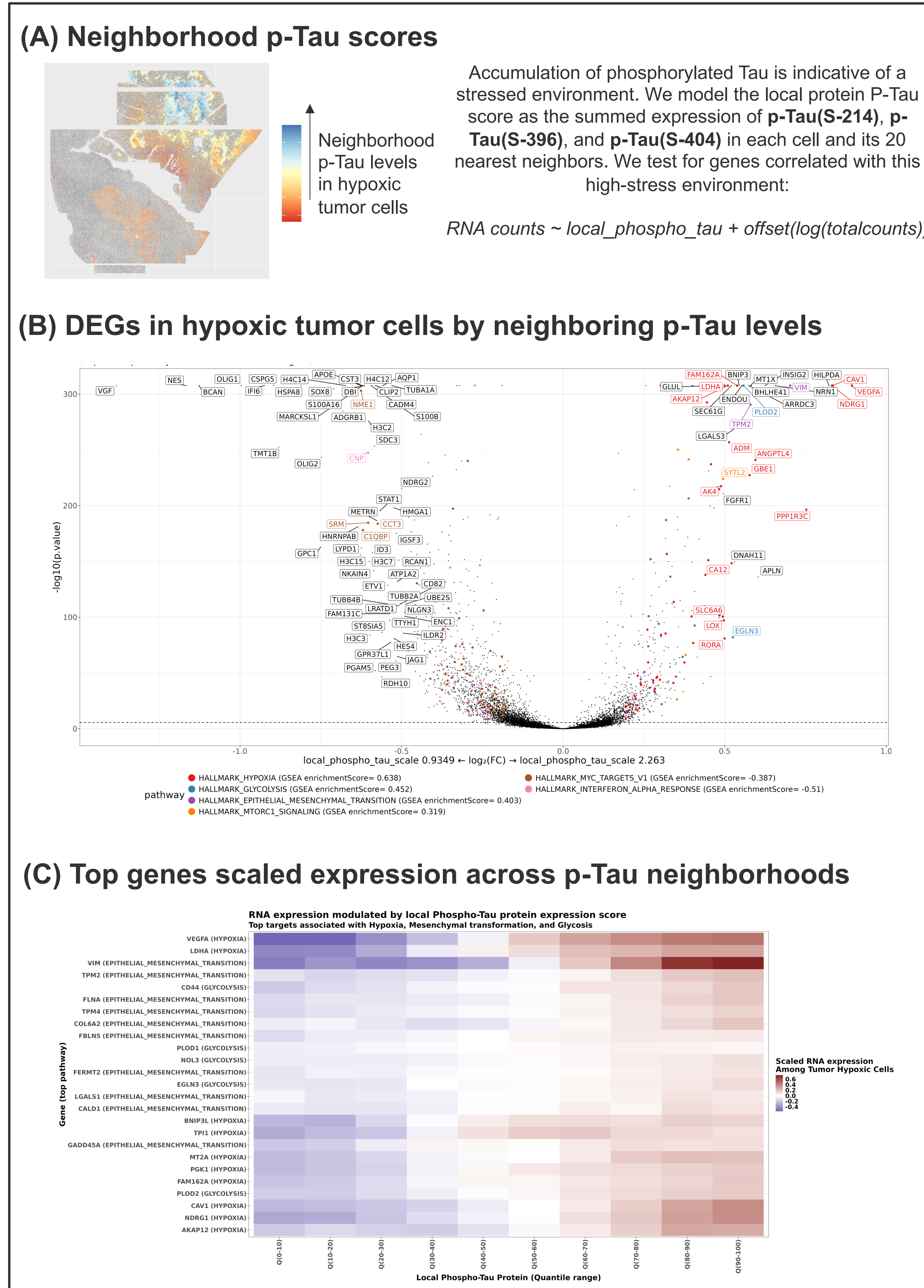


Fig 5. (A) Local protein-derived phospho-Tau score in hypoxic tumor cells. (B) Hundreds of genes were differentially expressed between high and low p-Tau regions. (C) Key genes showed a gradient of expression across p-Tau neighborhoods.

CosMx SMI Multiomics for Immune-Microenvironment Profiling

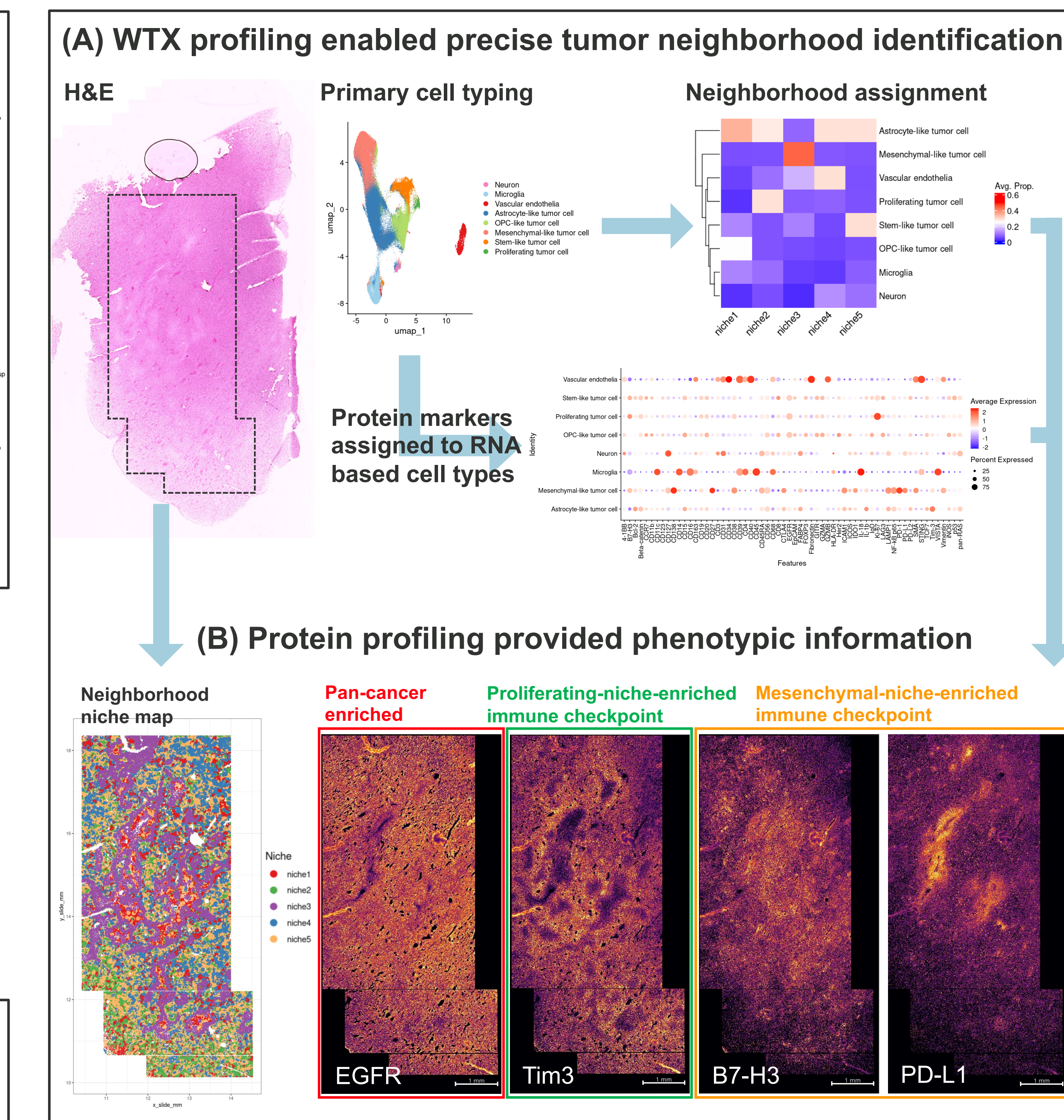


Fig 6. (A) WTX-based cell typing results provided a solid base to explore intratumor heterogeneity (e.g., spatial neighborhood driven by tumor cell composition), which can be integrated with protein expression profiles. (B) In this GBM sample, distinct immune checkpoint proteins were found to localize in different neighborhood niches, indicating region-specific immune evasion mechanisms.

Conclusions

- CosMx SMI enabled simultaneous profiling of over 18,000 transcripts and ~64 proteins from the same slide in a true multiomic approach.
- High RNA quality was maintained through the full multiomic processing pipeline.
- RNA and protein jointly contributed to granular cell typing of the glioblastoma sample.
- Tumor cells near elevated neighborhood p-Tau upregulated hypoxia, glycolysis, and EMT related genes.
- Tumor regions with different cell type compositions adopted distinct immune evasion strategies

CosMx SMI

