Novel spatial tagging of single nuclei spatializes single-cell ATAC-seq and V(D)J sequencing with gene expression for glioma cellular ecosystem profiling

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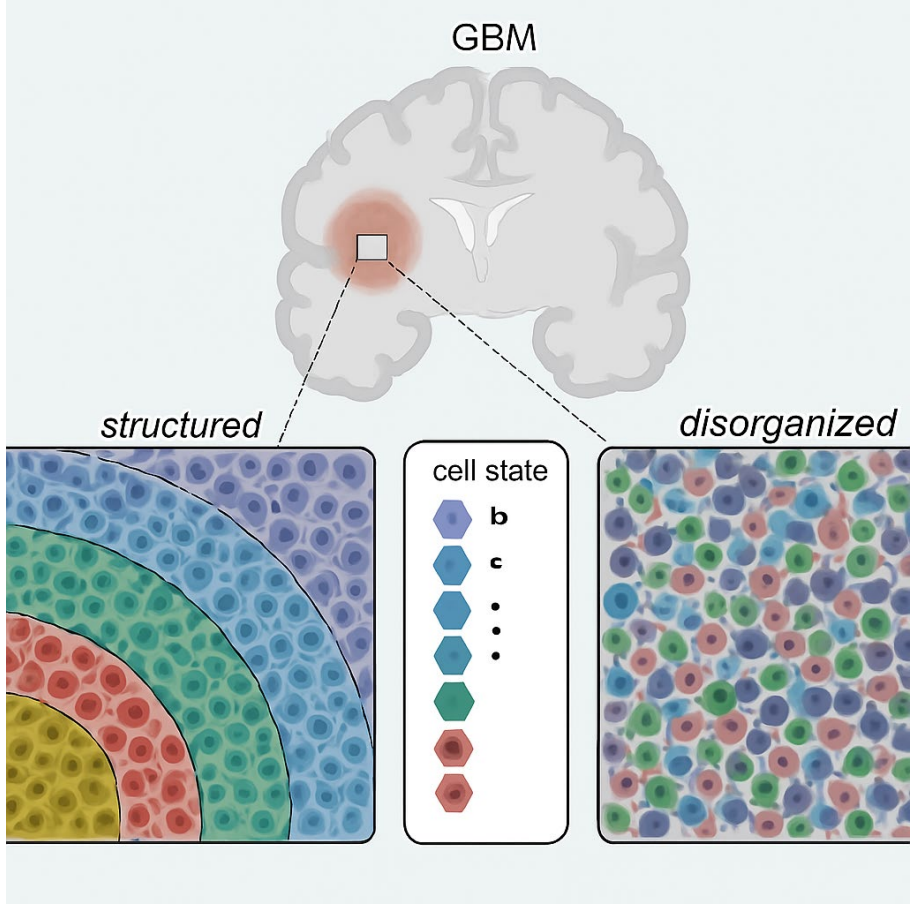
Glioblastoma (GBM) heterogeneity complicates therapeutic approaches



- Glioblastoma is currently an incurable disease, but spatial multiomic technology could help demystify spatial cell heterogeneity.
- Glioblastoma spatial architecture exhibits both disorganized arrangements and structured regions of malignant cells surrounded by diverse nonmalignant neighbors (Greenwald et al. 2024).

Delineating spatial organization elucidates cancer cell state coordination, leading to therapeutic opportunities.

Figure adapted from “Integrative spatial analysis reveals a multi-layered organization of glioblastoma.” (Greenwald et al. 2024, *Cell*) under a CC BY 4.0 license.

Adding a spatial dimension presents technical challenges

Key advances in glioblastoma research	Technology	Strength	Challenges
Defined inter/intra tumor malignant cell states (Tirosh et al. 2016; Neftel et al. 2019) and immune cell landscape (Mathewson et al. 2021)	scRNA-seq	High molecular complexity	Lack spatial delineation of tumor heterogeneity
Revealed regulatory programs that drive malignant states (Wang et al. 2024)	scRNA-seq + scATAC-seq	Multiomic capability	Lack spatial delineation of tumor heterogeneity
Identified large-scale spatial organization (Greenwald et al. 2024)	Conventional sequencing or probe-based spatial techniques	Spatial capability	• Limited resolution and missing cell types • Limited molecular complexity • No multiomic capability

E.g., cycling and immune cells

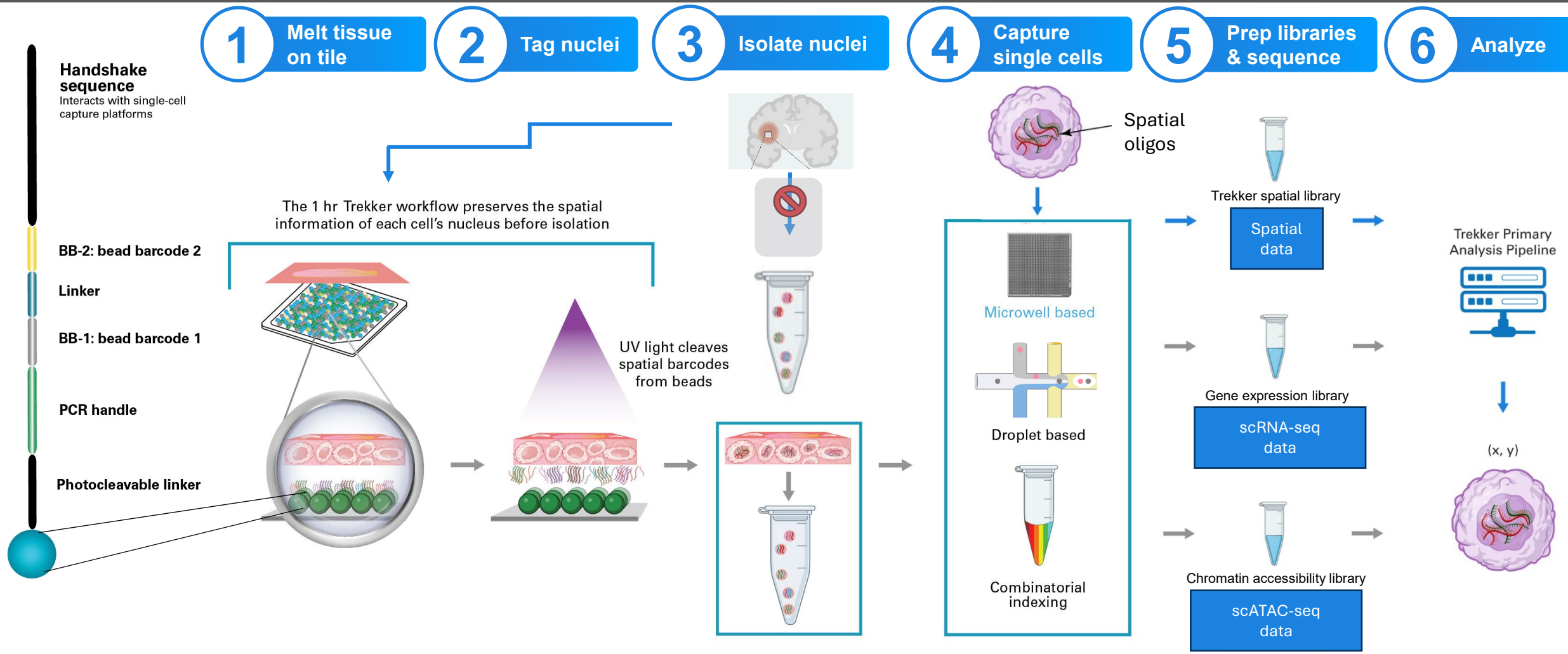
Greenwald et al. Integrative spatial analysis reveals a multi-layered organization of glioblastoma. *Cell* (2024).
Mathewson et al. Inhibitory CD161 receptor identified in glioma-infiltrating T cells by single-cell analysis. *Cell* (2021).
Neftel et al. An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. *Cell* (2019).
Tirosh et al. Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma. *Nature* (2016).
Wang et al. Single-cell multi-omics sequencing uncovers region-specific plasticity of glioblastoma for complementary therapeutic targeting. *Science Advances* (2024).

New technology solves these challenges

Technology	Challenges
scRNA-seq	Lack spatial delineation of tumor heterogeneity
scRNA-seq + scATAC-seq	Lack spatial delineation of tumor heterogeneity
Conventional sequencing or probe-based spatial techniques	• Limited resolution and missing cell types (e.g., **cycling**, immune) • Limited molecular complexity • No multiomic capability

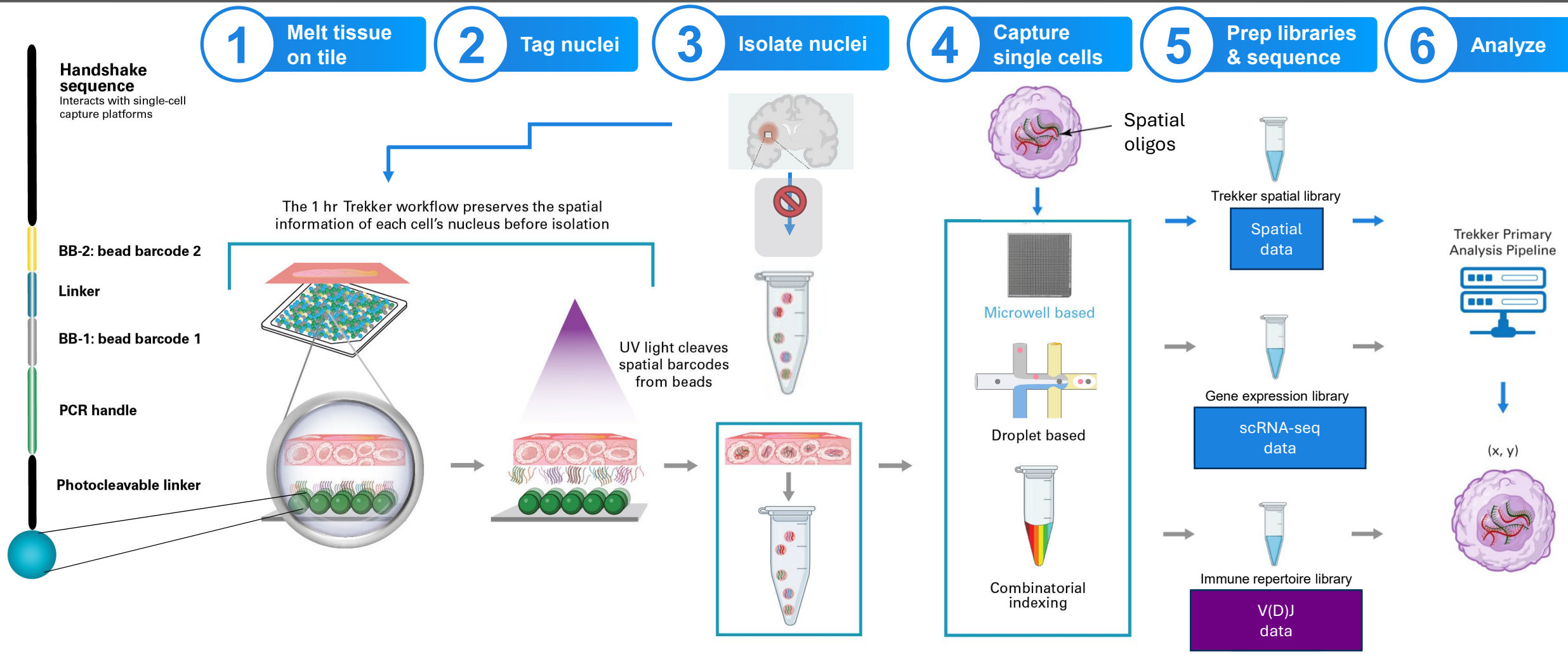
Technology	Strengths
Trekker™ technology (based on Slide-tags)	✓ True single-cell resolution ✓ High molecular complexity ✓ Multiomic capability ✓ Spatial capability

Glioblastoma spatial multiomic profiling using Trekker technology



Parts of this figure adapted from "Integrative spatial analysis reveals a multi-layered organization of glioblastoma." (Greenwald et al. 2024, *Cell*) under a CC BY 4.0 license.

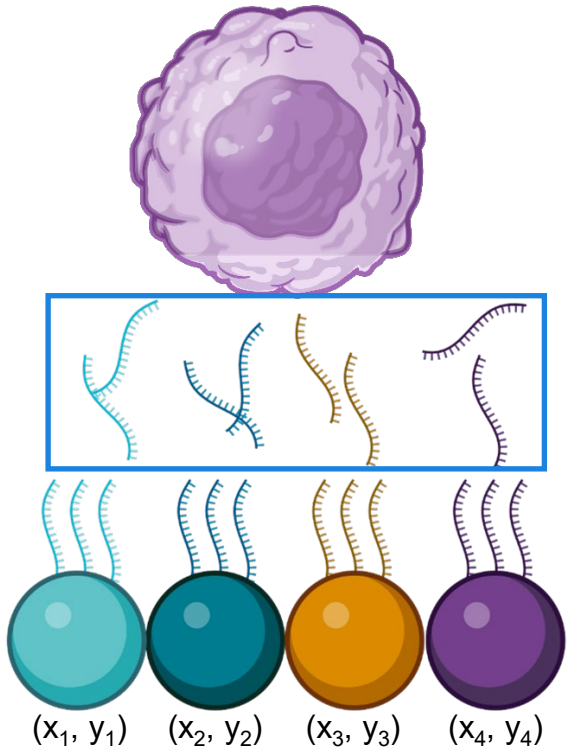
Glioblastoma spatial multiomic profiling using Trekker technology

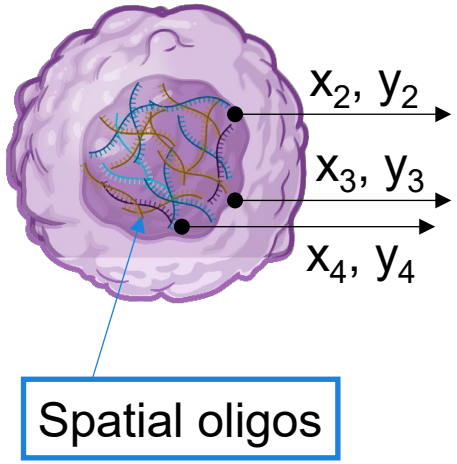


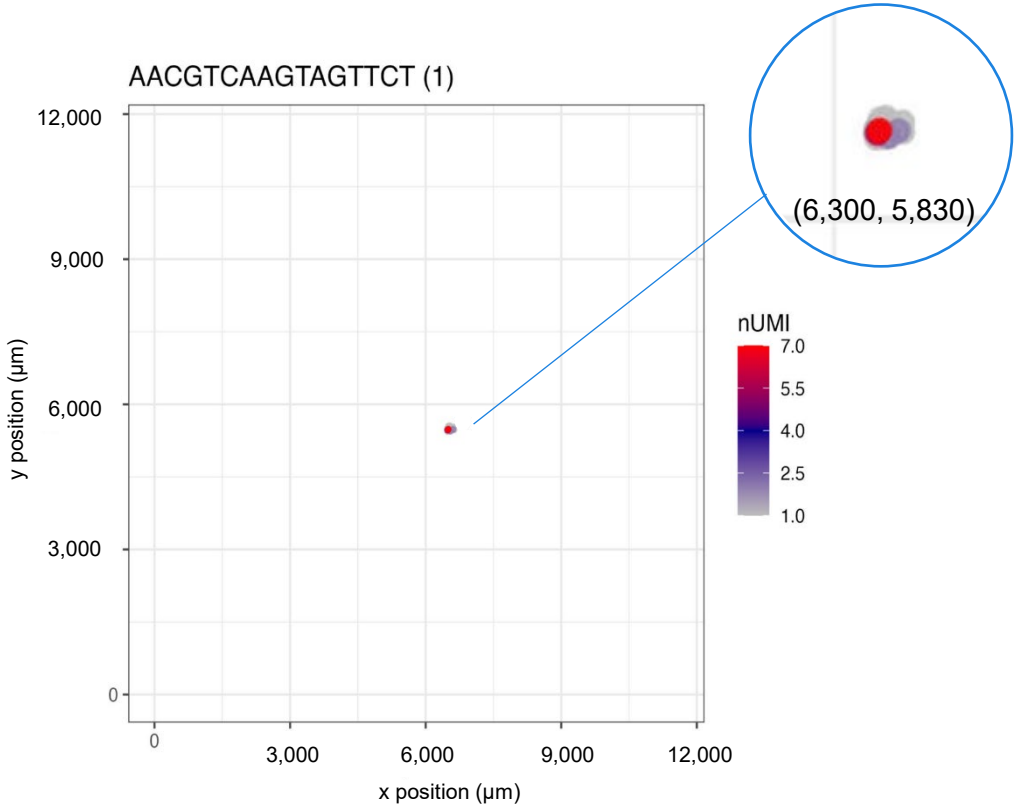
Parts of this figure adapted from "Integrative spatial analysis reveals a multi-layered organization of glioblastoma." (Greenwald et al. 2024, *Cell*) under a CC BY 4.0 license.

Spatial tagging enables spatial positioning of single cells

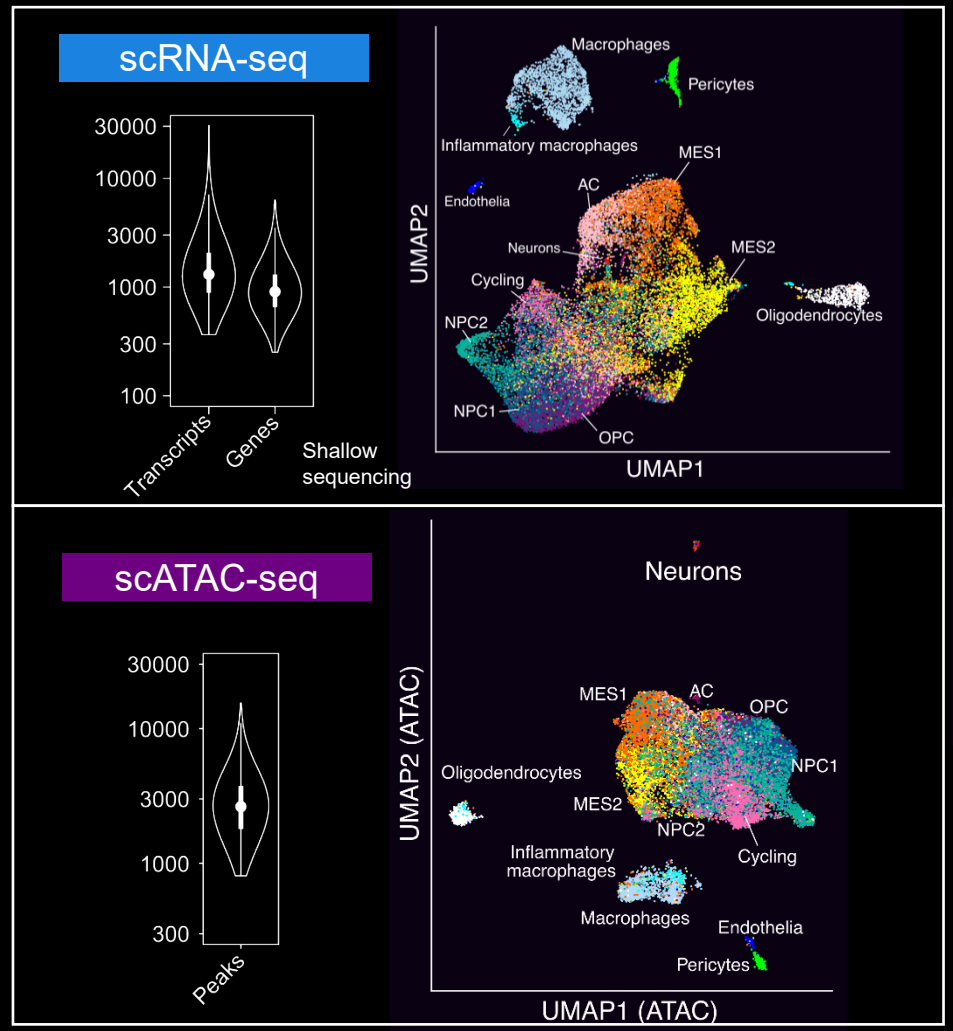
1. Oligos associated with an exact spatial location [(x, y)] diffuse into nearby nuclei
2. Analysis pipeline extracts sequences of all spatial oligos that tagged a nucleus
3. Pipeline constructs a spatial plot and identifies hot spot
4. Spatial coordinate of a cell is calculated as UMI-weighted centroid of all spatial oligos' coordinates





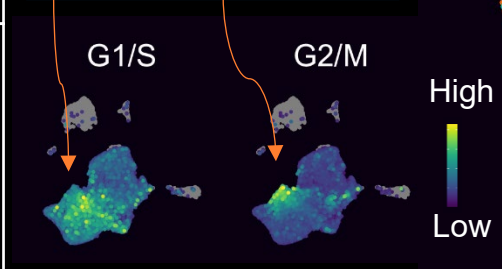


Trekker technology enables spatial positioning of high-quality single-cell RNA-seq and ATAC-seq data

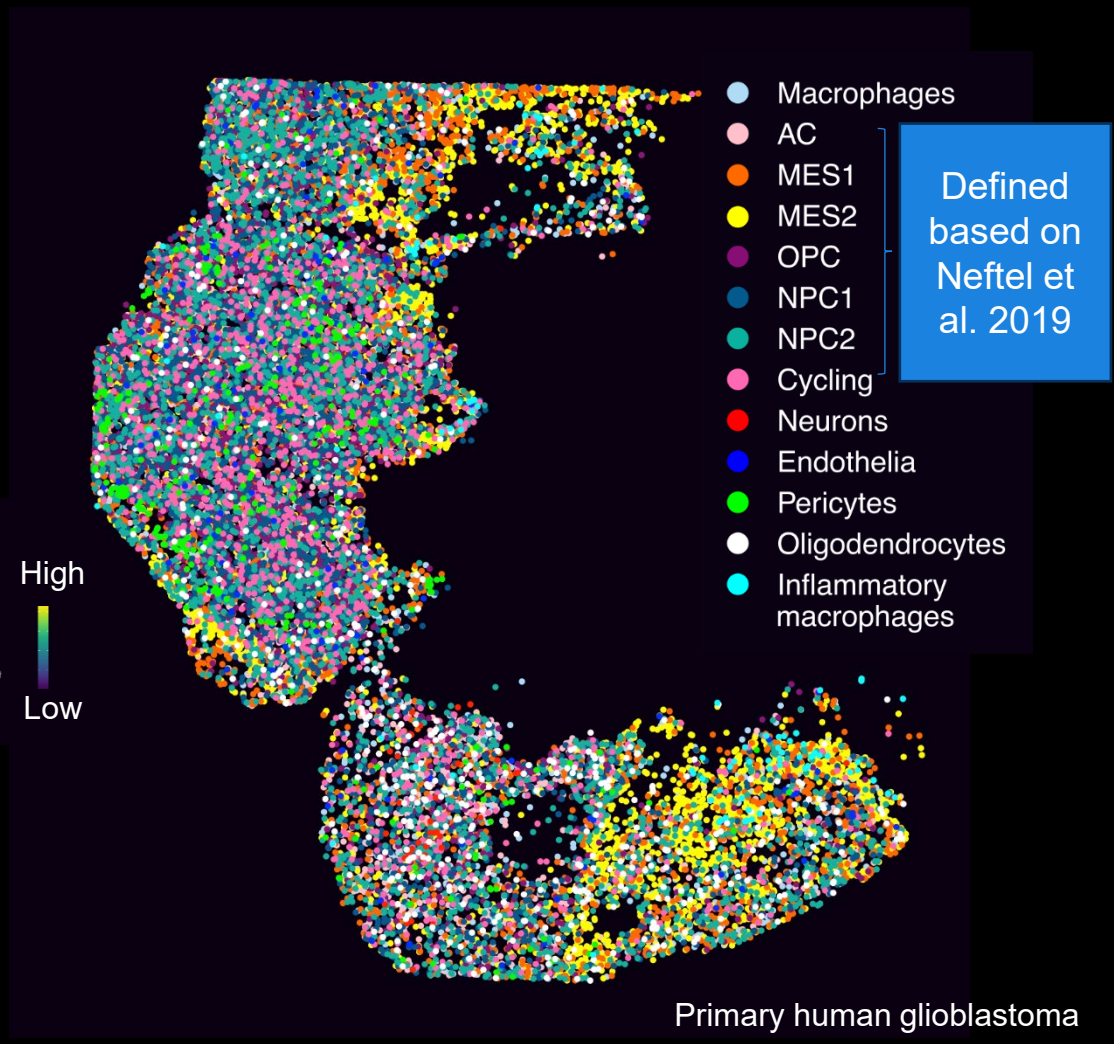


Six tumor subtypes are more resolved in RNA-seq data than in ATAC-seq data

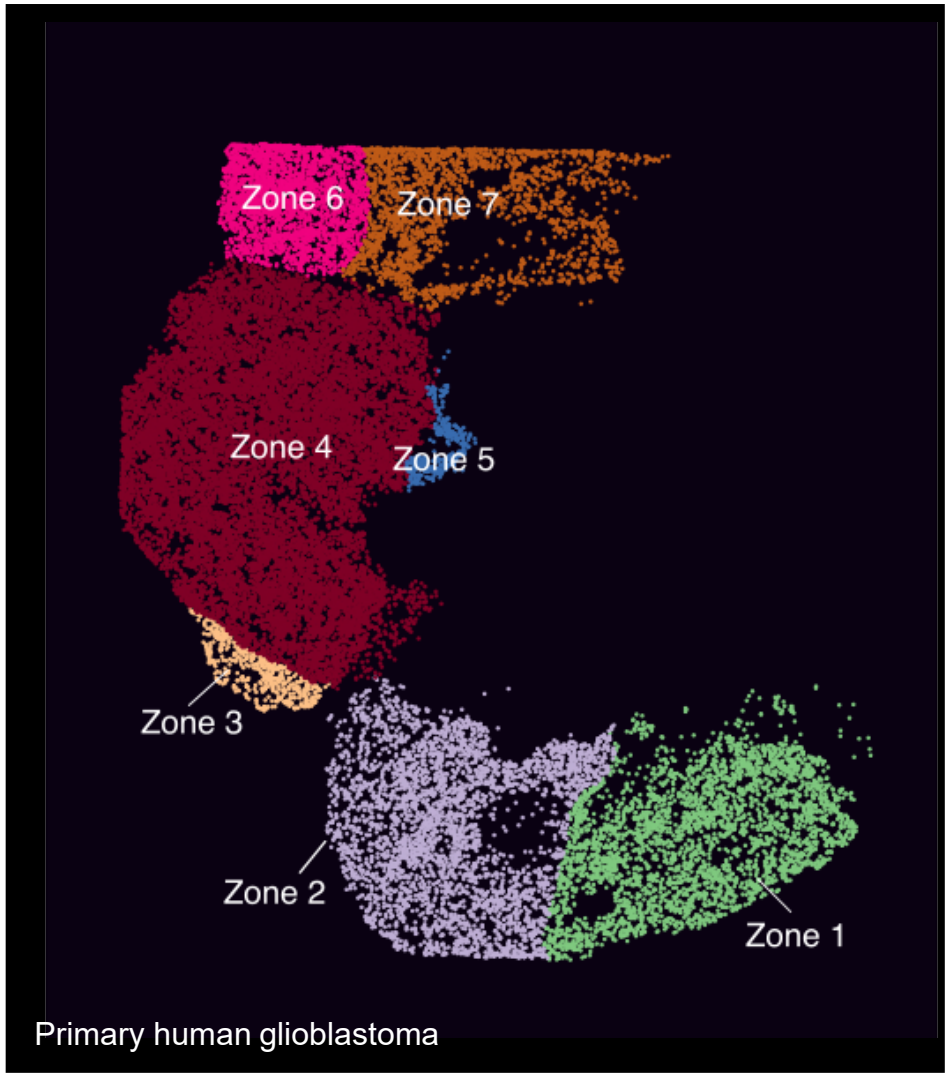
Cycling tumor cells are clearly distinguishable by phases

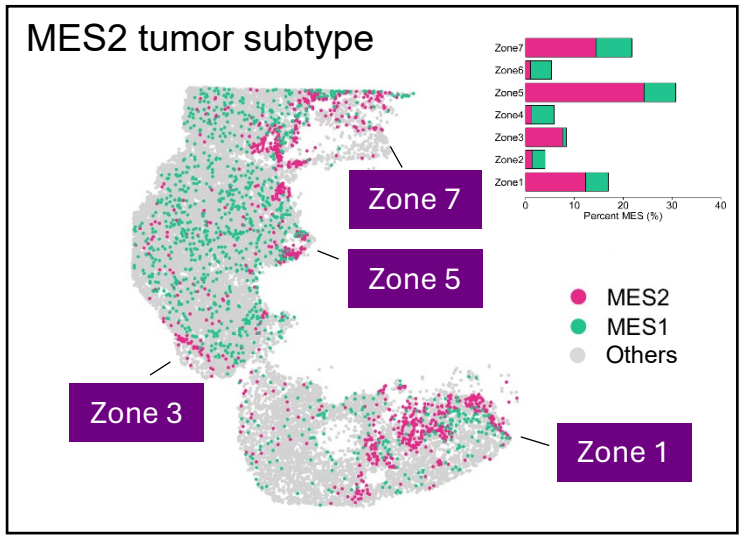


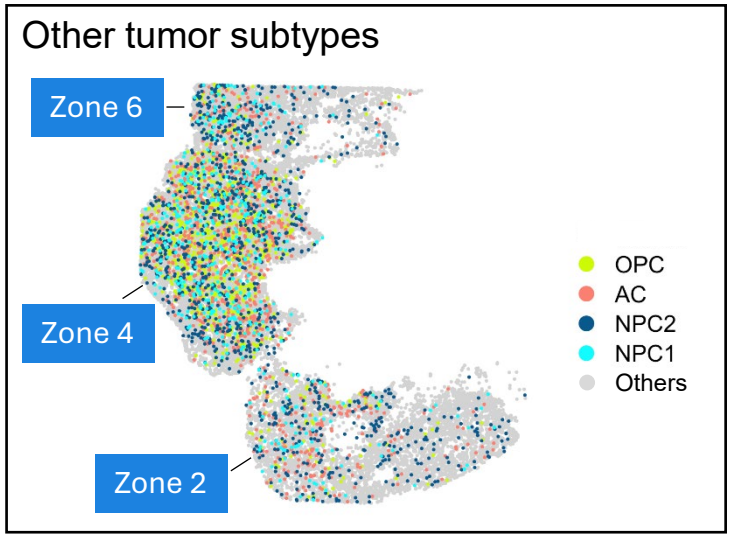
Neurons are more distinguishable in ATAC-seq data

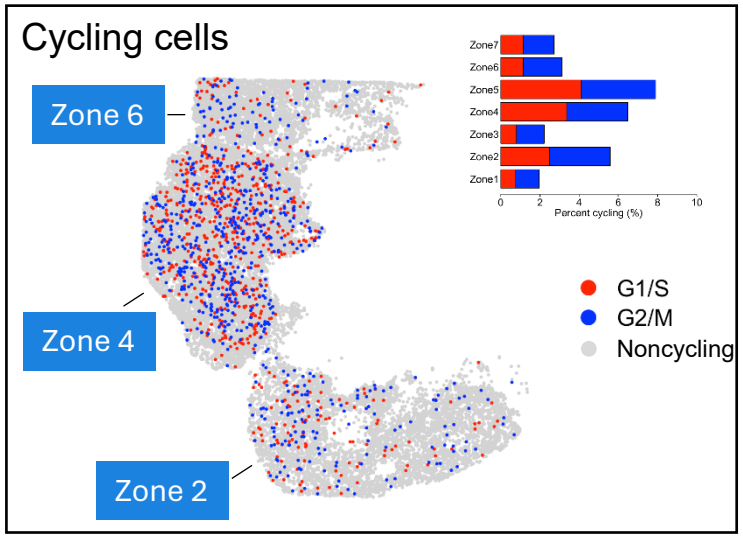


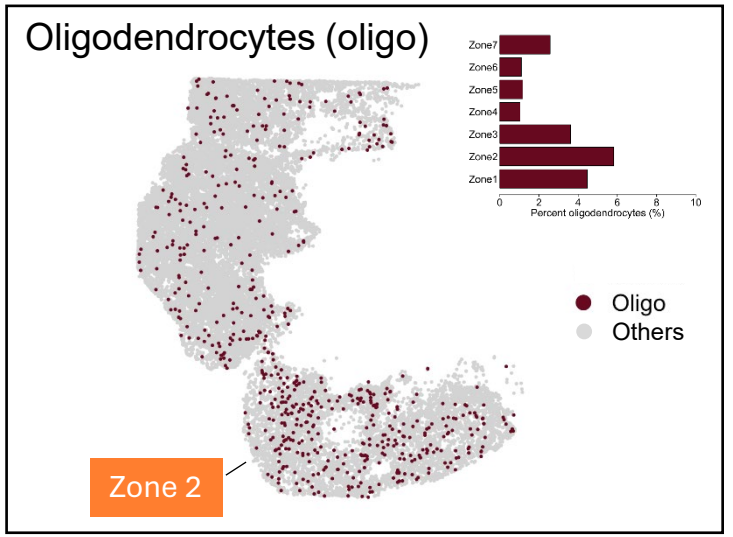
Seven distinct GBM tumor zones were identified from the spatial distribution of tumor subtypes and cycling cells



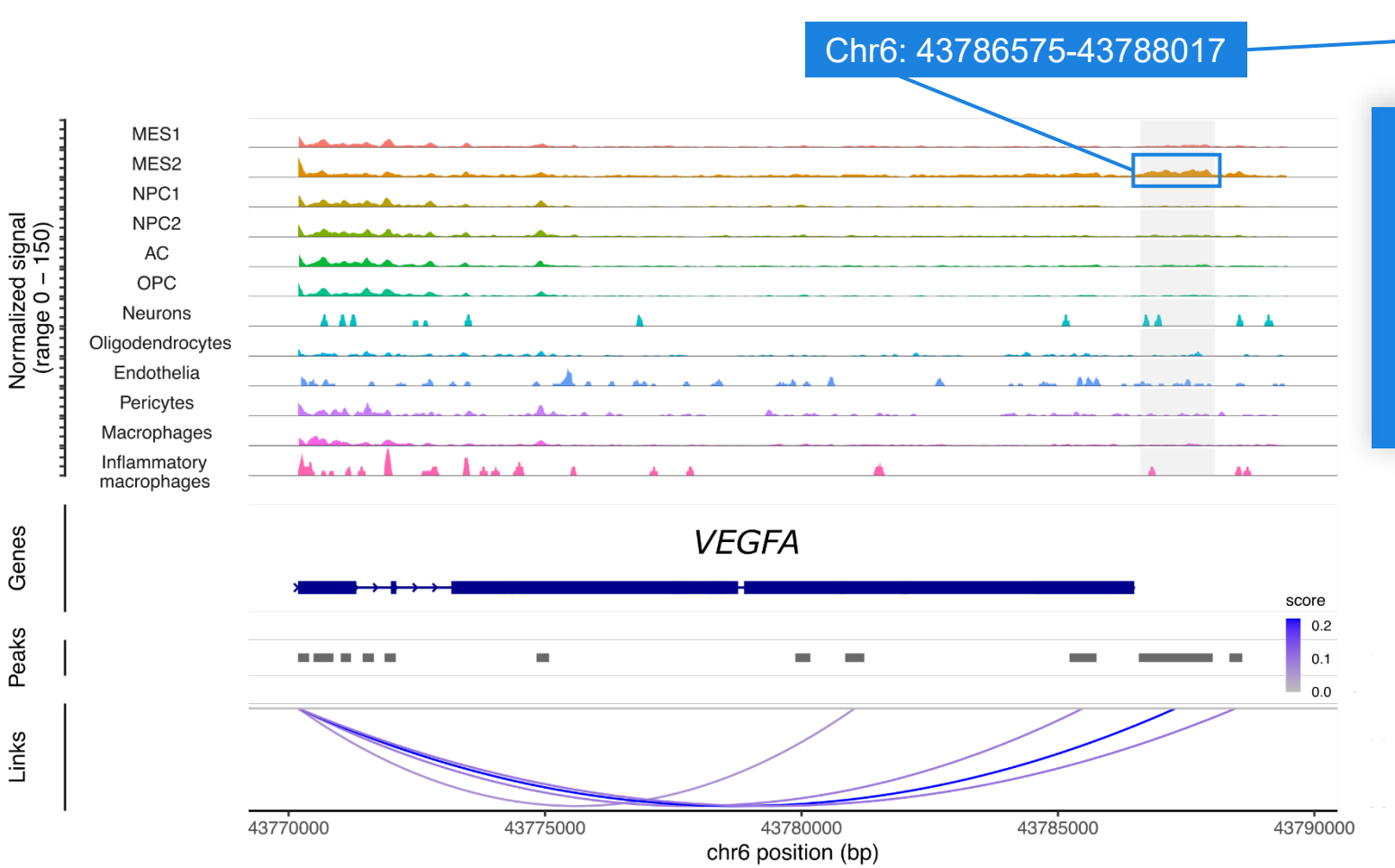




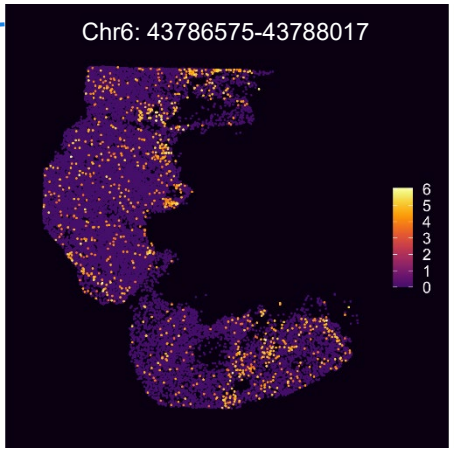


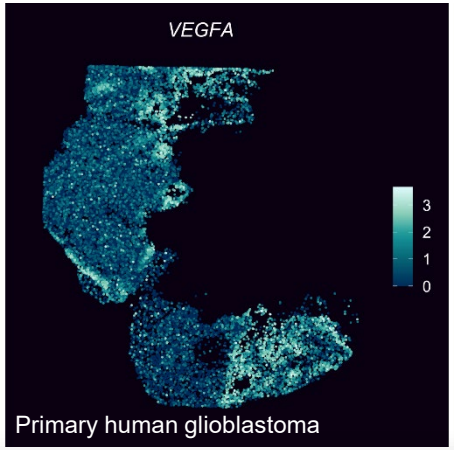


Multiomic analysis connects differential chromatin accessible regions and genes for spatial cell types



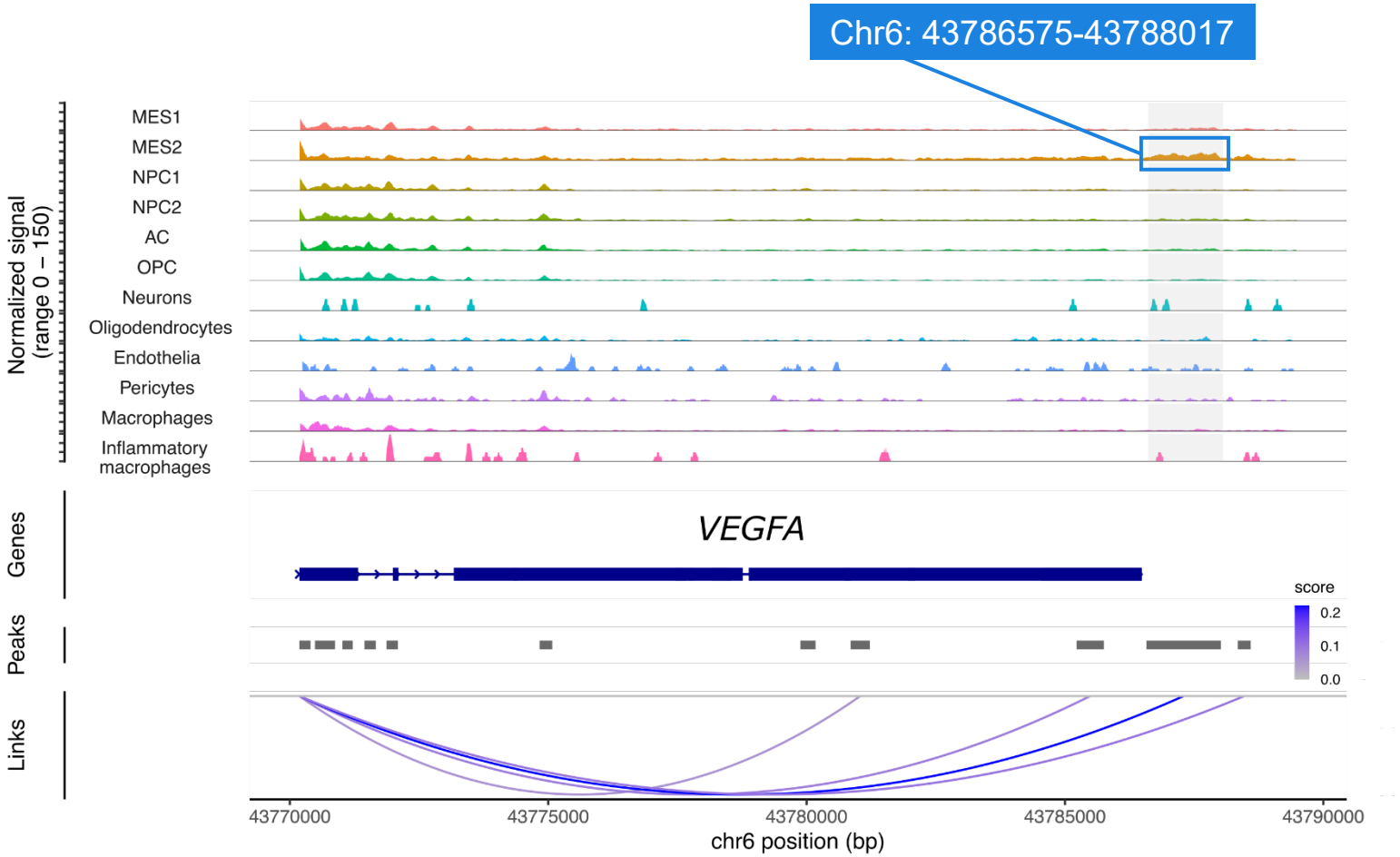
Top-defining MES2 chromatin accessible peak resides downstream but outside of the *VEGFA* annotated gene body





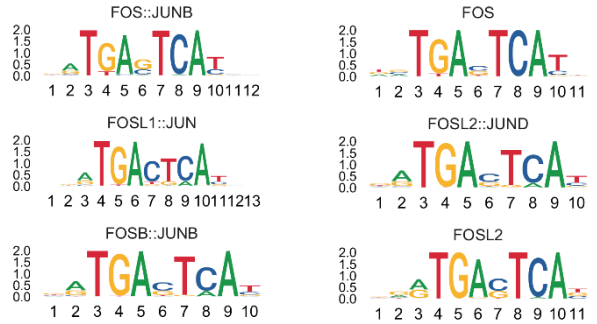
Chromatin accessibility map aligns closely with *VEGFA* expression map

Multiomic analysis connects differential chromatin-accessible regions and genes for spatial cell types

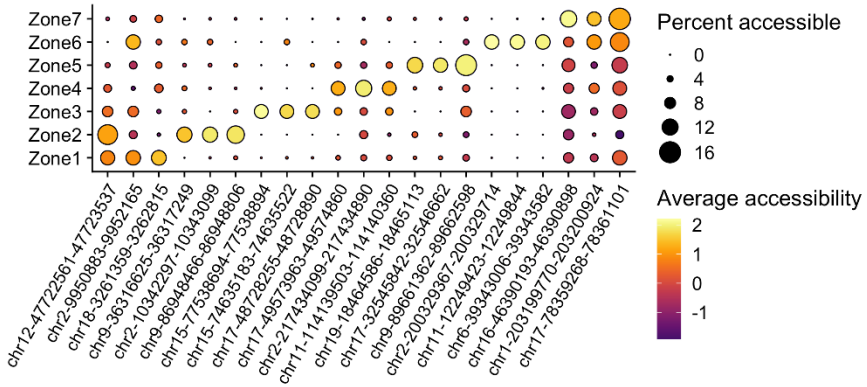


Further discoveries from ATAC-seq and spatial data

Enriched motifs in differential ATAC peaks in MES2 subtype

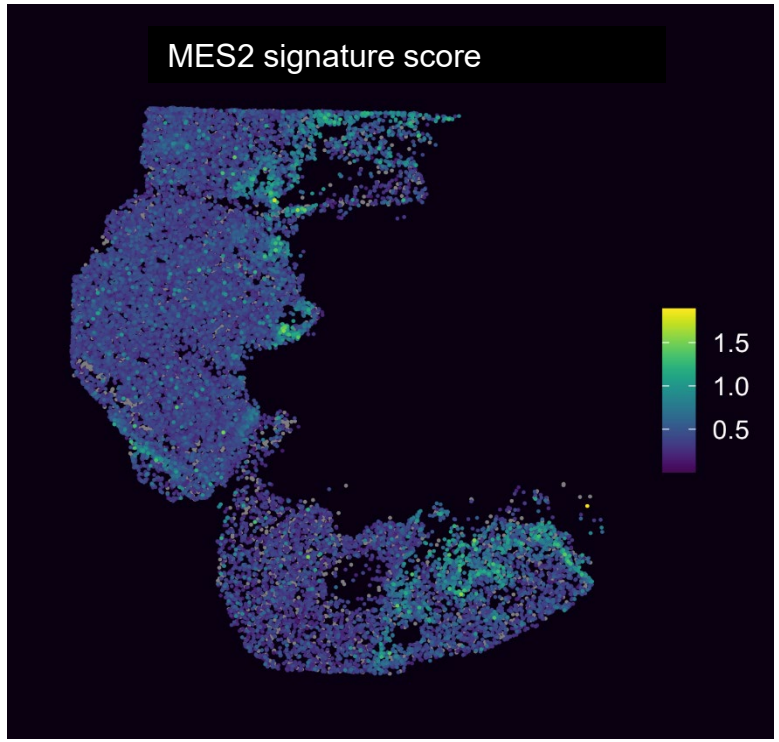


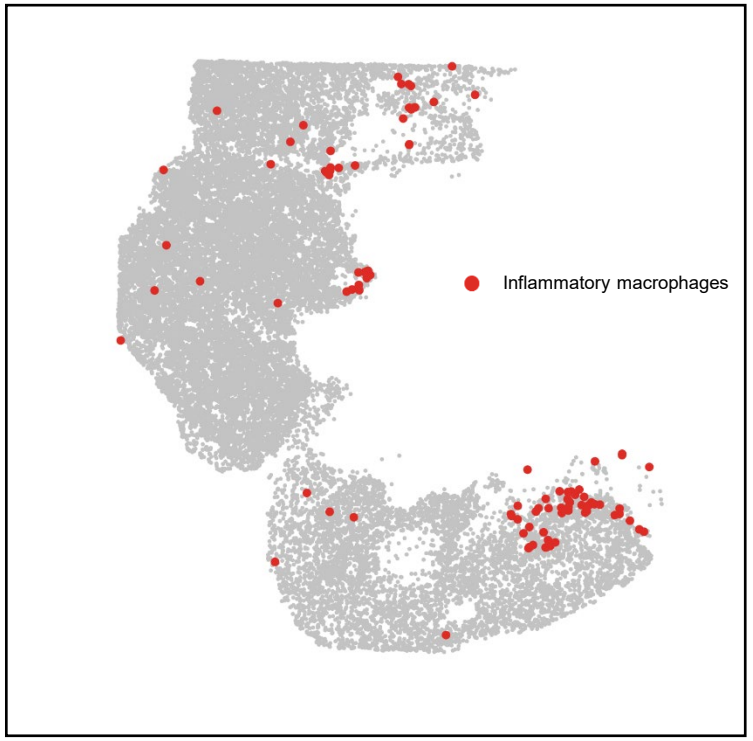
Spatially variable ATAC peaks in MES2 subtype

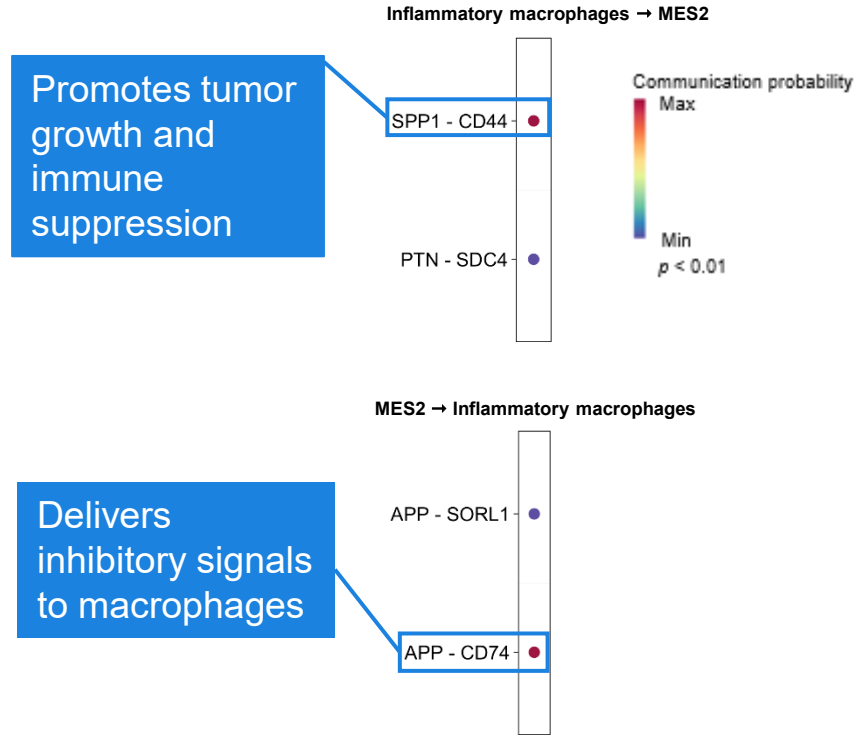


Spatially colocalized MES2 tumor cells and inflammatory macrophages exhibit immune-suppressive and tumor-promotive communications

Spatially aware ligand-receptor analysis enhances confidence in correct pair identification



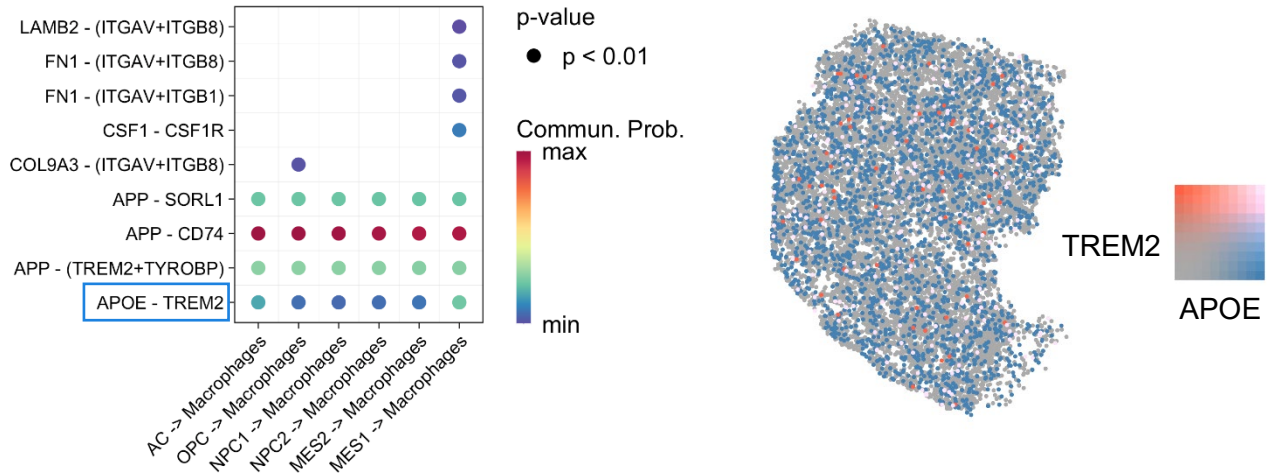




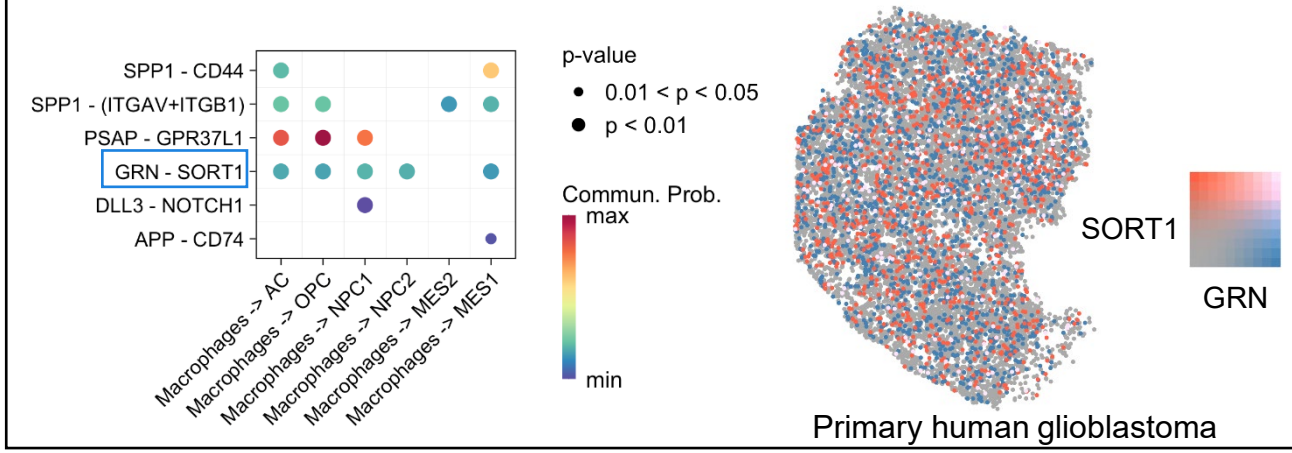
Identified ligand-receptor pairs could serve as candidates for therapeutic studies

Tumor, macrophages, and vasculature in Zone 4 exhibit immune-suppressive and angiogenic communication

Tumor (APOE) → macrophages (TREM2) communication



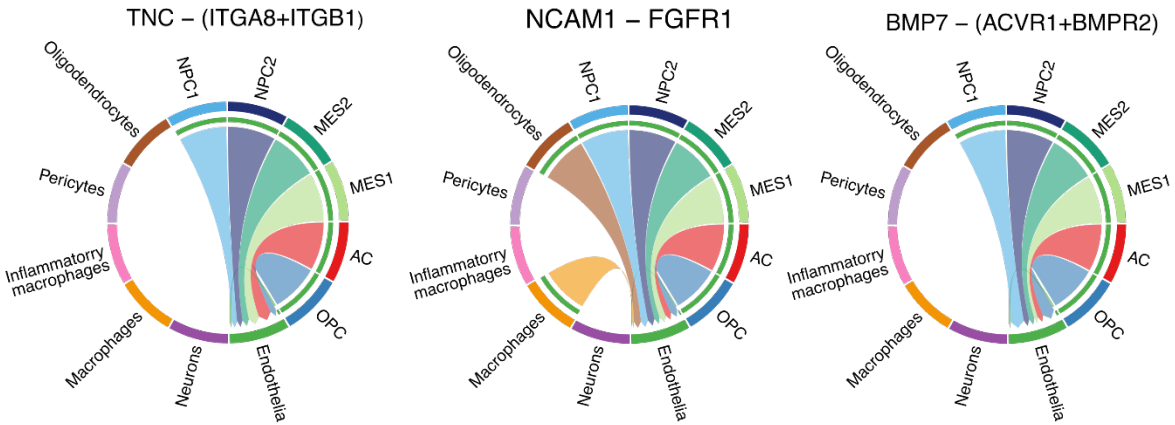
Macrophages (GRN) → tumor (SORT1) communication



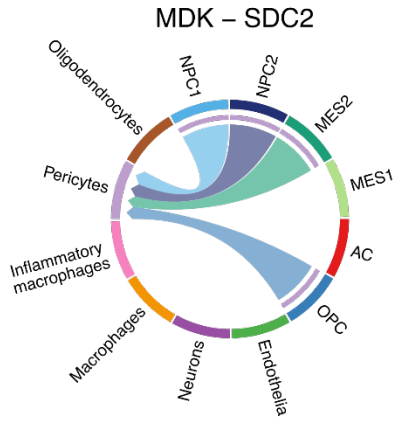
Primary human glioblastoma

Tumor → vasculature communication

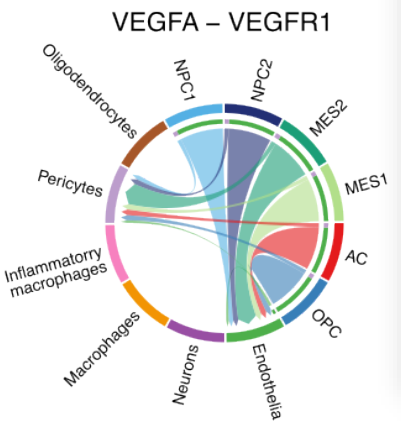
Endothelia



Pericytes



Both



Zone 4 is disorganized, has diverse tumor subtypes, and shows increased cell cycling & prominent vasculature

Multiomic spatial analysis reveals potential biomarkers for further GBM studies

Important insights enabled by Trekker technology

- **Mapped cycling tumor cells** that are undetectable with lower-resolution spatial technologies
- Discovered distinct spatial zones within glioblastoma
- Linked gene expression and chromatin accessibility within a spatial context
- Identified spatial marker genes, accessible chromatin regions, and cell communication across distinct tumor zones

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