

# Integrated spatial multiomics profiling with 1,200+ Discovery Proteome Atlas (DPA) and GeoMx® Whole Transcriptome Atlas (WTA) maps key tumor programs in Colorectal Cancer

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## Abstract

Colorectal cancer (CRC) exhibits spatially organized tumor and immune programs that are not captured by bulk profiling. To resolve this complexity, we applied integrated spatial multiomics using the GeoMx Whole Transcriptome Atlas (WTA) and Discovery Proteome Atlas (DPA) across one five micron fresh frozen section of CRC patient D107211. Tumor and matched normal regions were segmented into epithelial and microenvironment compartments and profiled for over 18,000 transcripts and 1,200 protein targets including 130+ post-translational modifications.

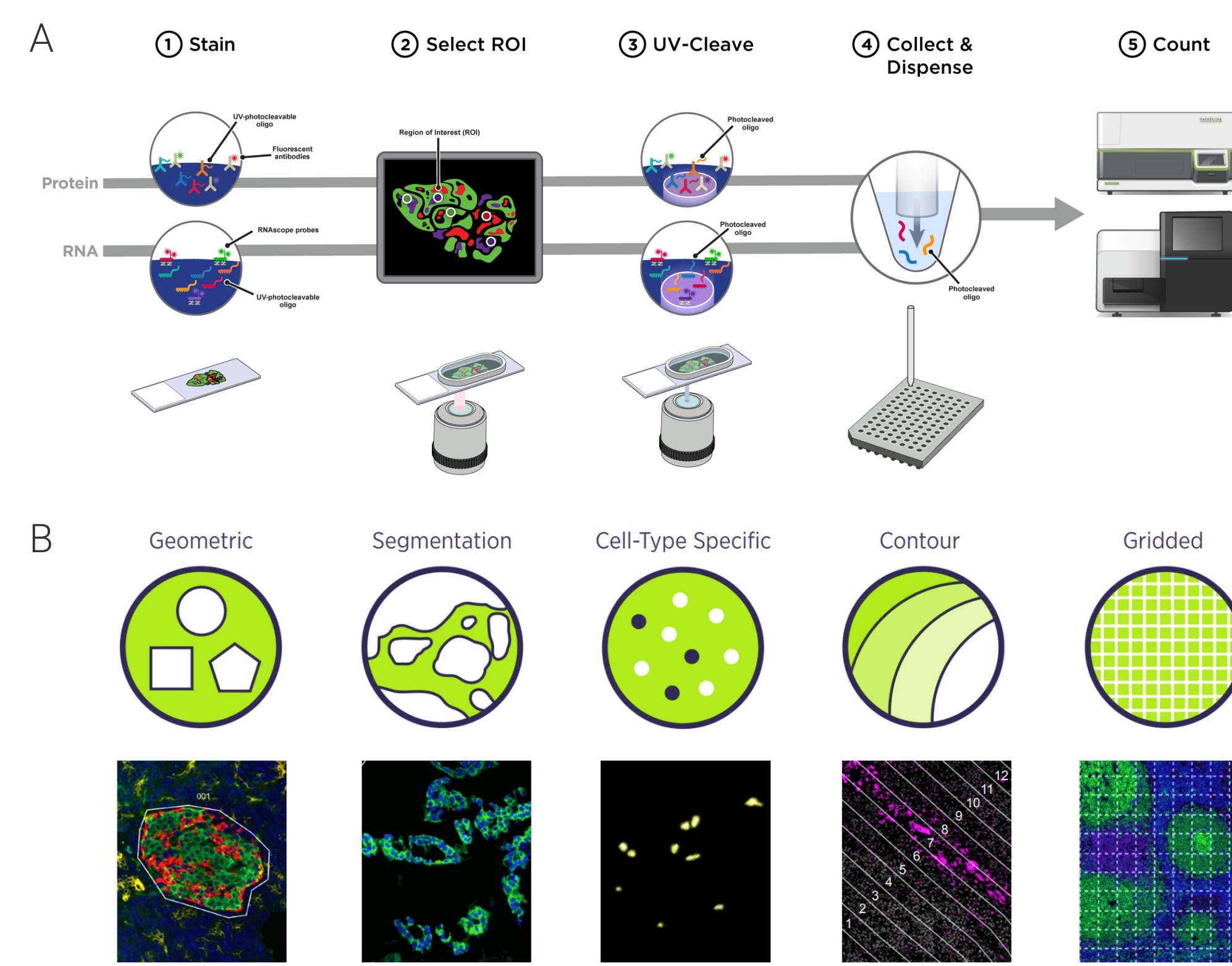
Spatial integration of RNA and protein data revealed a coordinated proliferative and metabolically active tumor phenotype. Tumor regions were enriched for MYC and E2F targets, G2/M checkpoint, OXPBOS, and MTORC1 signaling, supported by elevated protein markers of proliferation and replication stress, including Ki67, MCM2 pS41, and Histone H3 modifications. Metabolic reprogramming identified at the transcript level, including glycolysis and lipid metabolism, was validated by increased GLUT1, CA9, and glutaminase proteins. Oncogenic signaling pathways, including KRAS and PI3K-AKT-mTOR were activated.

Together, these results highlight the power of GeoMx-enabled spatial multiomics to integrate transcriptomic and proteomic measurements within the same tissue context, revealing coordinated tumor programs that cannot be resolved by single-modality approaches.

## GeoMx Digital Spatial Profiler (DSP) targets the cells that matter

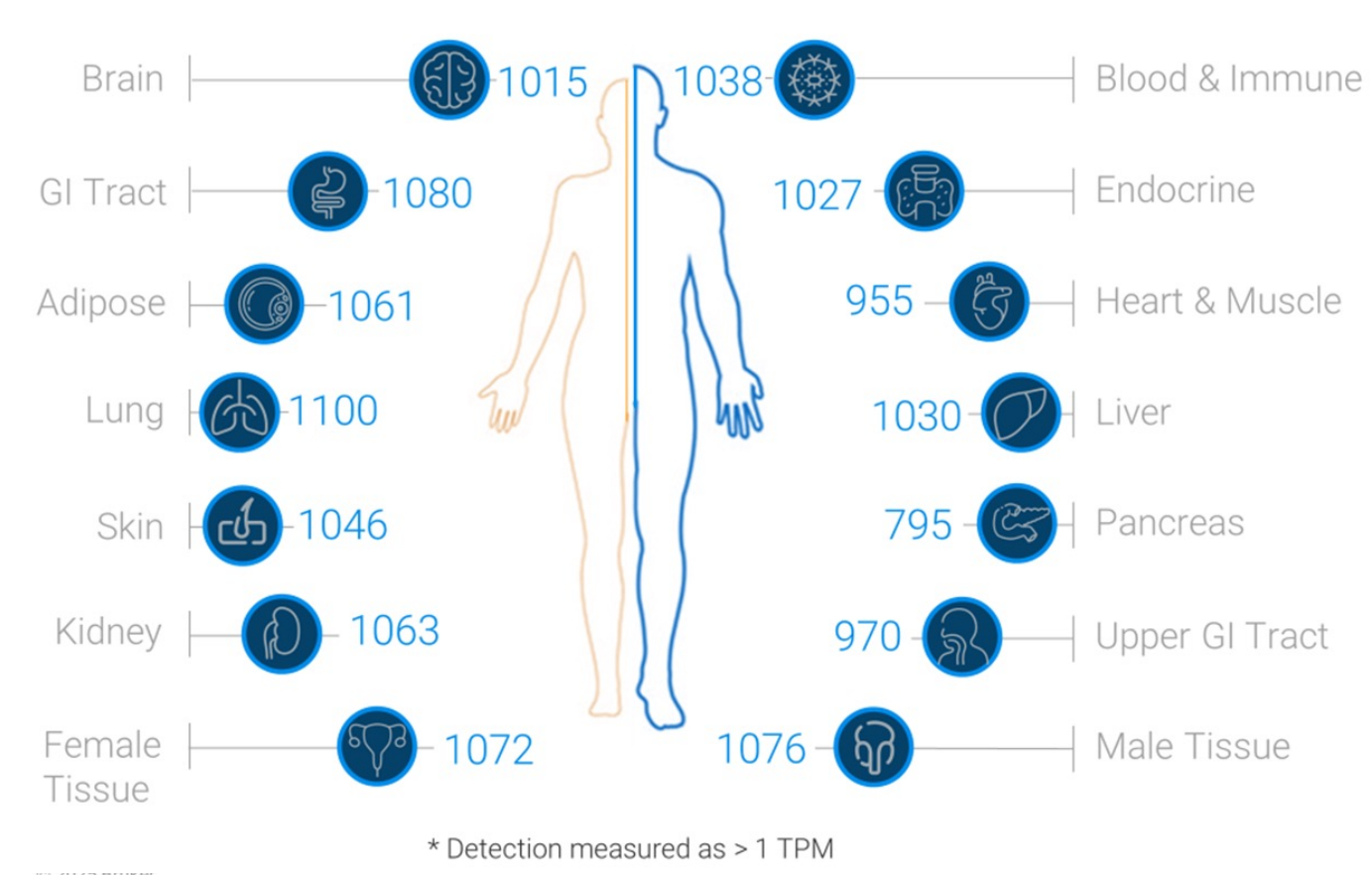


**Figure 1. GeoMx Digital Spatial Profiler (DSP) workflow and region-of-interest (ROI) strategies.** (A) GeoMx workflow includes slide prep and hybridization of high plex reagents, ROI selection guided by fluorescent markers, automated UV cleavage and collection of oligo tags, followed by library prep and sequencing. (B) User-defined ROI selection profiles cells based on tissue morphology and experimental question.



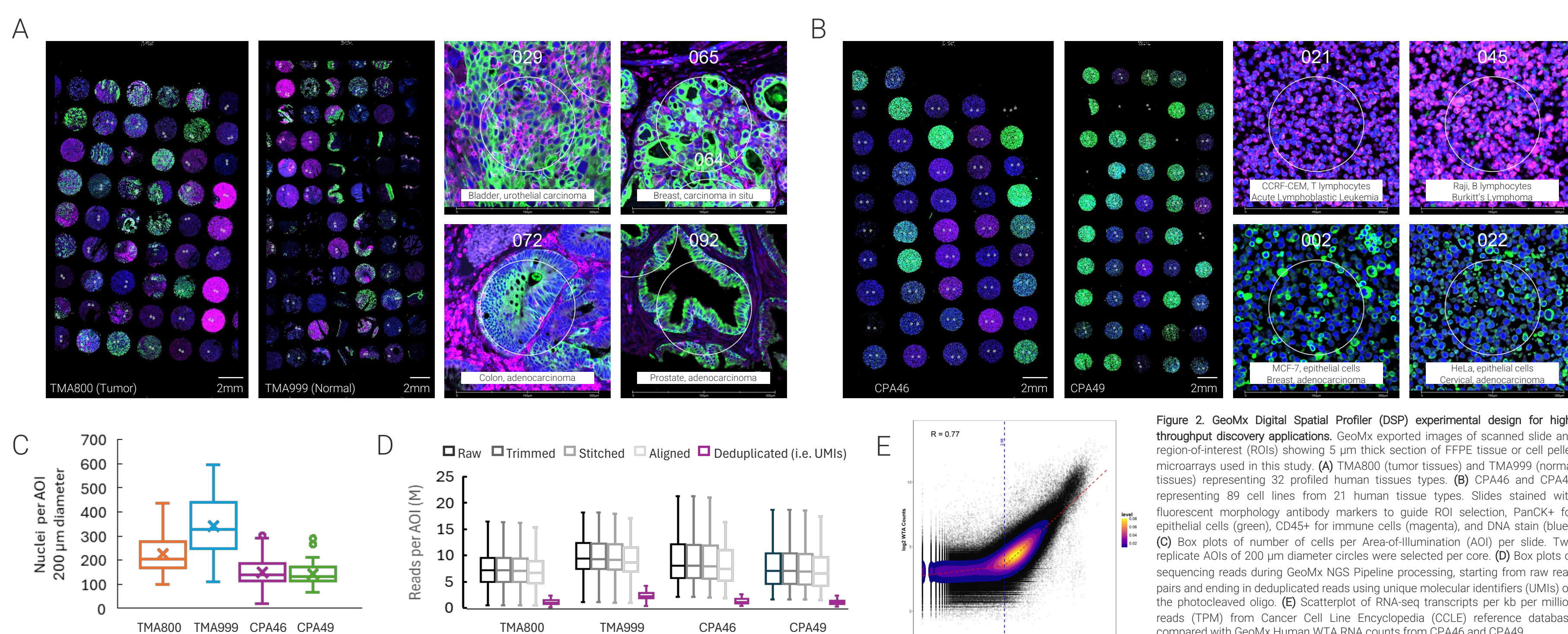
## Broad protein coverage across tissues and biology with Discovery Proteome Atlas (DPA)

Protein coverage by system, avg targets detected\*



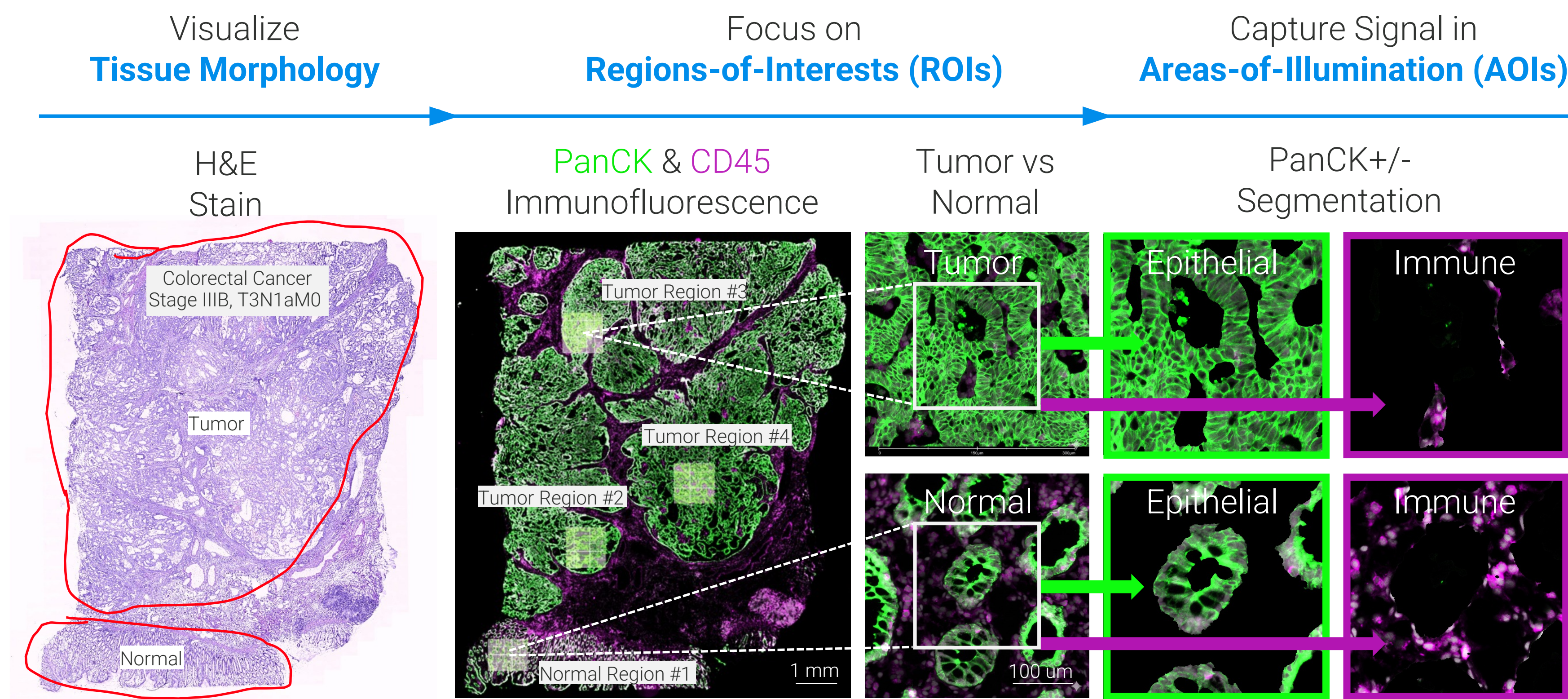
- 1,200+ protein targets
- 130+ post-translational modifications
- >99% of protein expressed in over 40 tissues
- Data-driven and curated content for Oncology, Immunology, and Neuroscience
- 77 curated pathways in Oncology and Immunology
- 79 curated pathways in Neuropathology & Neuroinflammation
- Multiomic workflows adds 18,000+ RNAs for measuring comprehensive functional biology *in situ*

## Designed for high-throughput multiomic discovery



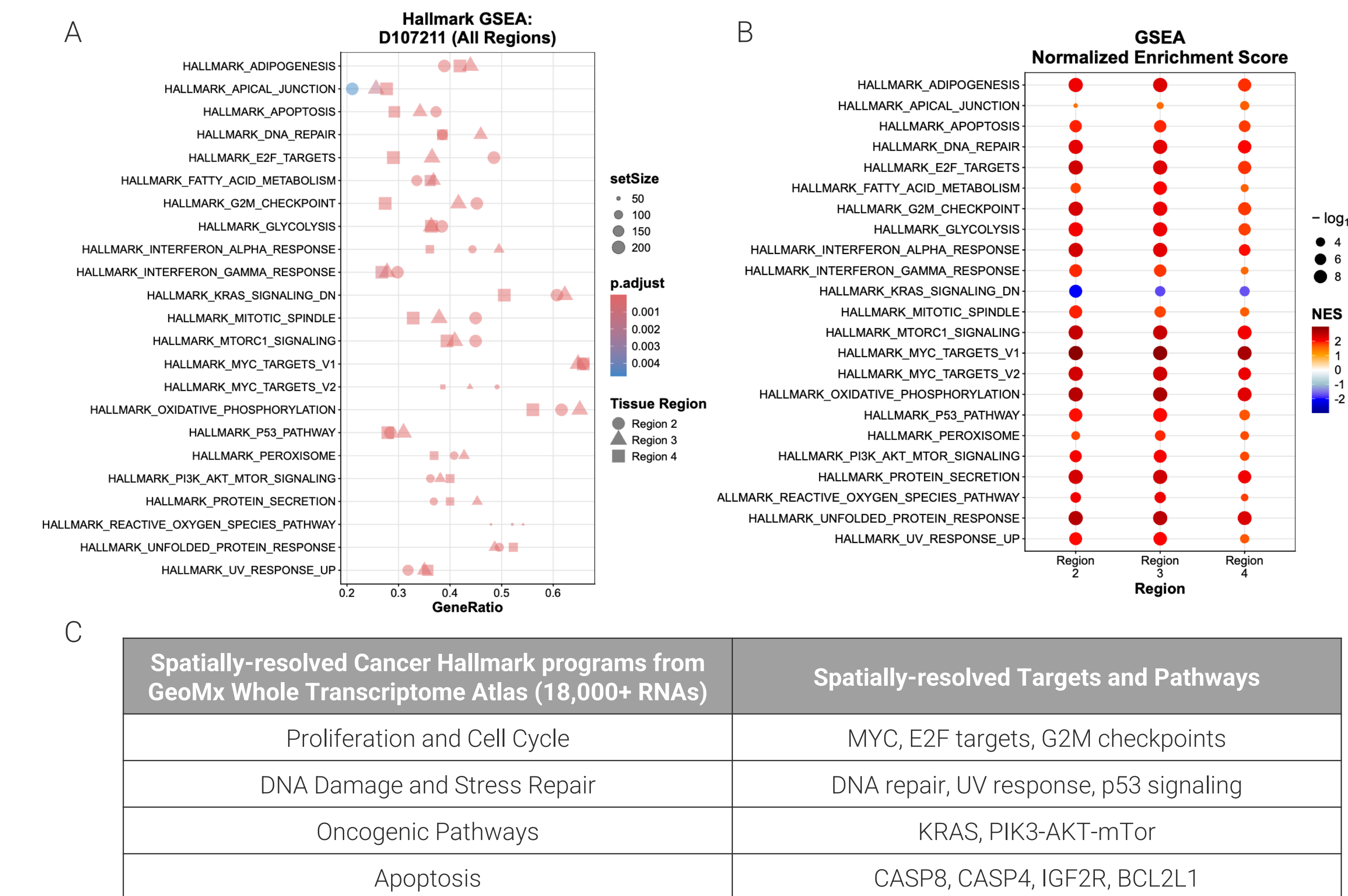
**Figure 2. GeoMx Digital Spatial Profiler (DSP) experimental design for high-throughput discovery applications.** GeMx-enabled images of a frozen slide and region-of-interest (ROI) showing 5 µm thick section of FFPE tissue or cell pellet microarray used in this study. (A) TMA800 (tumor tissues) and TMA999 (normal tissues) representing 32 profiled human tissues types. (B) CFA46 and CFA49 representing 80 cell lines from 21 human tissue types. Slides stained with fluorescent morphology antibody markers to guide ROI selection. PanCKs for epithelial (red) and CD45 for immune cells (magenta), and DAPI stain (blue). (C) Box plots of number of cells per Area-of-Illumination (AOI) per slide. Two replicate AOIs of 200 µm diameter circles were analyzed per slide. (D) Box plots of sequencing reads during GeoMx NGS Pipeline processing, starting from raw read pairs and ending in deconvoluted reads using unique molecular identifiers (UMIs) on the photolabile stage. (E) Scatterplot of RNA-seq transcripts per kb per million reads (TPM) from Cancer Cell Line Encyclopedia (CCLE) reference database compared with GeoMx human WTA bulk counts from CFA46 and CFA49.

## Spatial profiling guided by tissue morphology and cell type



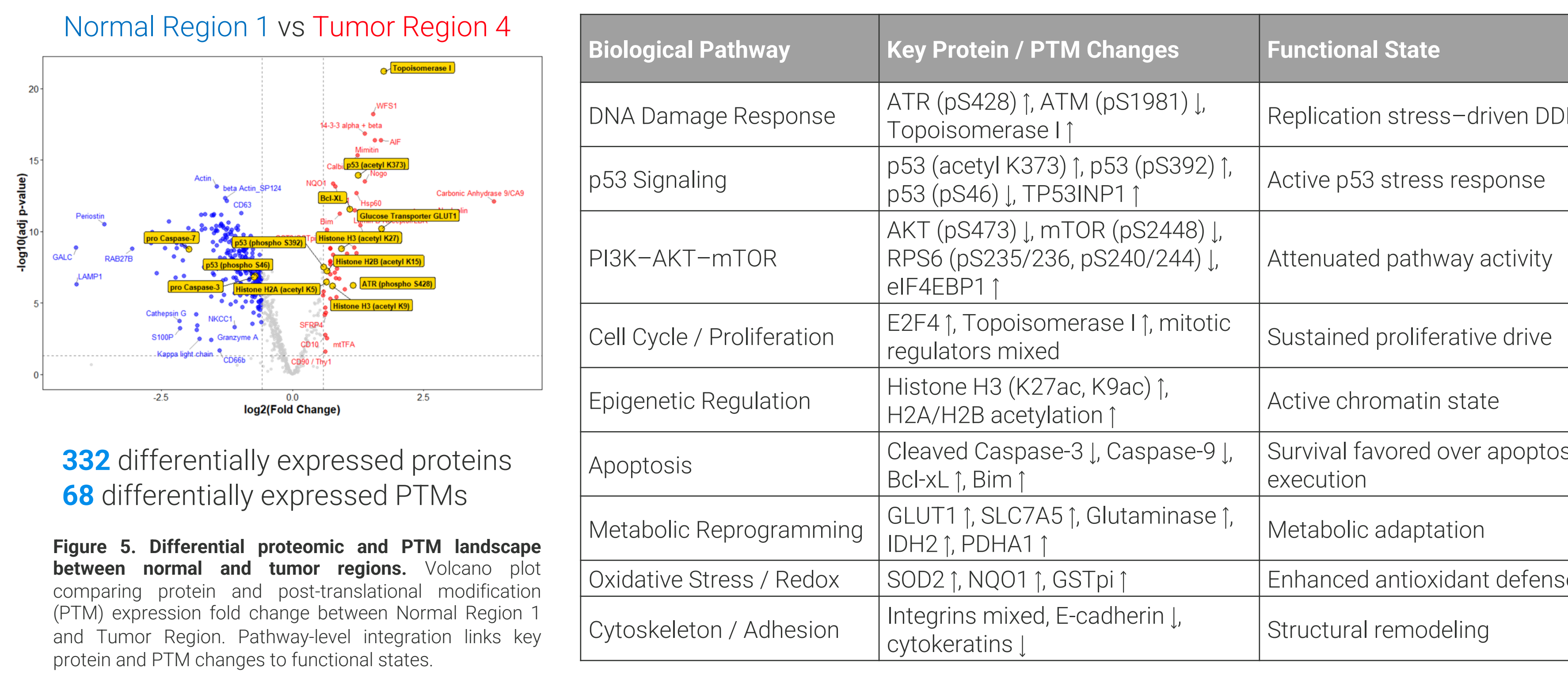
**Figure 3. GeoMx morphology-guided spatial profiling.** H&E post-staining of CRC patient D107211. PanCK (green) and CD45 (magenta) immunofluorescence are used to visualize tissue morphology and define regions of interest (ROIs), followed by compartment-specific segmentation into epithelial (PanCK+) and immune (PanCK-) areas of illumination (AOIs) for spatial profiling.

## Spatial mapping of Hallmarks of Cancer



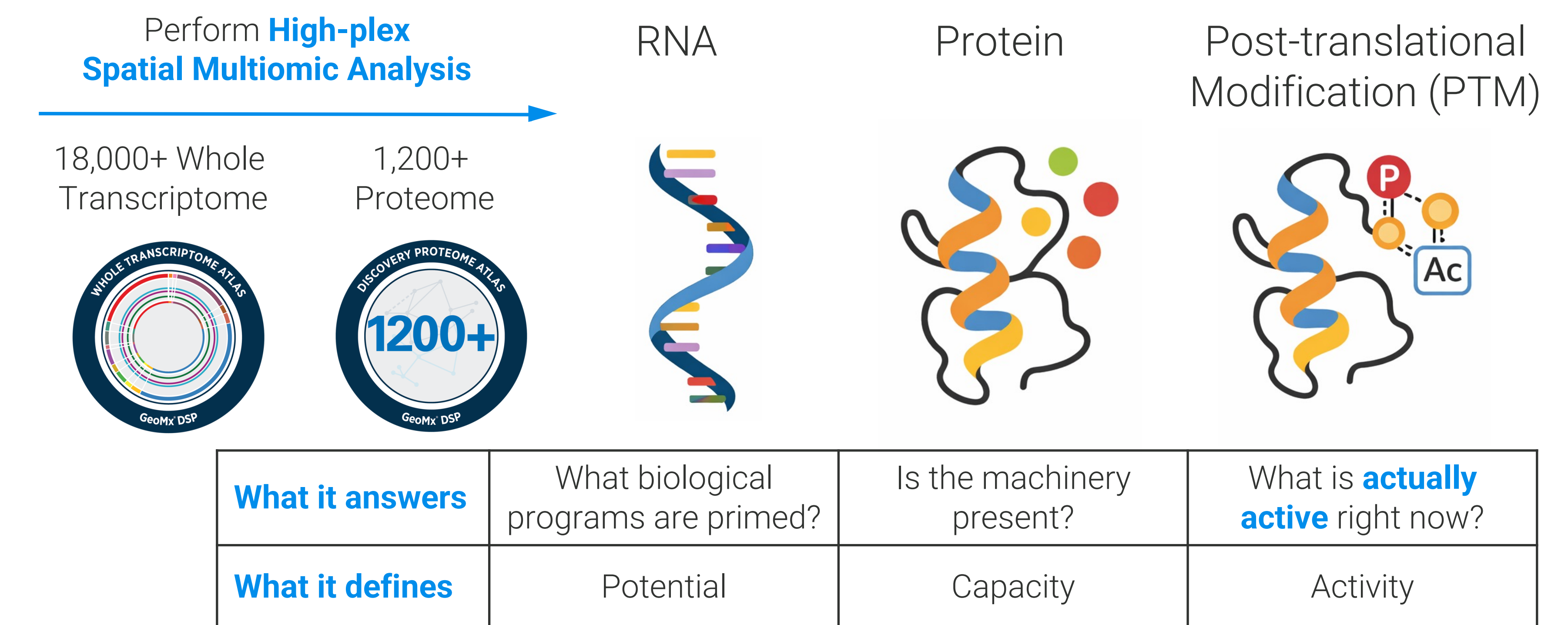
**Figure 4. Spatially-resolved cancer hallmark pathway activity across tumor regions.** (A) Hallmark Gene Set Enrichment Analysis (GSEA) of PanCK+ tumor segments comparing each tumor region to normal using GeoMx Whole Transcriptome Atlas (WTA) profiling. (B) Normalized enrichment scores reveal region-specific activation of hallmark pathways relative to normal. (C) Summary of tumor-enriched pathways by whole transcriptome profiling, highlighting biological questions that extend beyond RNA-based analysis alone.

## Proteome and PTM signaling define tumor functional state



**Figure 5. Differential proteomic and PTM landscape between normal and tumor regions.** Volcano plot comparing protein and post-translational modification (PTM) expression fold change between Normal Region 1 and Tumor Region. Pathway-level integration links key protein and PTM changes to functional states.

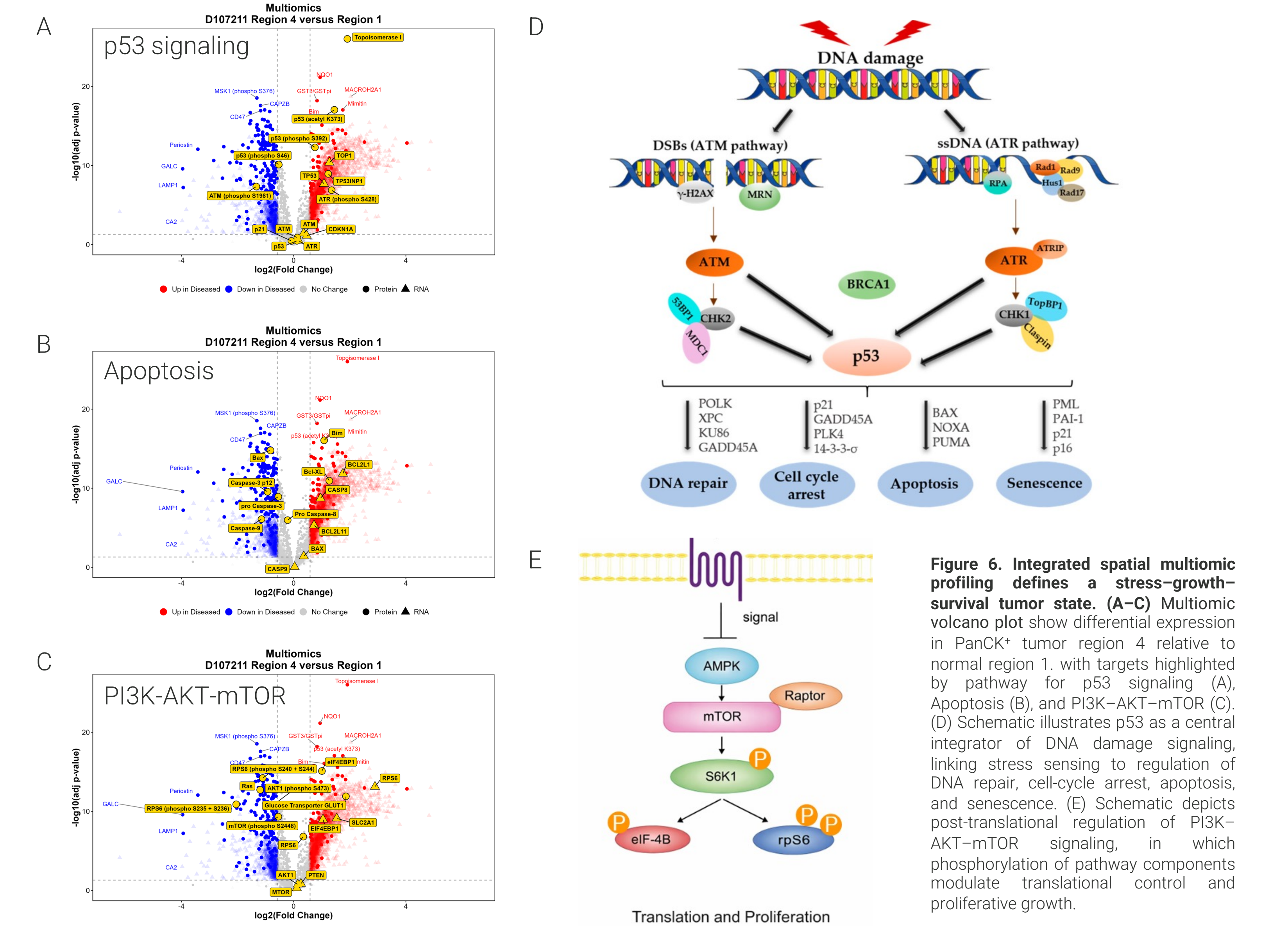
## Understanding what is primed, present, and active in space



RNA pathway enrichment ≠ pathway activation

## Integrated multiomic spatial profiling reveals stress-growth-survival tumor state

Model: Tumor region is a hypermetabolic, mTOR-driven state under active DNA damage stress, with engaged but ineffective p53 signaling, and a critical survival dependency on Bcl-xL preventing apoptosis.



**Figure 6. Integrated spatial multiomic profiling defines a stress-growth-survival tumor state.** (A-C) Multiomic volcano plot show differential expression in PanCK+ tumor region 4 relative to normal region 1, with targets highlighted by pathway for p53 signaling (A), Apoptosis (B), and PI3K-AKT-mTOR (C). (D) Schematic illustrates p53 as a central integrator of DNA damage signaling, linking stress sensing to regulation of DNA repair, cell-cycle arrest, apoptosis, and senescence. (E) Schematic depicts post-translational regulation of PI3K-AKT-mTOR signaling, in which phosphorylation of pathway components modulate translational control and proliferative growth.

## Conclusions

1. GeoMx DSP targets the cells that matter through morphology-guided "microdissection" by light, resolving tissue heterogeneity within a single 5-µm section. This approach enables separation of tumor and tumor microenvironment and direct comparison of tumor regions to normal tissue.
2. GeoMx DSP platform delivers the highest-plex multiomic profiling in spatial biology, enabling comprehensive functional insight through simultaneous measurement of 18,000+ transcripts and 1,200+ proteins. In this study, spatial mapping of cancer hallmark pathways defined functional tumor states that extend beyond RNA-based pathway priming alone.
3. The Discovery Proteome Atlas profiles >130 post-translational modifications, including >100 phosphorylation sites, enabling assessment of protein activity states. Integrated high plex multiomics defines a CRC stress-growth-survival state and reveals targetable dependencies including PI3K-AKT-mTOR, BCL-xL, ATR, and topoisomerase.

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