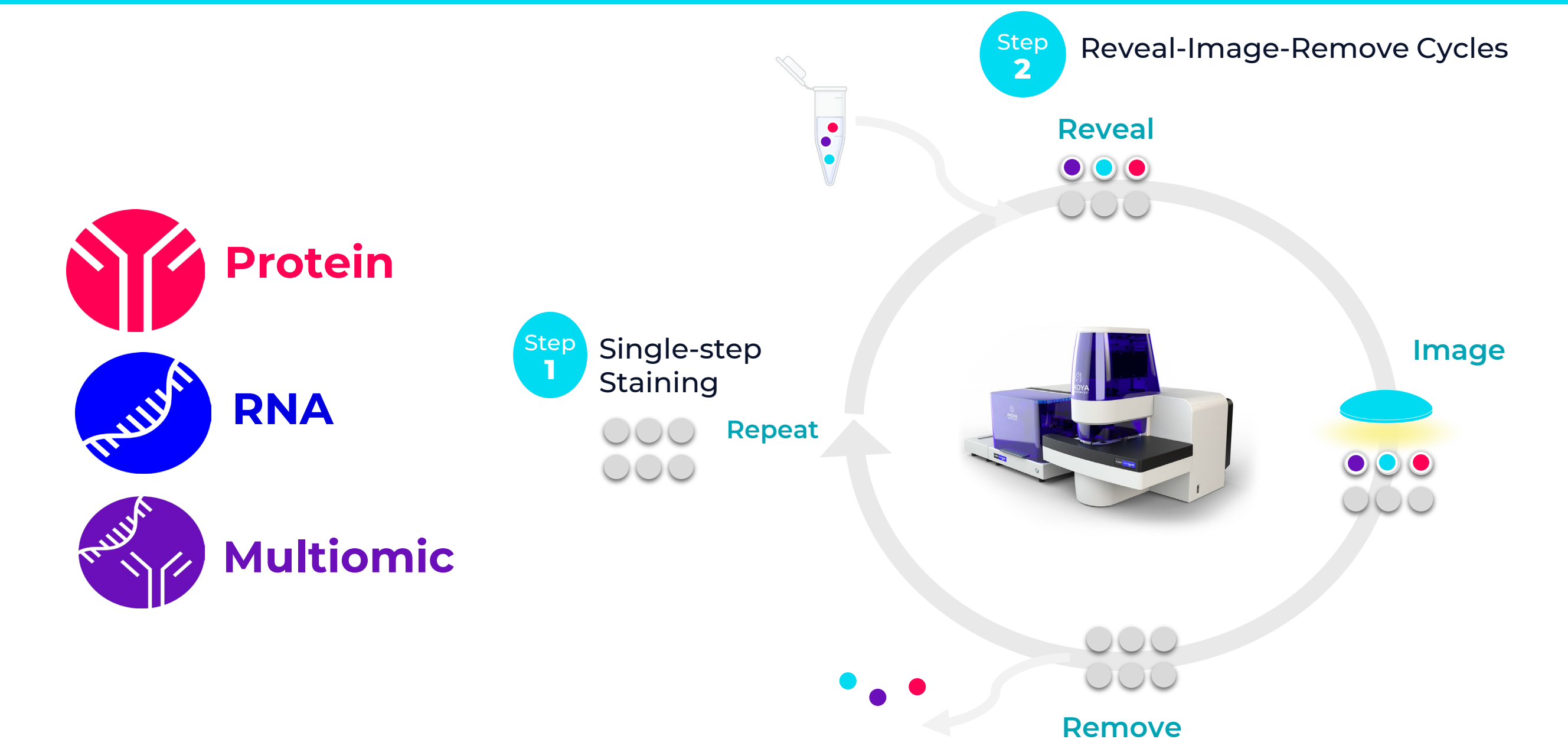


Rapid whole-slide spatial analysis of FFPE tissues enables the discovery of key cellular niches

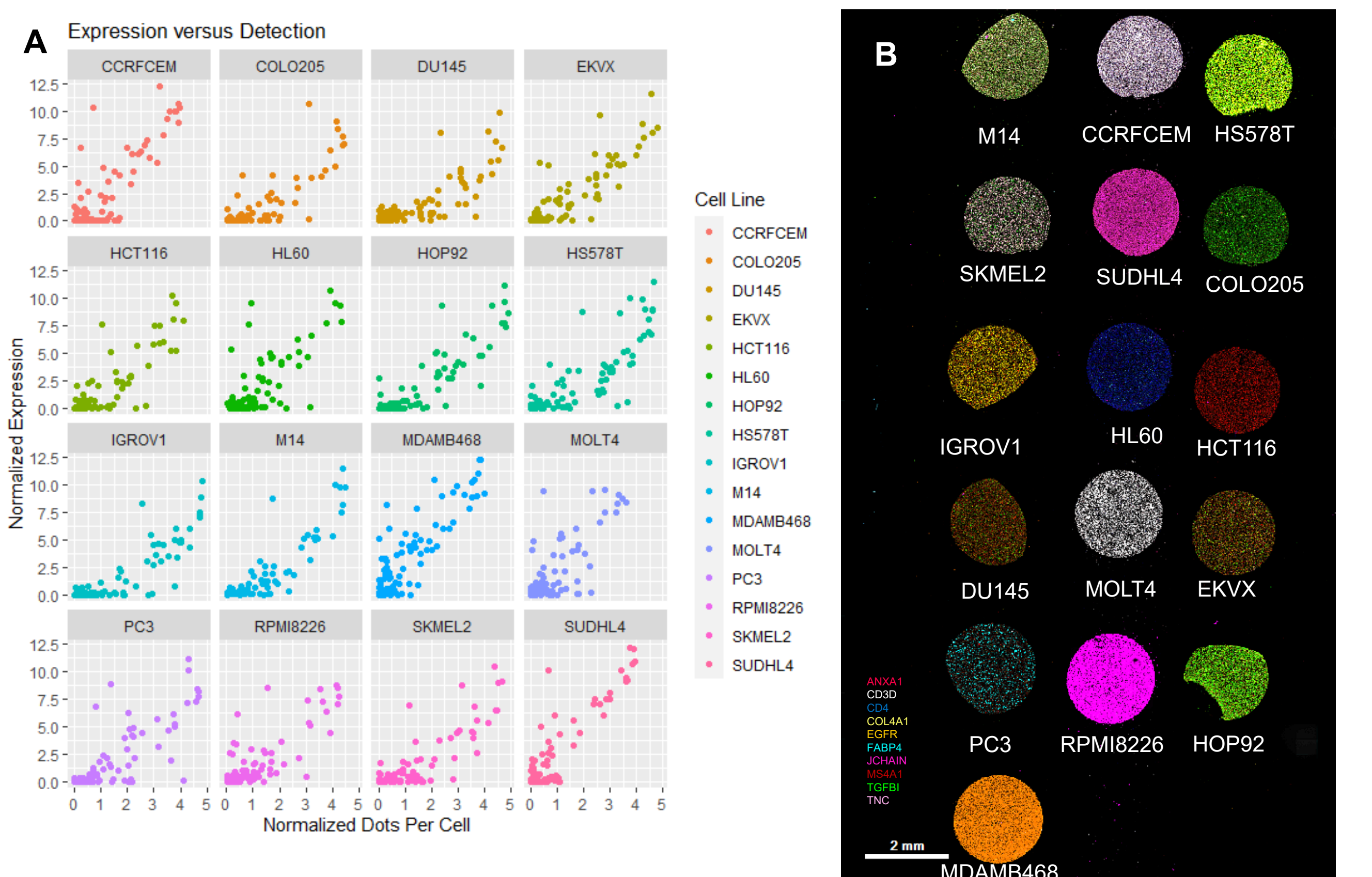
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1. High-Speed, True Multiomic Spatial Phenotyping

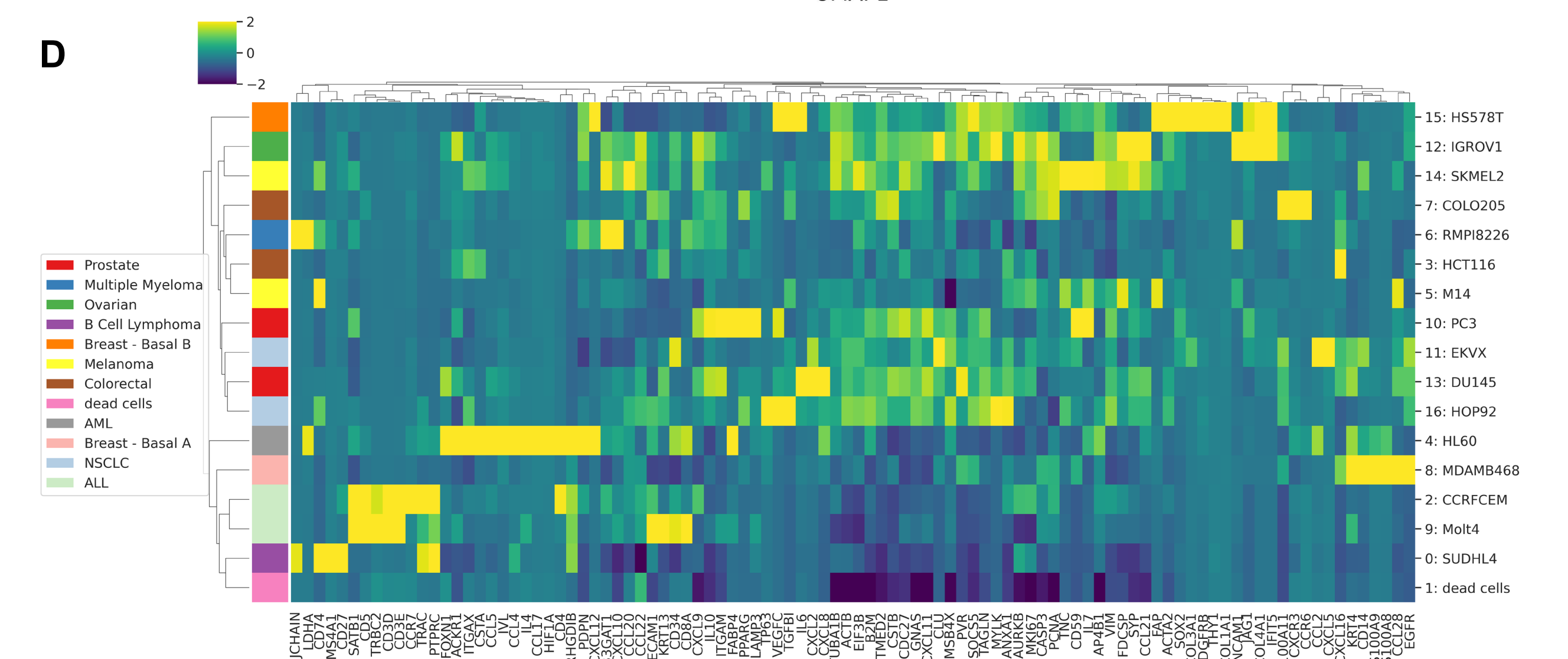


Spatial biology analyses are critical to understanding key cellular interactions. While there have been tremendous advancements in spatial technologies, there was always a trade-off between plex, resolution, and speed. Akoya's PhenoCycler®-Fusion spatial biology platform solves this tradeoff by enabling rapid spatial phenotyping of whole FFPE slides using protein targets, RNA targets, or a combination of both. The PhenoCycler-Fusion utilizes high-speed whole-slide imaging at single-cell resolution, proprietary data compression algorithms, and an ecosystem of software partners to streamline various analysis needs. This workflow is inherently unbiased, and simultaneous detection of RNA and protein provides a comprehensive view of tumor biology. Here, we demonstrate the robustness and scalability of our true multiomics chemistry by spatial phenotyping 46 samples, 23 tissue types, and more than a million cells. Protein and RNA biomarkers play complementary roles in defining cell phenotypes and cell states, respectively, in a tissue sample. Proteins are the key effectors of cell function and detecting cell surface protein markers can serve as a ground truth for cell identity. RNA markers such as cytokines and chemokines can provide an insight into signaling pathways that result in changes in cell states, arising from interactions within the tumor microenvironment. Measuring both analytes within the same tissue can give researchers an accurate picture of tumor progression and response to therapy.

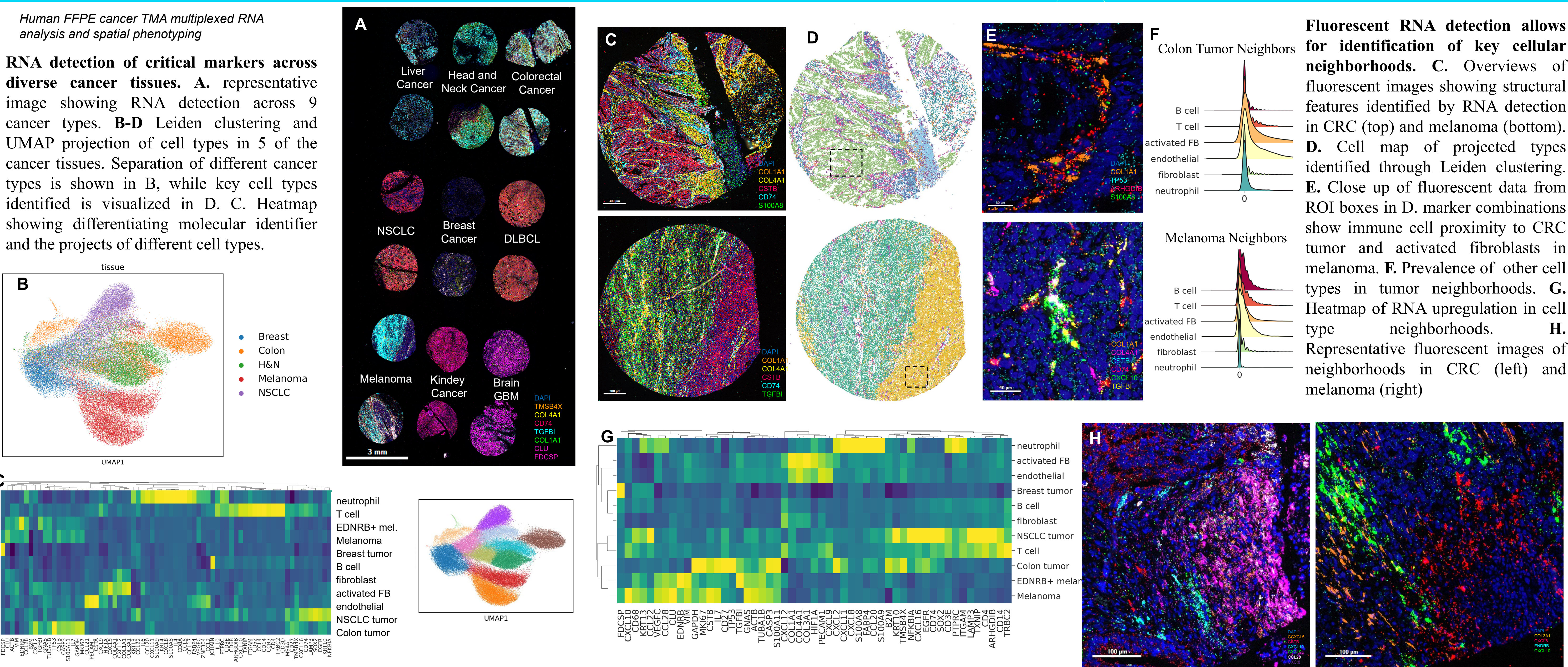
2. RNA only and true multiomics workflows enable high-plex detection of targets in FFPE samples



RNA detection is robust, sensitive, and specific. RNA detection was performed on a cell pellet array featuring 16 cell lines for a panel of 100 targets. Following RNA barcoding, cyclic detection of labeled RNA species was performed on the PhenoCycler-Fusion. **A.** Plots comparing sequencing data to the average number of detection events for each gene per cell across the 16 cell lines. Detection events and sequencing data were transformed through the operation $\log_2(n+1)$ prior to plotting. **B.** A representative panel showing differential detection of markers across the 16 cell lines. **C.** Projection of cell type clusters data using UMAP shows clear separation of cell lines using RNA detection. Cells were segmented and RNA detection events for each marker were assigned. Leiden clustering was then performed on segmented data. **D.** Heat Map showing differential RNA detection of key molecular identifiers allowing differentiation of the 16 cell lines during clustering.

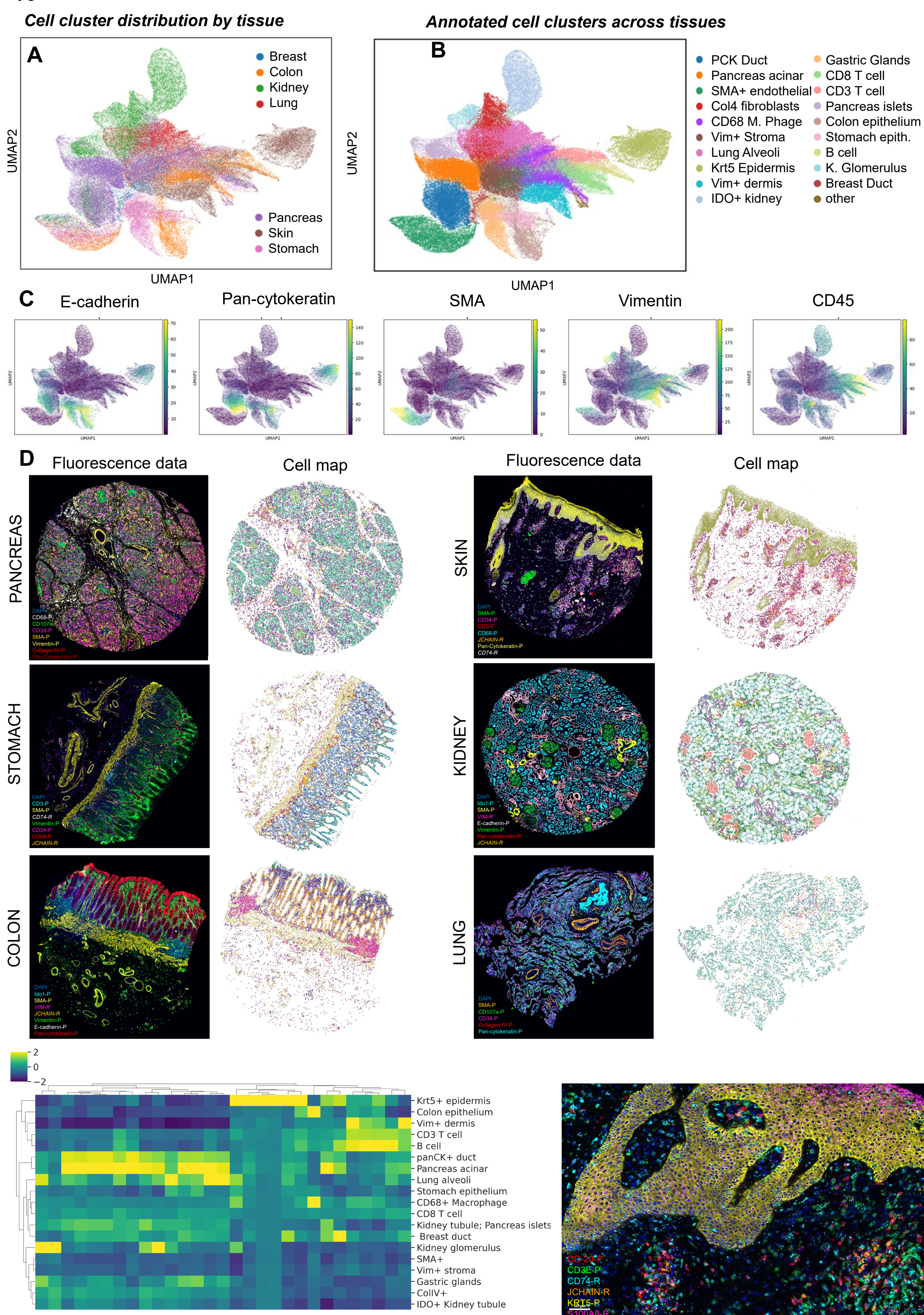


3. Spatial phenotyping within cancer FFPE tissues reveals key cell types and niches using RNA chemistry

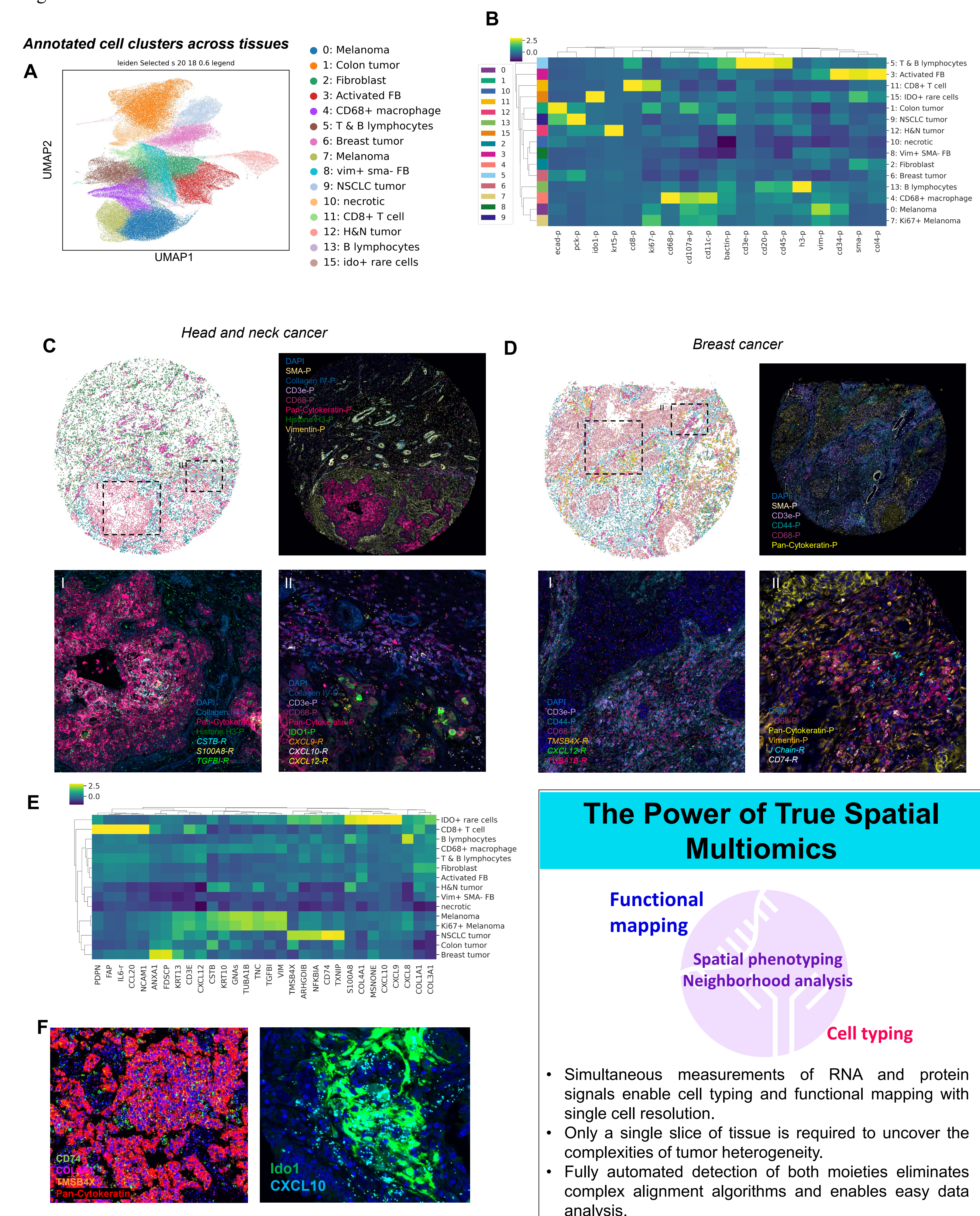


4. True spatial multiomics enables accurate spatial phenotyping and neighborhood identification across variety of normal and cancer human FFPE tissues

Normal FFPE Human Tissues. **A.** Different tissues separated by clustering, highlighting the differences in expression across targets. **B.** 19 key cell types are identified based on initial protein clustering. **C.** Key protein differentiators cluster into different locations, validating the staining process within the multiomic workflow. **D.** Cell types are distinguished based on differential expression of key protein targets. **E.** Tissue architecture is generated with spatial phenotyping across a variety of tissue types and matches raw fluorescence distribution.



Cancer FFPE Human Tissues. **A.** UMAP of key cell types identified across different cancers using multiomic panel. **B.** Differential expression of key cell types across panel and tissues. **C.** Cell type map and example staining combinations for H&N FFPE core. **D.** Cell type map and example staining combinations for Breast cancer core. **E.** Heat map of key RNA target proximity to cell types of interest with images of example fluorescence validation of trends. **F.** representative RNA neighborhoods



The Power of True Spatial Multiomics



- Simultaneous measurements of RNA and protein signals enable cell typing and functional mapping with single cell resolution.
- Only a single slice of tissue is required to uncover the complexities of tumor heterogeneity.
- Fully automated detection of both moieties eliminates complex alignment algorithms and enables easy data analysis.