

Spatial Atlases of Immunological Development within the Lymph Node

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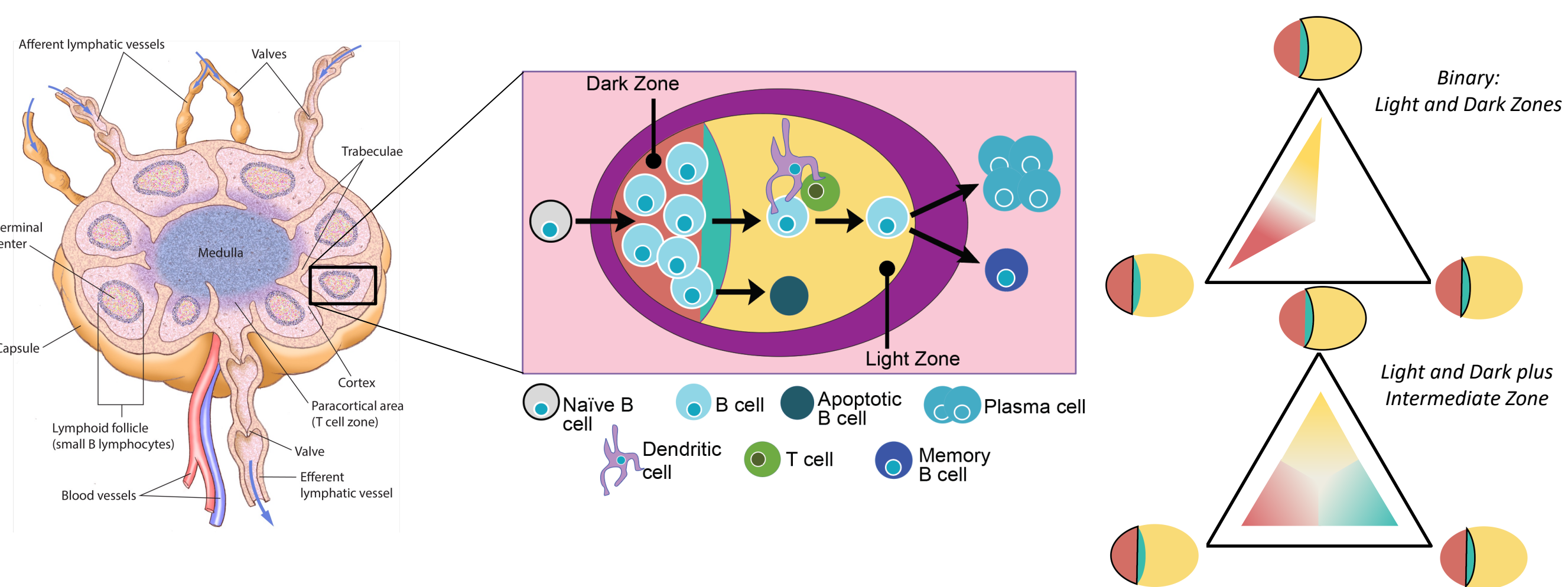
Introduction

Immunological mechanisms regulating detection and clearance of cancer, including CTLA-4 and PD-L1, were discovered by studying the natural physiological processes regulating immune cell maturation, attenuation, and dissemination throughout the body. Single-cell atlases mapping immune cells provide hints to these aspects of immunology but lack essential spatio-temporal relationships between cells. With the advent of spatial 'omics we can resolve thousands of RNA or more than 60 protein molecules simultaneously *in situ*, enabling direct insight into the dynamics occurring as immune cells mature and migrate through tissue.

The cells within the lymph node exist in a well-characterized spatial arrangement. At a finer spatial scale, B cells mature in regions known as germinal centers with distinct biology captured in Dark Zone (DZ) and Light Zone (LZ). In their review of germinal center biology, Young and Brink¹ noted that one-third of GC B cells cannot be classified as pure LZ or DZ phenotypes and posited that "Intermediate Zone" (IZ) GC B cells exist at the DZ-LZ spatial transition. This hypothesis can be explicitly tested with a spatial 'omics approach.

In our previous work, we used GeoMx® Digital Spatial Profiling (DSP) to profile key structures within the lymph node (tonsil) including the germinal center, mantle zones, medulla, and paracortex. These data can be viewed interactively or downloaded publicly (see QR code below). Herein we combined DSP with CosMx™ Spatial Molecular Imaging (SMI) to map serial sections of a single FFPE block of normal lymph node to characterize the spatial transcriptomic landscape. In addition, we tested whether the cellular and expressional composition at the DZ-LZ interface showed distinct biology.

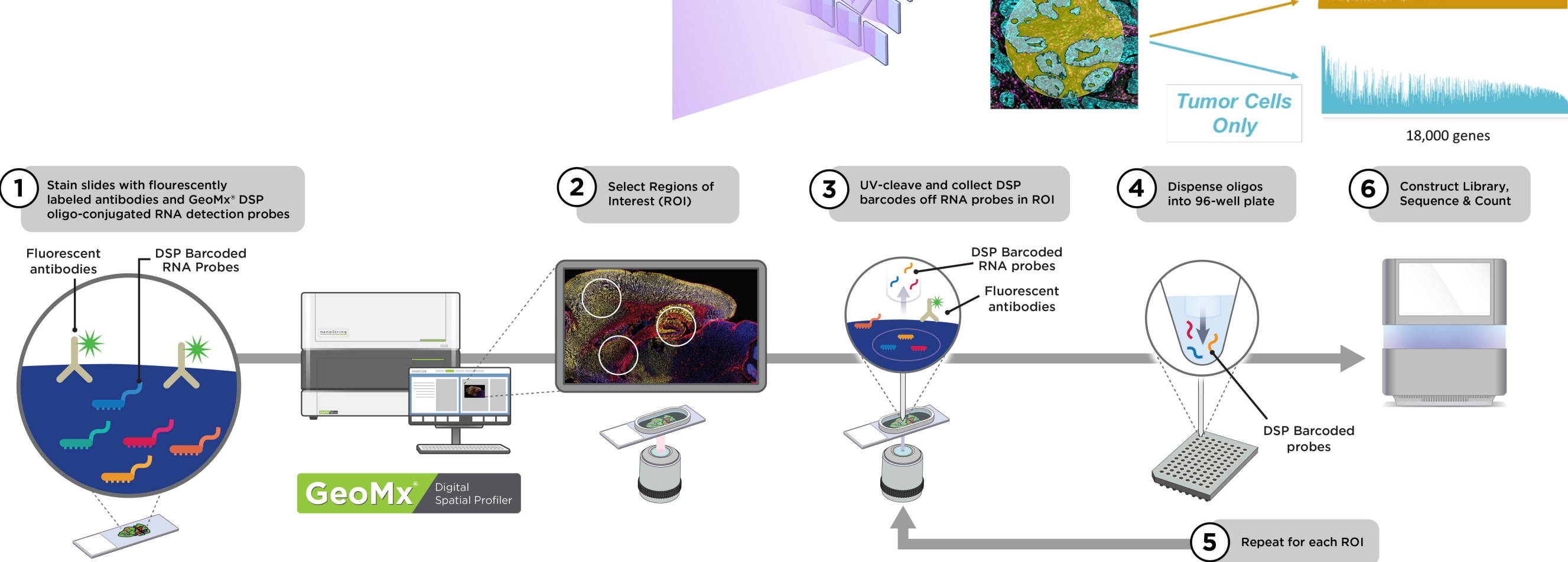
Elucidating Lymph Node Biology: B-cell Maturation



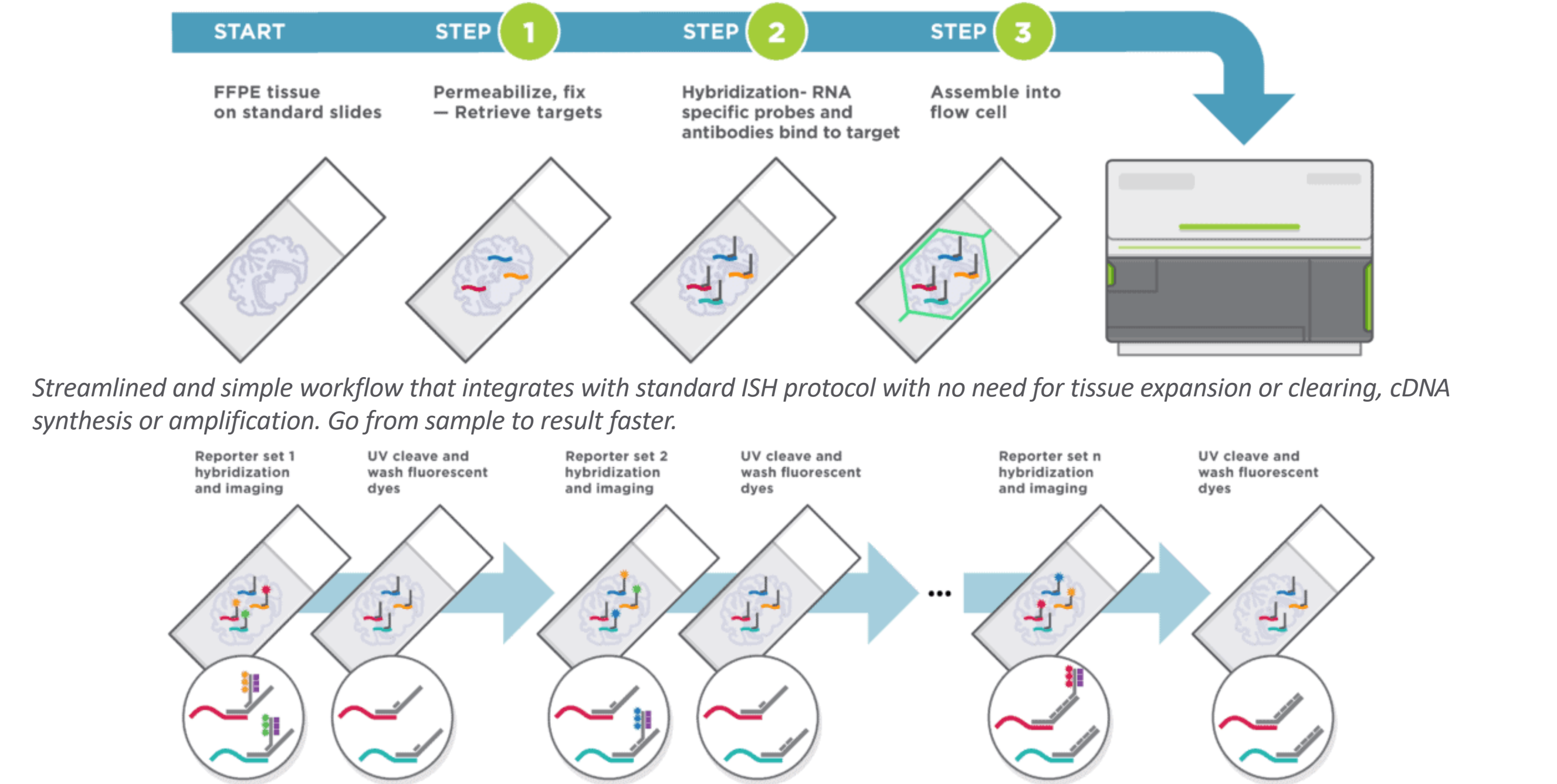
Schematic of lymph node and B-cell maturation within a germinal center. B cells mature and proliferate in the germinal center. Dark Zone is the location of proliferation. Selection by interacting T cells and APCs occurs in the Light Zone. Spatially disruptive scRNAseq approaches suggest B cells containing both DZ and LZ characteristics are found. By including spatial information, we can test if the "Intermediate Zone" contains distinct biology.

Technology Overview: GeoMx® DSP and CosMx™ SMI

GeoMx captures tissue morphology
GeoMx hardware and workflow are both intimately linked to profile tissue structures and morphological compartments



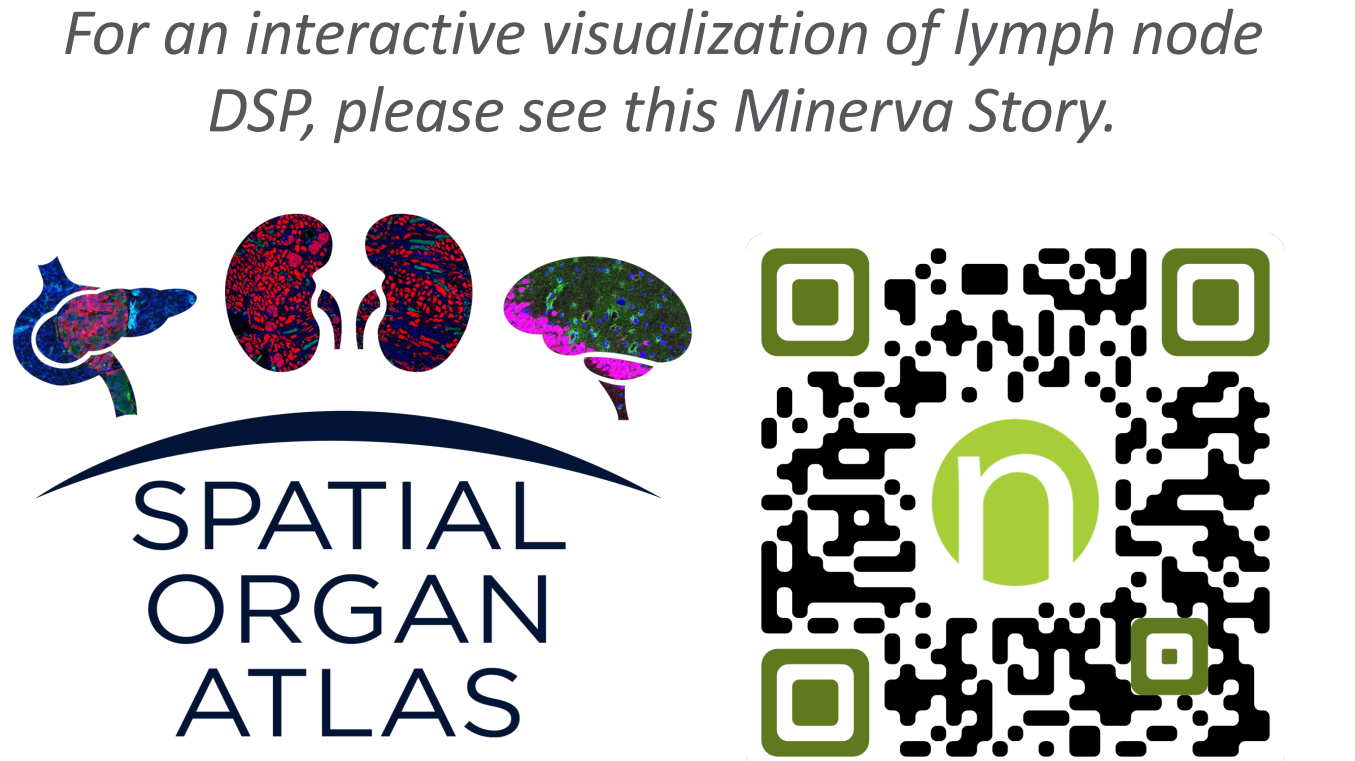
CosMx SMI is the first high-plex in situ analysis platform to provide spatial multiomics with formalin-fixed paraffin-embedded (FFPE) and fresh frozen (FF) tissue samples at cellular and subcellular resolution
CosMx SMI is an integrated system with mature cyclic fluorescent in situ hybridization (FISH) chemistry, high-resolution imaging readout, interactive data analysis and visualization software.



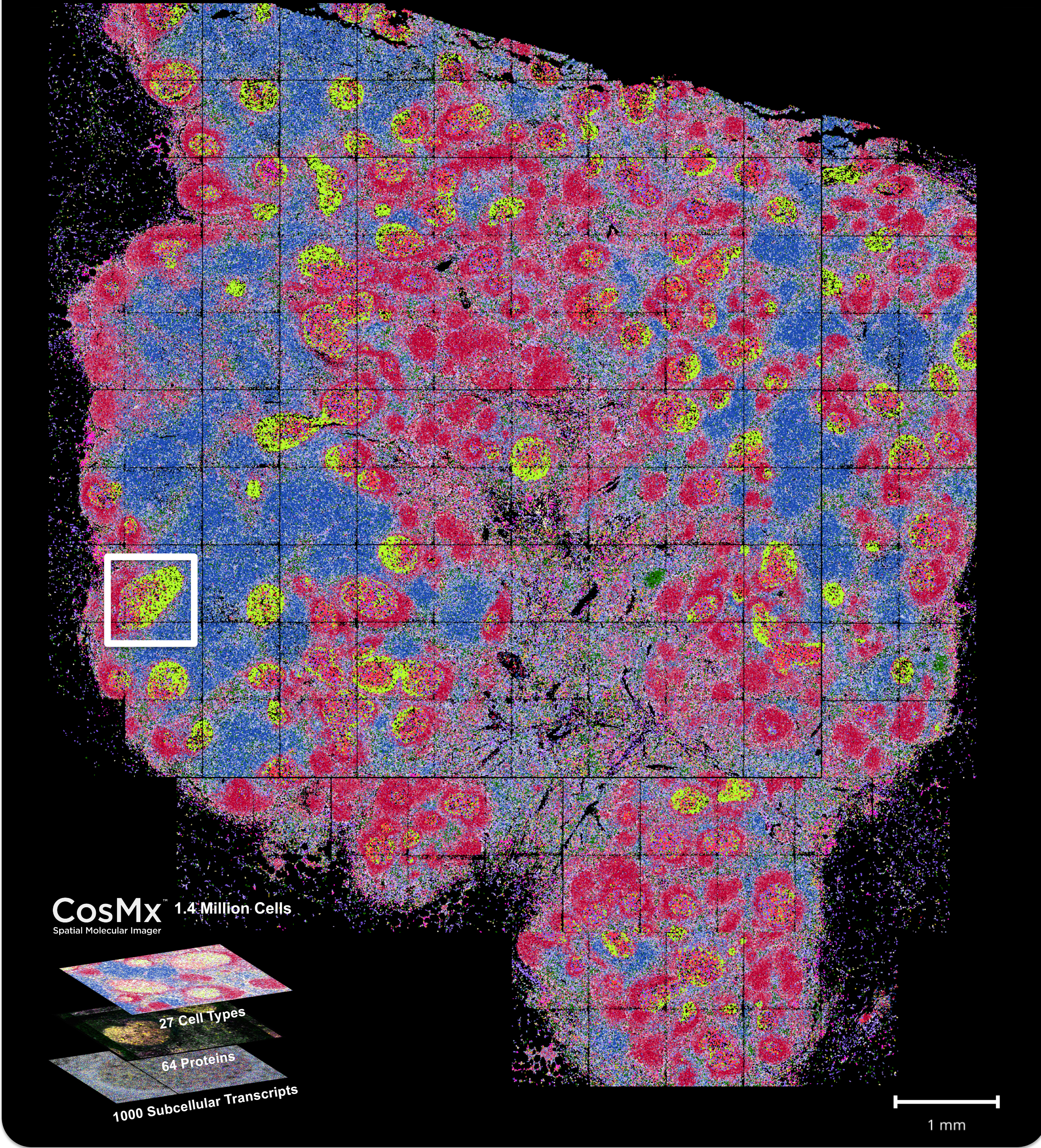
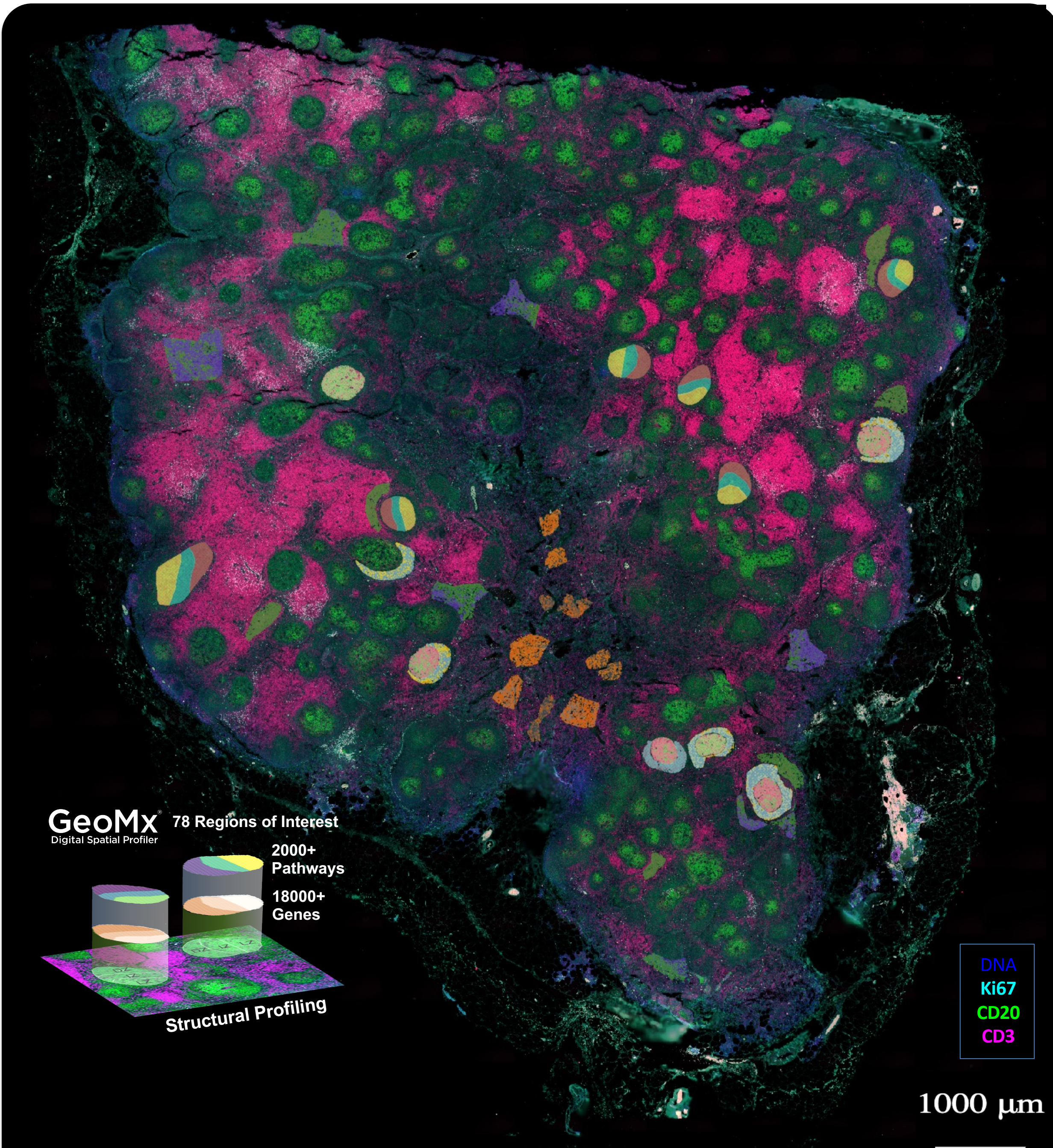
Robust hybridization chemistry that provides higher sensitivity and supports high-plex assays in your tissue samples to uncover deeper biological insights.

References and Links

- Young and Brink. 2021. The unique biology of germinal center B cells. *Immunity*. Issue 54: 1652-1664.
- Danaher *et al.* Pre-print. Insitutype: likelihood-based cell typing for single cell spatial transcriptomics. <https://www.biorxiv.org/content/10.1101/2022.10.19.512902v1>



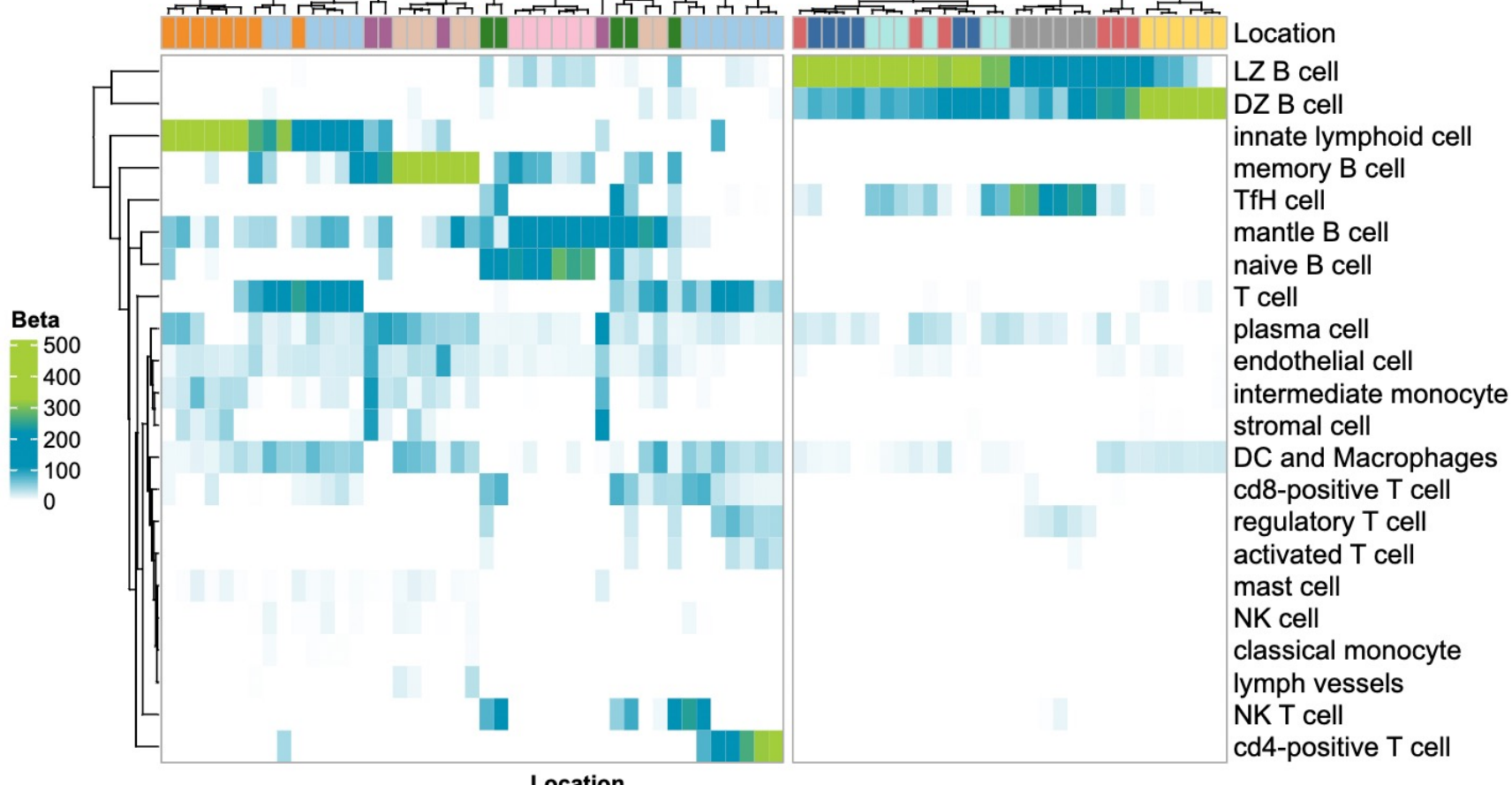
High Plex, Multi 'omic Spatial Atlas of a Healthy Lymph Node



Above: GeoMx DSP profiling of key structures within the lymph node. Subset of germinal centers were profiled as 3 zones representing LZ, DZ, and the "intermediate" zone. **Below:** CosMx SMI data of a serial section showing 23 cell types identified with *insitutype*². Cell types are displayed by UMAP. **Insets:** View of interacting cell types of a single germinal center and orange and blue transcripts in XY space showing the interaction of IL18 in APCs with CD48 in DZ B cells, respectively. Ligand/Receptor analysis showed a positive association with these molecules while GeoMx DSP showed IL18 signaling was more pronounced in the Intermediate Zone.

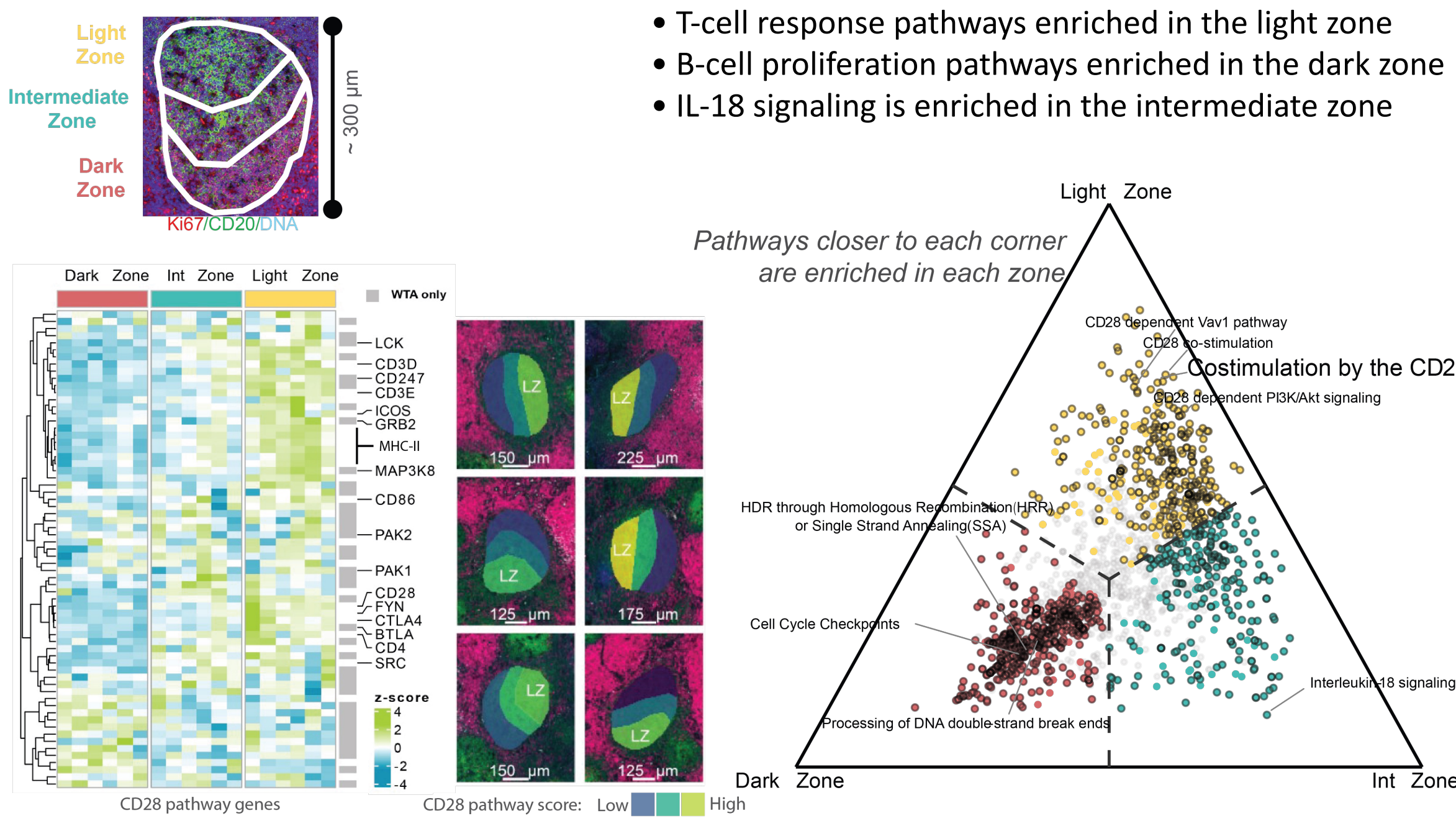
CosMx™ SMI-identified Cell Types can be Mapped to GeoMx® DSP ROIs

Cell types found using CosMx SMI can be used to inform the cellular composition of GeoMx DSP ROIs. The heatmap shows abundance of a given cell type by GeoMx DSP-defined location. As expected, the abundance of Light and Dark Zone-specific B cells were highest in their respective ROIs. TFH cells were the most abundant in LZ germinal centers and in germinal centers with DSP collected only the subset of T cells. Memory B cells were highest in the paracortex, likely as they exited the germinal centers.

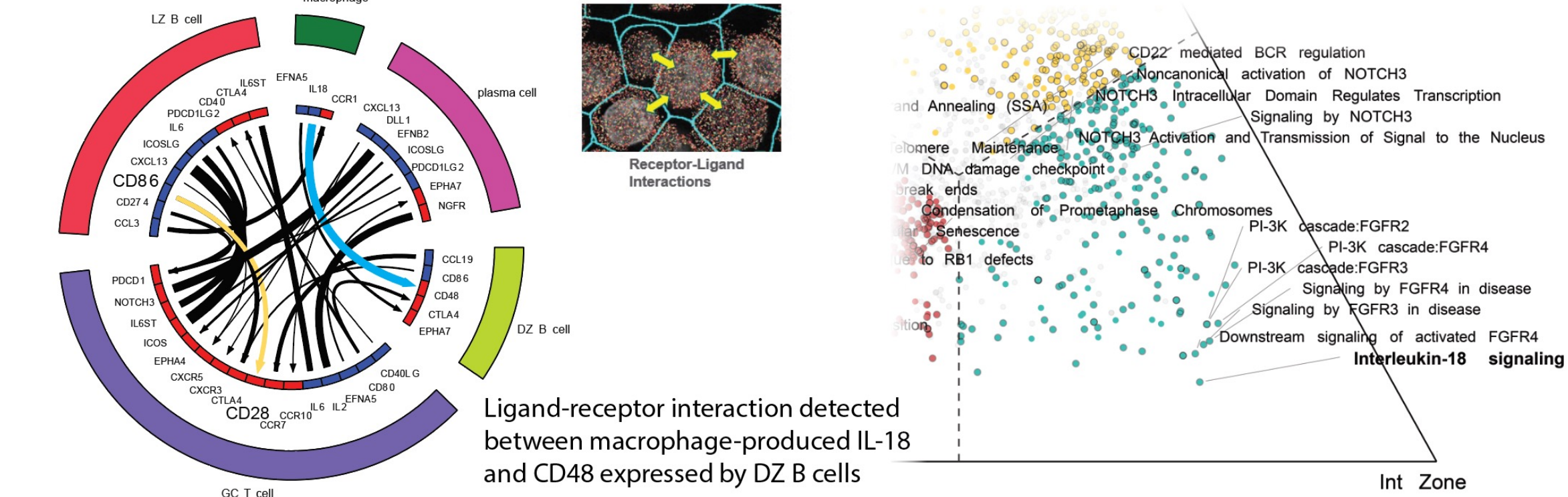


- Location**
- Blue: Germinal Center (B cell AOI)
 - Orange: Germinal Center (Dark Zone)
 - Green: Germinal Center (Int Zone)
 - Red: Germinal Center (Light Zone)
 - Grey: Germinal Center (T cell AOI)
 - Yellow: Mantle (B cell AOI)
 - Purple: Mantle (T cell AOI)
 - Dark Blue: Medulla (B cell AOI)
 - Light Blue: Medulla (T cell AOI)
 - Dark Green: Paracortex (B cell AOI)
 - Light Green: Paracortex (T cell AOI)

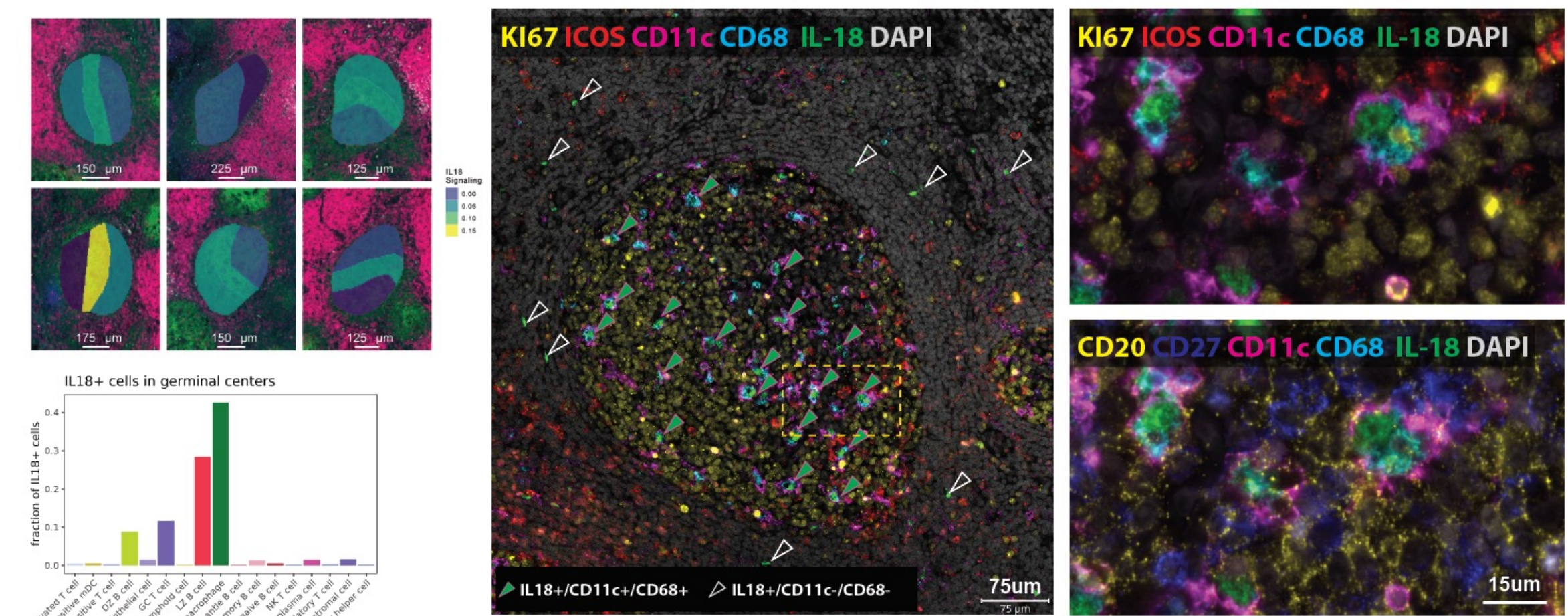
GeoMx® DSP Provides Whole-transcriptome Measurement of Lymph Node Spatial Biology



CosMx™ SMI Yields a High-resolution Map of Germinal Center Cell Interactions

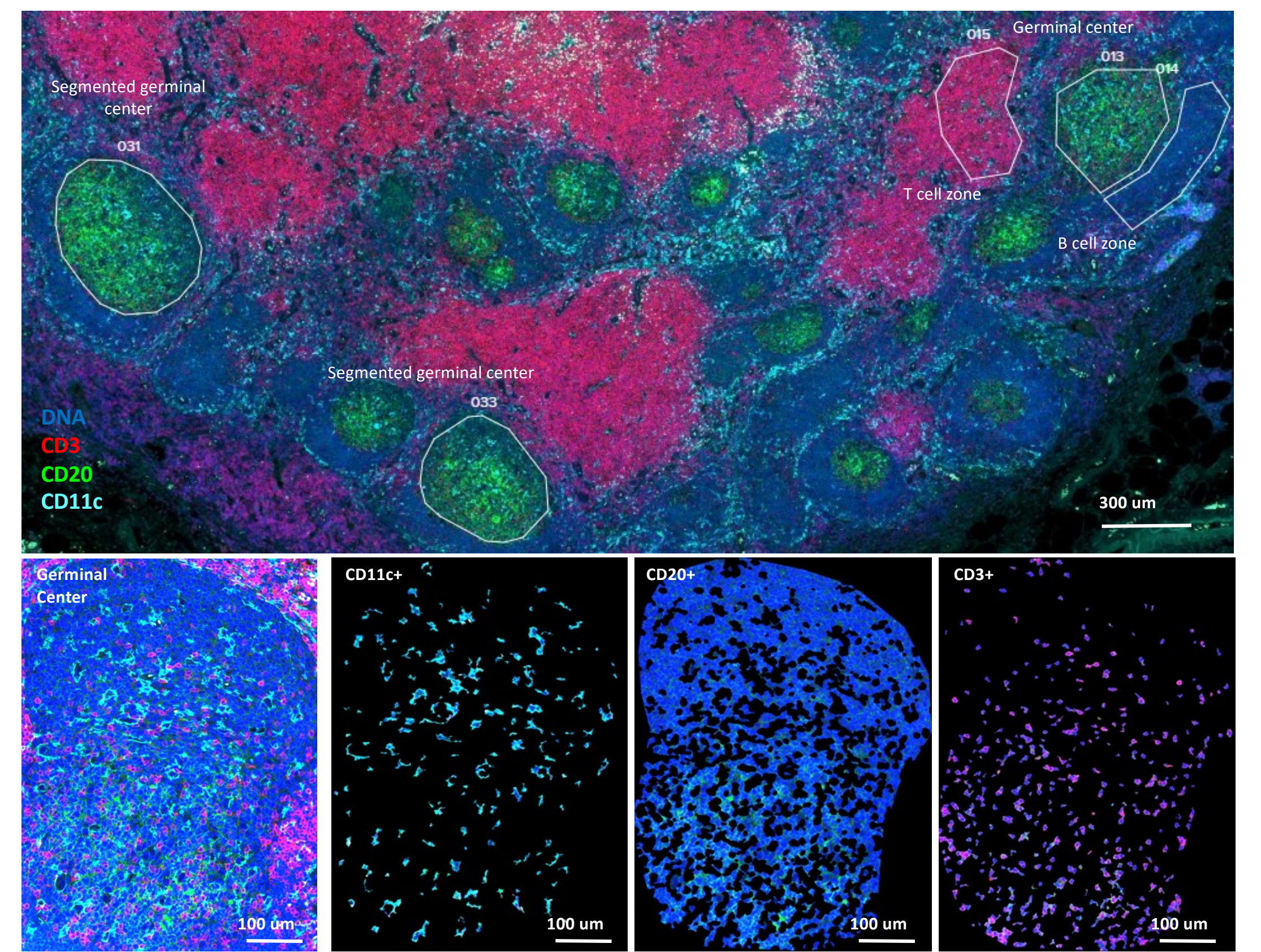


Interleukin 18 Signaling at the Intermediate Zone may Drive B-cell Differentiation



With both GeoMx and CosMx RNA: evidence of **IL-18 signaling occurring from macrophages to B cells**. Likely as they exit the dark zone to help differentiate them into memory B cells and plasmablasts.
CosMx protein: IL-18 is expressed across the tissue, but **expressed specifically by macrophages** inside the germinal centers.

A Reference for Lymph Node Biology



Spatial organ atlas profiling of lymph node expands the study to multiple ROI strategies and provides a freely downloadable study to serve as a reference for normal lymph node biology

As an example of results found in the spatial organ atlas profiling of lymph node we are showing differential expression of CD11c+ vs CD3+ cells from germinal center, again highlighting the novel role IL18+ CD11c+ cells found in this structure

