Zachary Lewis, Tiên Phan-Everson, Gary Geiss, Yan Liang, Emily Brown, Stephanie Zimmerman, Tyler Hether, Jason Reeves, Shanshan He, Julian Preciado, Michael Patrick, Mithra Korukonda, Edward Zhao, Liuliu Pan, Stefan Phelan, Charlie Glaser, Anna Pavenko, Ruchir Bhatt, Carl Brown, Dwayne Dunaway, Joseph Phan, Jianji Chen, Alyssa Rosenbloom, Brian Filanoski, Rhonda Meredith, Kan Chantranuvatana, Brian Birditt, Giang Ong, Hye Son Yi, Erin Piazza, Vikram Devgan, Patrick Danaher, Michael Rhodes, Joseph M Beechem

## Abstract

Spatial biology, together with a very new focus on high plex protein-detection technology, is the next frontier of molecular research because it provides biological insight in the context of tissue, cellular location, subcellular organization, and (ultimately) function. The CosMx™ Spatial Molecular Imager demonstrates simultaneous single-cell and sub-cellular detection of over 100 proteins on standard, biobanked, FFPE tissue samples. This high plex protein panel detects key drivers of cancer progression and immune cell activation states. Key to CosMx protein technology is an antibody-oligonucleotide-conjugate 64-bit encoding method. The encoding scheme is enabled by a 20nm hybridization-based optical barcode. The CosMx system uses a fully automated, cyclic microfluidics imaging system, high-resolution optics, and 3D capability. CosMx oligo-labeled antibodies undergo rigorous QC testing, site-specific oligo labeling chemistry, and size-exclusion purification to select for pure antibody-oligo imaging reagent with no unconjugated antibody or free oligonucleotide contamination. The CosMx protein assay reagents were validated on multi-organ FFPE tissue microarrays and 52 human FFPE cell lines, including overexpression lines for key targets and cellular activation states, such as GITR, CD278, PD-L1, and PD-1. Benchmarking to multiple orthogonal datasets (e.g., the Human Protein Atlas, Cancer Cell Line Encyclopedia, low-plex IHC) demonstrates that the assay is highly sensitive and specific. CosMx SMI Protein produces protein localization maps for each target that can be integrated with CosMx SMI RNA's 1000-plex RNA detection, generating an unprecedented information-rich, multi-omic view of spatial biology that could usher in a completely new understanding of health and disease.

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

## CosMx SMI Protein: multiplex detection by fast cyclic imaging
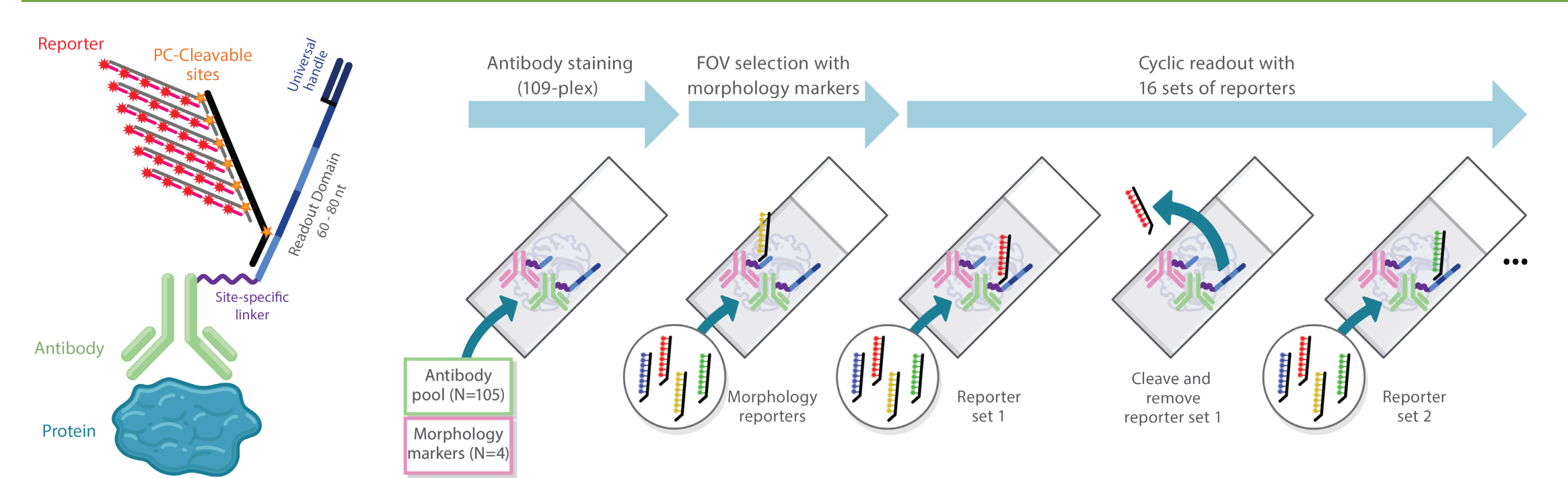
The Spatial Molecular Imaging platform uses automated fluidics and imaging to hybridize and visualize 16 sets of four fluorescent reporters, enabling **>100-plex protein** or **1000-plex RNA** readout.

## Elucidating lymph node biology: B-cell maturation
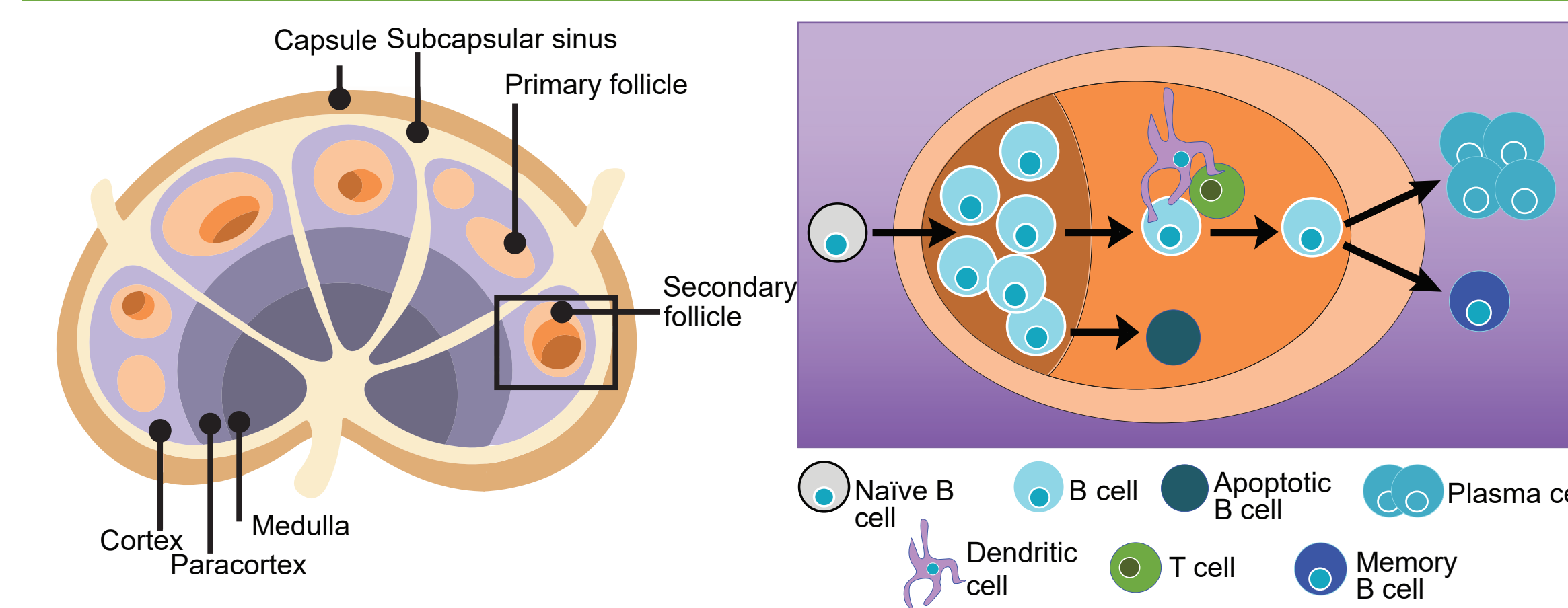
B cells mature and proliferate in the **germinal center**
**Dark zone:** somatic hypermutation and proliferation
**Light zone:** selection by interactions with T cells and APCs
"Intermediate" B cells have been identified by scRNA-seq

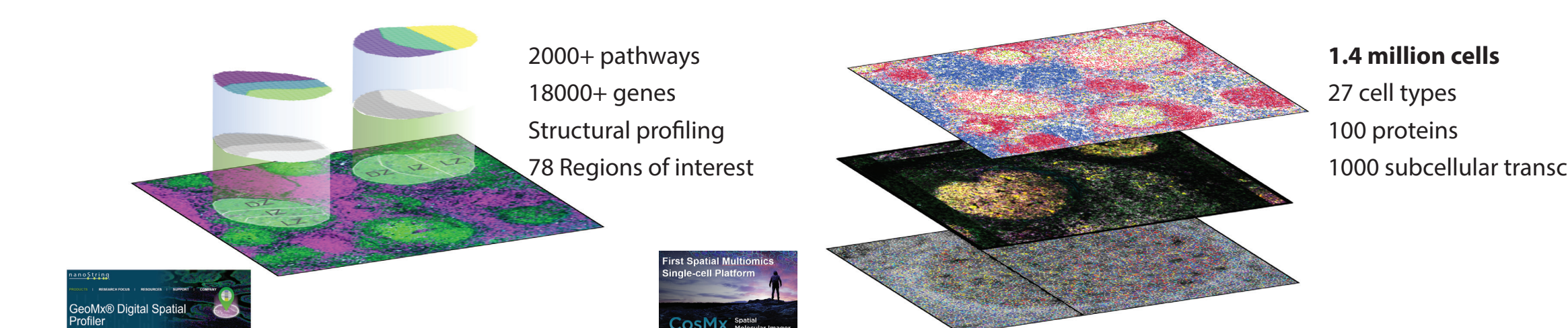*Is there a spatially resolved zone where these "intermediate" B cells reside?*

## A multiomic spatial approach to investigate immune cell development and B-cell maturation

### Serial sections profiled with:

**GeoMx®**
*Whole Transcriptome Atlas*
18,000+ genes

**CosMx SMI RNA**
1000 genes
1.4M cells

**CosMx SMI Protein**
105 proteins
1.4M cells
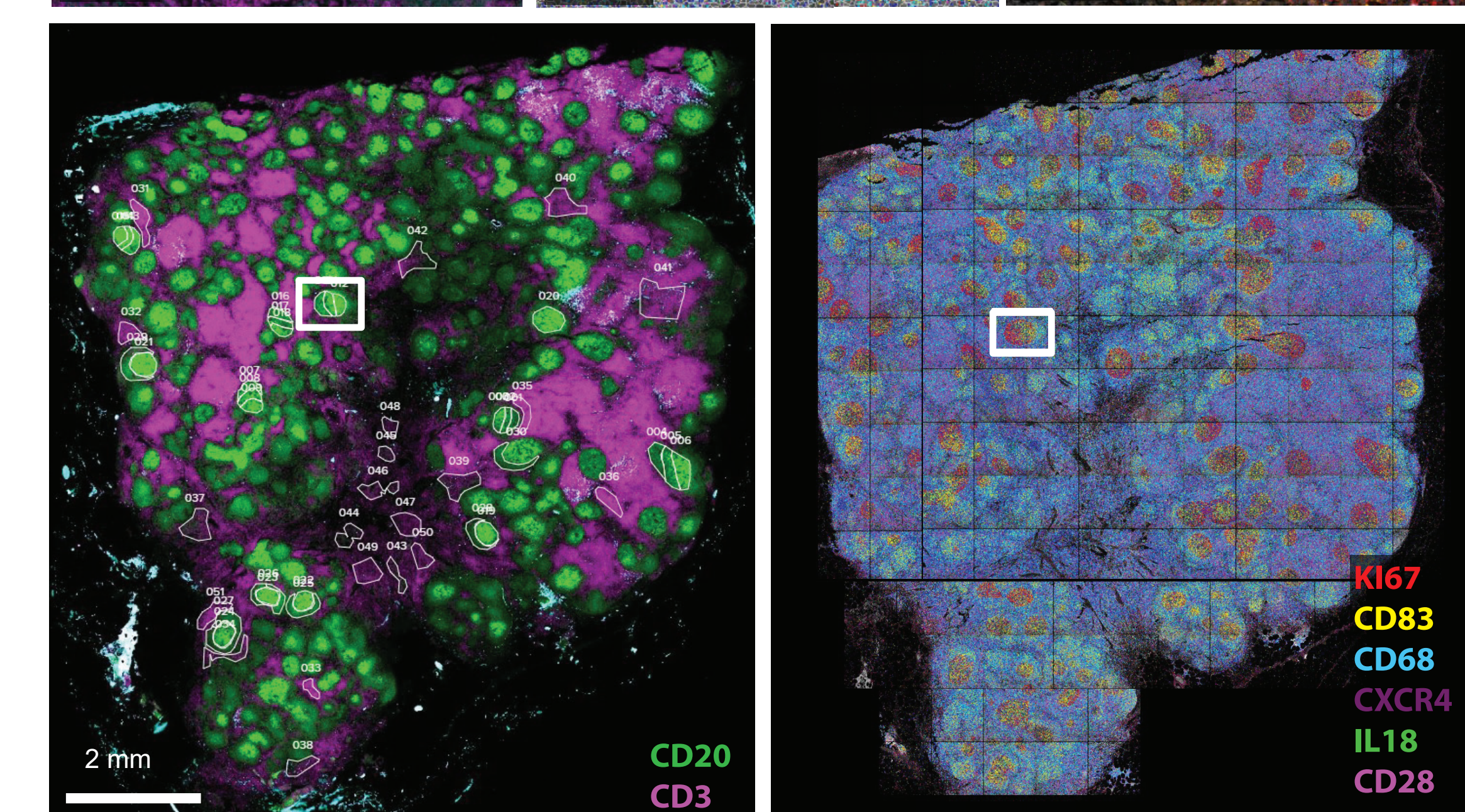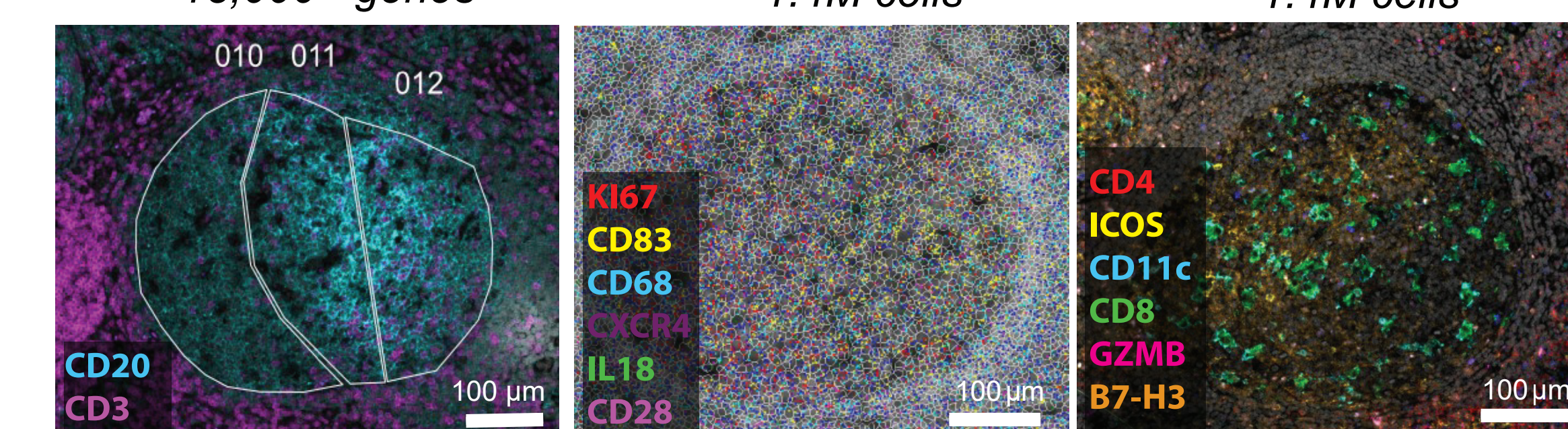
Integration of GeoMx whole-transcriptome atlas and CosMx RNA & Protein assays for **deep insights into B-cell maturation and antibody mediated immunity**
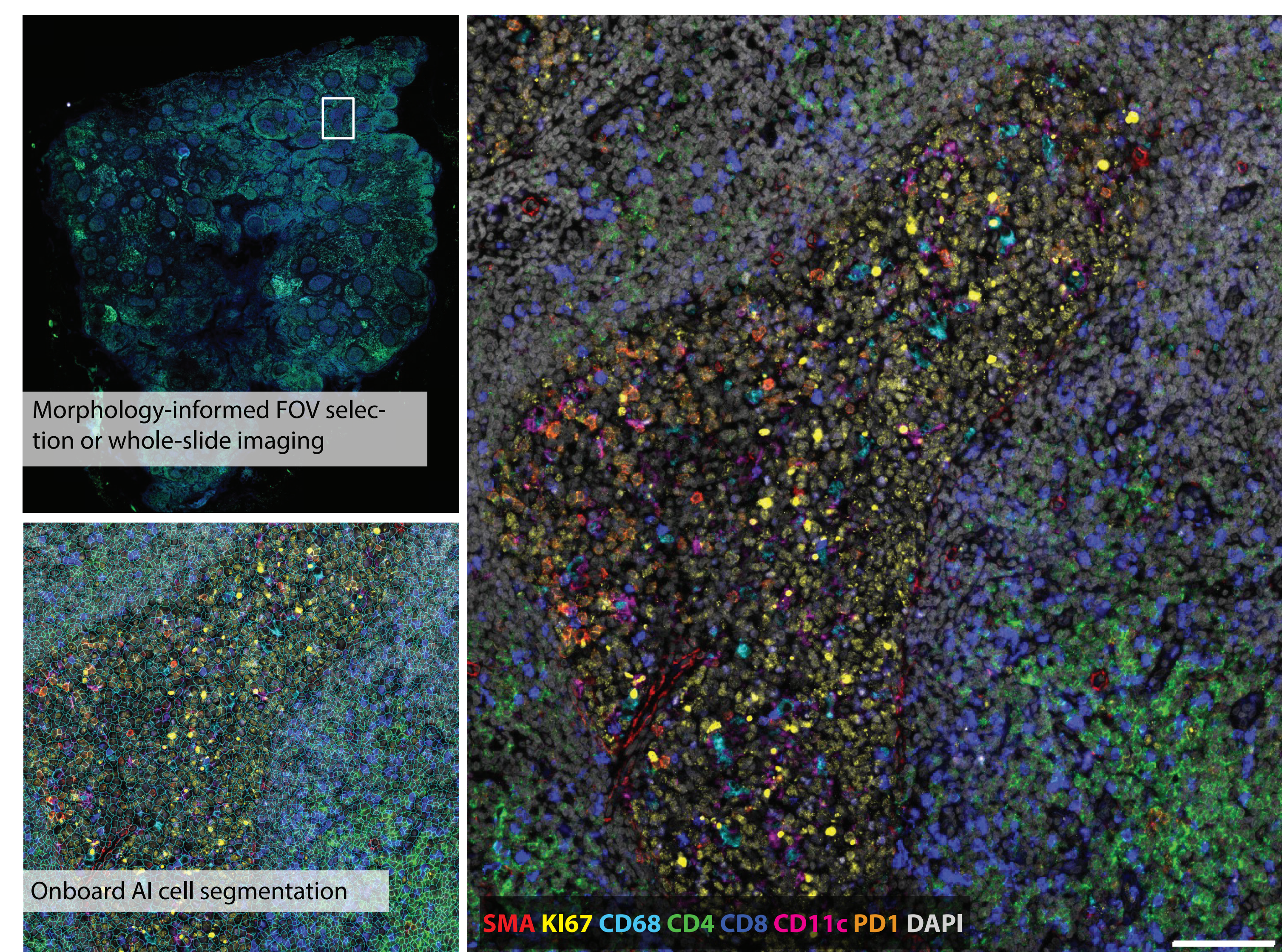
2000+ pathways
18000+ genes
Structural profiling
78 Regions of interest

1.4 million cells
27 cell types
100 proteins
1000 subcellular transcripts

## Large tissue scans for discovery-based research

Morphology-informed FOV selection or whole-slide imaging

Onboard AI cell segmentation

SMA KI67 CD68 CD4 CD8 CD11c PD1 DAPI

## 109 proteins and 1000 RNAs in 1.4 million cells

Single-cell **protein** expression

CD4 CD8 CD40 KI67 TBET HLA-DRA
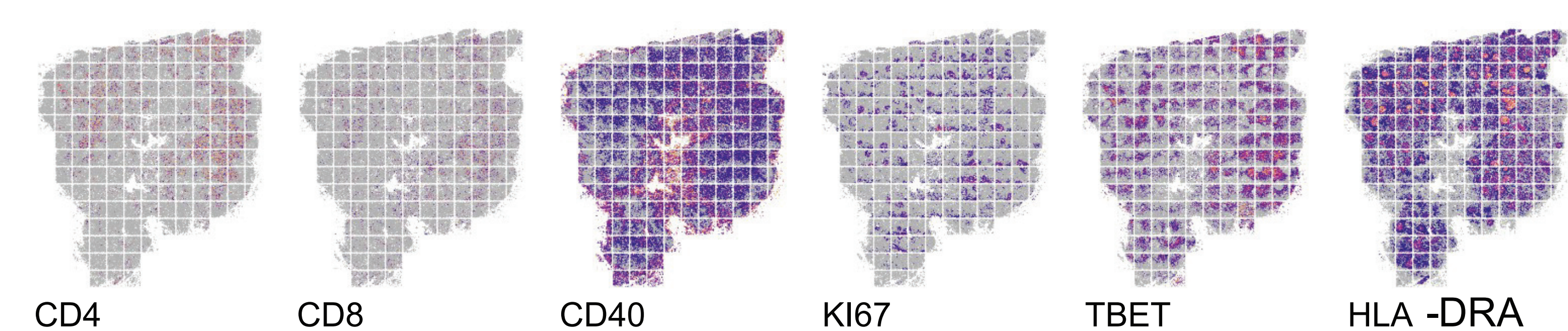
Single-cell **RNA-based** cell typing

activated T cell
cd141-positive mDC
cd1c-positive mDC
cd4-positive T cell
cd8-positive T cell
classical monocyte
DZ B cell
endothelial cell
GC T cell
innate lymphoid cell
intermediate monocyte
lymph vessels
LZ B cell
macrophage
mantle B cell
mast cell
mature NK T cell
memory B cell
naive B cell
NK cell
NK T cell
pDC
plasma cell
regulatory T cell
stromal cell
T cell
T follicular helper cell

1mm

## Subcellular insight into protein localization

CD4 PD1 CD68 ICOS CD8 GZMB DAPI

KI67 HLA-DRA CD20 DAPI

SMA KI67 CD31 CD34 B-cat FN1 DAPI

KI67 CD11c CD68 CD11b DAPI
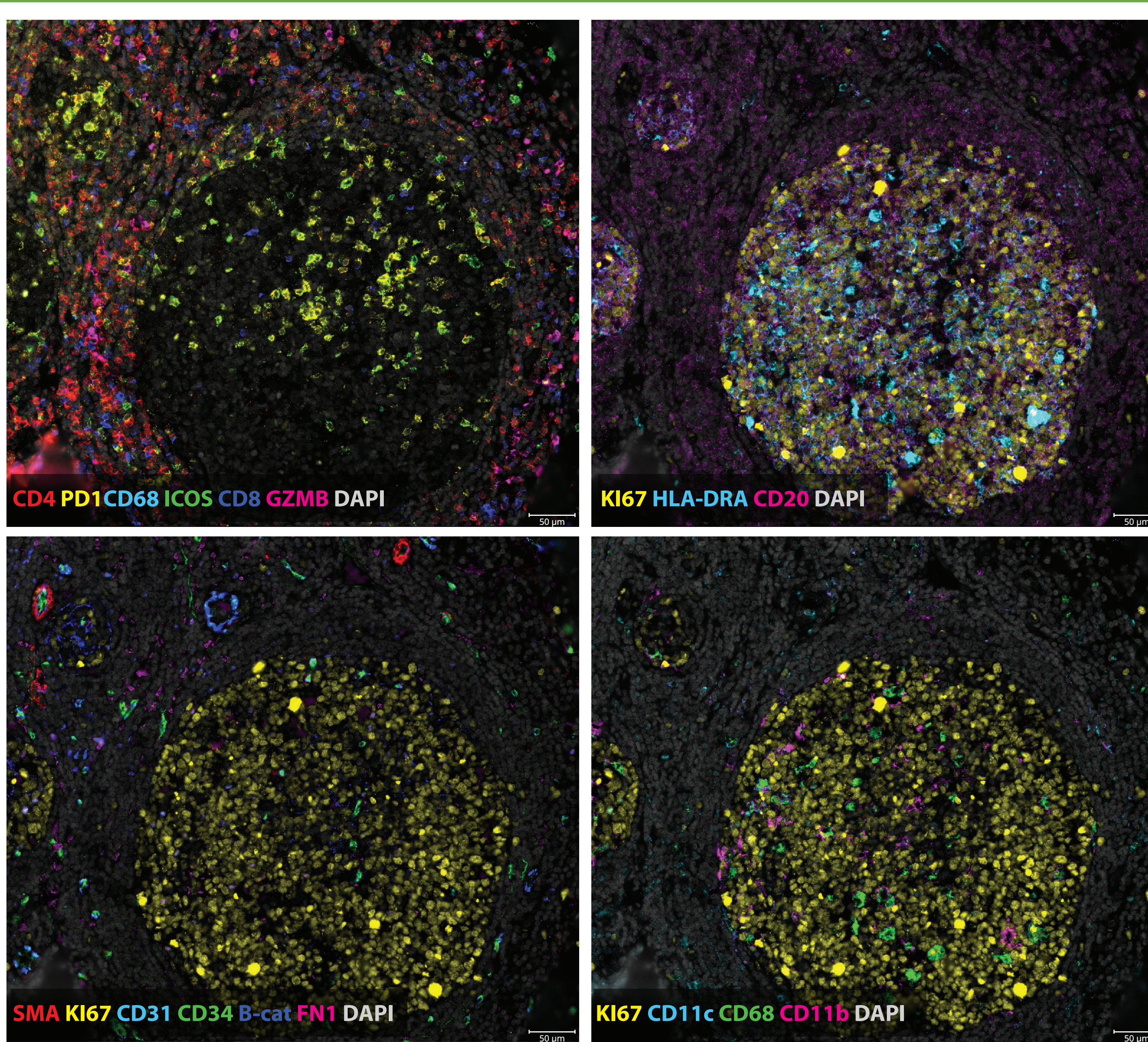
## Light and dark zone B cells have differential RNA expression

RNA-based cell typing demonstrates differential expression between immune cell populations

GC TfH cell

LZ B cell

KI67 CD83 CD48 IL18 CXCR4 CD86

DZ LZ

GeoMx WTA matched germinal centers

Differential expression of LZ B cell (CD83) and DZ B cell (CXCR4) transcripts

CosMx RNA shows CD86 from LZ B cells colocalize with CD28+ GC TfH cells
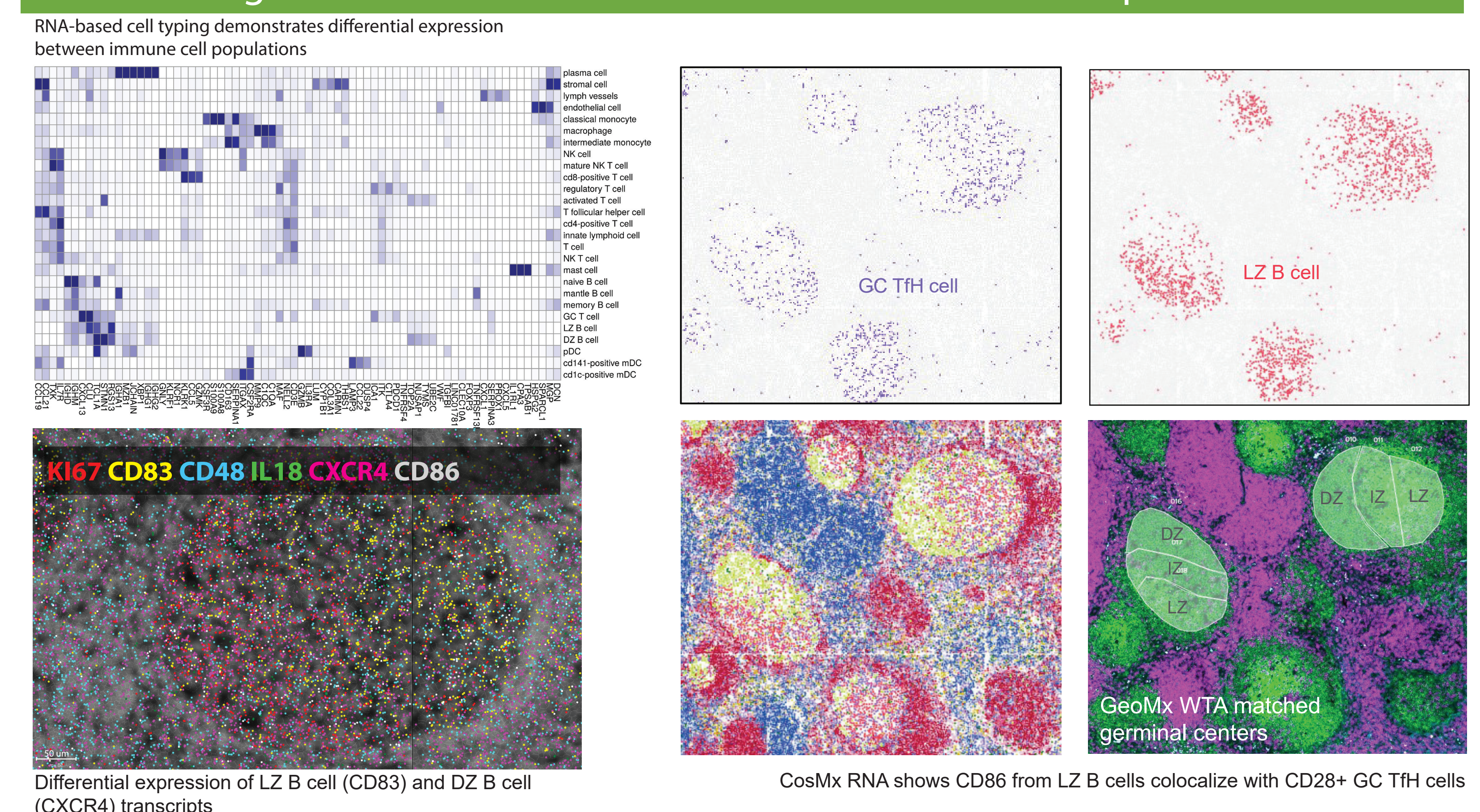
## GeoMx provides whole-transcriptome measurement of lymph node spatial biology

3-zone profiling with GeoMx

Light Zone
Intermediate Zone
Dark Zone

KI67/CD20/DNA
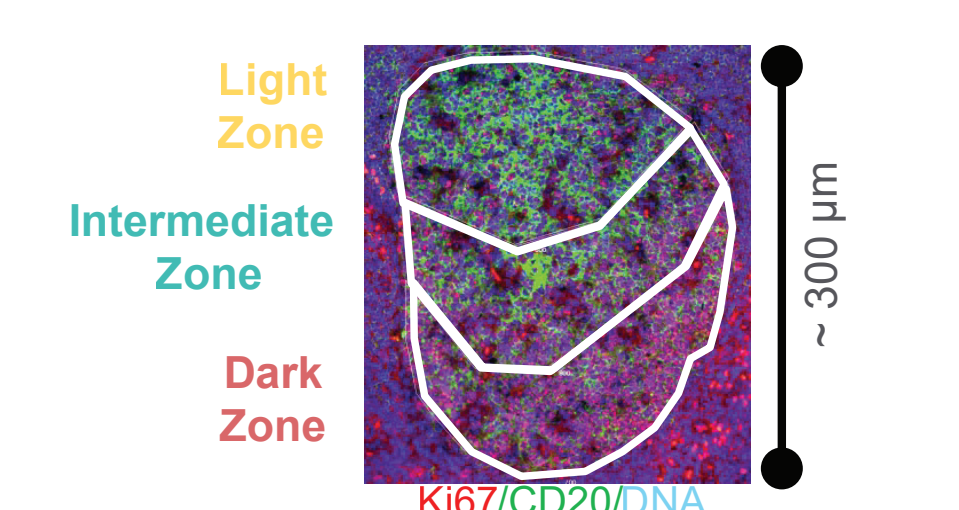
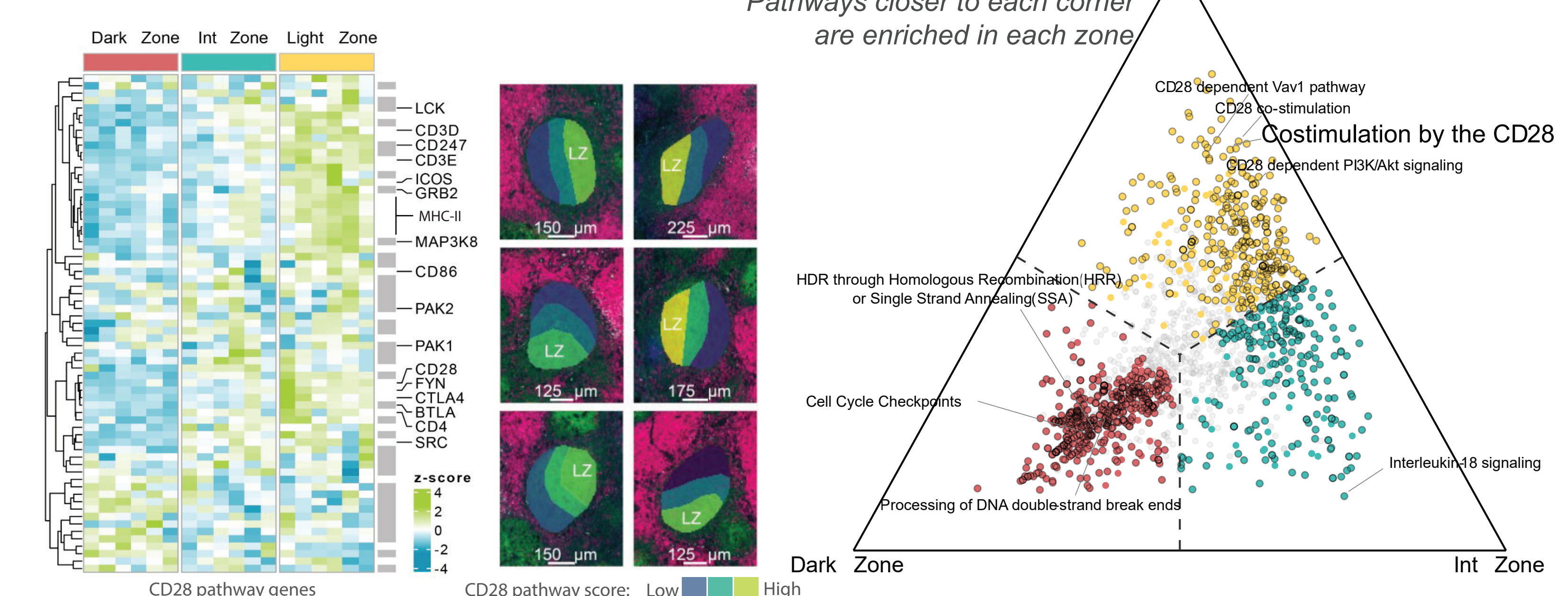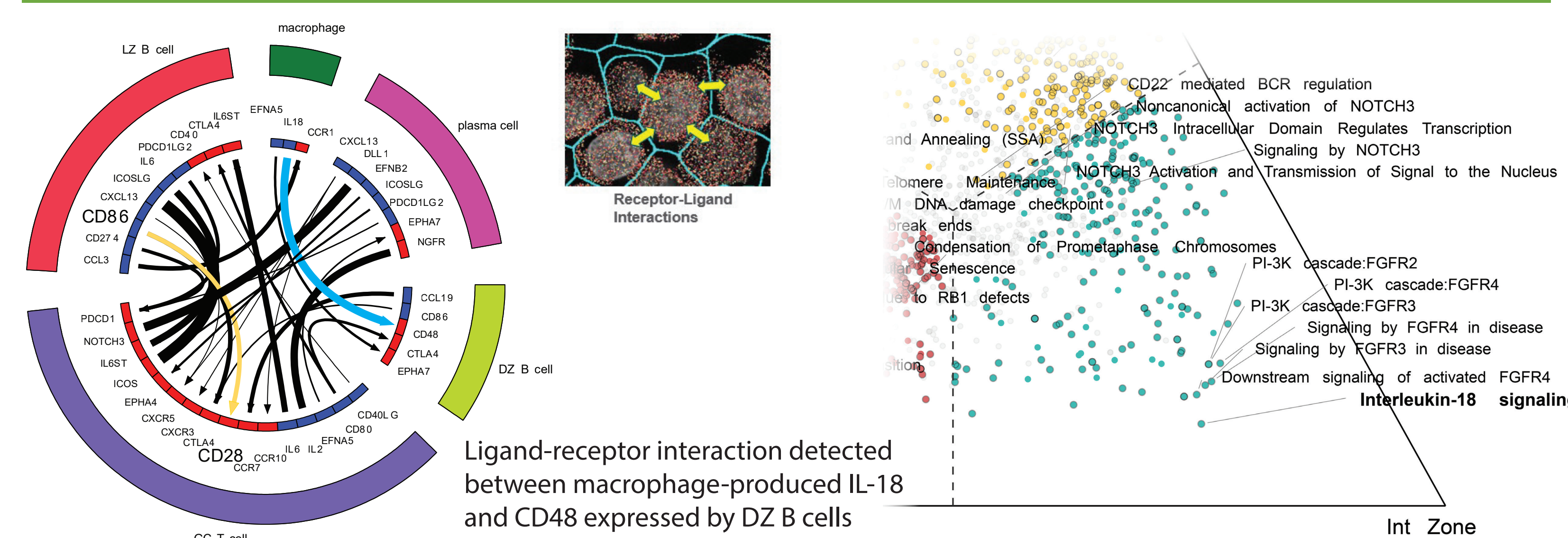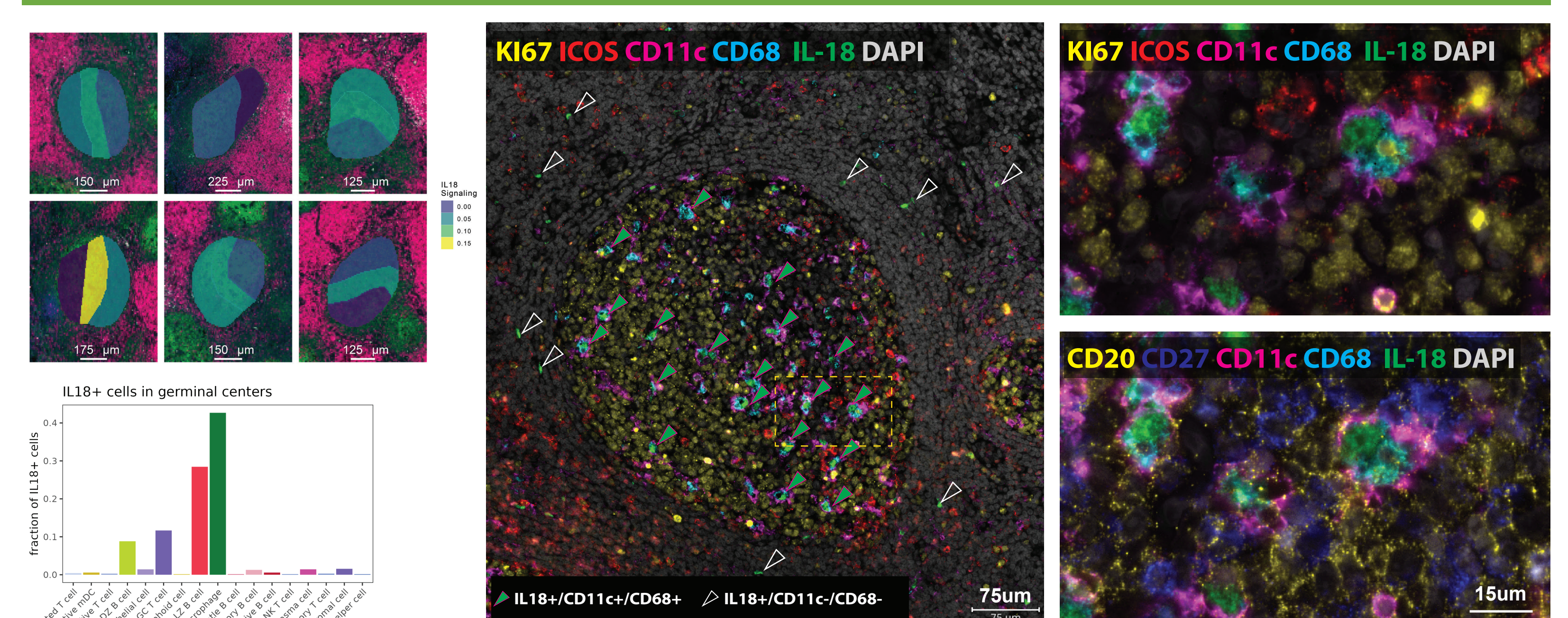- T-cell response pathways enriched in the light zone
- B-cell proliferation pathways enriched in the dark zone
- IL-18 signaling is enriched in the intermediate zone

*Pathways closer to each corner are enriched in each zone*

Light Zone

CD28 dependent Vav1 pathway
CD28 co-stimulation
Costimulation by the CD28
CD28 dependent PI3K/Akt signaling

HDR through Homologous Recombination (HRR) or Single Strand Annealing (SSA)

Cell Cycle Checkpoints

Interleukin-18 signaling

Processing of DNA double-strand break ends

Dark Zone                    Int Zone

CD28 pathway genes
CD28 pathway score: Low | High

## CosMx yields a high-resolution map of germinal center cell interactions

LZ B cell
macrophage
plasma cell

CD86

CD28

DZ B cell

GC T cell

Receptor-Ligand Interactions

Ligand-receptor interaction detected between macrophage-produced IL-18 and CD48 expressed by DZ B cells

CD22 mediated BCR regulation
Noncanonical activation of NOTCH3
NOTCH Intracellular Domain Regulates Transcription
Signaling by NOTCH3
Annealing (SSA)
Activation and Transmission of Signal to the Nucleus
DNA damage checkpoint
PI-3K cascade:FGFR4
Condensation of Prophase Chromosomes
PI-3K cascade:FGFR3
Senescence
Signaling by FGFR4 in disease
response to RB1 defects
Signaling by FGFR3 in disease
Downstream signaling of activated FGFR4
**Interleukin-18 signaling**

Int Zone

## Interleukin 18 signaling at the intermediate zone may drive B-cell differentiation

IL18+ cells in germinal centers

KI67 ICOS CD11c CD68 IL-18 DAPI

KI67 ICOS CD11c CD68 IL-18 DAPI

CD20 CD27 CD11c CD68 IL-18 DAPI

IL18+/CD11c+/CD68+     IL18+/CD11c-/CD68-

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.
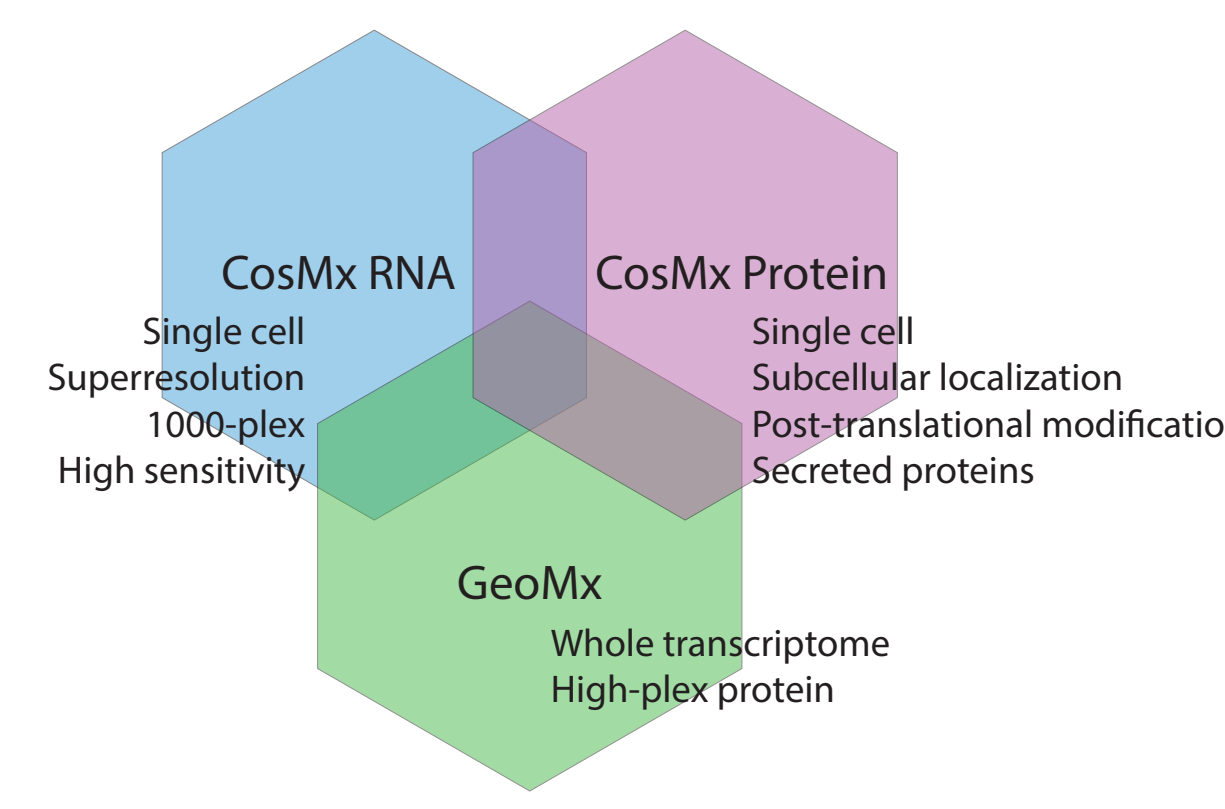
CosMx protein: IL-18 expressed across the tissue, but **expressed specifically by macrophages** inside the germinal centers.

## Conclusions

The **integration of GeoMx and CosMx assays** provides a complete picture of the spatial aspects of immune cell interaction and maturation in a section composed of **1.4 million cells**.

CosMx reveals **unique germinal center cell types**, **ligand-receptor interactions** and **protein localization**.

These **cell-level** differences in spatial protein and gene expression drive the **transcriptome-level** differences observed across regions of the germinal center using GeoMx.

CosMx RNA
Single cell
Superresolution
1000-plex
High sensitivity

CosMx Protein
Single cell
Subcellular localization
Post-translational modification
Secreted proteins

GeoMx
Whole transcriptome
High-plex protein