

# Spatial Single Cell characterization of SIV reservoirs in lymphoid tissues and B cell follicles in rhesus macaques

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## Abstract

HIV cannot be eradicated by antiretroviral therapies (ART) alone. Although lifelong suppression of HIV replication with ART seems possible, side effects, resistance and cost contribute to the necessity of finding an 'HIV Cure'.

### Challenge:

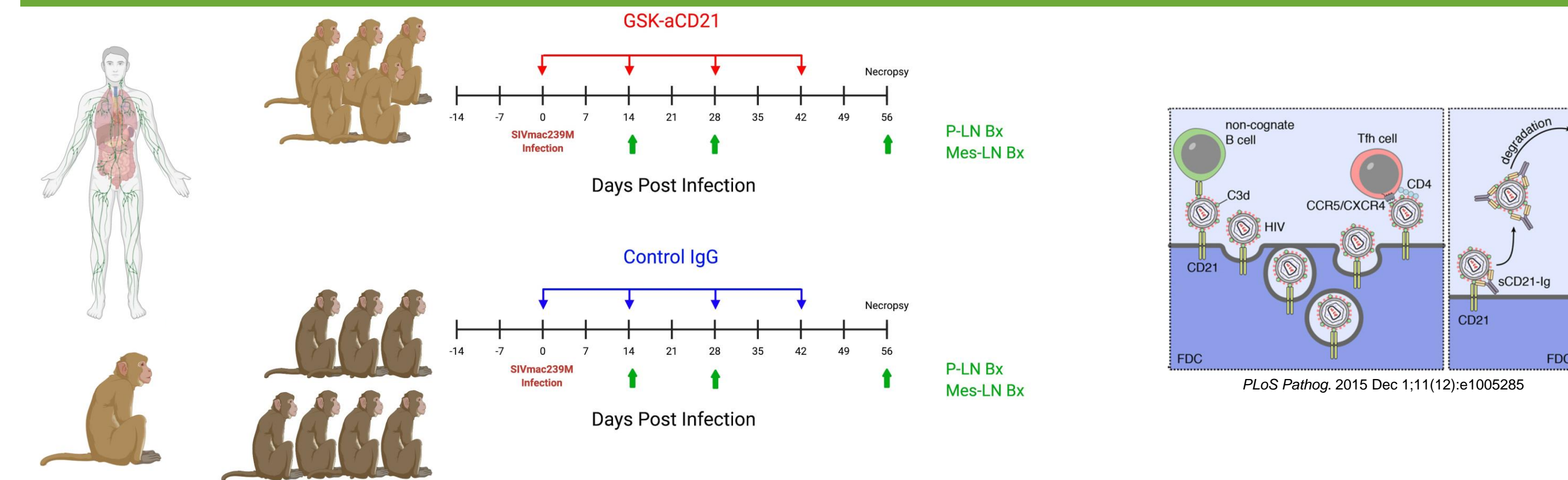
- The major obstacle to eradicating HIV is the persistence of viral reservoirs despite extended ART, which can give rise to recrudescence infection when ART is stopped.
- An understanding of complex spatial host-viral interactions *in situ* that retains important contextual insight into the cellular immune neighborhoods and inflammatory landscapes is needed to define mechanisms driving HIV persistence.

### Results:

- Spatial profiling of lymph nodes from chronically SIVmac239M infected nonhuman primates (NHP; rhesus macaques) with was performed on FFPE tissue sections using NanoString® GeoMx® and CosMx™ platforms.
  - Characterized 1563 RNA or 78 proteins targets at a multicellular level with GeoMx.
  - Characterized 1000 RNA targets (including viral RNAs) at a single cell level with CosMx.
- GeoMx profiling of B cell follicle TME in SIV+ lymph nodes demonstrated that regions with active viral infection and storage revealed:
  - Altered genes including IL-4, IL-17, and HLA signaling, interferon/inflammation, and B cell development pathways
  - >200 differentially expressed genes that would not have been observed with non-spatial approaches.
- CosMx profiling of serial FFPE sections from the same samples augmented the GeoMx observations and provided further elucidation of the transcriptional profile at single cell resolution.
  - Measurement of SIV viral particles with subcellular resolution enables discrimination of productively infected cells from uninfected SIV reservoirs.
  - Spatial context analysis on individual cells accurately reproduces the tissue architecture and immune neighborhoods of the lymph node.
  - First measurements of the microenvironment alterations surrounding individual SIV infected cells within the different lymph immune neighborhoods.

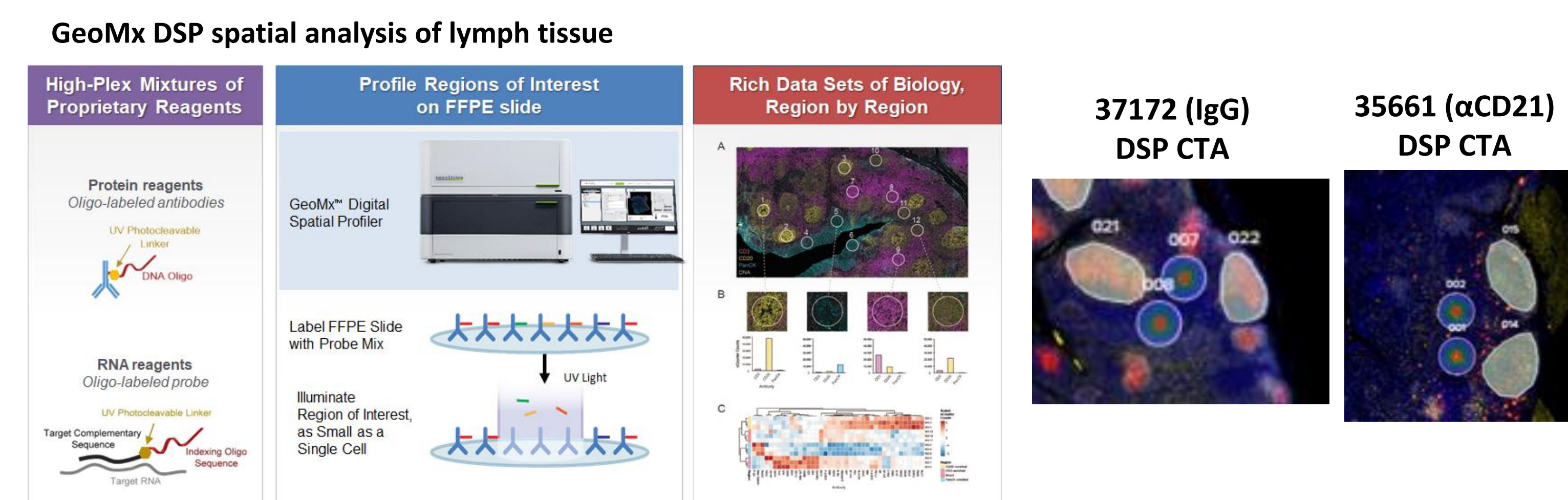
This spatial profiling provides insight beyond what is available from bulk analyses and shows the role of viral reservoirs in the context of their cellular immune neighborhoods. These results improve our understanding of NHPs as a model system for HIV and may lead to potential treatment options to eliminate the entirety of the viral reservoir in the infected individual.

## Sample and study design

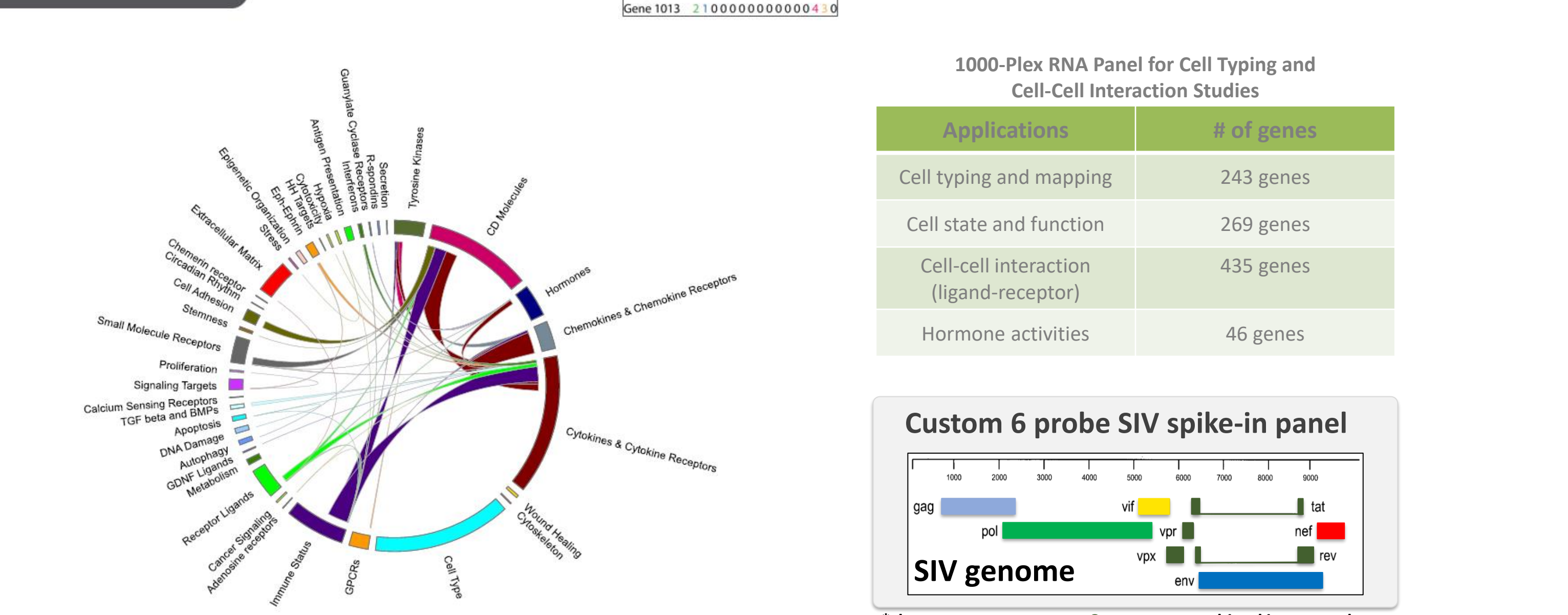
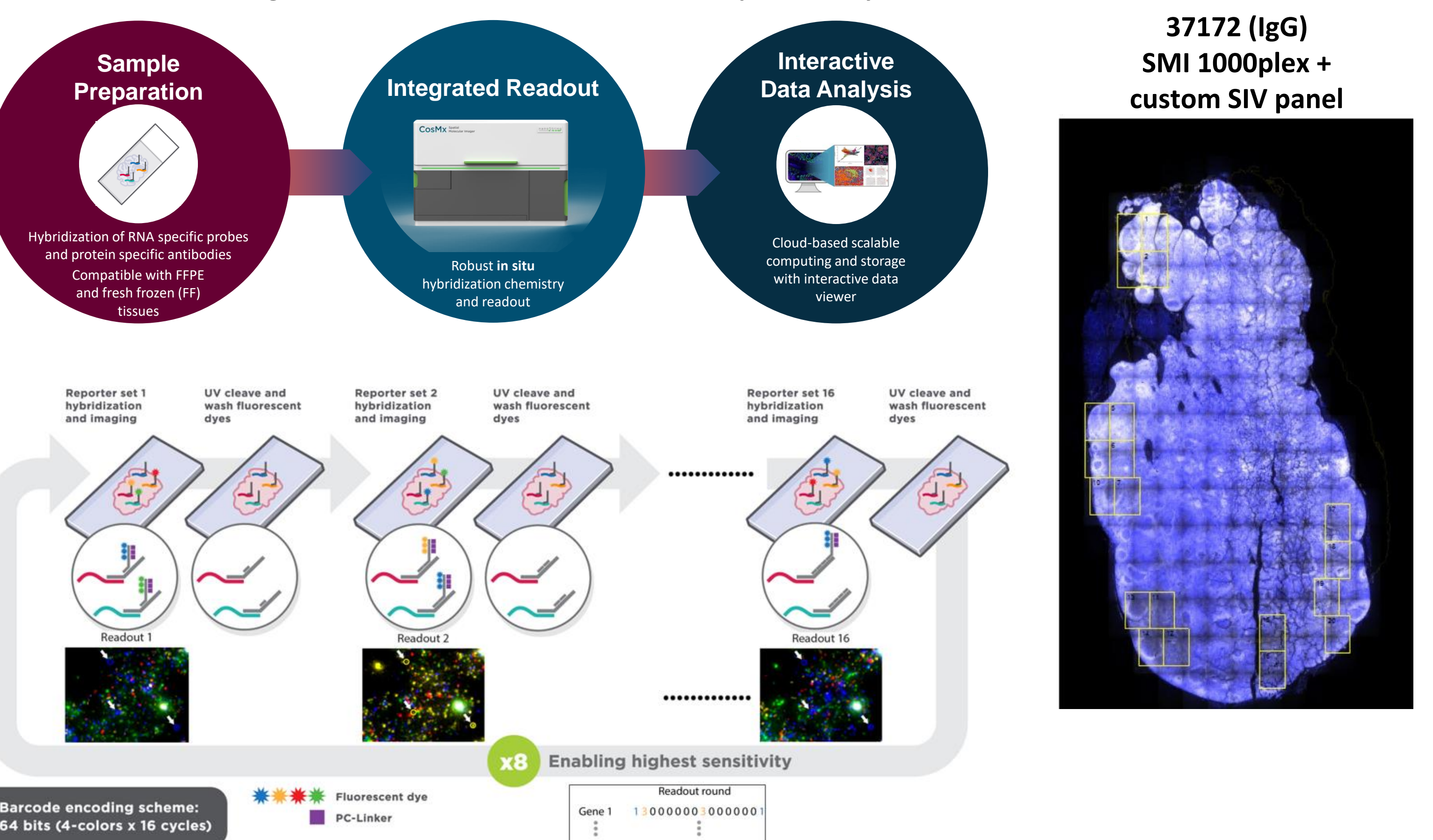


SIV infection of rhesus macaques (RM) is important model of HIV infection of humans. RMs were placed into two study groups (as shown above), the day 56 (week 8) post SIV infection (and treatment) lymph nodes were used to make FFPE blocks. Serial 5 µm sections of FFPE lymph nodes were utilized for spatial transcriptomic analysis on two nanoString Technologies platforms; the GeoMx DSP and the CosMx SMI. Samples include 6 SIV infected lymph nodes where three had been treated with an αCD21 blocking antibody (red arrows) and three with an IgG (black arrows) control antibody. The SMI panel consisted of the RNA 1K-plex panel with a custom spike-in probe set for 9 SIV genes. The tissues were stained with a morphology kit including B2M/CD298, PanCK, CD45, and CD20 antibodies to help select applicable Field of Views (FOVs) for analysis.

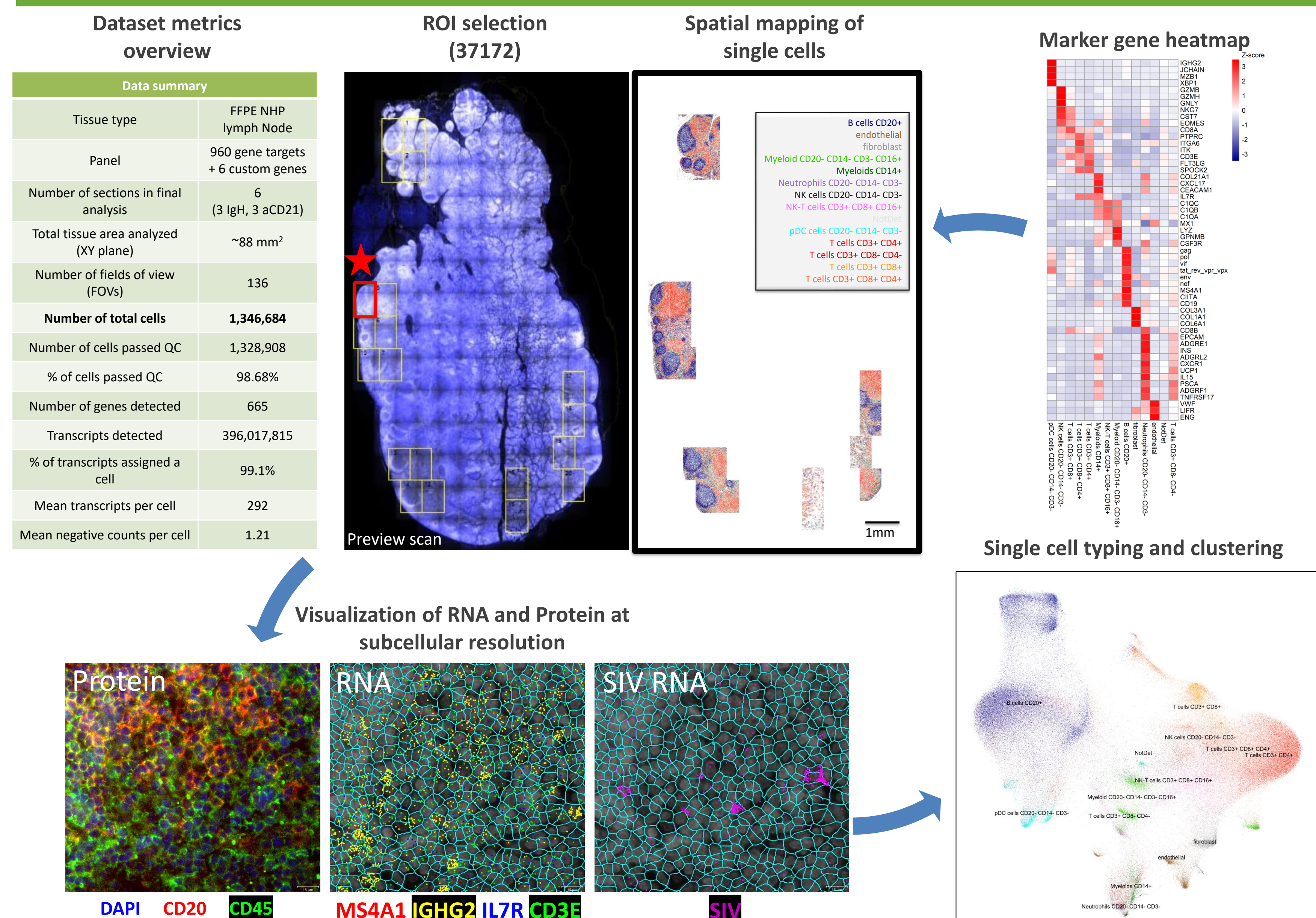
## Overview of the CosMx and GeoMx assays



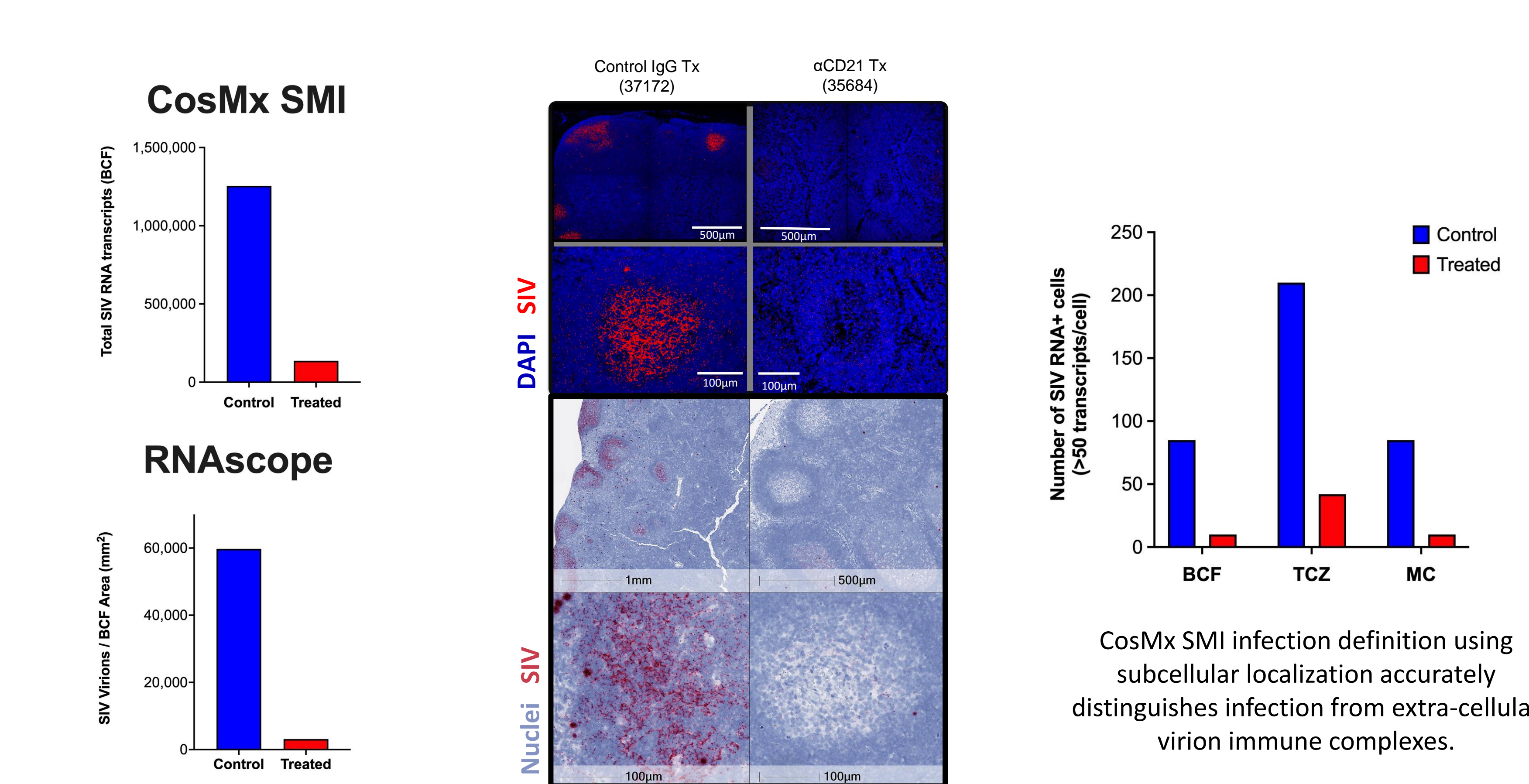
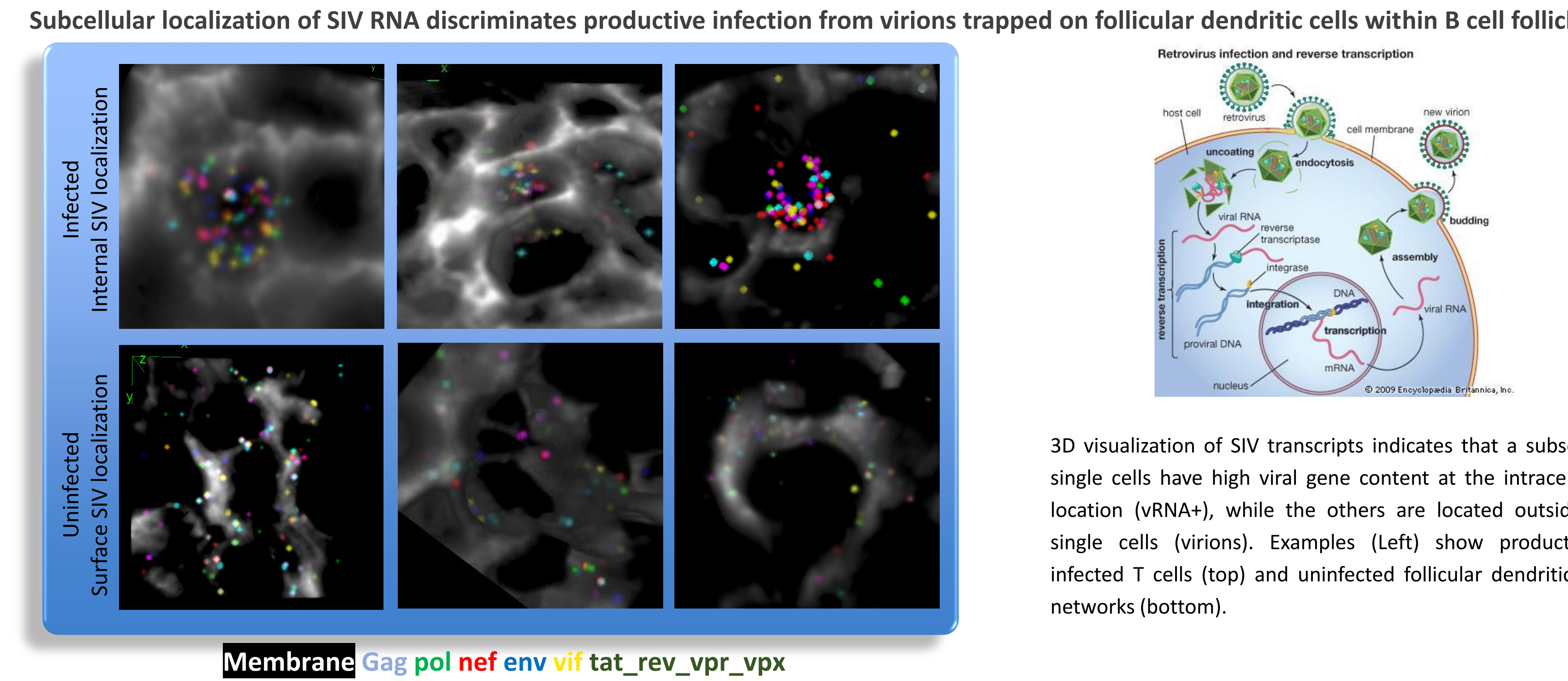
### CosMx SMI is a single instrument solution for subcellular spatial analysis



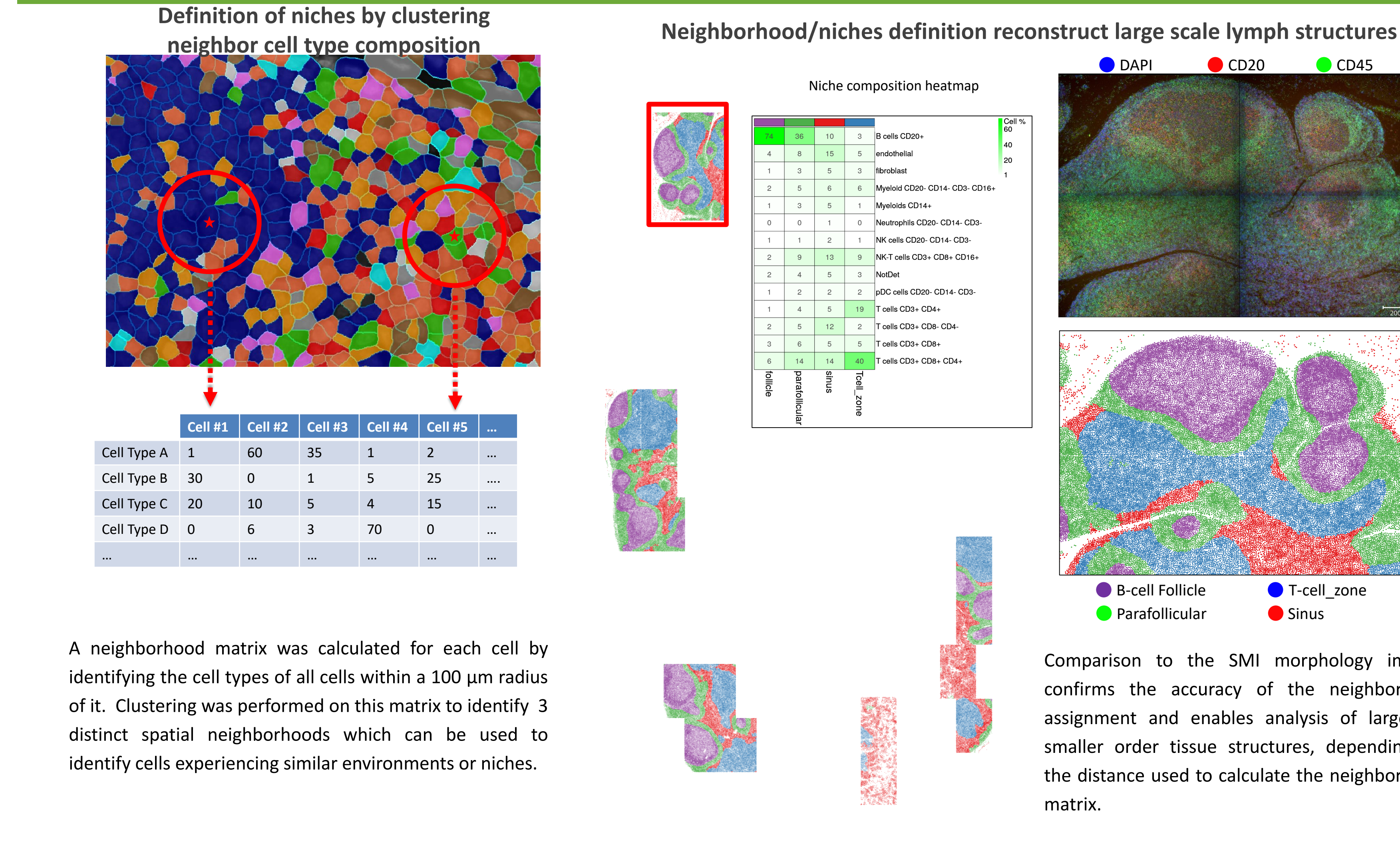
## Workflow: SMI visualizes spatially resolved 1K genes for 6 SIV+ NHP lymphoid tissues



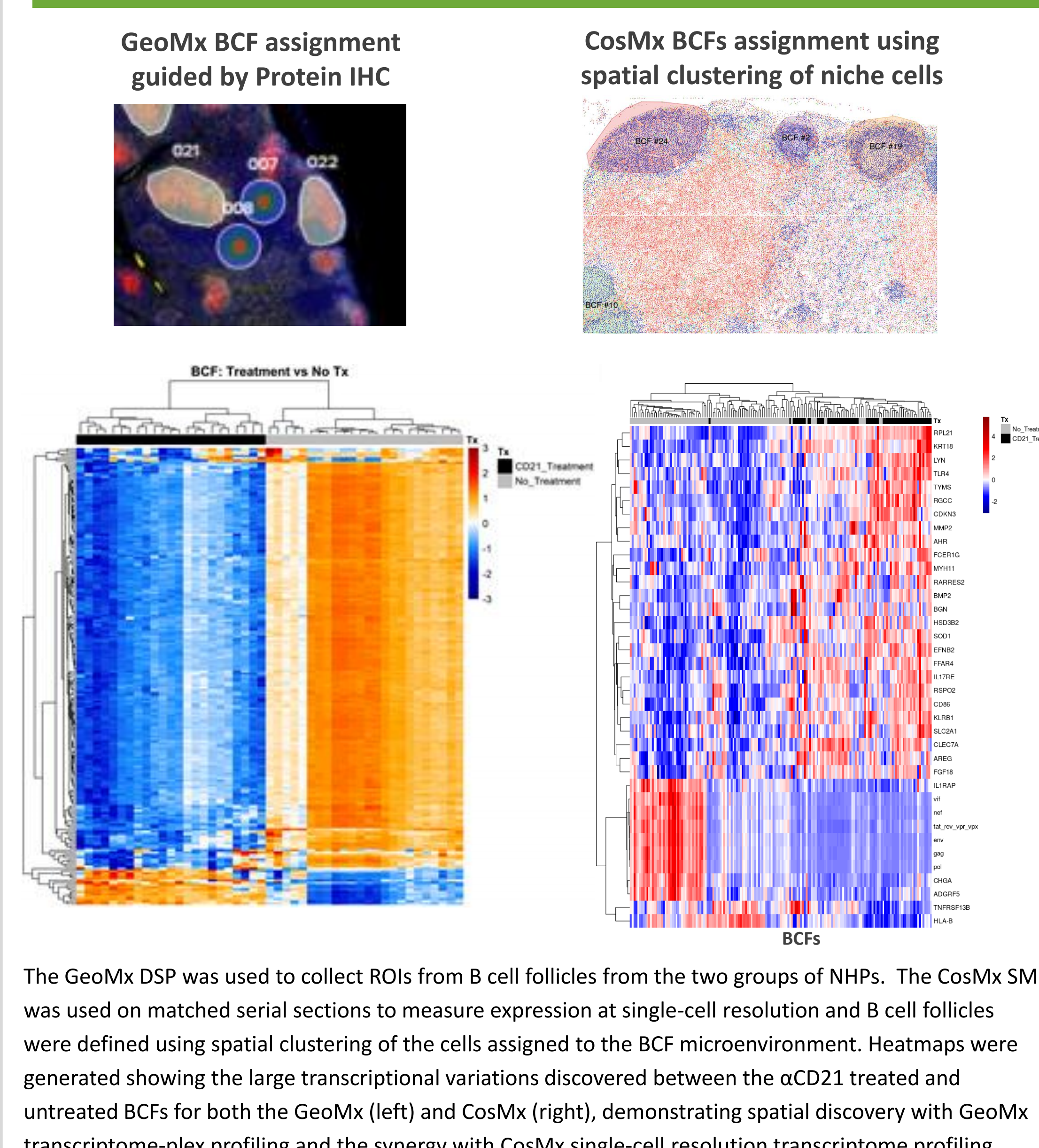
## SMI visualizes SIV genes in 3D



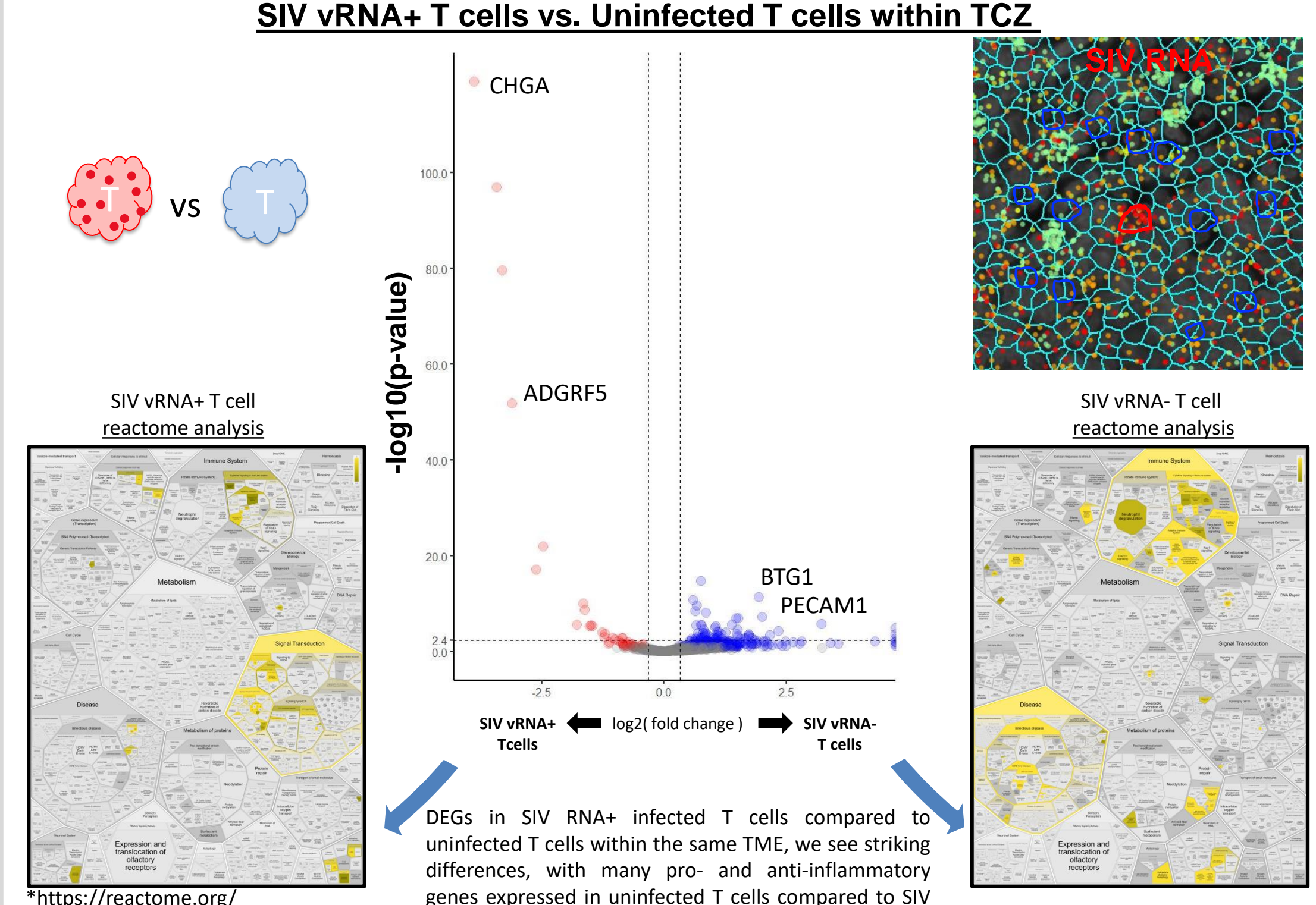
## Spatial neighborhood clustering for analysis within tissue niches



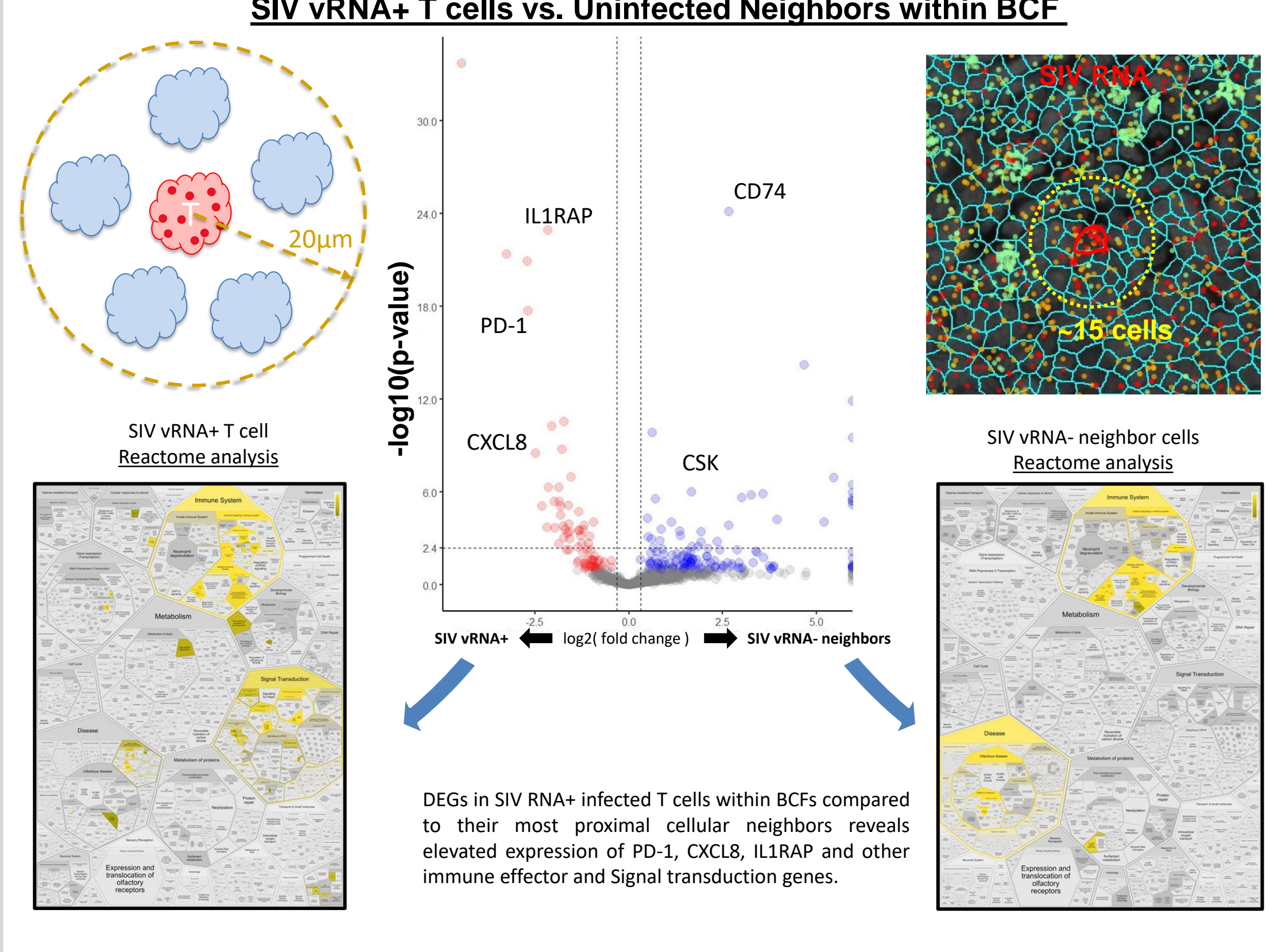
## Profiling of B-cell follicles by GeoMx DSP and CosMx SMI



## CosMx: Differential expression at single cell level within unique tissue niches



## SIV vRNA+ T cells vs. Uninfected Neighbors within BCF



## Conclusions

- The CosMx Spatial Molecular Imager (SMI) is a single instrument solution for subcellular spatial analysis: SMI provides sub-cellular resolution of 1000+ plex transcriptomic information and SIV viral genes.
- Both GeoMx and CosMx SMI spatial platforms provide complementary spatial information: GeoMx DSP profiles up to the whole transcriptome, while CosMx SMI provides sub-cellular resolution of biological targets.
- Sub-cellular resolution spatial transcriptomics enables discrimination of single-infected cells from virions located in the extracellular space of single cells (reservoirs).
- Using single cell spatial transcriptomic analysis we performed the first characterization of SIV infection effects on BOTH the individual infected cells and their local microenvironment.

