Neighborhoods & Niches: Spatial Characterization of SIV Reservoirs in NHP Lymphoid Tissues

CASE STUDY

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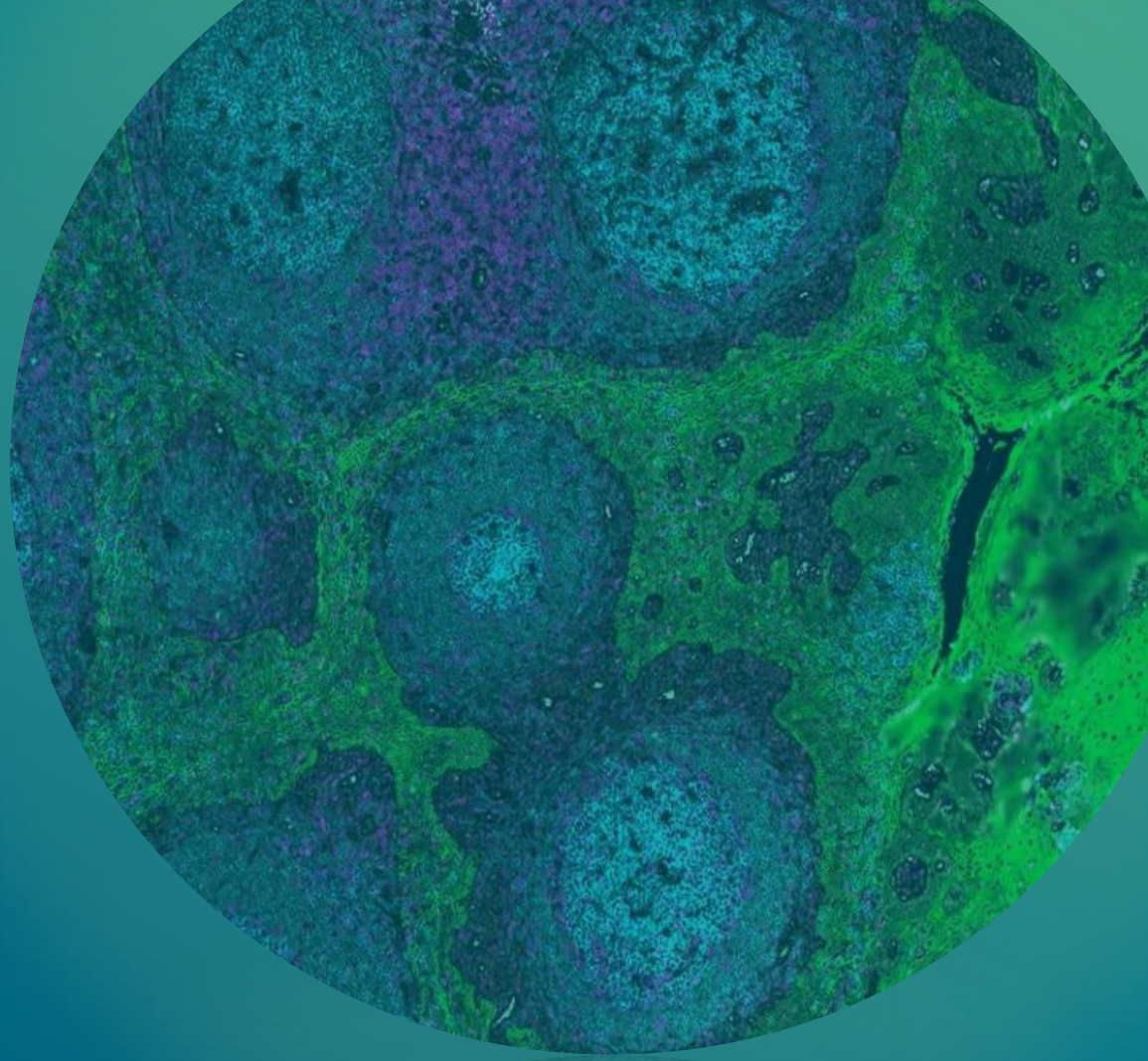

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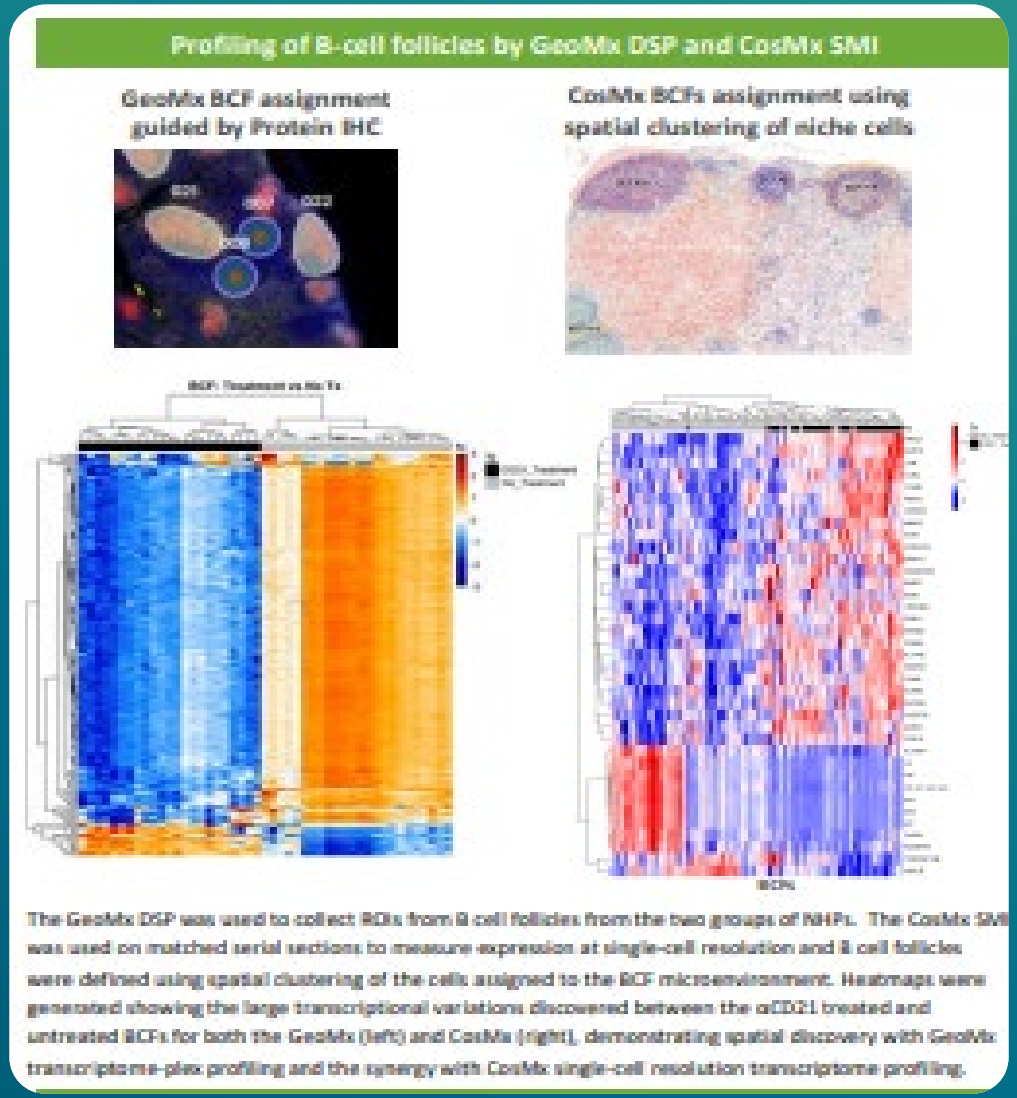
Spatial Landscape of productively infected from uninfected Cells in SIV reservoirs

- Spatial imaging and profiling provide **insight beyond what is available from bulk analyses** and show 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



The GeoMx DSP was used to collect ROIs from B cell follicles from the two groups of NHPs. The CosMx SM was used on matched serial sections to measure expression at single-cell resolution and B cell follicles were defined using spatial clustering of the cells assigned to the BCF microenvironment. Heatmaps were generated showing the large transcriptional variations discovered between the αCD21 treated and untreated BCFs for both the GeoMx (left) and CosMx (right), demonstrating spatial discovery with GeoMx transcriptome-plex profiling and the synergy with CosMx single-cell resolution transcriptome profiling.

Spatial Characterization of SIV Reservoirs

Scan here to download or learn more.

Background

- Persistent cure against HIV infection is still missing

Research question

- What is the impact of αCD21 treatment, attenuating the binding and retainment of virions on Follicular Dendritic cells (FDCs) in the lymph nodes?
- What are the spatial host-viral interactions that allow HIV reservoirs to persist in lymph nodes?

Experimental design

- Compare transcriptional profiles in infected versus uninfected lymph nodes (FDCs)

Study Details	
Research area:	Infectious Diseases, HIV
Organism and tissue:	NHP (Rhesus macaques) lymph node
Sample type:	FFPE (5um sections)
Instrument:	GeoMx®, CosMx™
Analyte:	RNA
Readout:	NGS, CosMx
Assay:	GeoMx: CTA, CosMx: 1000-plex SMI panel with spike-in for 9 SIV genes probes
Journal, Year:	Presentation at AGBT 2022

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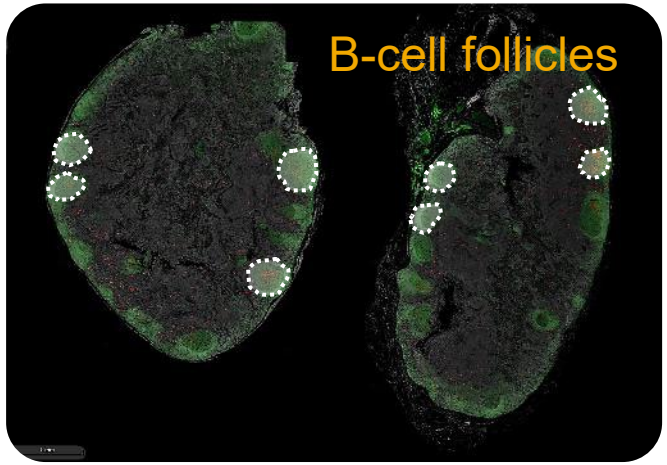
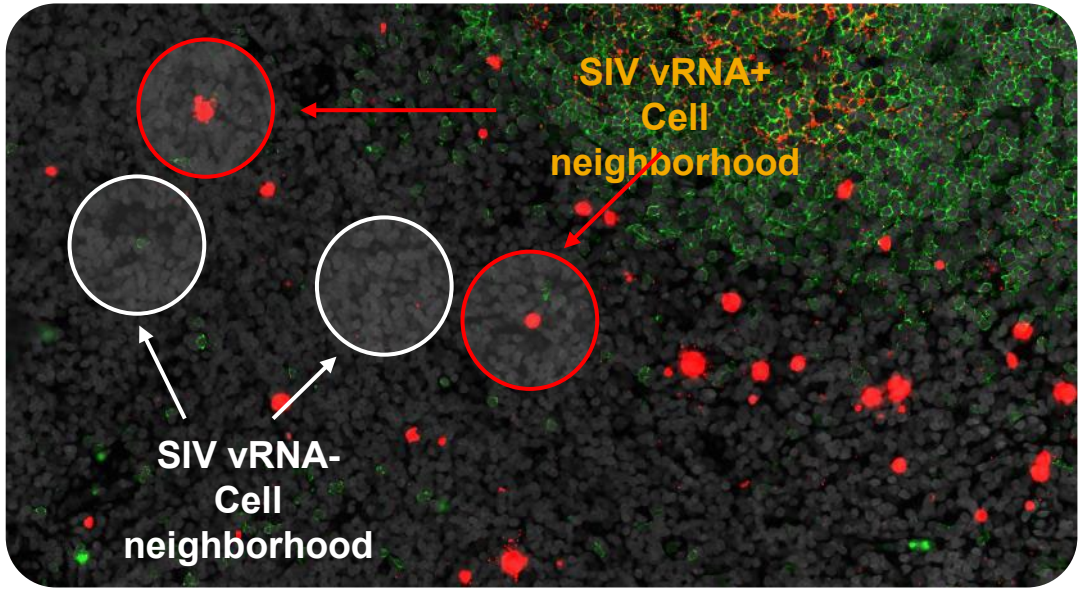
Surveying the Right Neighborhoods to Understand the Biology of SIV Infected Cells in Treated Vs Control Animals

Morphology markers for ROI selection & profiling with the GeoMx® DSP platform

Segment	Target	Channel
Syto 13	DNA	488nm
B-cells	CD20	532nm
Viral particles	SIV vRNA	647nm

- αCD21 treated versus control animals
- Virus positive versus virus negative regions





CD20 / SIV vRNA / Syto 13

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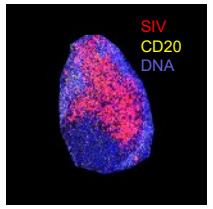
Immune Profiles of SIV RNA+ and adjacent SIV- Cellular Neighborhoods

Control



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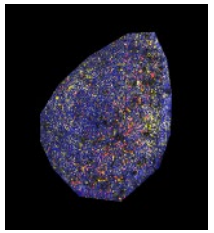
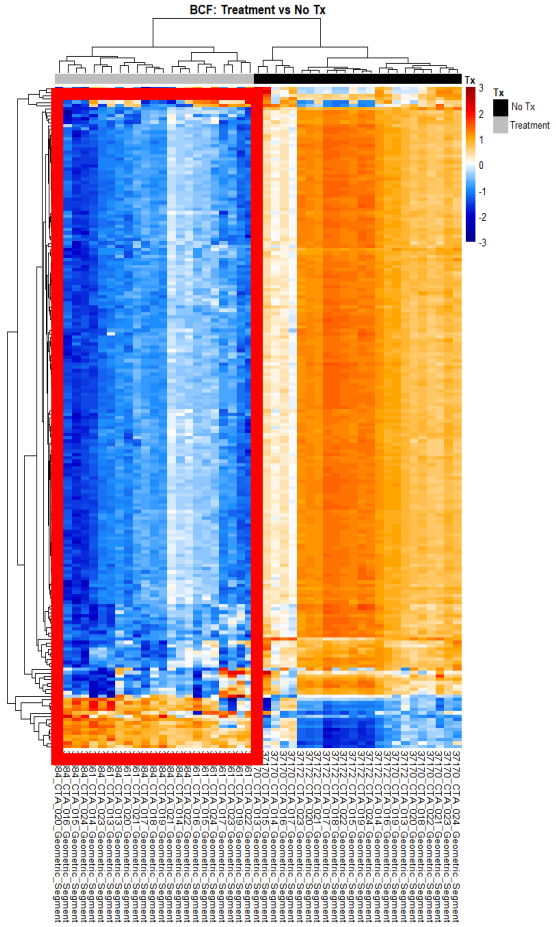




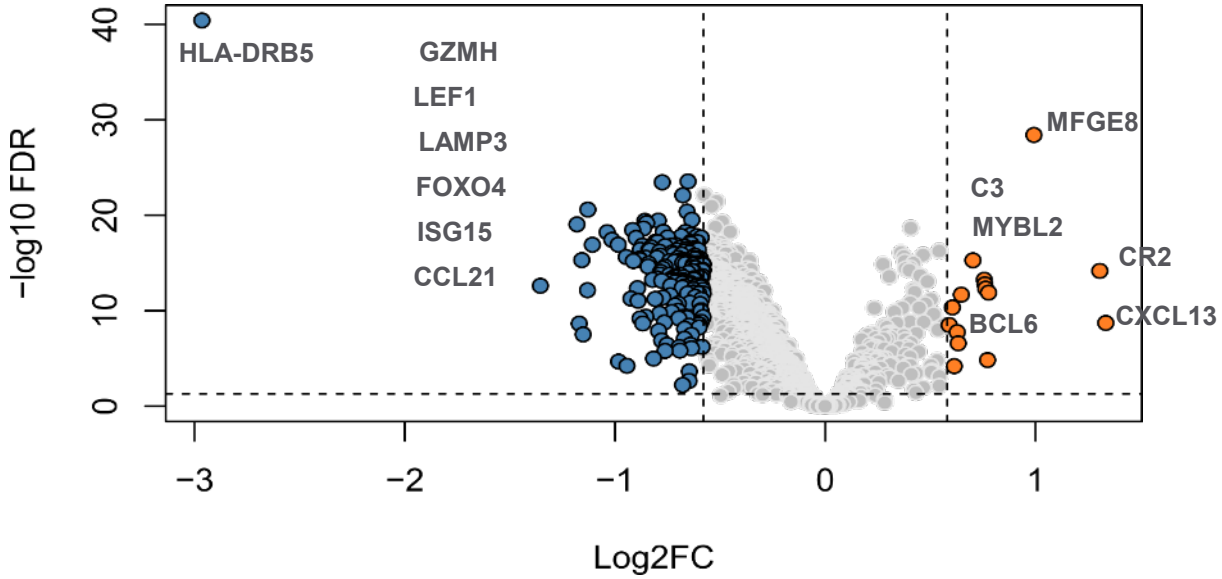
Treatment results in substantial

Down-Regulation:

- MHC-I and MHC-II PRES.
- DC FUNCTION
- CTL/NK CELL FUNCTION
- T CELL ACTIVATION
- TYPE 1 IFN RESPONSES

Up-Regulation:

- CELL ADHESION
- CELL MIGRATION
- COMPLEMENT
- CELL PROLIFERATION
- B CELL SIGNALING



GeoMx profiles reveal gene expression changes with treatment

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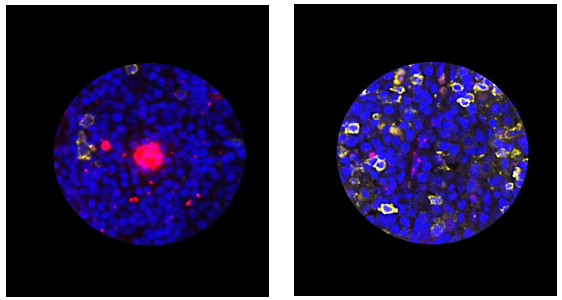
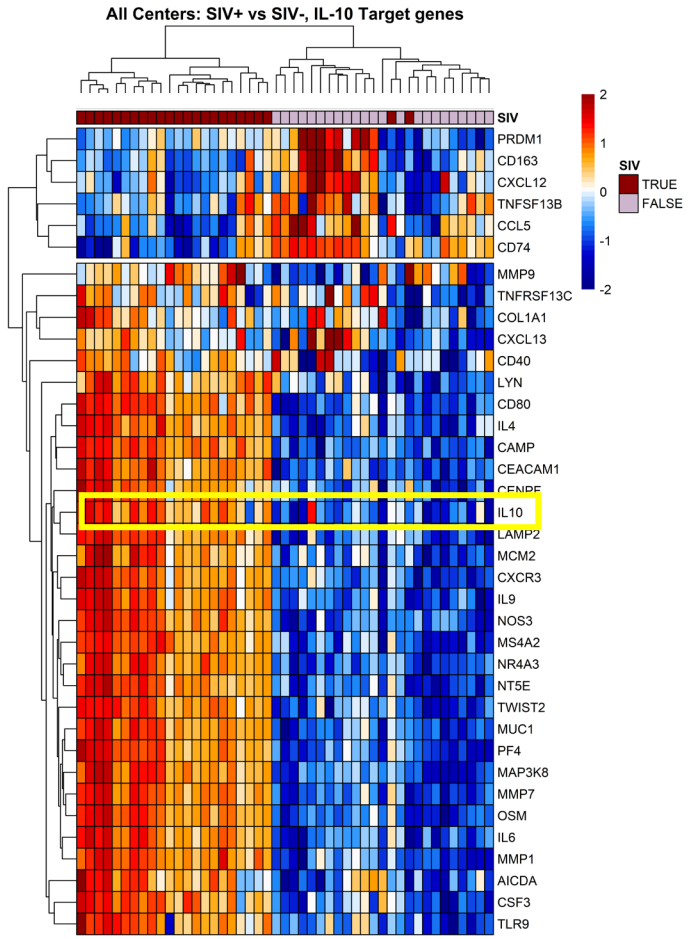
GeoMx Profiling Reveals Immune Environments of Infected Cells

SIV+ Center **SIV- Center**

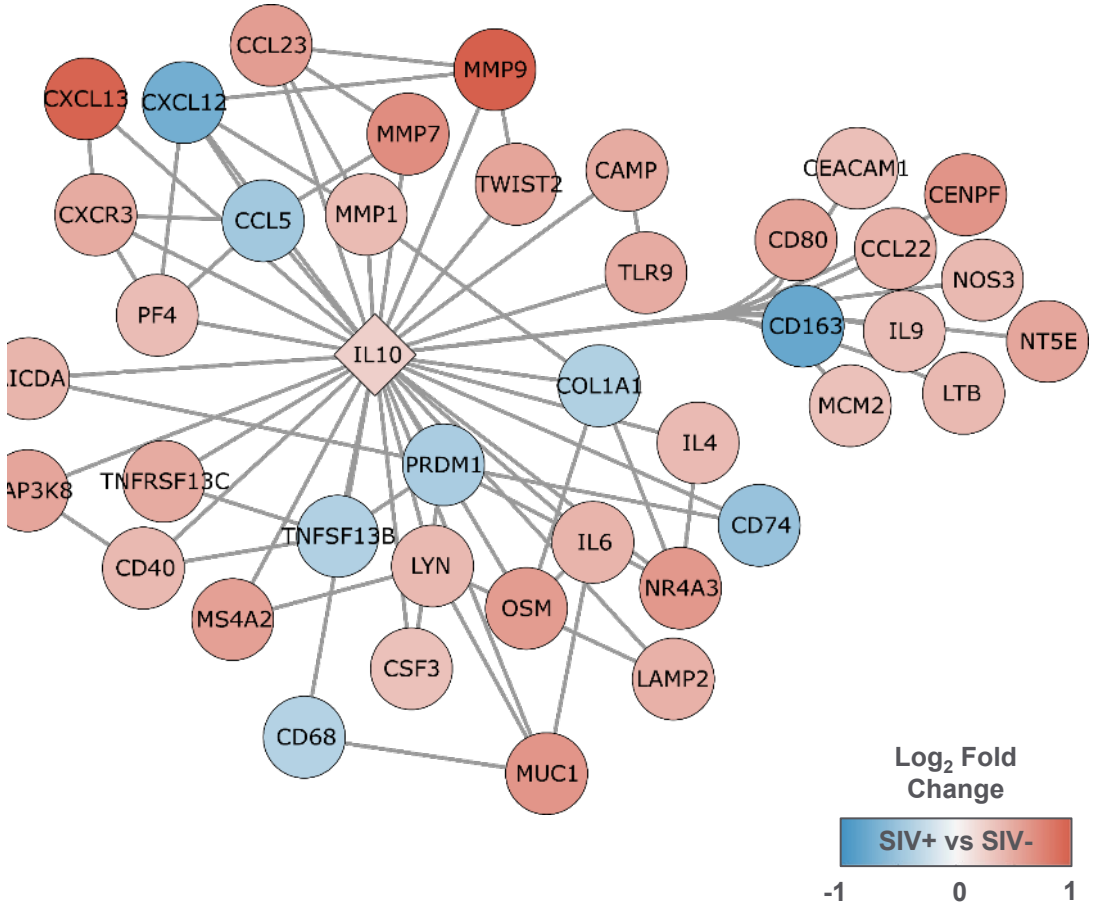


IL-10 was a significant upstream regulator ($p=2.14 \times 10^{-23}$) for genes DE between SIV+ and SIV- centers.

Heatmap of IL-10 and its targets shows that these genes are primarily up-regulated in SIV+ centers.



Upstream Regulator & Targets: Ingenuity Pathway Analysis



Differential Expression Cutoffs: adjusted p-value < 0.05, absolute Fold Change > 1

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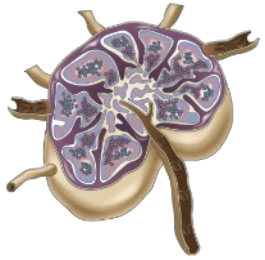
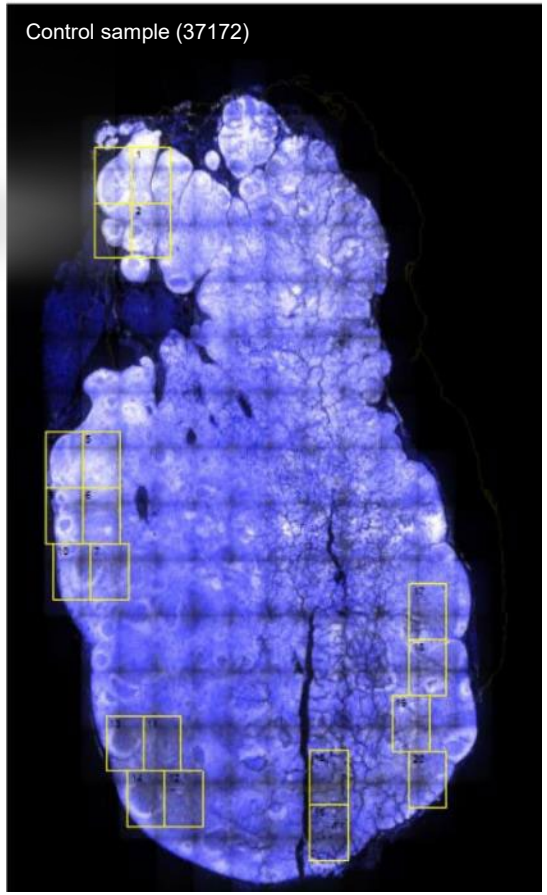
Spatial Molecular Analysis of Six SIV + Neighborhoods at Single and Sub-Cellular Resolution with CosMx™ SMI

CosMx™
Spatial Molecular Imager

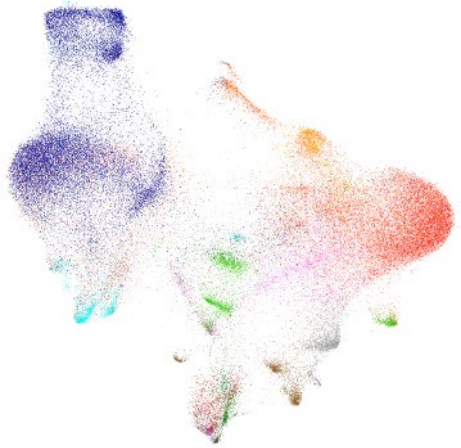


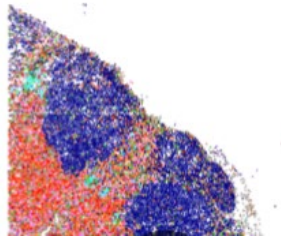
FOV selection



CosMx SMI Analysis / cell segmentation

UMAP projection

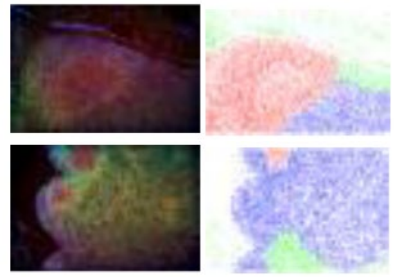




Cell typing

- CD14+ Myeloid cells
- CD20+ B cells
- CD16+ Myeloid cells
- Neutrophils
- NK cells
- pDCs
- CD4+CD8- T cells
- CD8+CD4- T cells
- CD4-CD8- T cells
- CD4+CD8+ T cells
- NK T cells
- Endothelial cells
- Fibroblasts
- Not Determined

Neighborhood Maps



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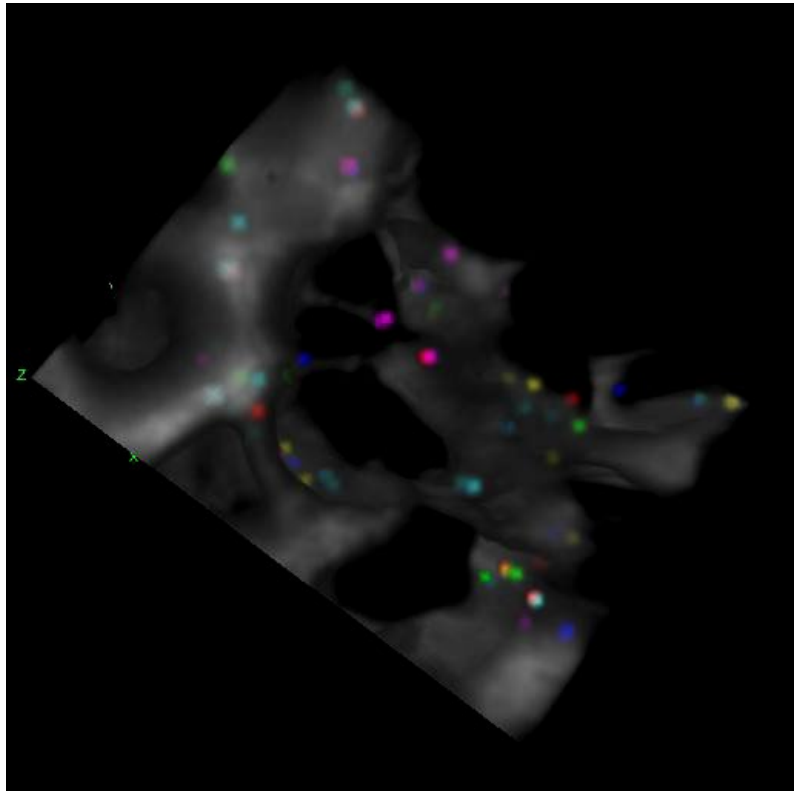
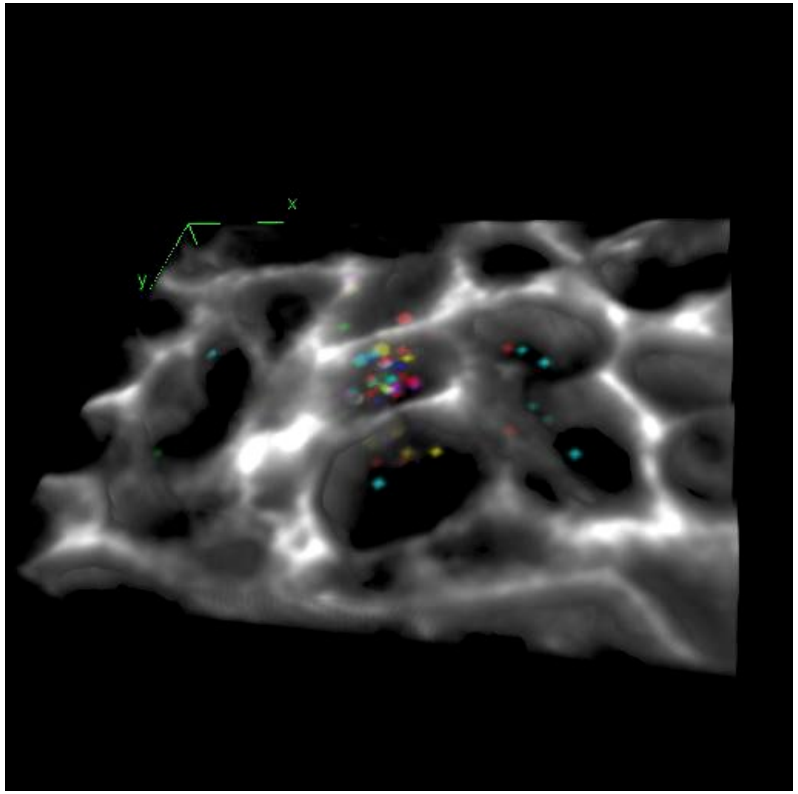
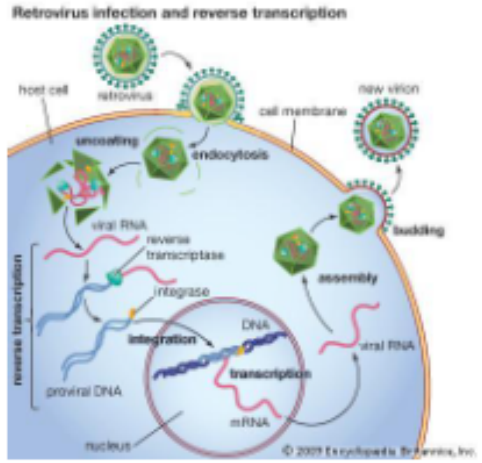
CosMx™ Resolves Infection Status at Single & Sub-cellular Level

3- Dimensional SMI Subcellular localization of SIV RNA discriminates productive infection from virions trapped on follicular dendritic cells within B cell follicles







3D visualization of SIV transcripts indicates that a subset of single cells have high viral gene content at the intracellular location (vRNA+), while the others are located outside of single cells (virions). Examples (Left) show productively infected T cells (top) and uninfected follicular dendritic cell networks (bottom).

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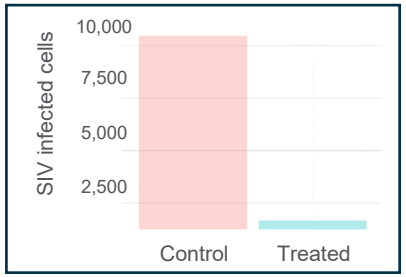
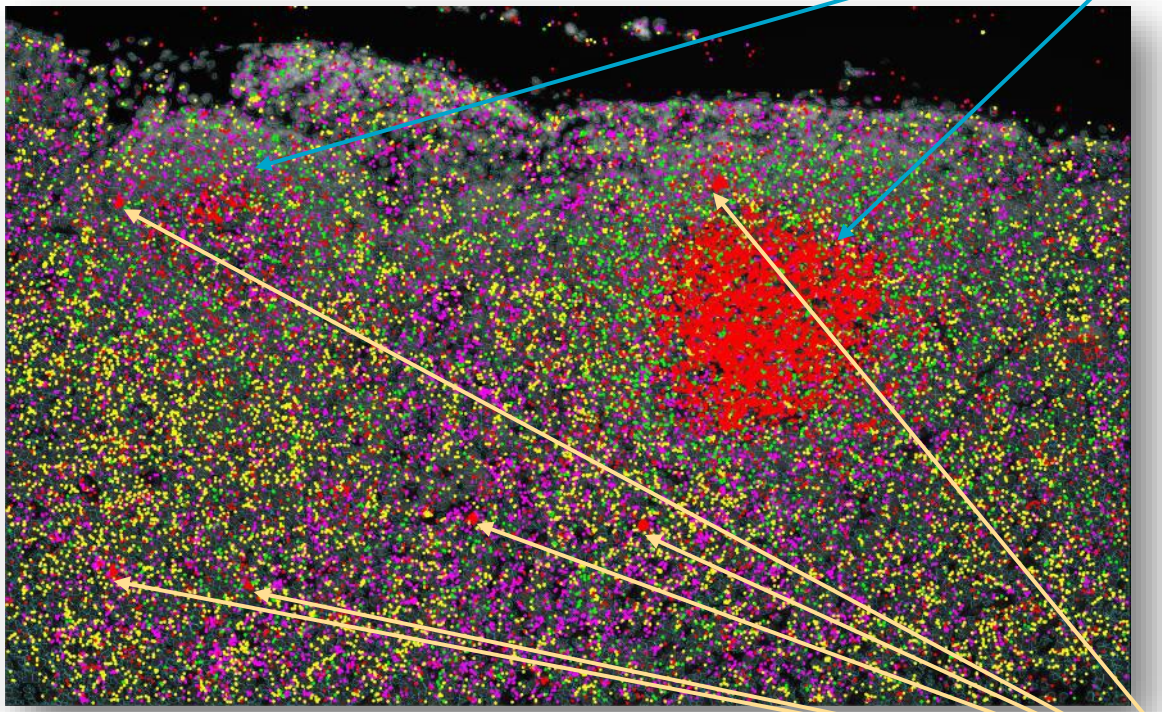
SIV Infected Cells are Significantly Reduced in Treated Tissue

SIV genes (env, nef, pol, …)
MS4A1 (B-cell marker)
CD3 genes (T-cell marker)
MX1 (cellular antiviral response gene)

Viral reservoir in B-cell follicles



Untreated Control



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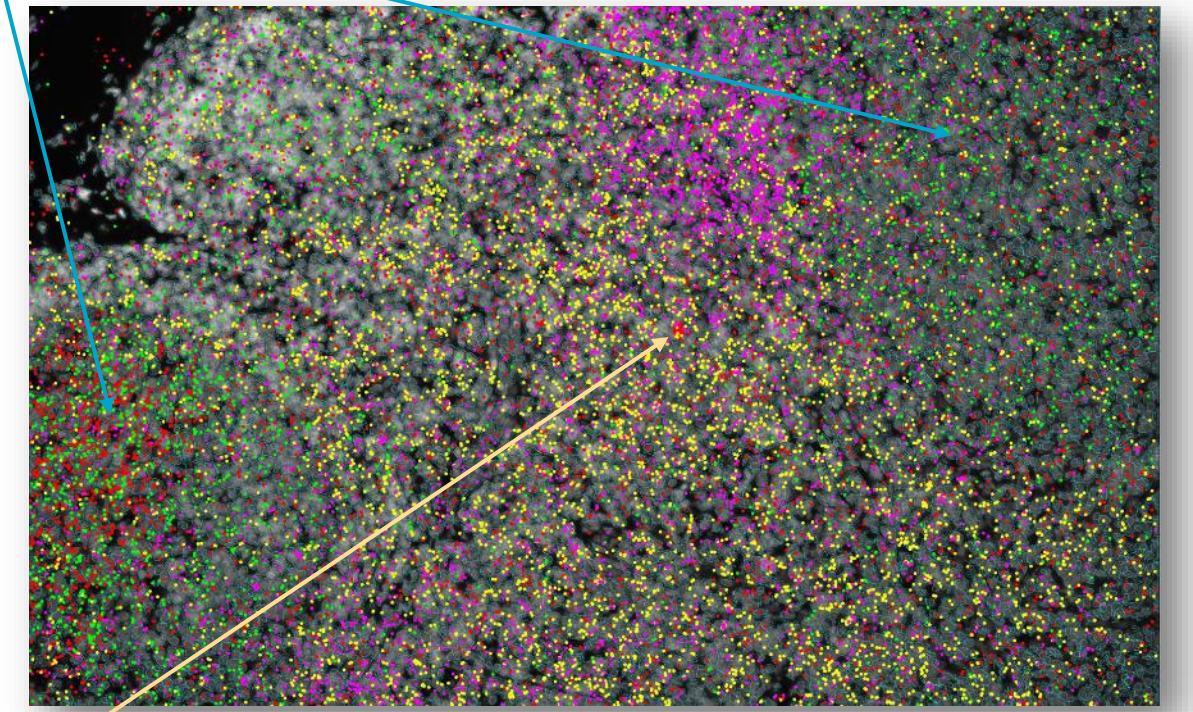

Productively infected cells

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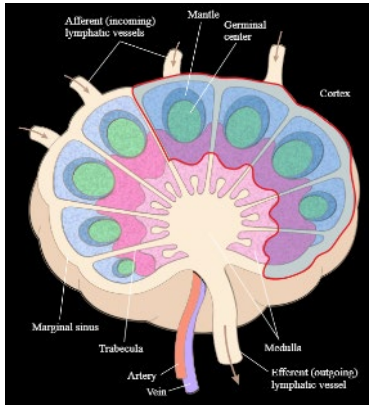
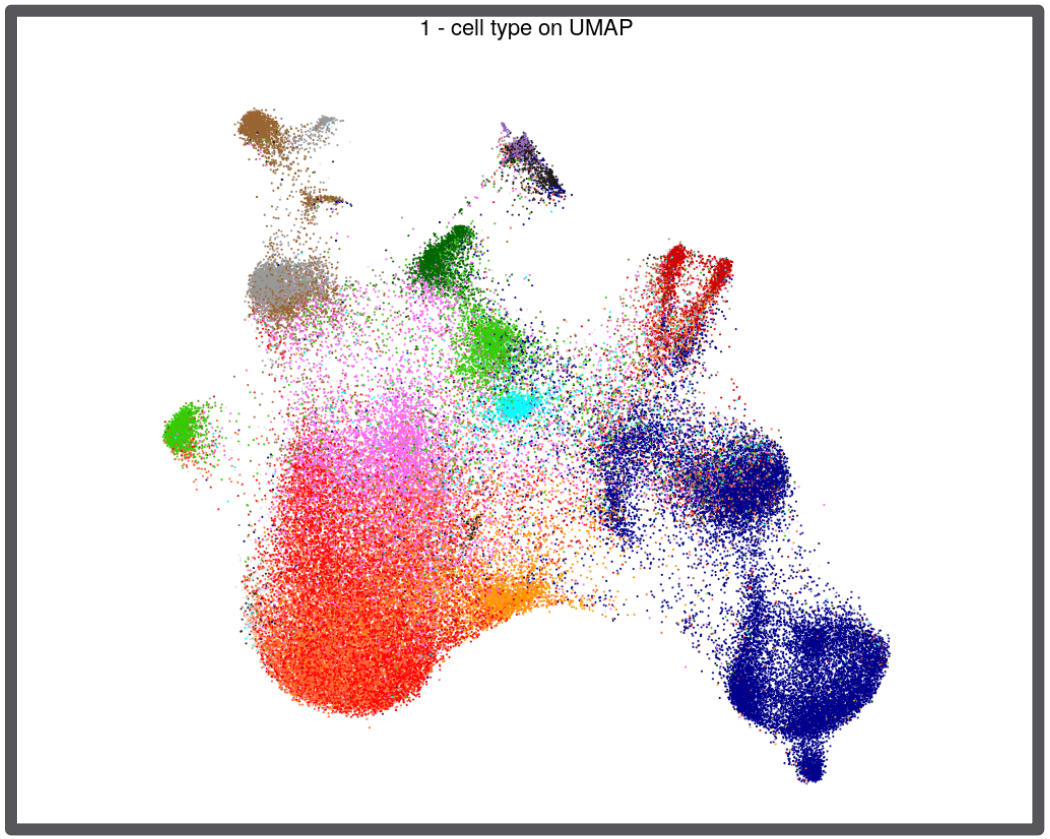
Single Cell Atlases of Treated Vs Control Samples Predict SIV Infection Status of Tissues

Visualizing B-cell enriched follicle regions Vs T-cell zones in TME niches



Anti-CD21 Treated LN



Cell type

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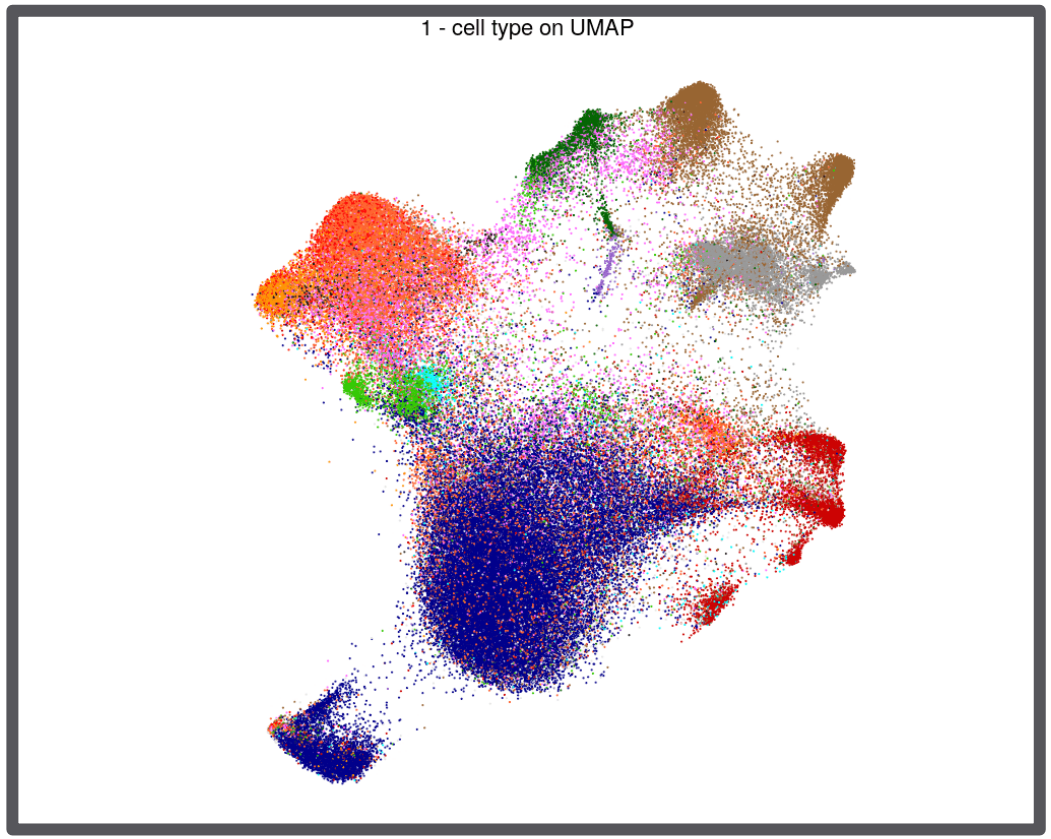
- CD4-CD8- T cells
- CD4+CD8+ T cells
- NK T cells
- Endothelial cells
- Fibroblasts
- Not Determined

TME Niche

- follicle
- parafollicular
- sinus
- Tcell_zone

Control LN



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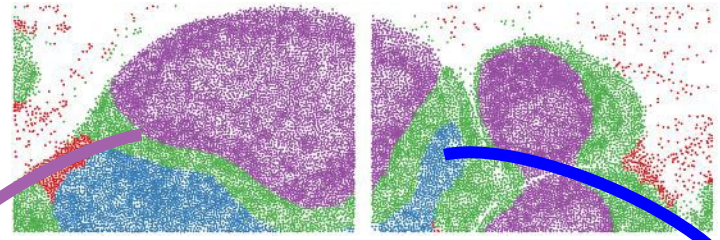
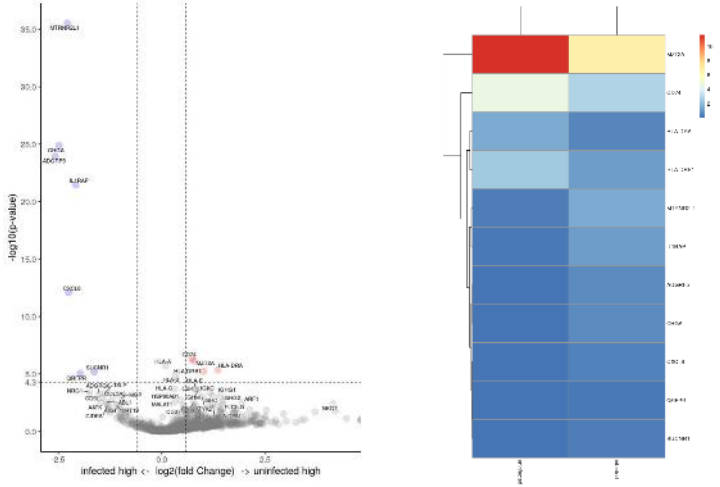
CosMx™ Measures Infection-Associated Gene Expression Changes in Different Tissue Microenvironments

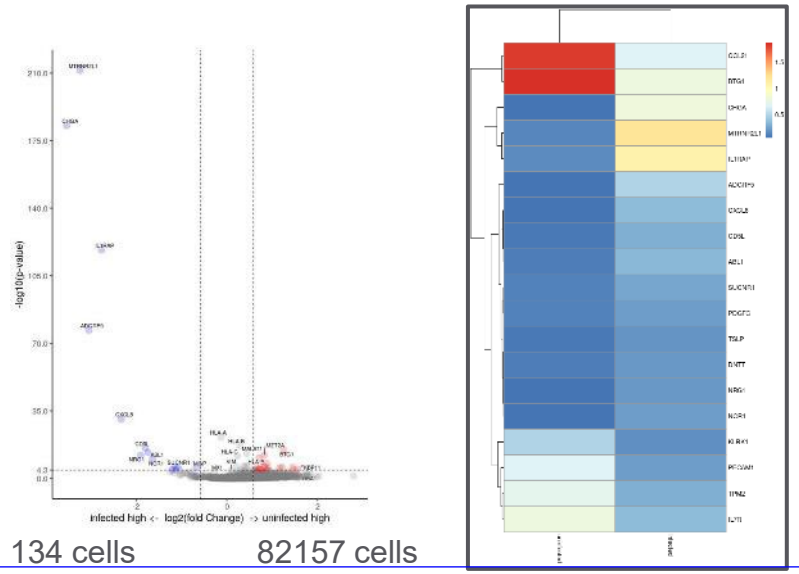


DE between individually identified **infected vs uninfected** cells **inside the BCFs**

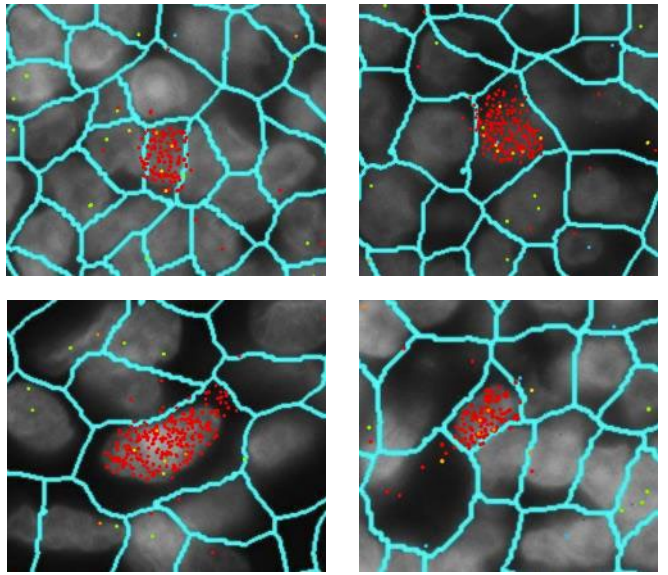


61 cells 19042 cells

DE between individually identified **infected vs uninfected** cells **inside the T Cell Zone**



134 cells 82157 cells



Infection upregulated genes

- IL1RAP
- MTRNR2L1

SIV genes

Infection downregulated genes

- FKBP11
- CRP

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Key Takeaways

Results

- Identified genes associated with viral persistence and treatment response (IL10 and targets)
- Resolved infection-associated gene expression at the level of tissue microenvironments
- Differentiated in situ between productively infected cells versus surface bound

Conclusions

- Able to identify unique biomarkers associated with viral persistence
- Targeted interventions may eliminate persistence in an HIV cure strategy

GeoMx/CosMx Edge

- Gene Expression Profiling and Single Cell Imaging together allow a comprehensive and accurate spatial survey of SIV infected and non-infected tissue neighborhoods at all scales, thus allowing for the first time, an ability to discriminate between productively infected Vs. uninfected SIV reservoirs at a subcellular resolution

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