

Visualization of HIV reservoir: an imaging perspective

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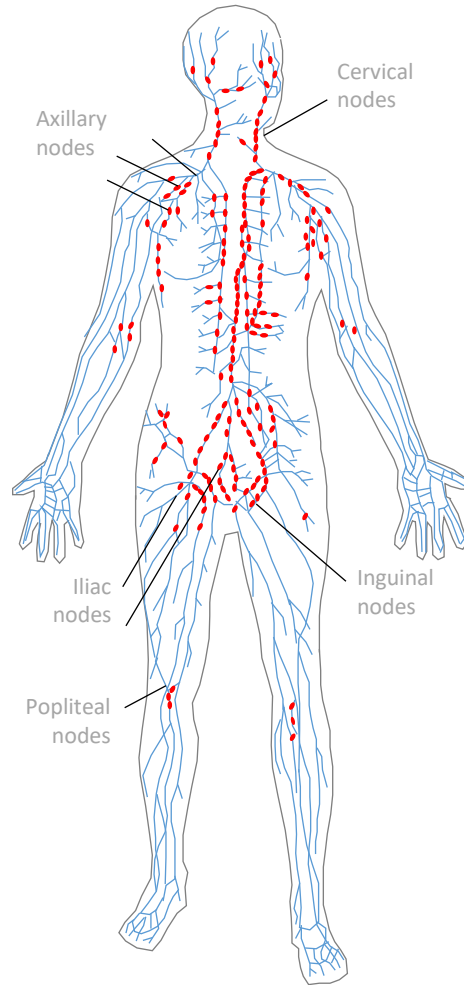
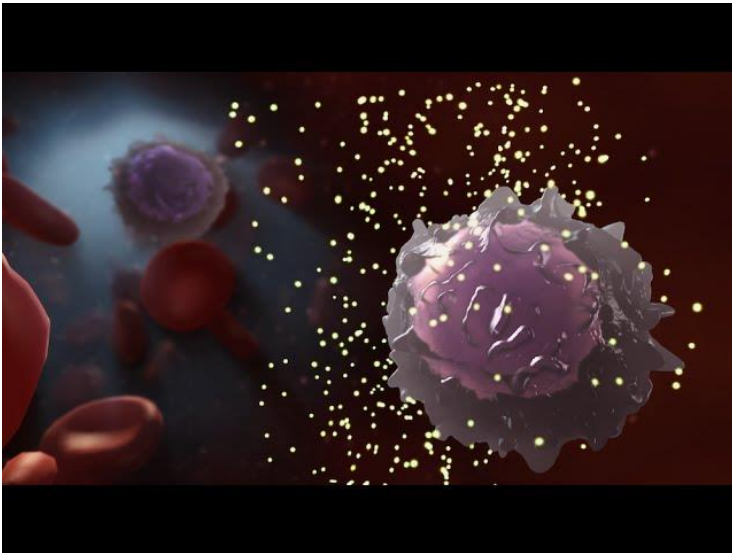


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50 µm

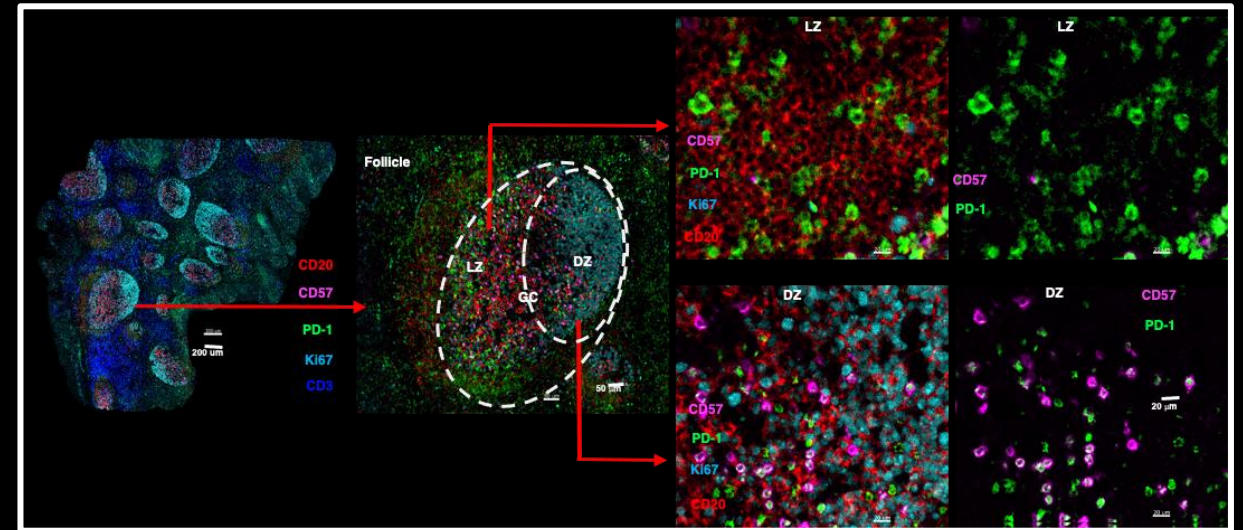
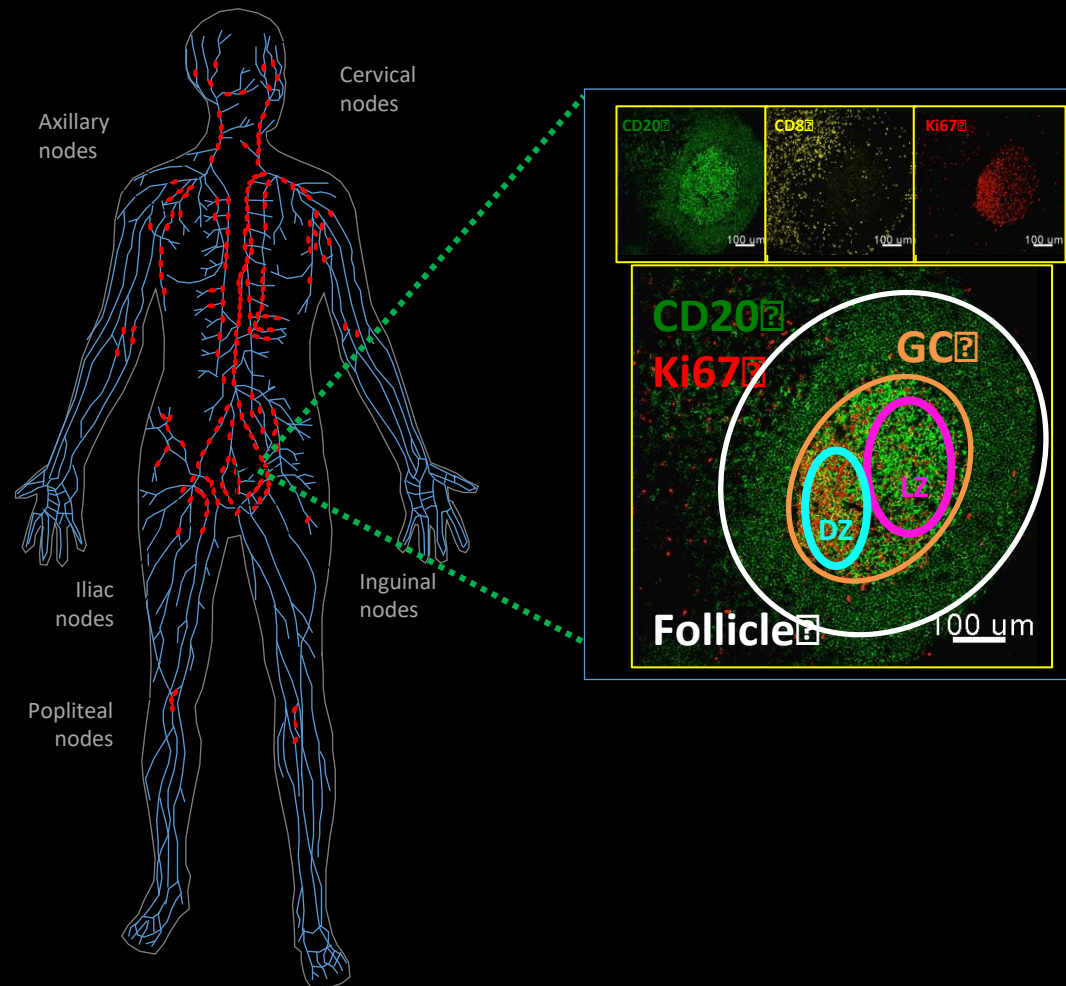
HIV reservoir microenvironment

**HIV silence / re-activation:
Intrinsic vs microenvironment factors**

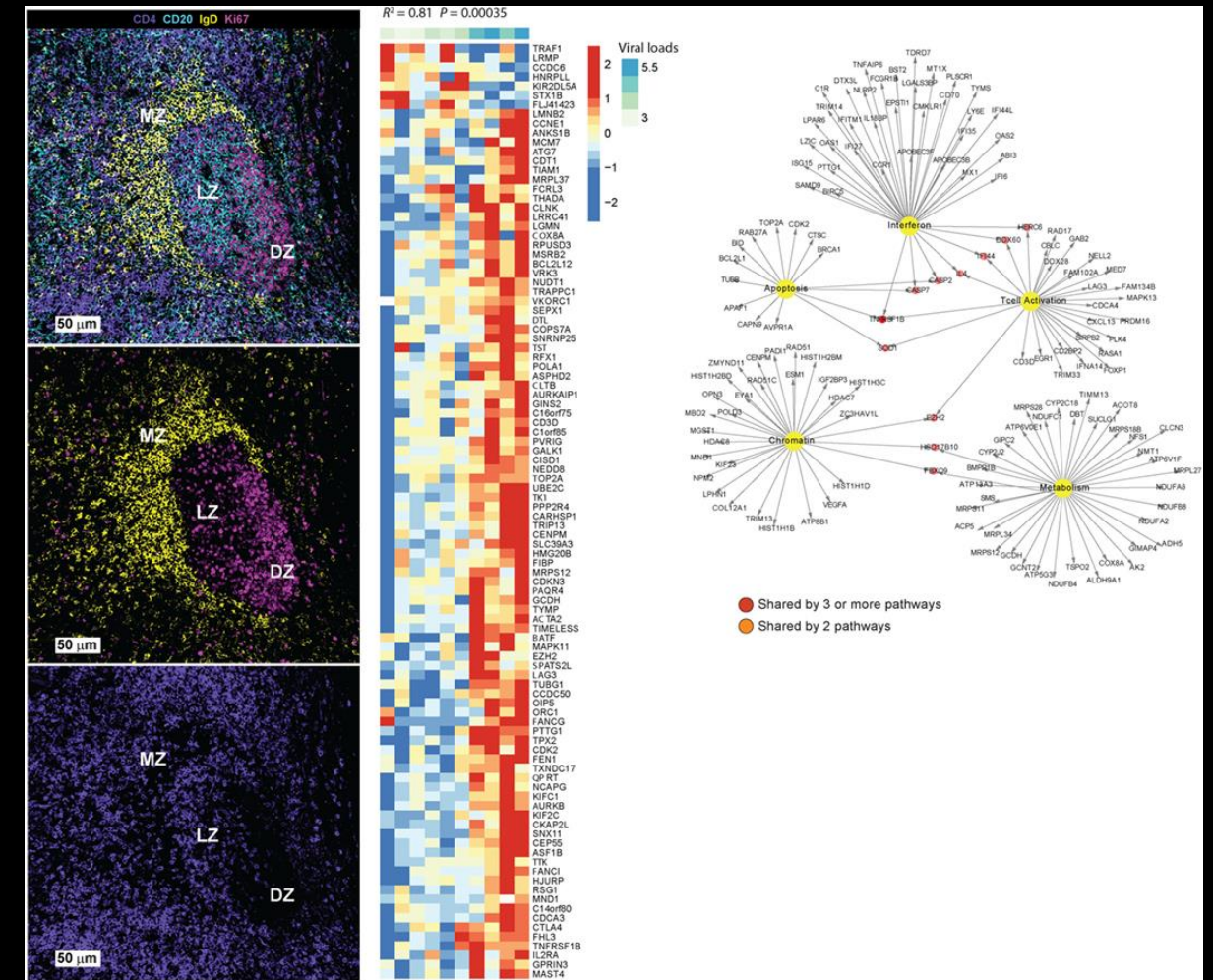
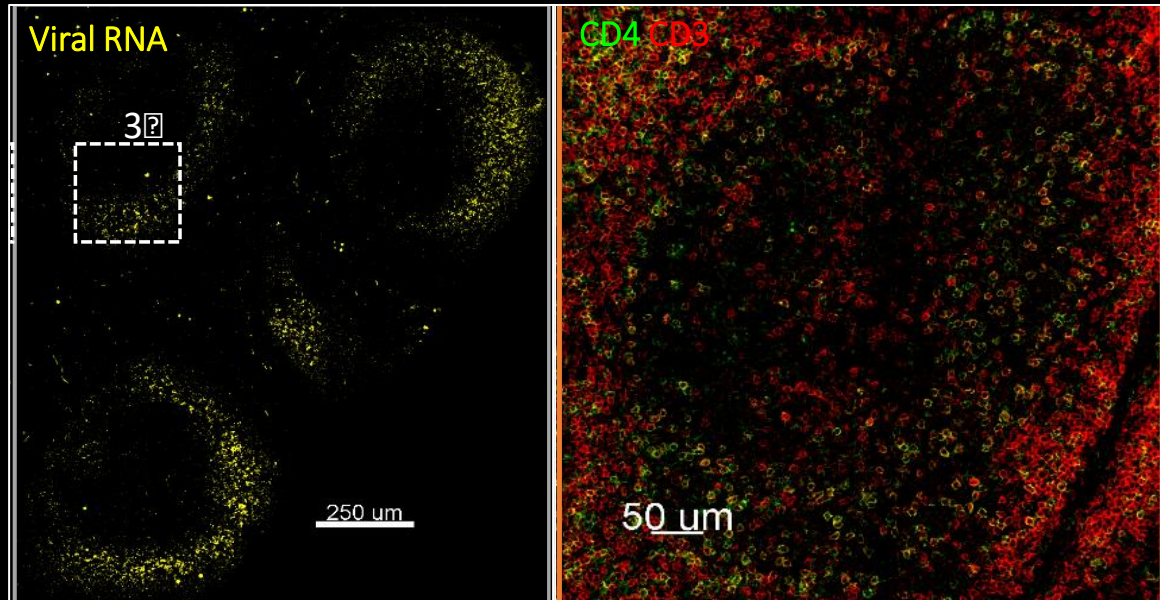


1. Microenvironment varies among different
 - i) tissues (even LNs)
 - ii) areas within the same tissue
2. In situ effector (CTLs, NKs) mechanisms are also different between areas/tissues

Lymph Nodes: a major HIV reservoir anatomical site

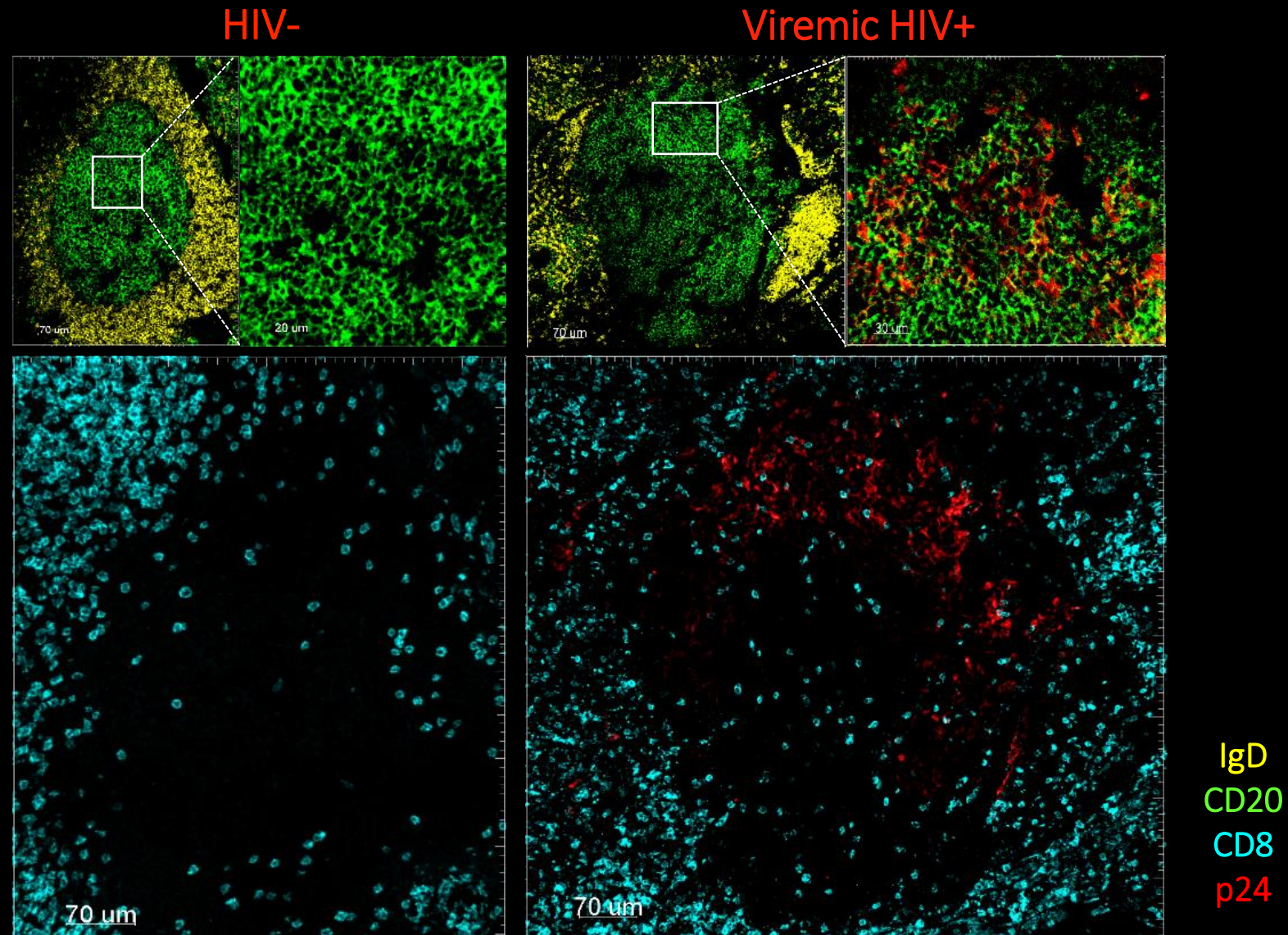


T_{FH} cells hold actively transcribed and latent virus

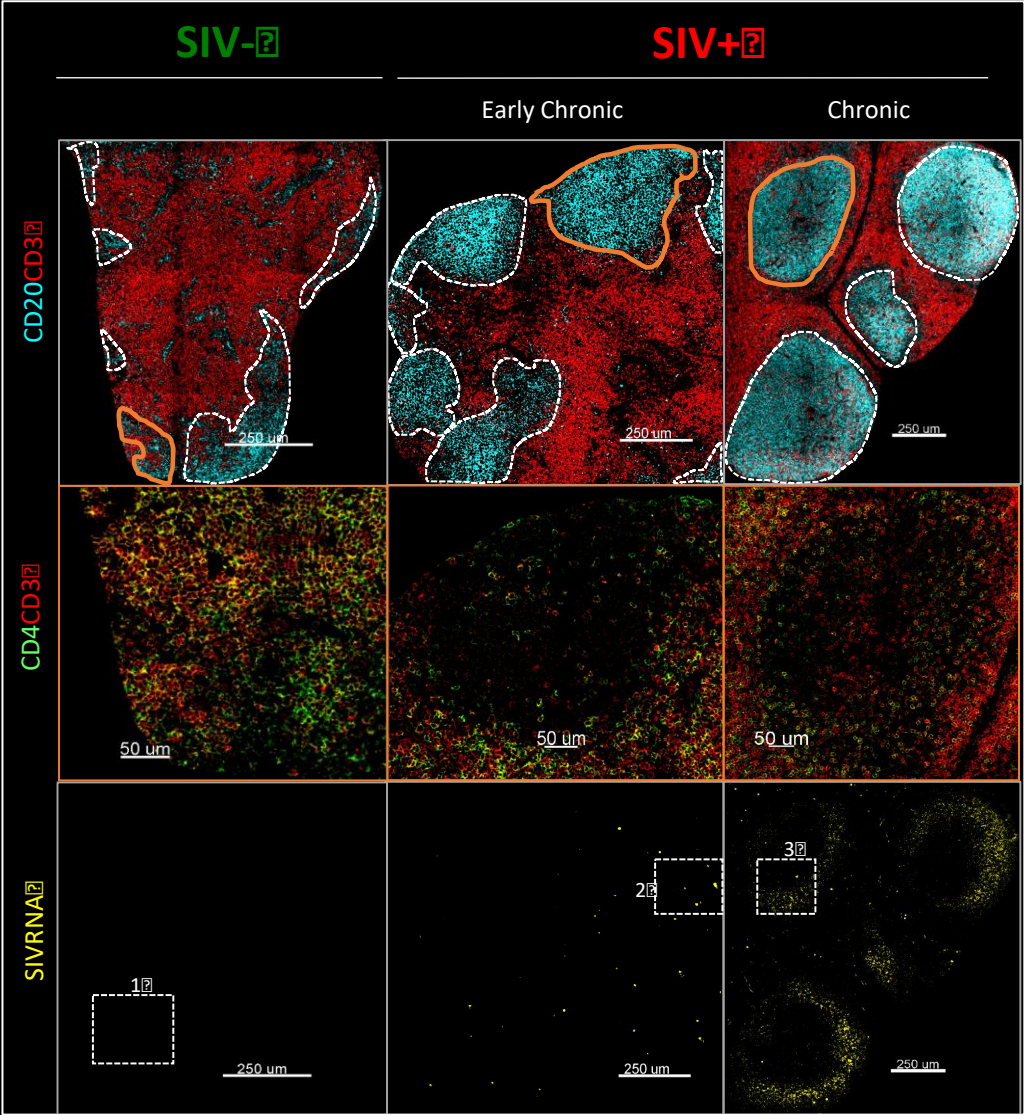
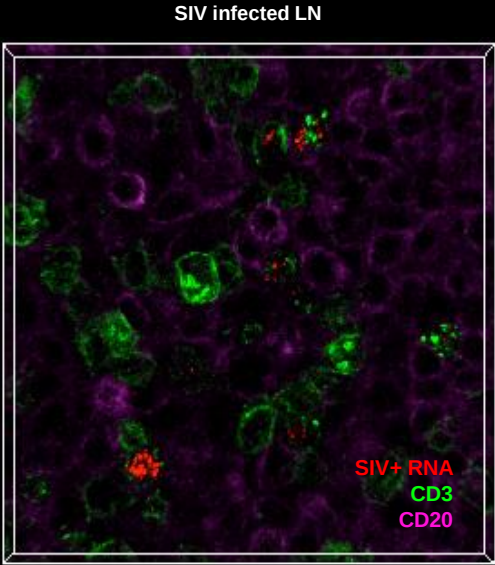
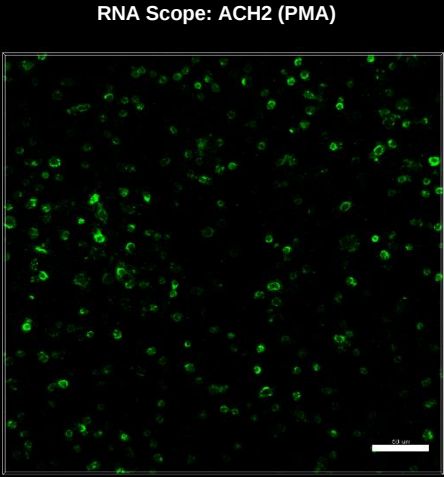
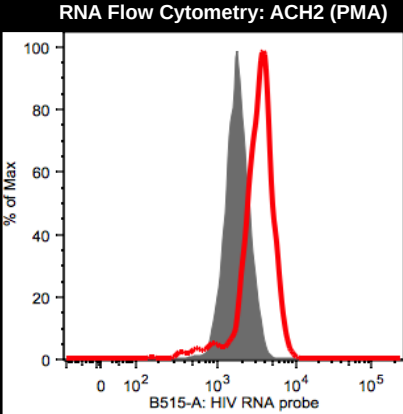


TFH gene signature correlated to viral load

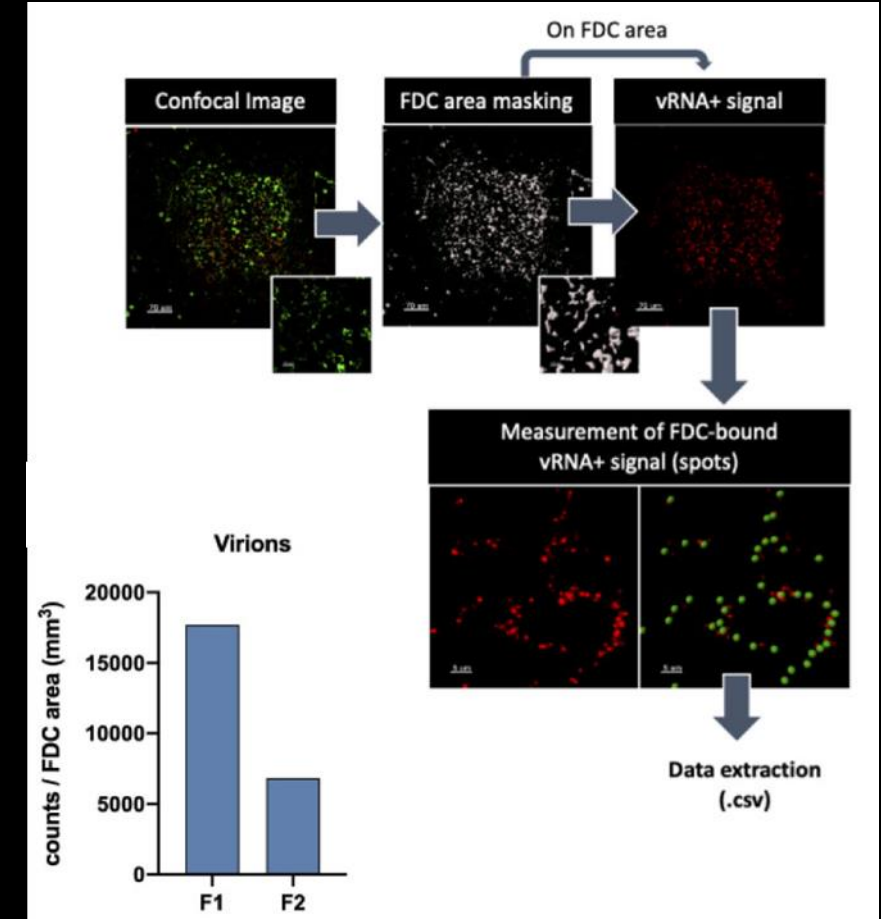
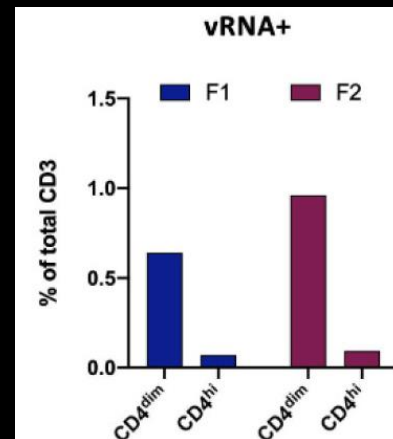
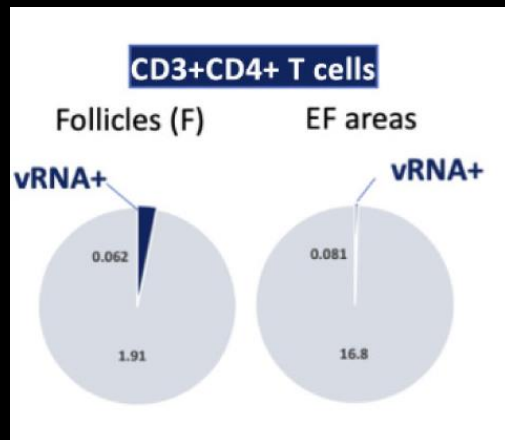
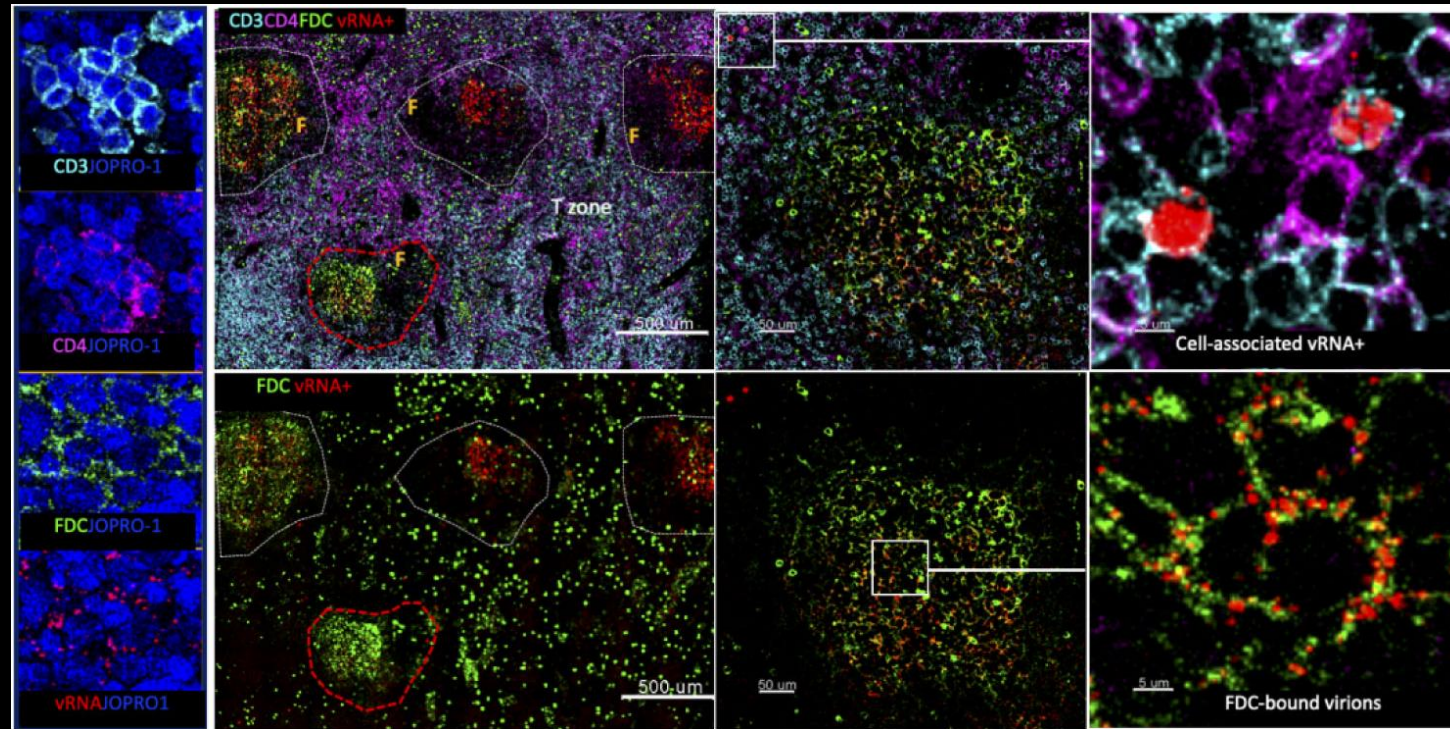
Follicular CD8 T cells as a component of viral reservoir microenvironment



Detection of the virus: RNAscope

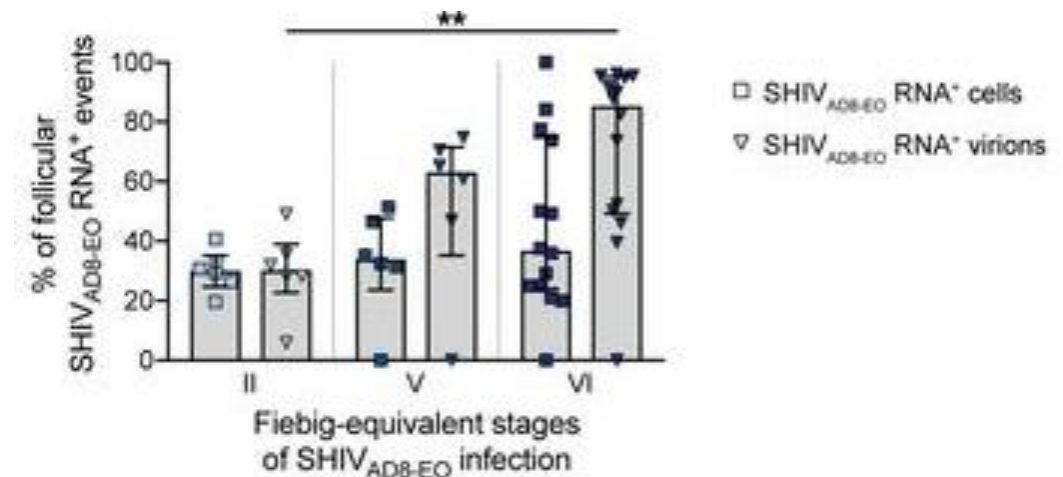
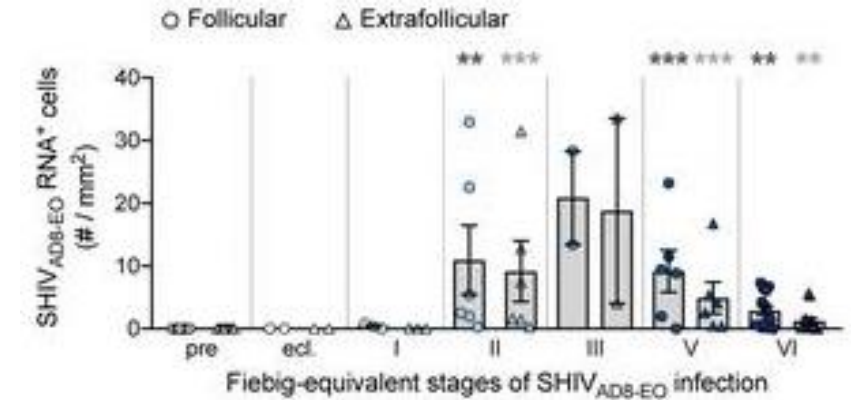
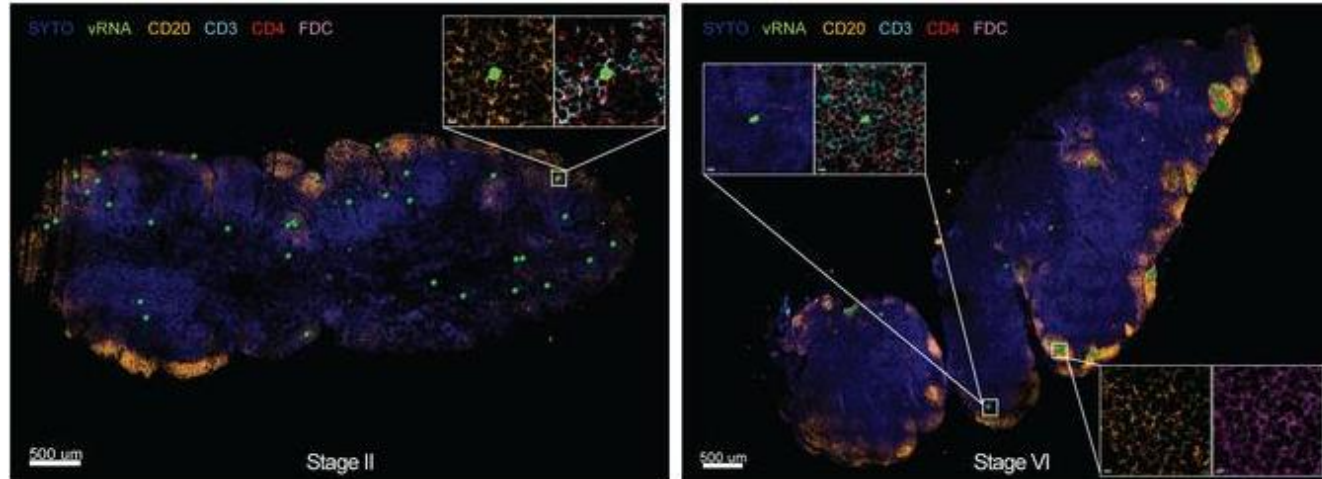


Lymph Nodes: enumerating actively transcribed virus (virions / cells)

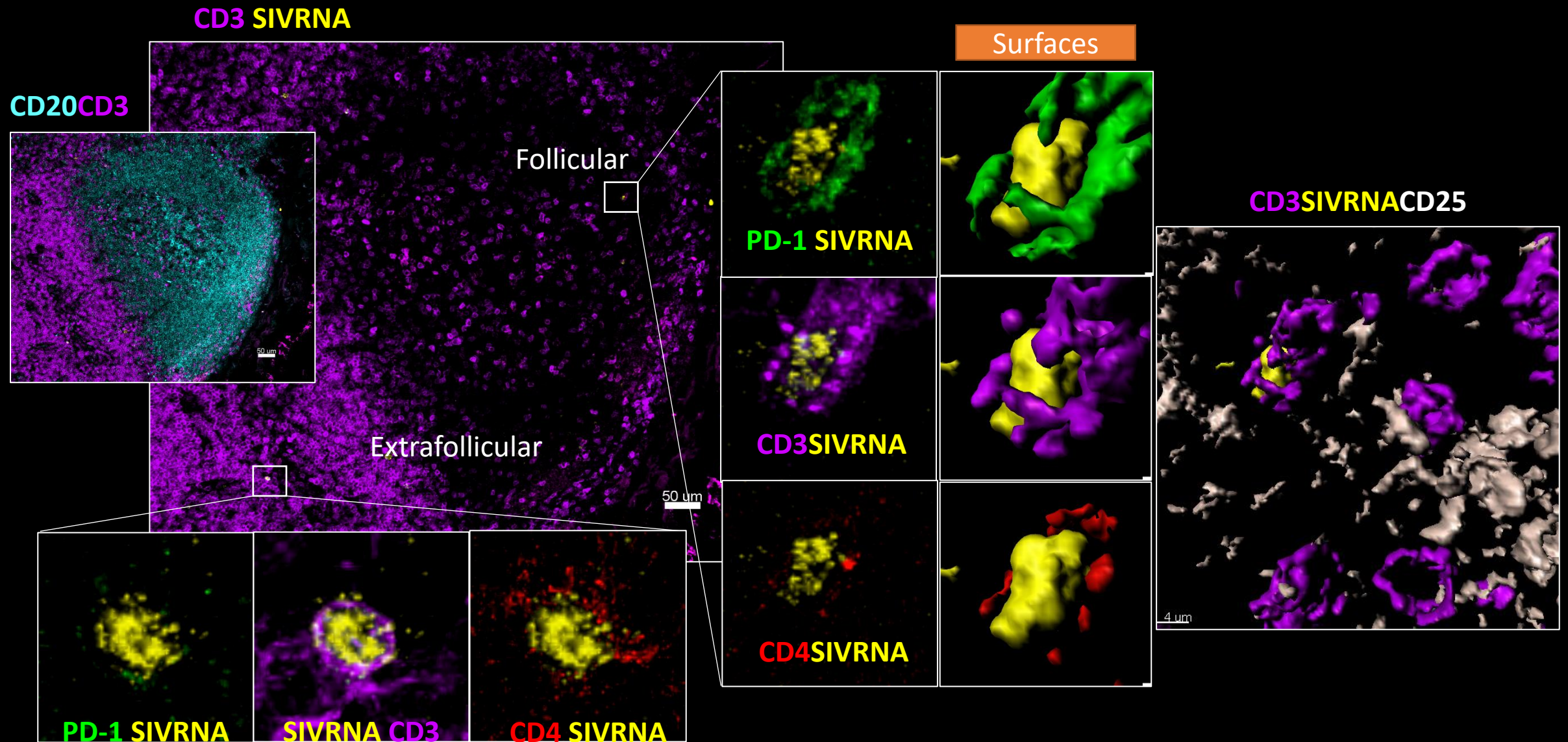


Lymph Nodes: enumerating actively transcribed virus (virions / cells)

Time point (pc)	d 0	d 3	d 7	d 10	d 14	d 17	wk 3	wk 4	wk 5	wk 6	wk 7	wk 8	wk 12	wk 16	wk 20
Intrarectal challenge															
HH7	-	Eclipse	Eclipse	Eclipse	I	I	II	V	V	VI	VI	VI	VI	VI	VI
DGEM	-	Eclipse	I	I	II	IV	V	V	VI	VI	VI	VI	VI	VI	VI
HZ2	-	Eclipse	Eclipse	I	I	II	III	V	V	VI	VI	VI	VI	VI	VI
DGAM	-	Eclipse	I	I	II	II	V	V	VI	VI	VI	VI	VI	VI	VI
HR8	-	Eclipse	I	I	II	III	V	VI	VI	VI	VI	VI	VI	VI	VI
DG81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

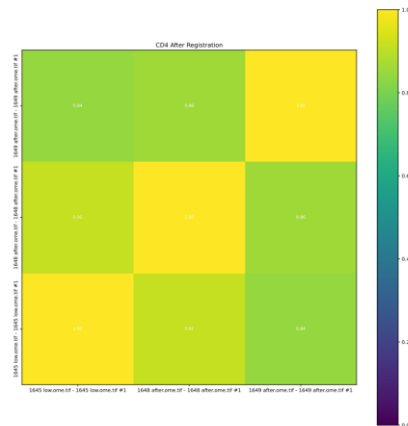
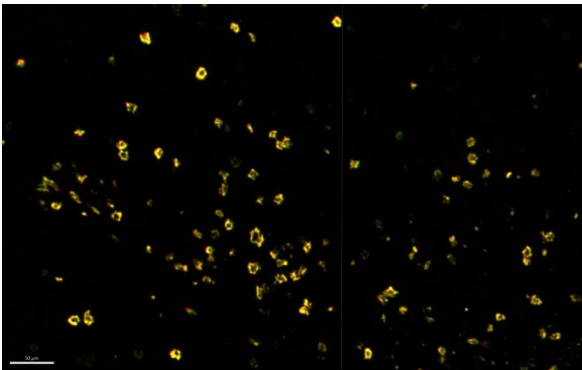
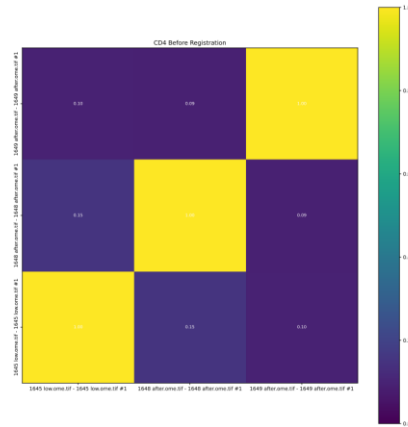
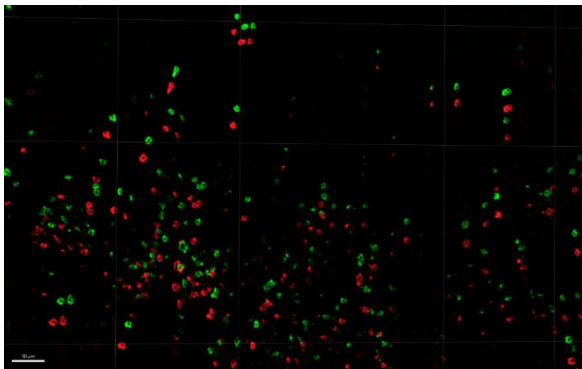


Combining mRNA and protein detection for the identification of HIV/SIV infected cells at tissue level

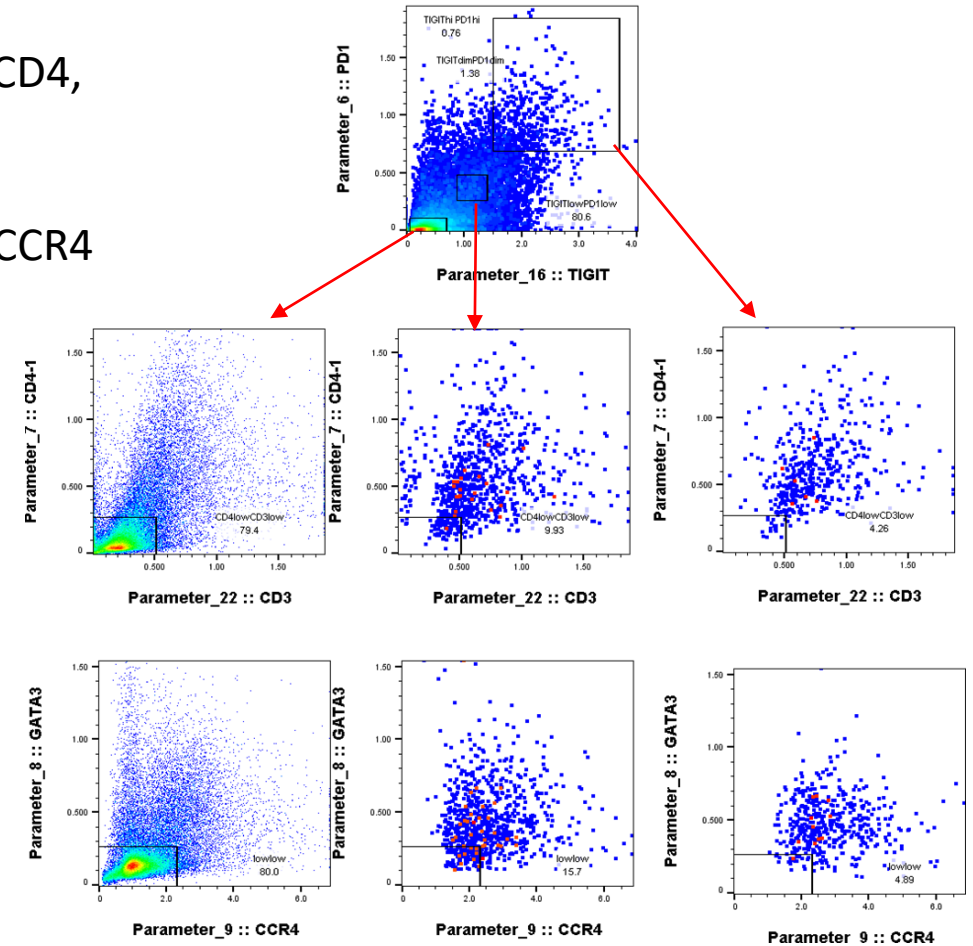


Development of 'big' imaging panels for characterization of CD4 T cells and surrounding environment

Constructing imaging panels allowing for the in depth characterization of:
CD4 T cells,
CD8 T cells and
innate immunity cells



Cy1: PD1, CD4,
GATA3
Cy2: TIGIT
Cy3: CD3, CCR4



Connecting *in situ* Phenotype with cell morphological characteristics (especially for innate immune cells in the HIV reservoir microenvironment)

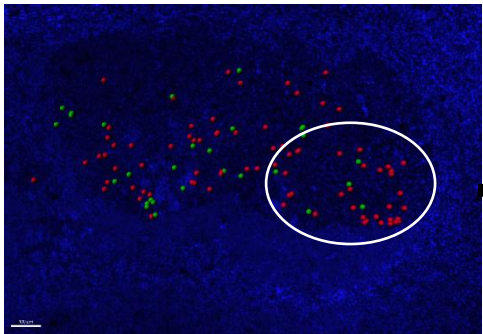
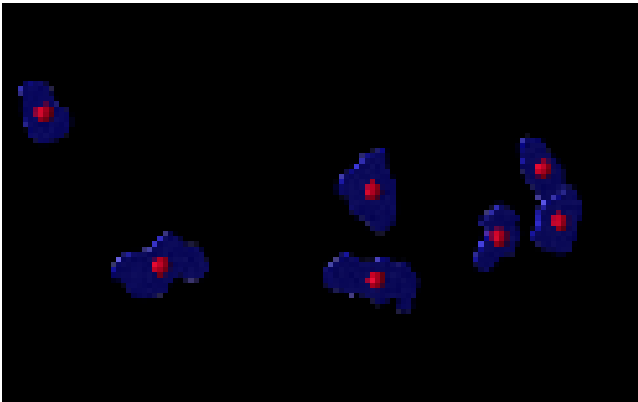
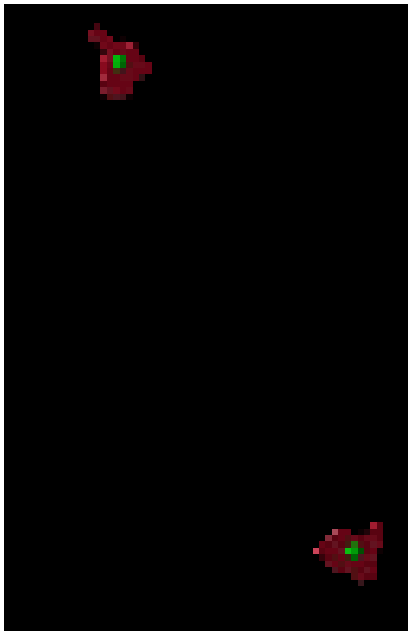
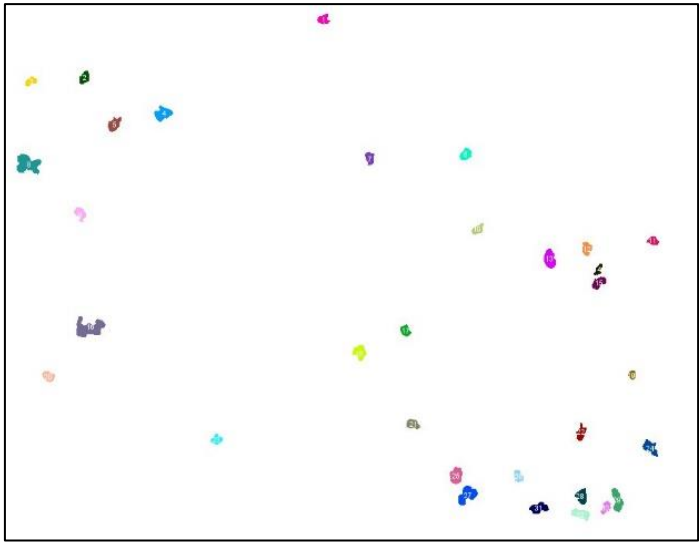


Image J



	Area	Mean	Min	Max	Circ.	Skew	Kurt	%Area	AR	Round	Solidity
1	142	255	255	255	0.647	NaN	NaN	100	1.211	0.826	0.807
2	153	255	255	255	0.768	NaN	NaN	100	1.631	0.613	0.908
3	117	255	255	255	0.770	NaN	NaN	100	1.709	0.585	0.836
4	285	255	255	255	0.674	NaN	NaN	100	1.362	0.734	0.846

Development of ‘big’ imaging panels for characterization of CD4 T cells and surrounding environment

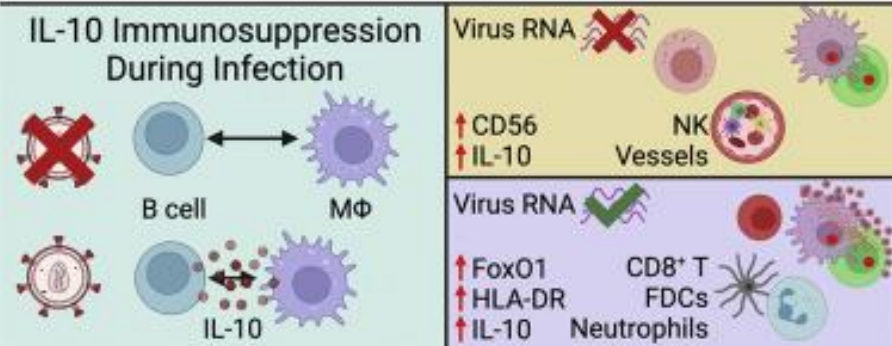
Focus 1: comprehensive characterization of CD4 T cells

Focus 2: aiming the surrounding cells

PANINI: Robust DNA, RNA and Protein Imaging in Tissues



Application to Study SIV Infection in NHP Models in situ



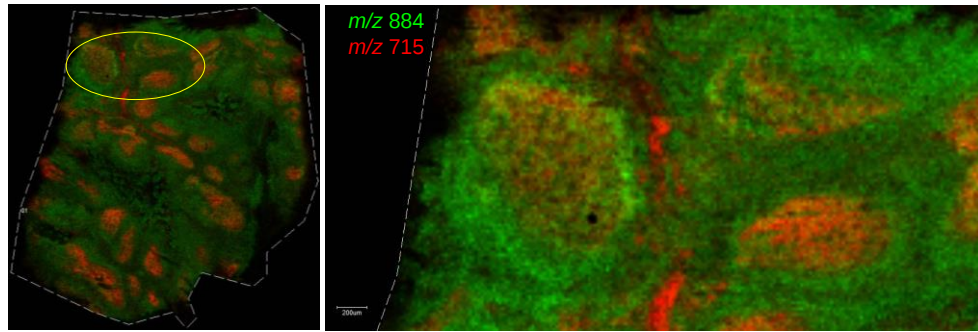
Combined protein and nucleic acid imaging reveals virus-dependent B cell and macrophage immunosuppression of tissue microenvironments

Viral reservoir microenvironment

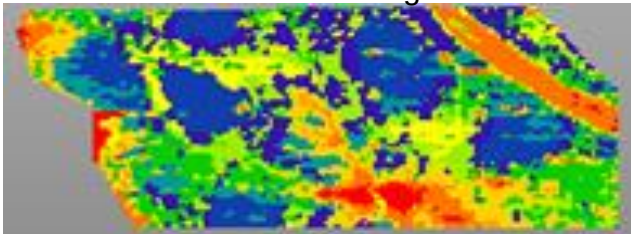
Novel molecules / pathways

Non-hypothesis driven discoveries

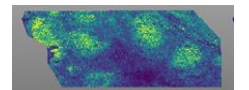
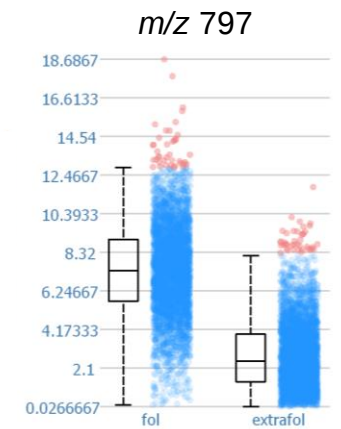
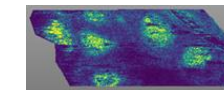
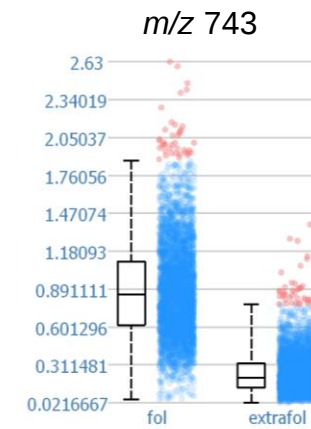
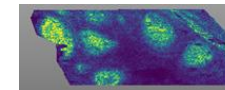
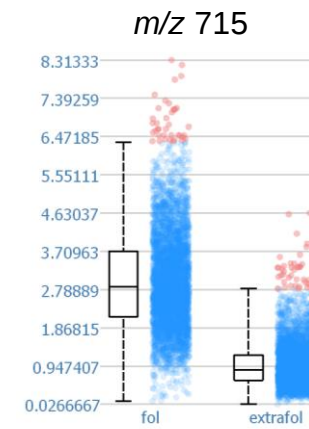
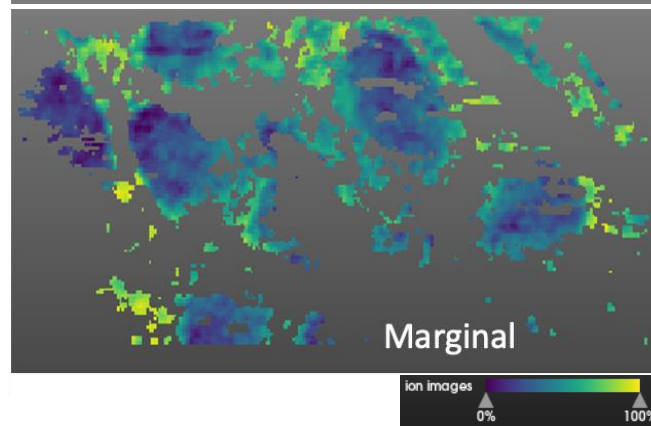
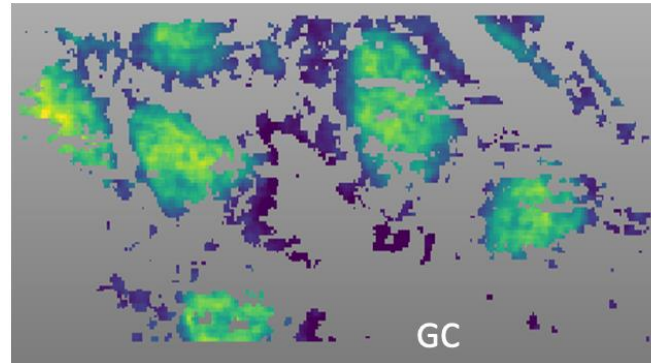
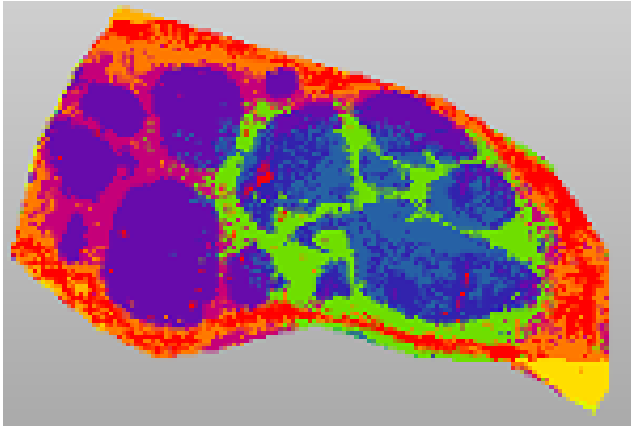
In situ lipidome / metabolite / protein profiling using MALDI-TOFF Imaging



Negative ion mode

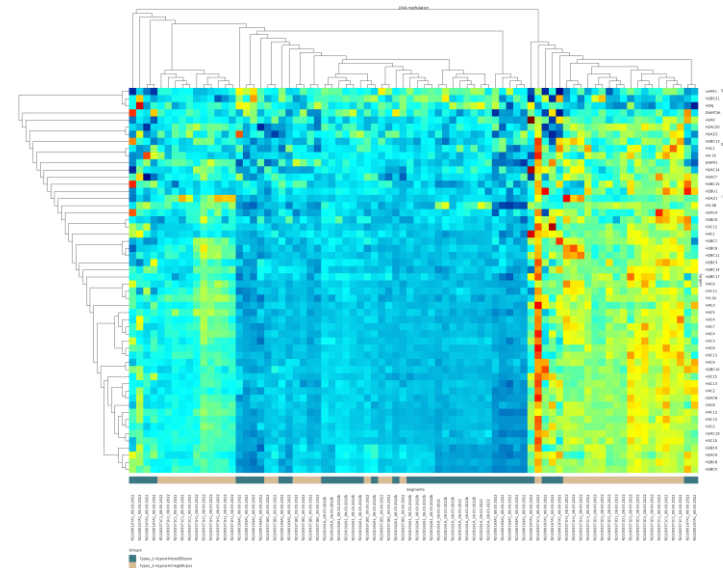
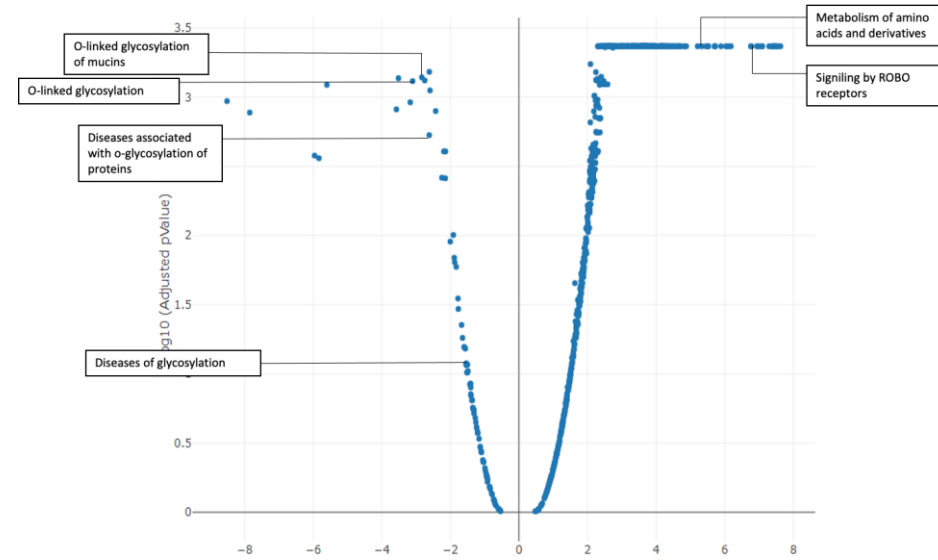
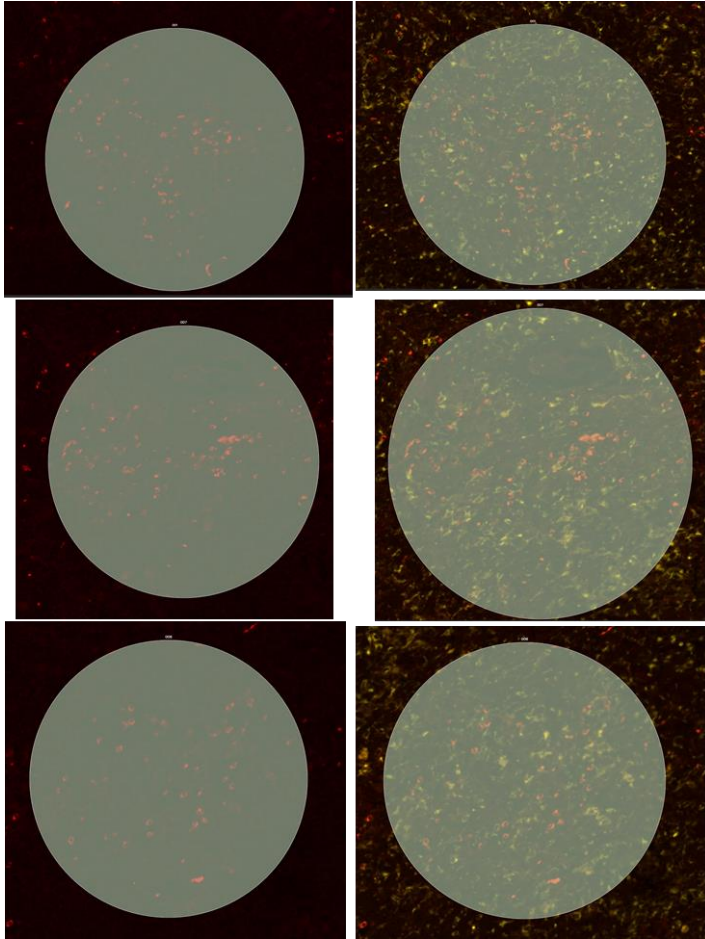


Positive ion mode

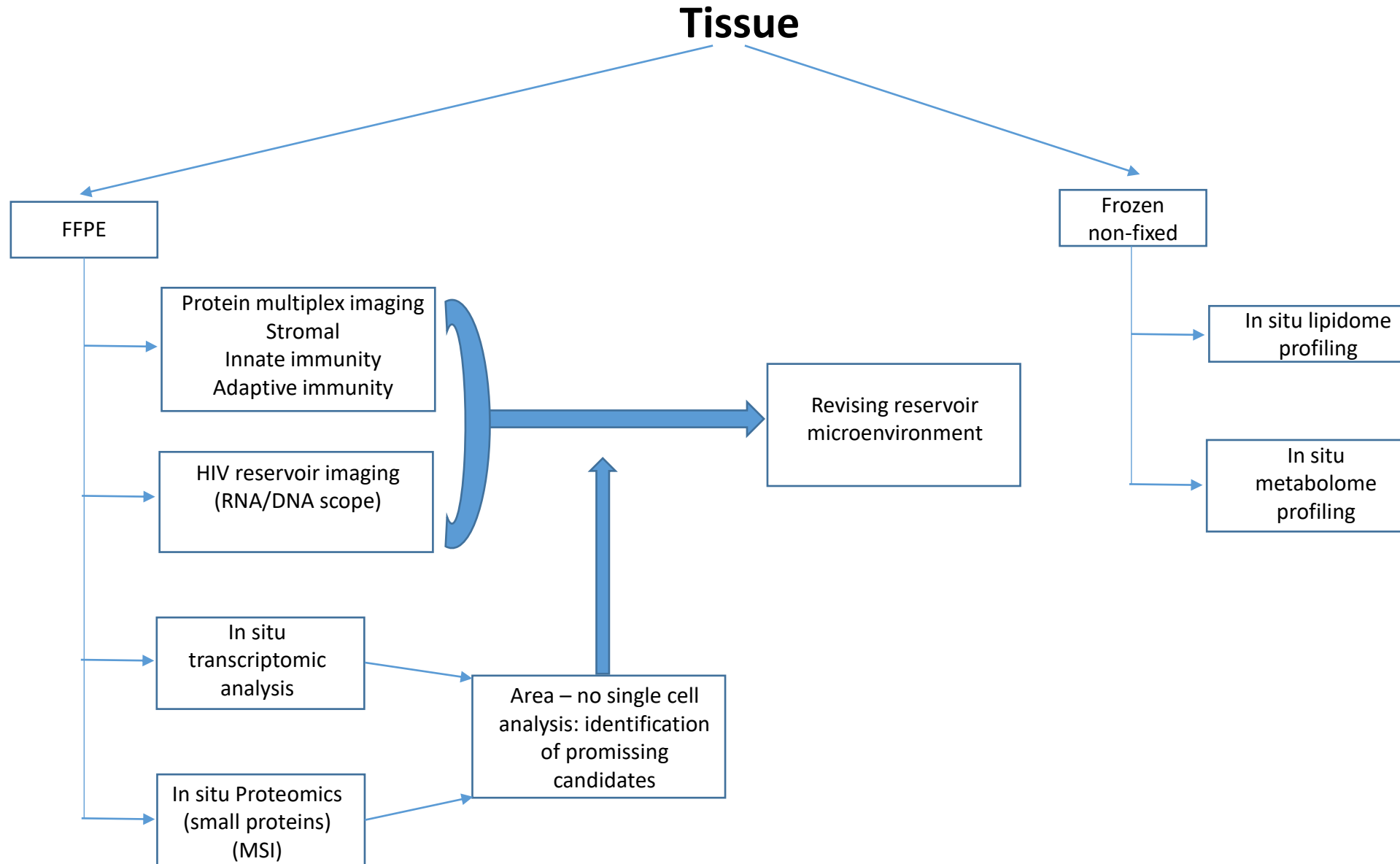


In situ tissue transcriptomic profiling

Neoplastic cells **Macrophages**



Experimental Approach



Laboratory of Tissue Investigation, CHUV

Kalliopi Ioannidou, PhD
Spyros Georgakis, PhD
Cloe Brenna (PhD student)
Maddalena Maria Perra (PhD student)
Michael Orfanakis (PhD student)
Amanda Chantziou (MSc student)
Emilie Lingre (research technician-40%)
Damien Maisson (research technician-20%)

Tissue Analysis Core Laboratory, VRC, NIAID, NIH

Giulia Fabozzi (Staff Scientist)
Benjamin Oyler (Staff Scientist)
Eirini Moysi (Research Fellow)
Adam Molyvas (Postdoc fellow)
Jeferson Valencia (Postdoc fellow)
Iris Martin (BSc student)



Dale and Betty Bumpers
Vaccine Research Center
National Institute of Allergy and Infectious Diseases
National Institutes of Health