

# Characterization of the SIV tissue reservoir transcriptional environment at the single focus level during ART and post ATI

10th International Workshop on HIV Persistence during Therapy

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10<sup>th</sup> EDITION

# HIV PERSISTENCE DURING THERAPY™

Reservoirs & Eradication  
Strategies Workshop



DECEMBER 13-16, 2022  
[www.hiv-persistence.com](http://www.hiv-persistence.com)

FLORIDA  
**Miami** USA

## CONFLICTS OF INTEREST

None

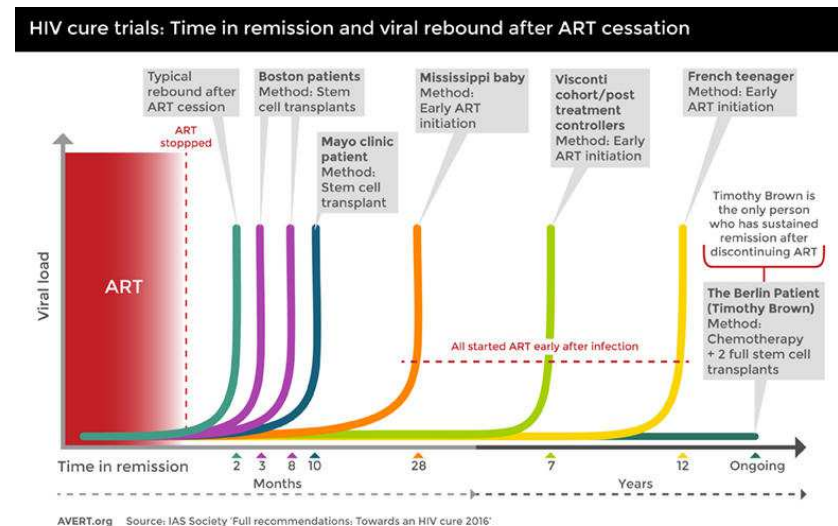
[www.hiv-persistence.com](http://www.hiv-persistence.com)

## Reservoir main questions

Current ART quickly suppress HIV to levels undetectable in the blood.

However, the virus is not eradicated from the body and in most cases comes roaring back if the drugs are stopped.

- Where the reactivated virus comes from?
- How is the reservoir maintained?
- What are the characteristics of the cells that harbor the reservoirs?
- How is the virus able to retain the variability that it needs to survive?
- How is the virus able to circumvent immunity in tissues to rebound?



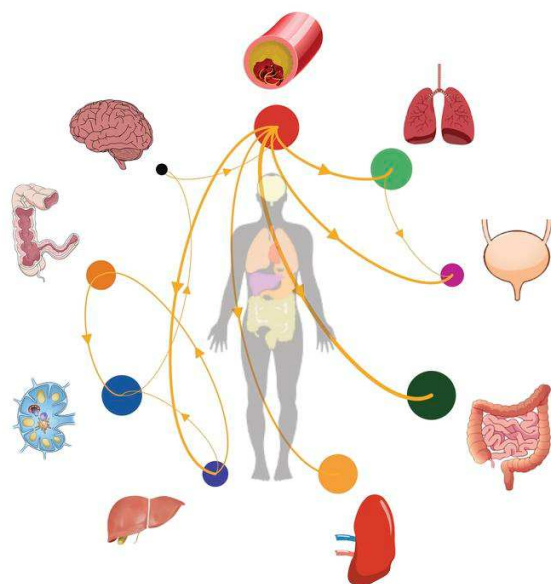
## The Reservoir is everywhere (and now we can find it!)



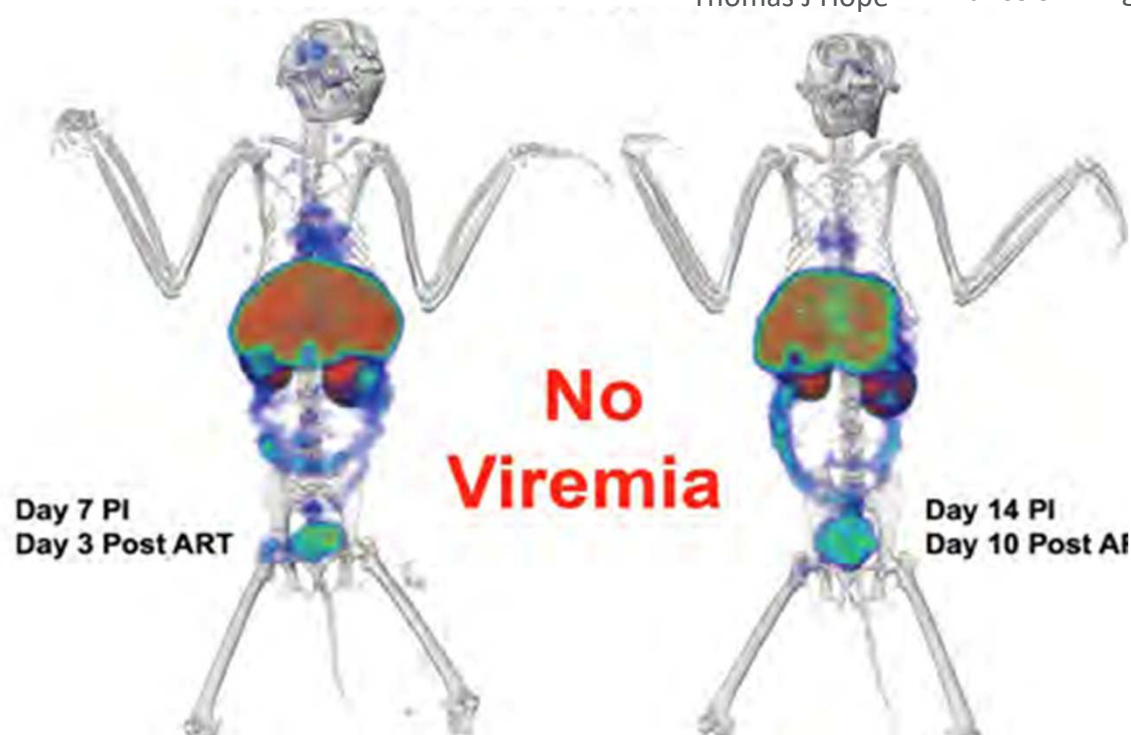
Thomas J Hope



Francois Villinger

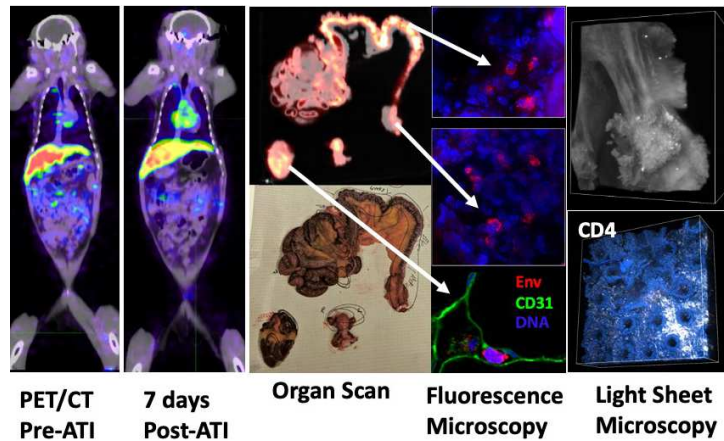


Chaillon et al. *J Clin Invest.* 2020



# Understanding SIV reservoirs and rebound

## PET-CT guided necropsy and multiscale imaging



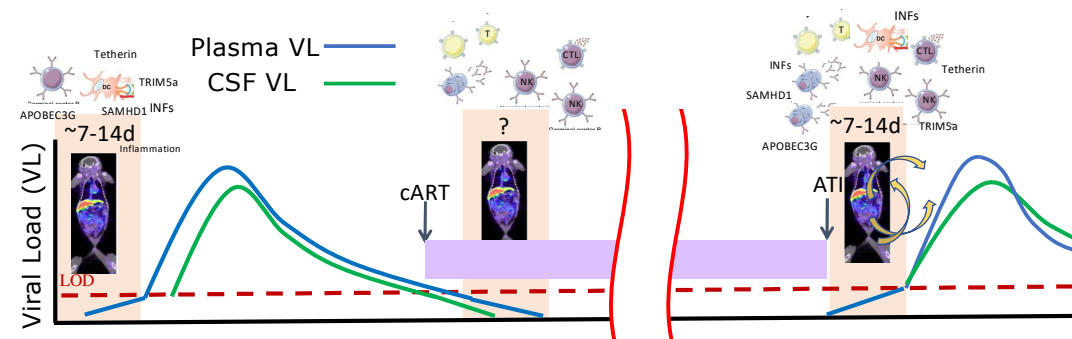
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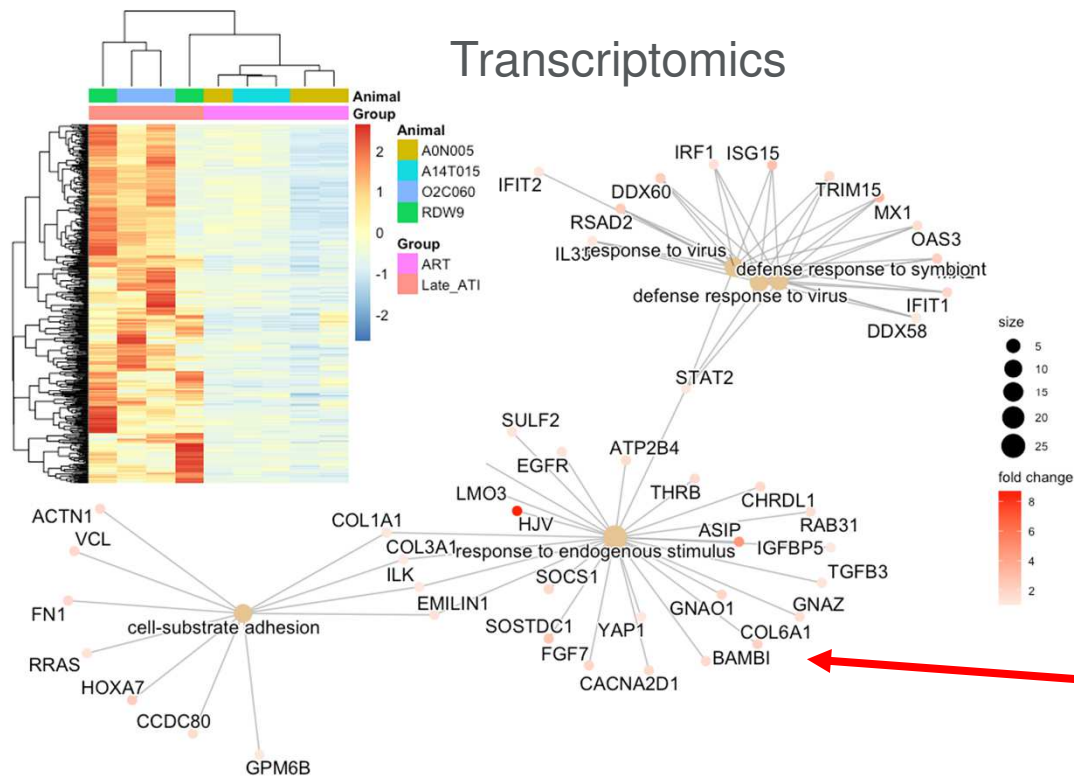
Elena Martinelli



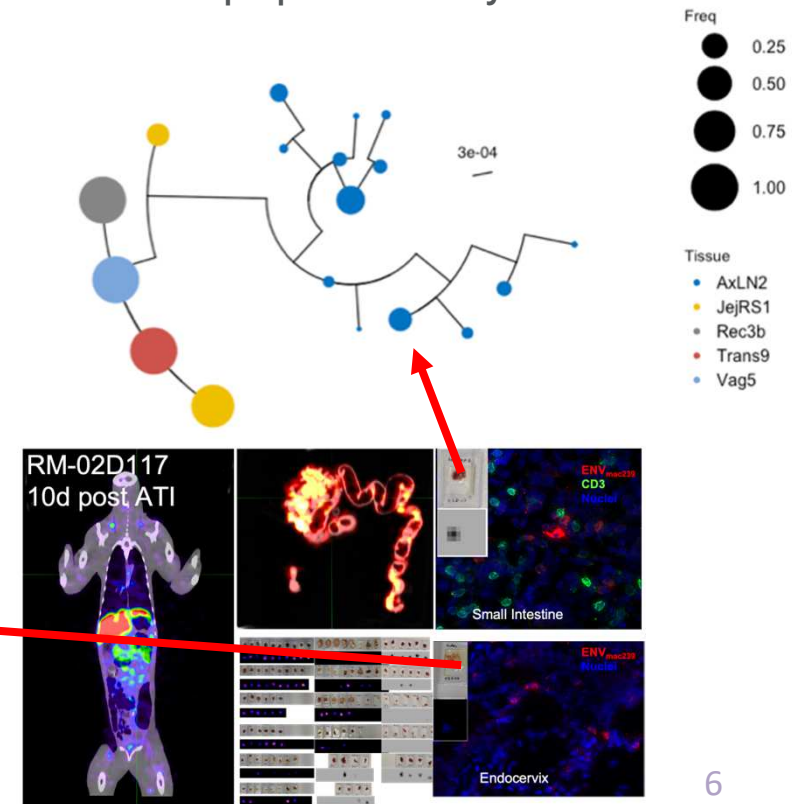
Francois Villinger



# Virus-host system in SIV reservoirs



## Intra-host population dynamics





# PET-CT-Guided Spatial Transcriptomics of SIV Tissue Reservoirs

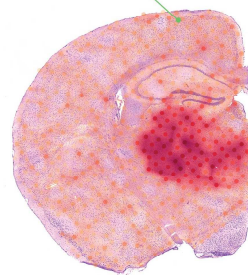
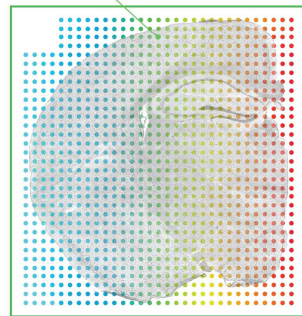
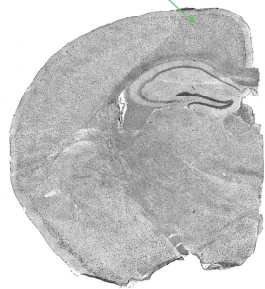
10x  
GENOMICS®

Visium Spatial Gene Expression

Tissue  
Section

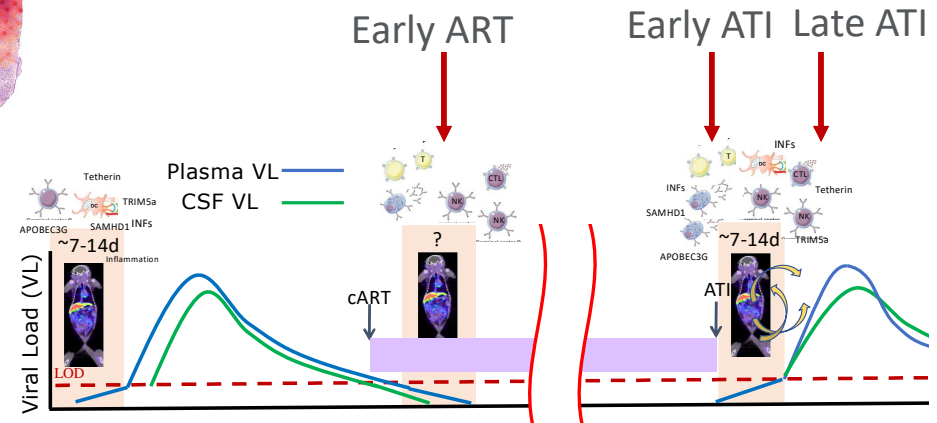
Spatial  
Transcriptomic  
Map

Visualize Expression  
of any **mRNA**



High dose (mac239) challenge  
vaginal and rectal with biopsies.  
Early reservoir: ART initiation 4  
days post-challenge.  
ATI: 6-8 months post ART  
initiation.

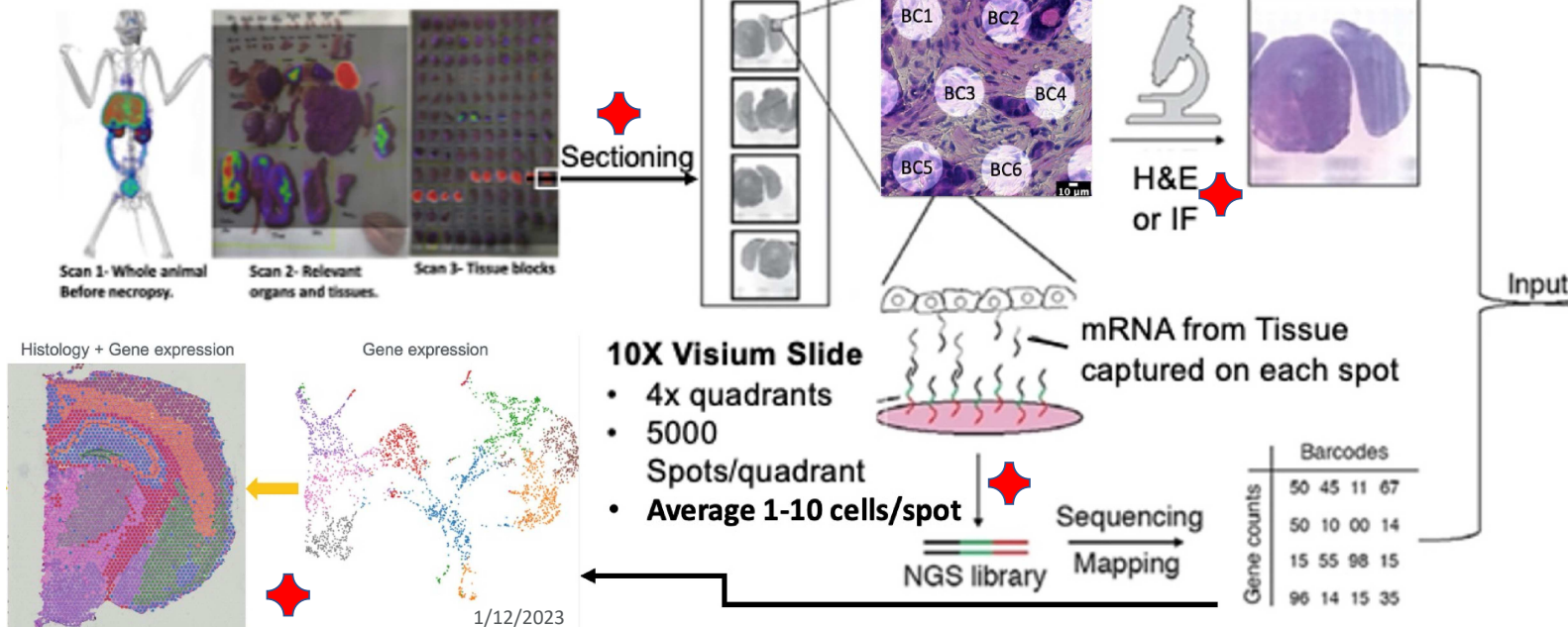
**N**Northwestern  
Medicine®



# PET-CT-Guided Spatial Transcriptomics of SIV Tissue Reservoirs

## Steps optimized

- Screening of tissues
  - PET/CT
  - Infected vs Non-infected
  - RNA Quality
  - Tissue Quality



Elena Martinelli, PhD



Muhammad Shoaib Arif, PhD

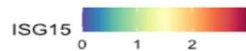
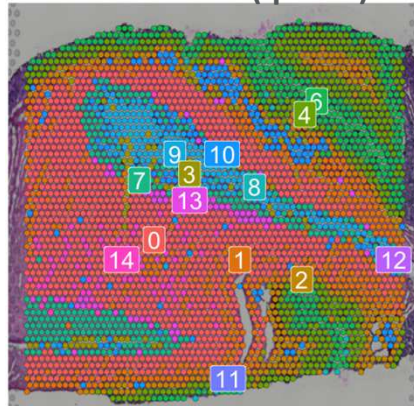


Christopher Thuruthiyil

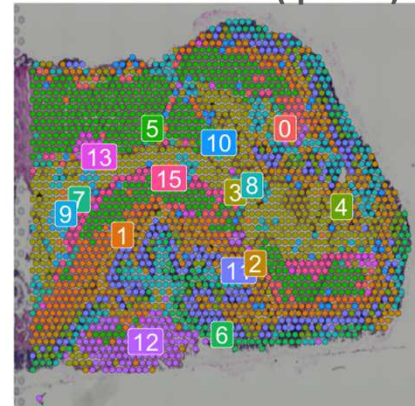


# First Experiment: 10x Spatial transcriptomics identify ISGs upregulated in SIV infected tissues

Trans Colon (qPCR -)

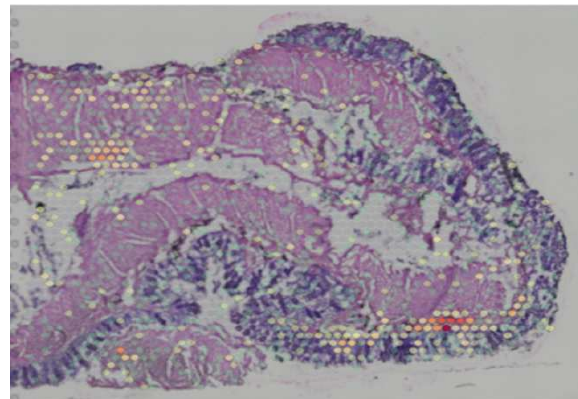
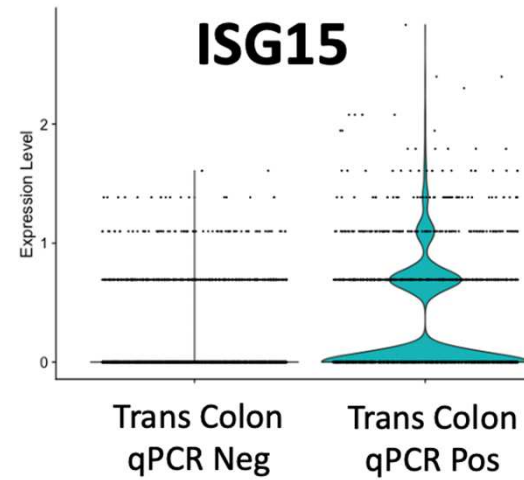


Trans Colon (qPCR +)

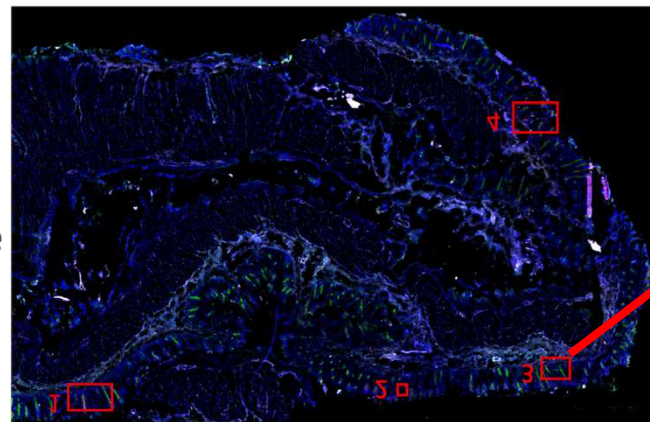


**ISG15**

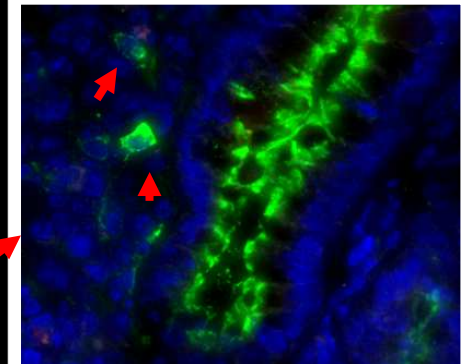
**18 days ATI  
T.Colon**



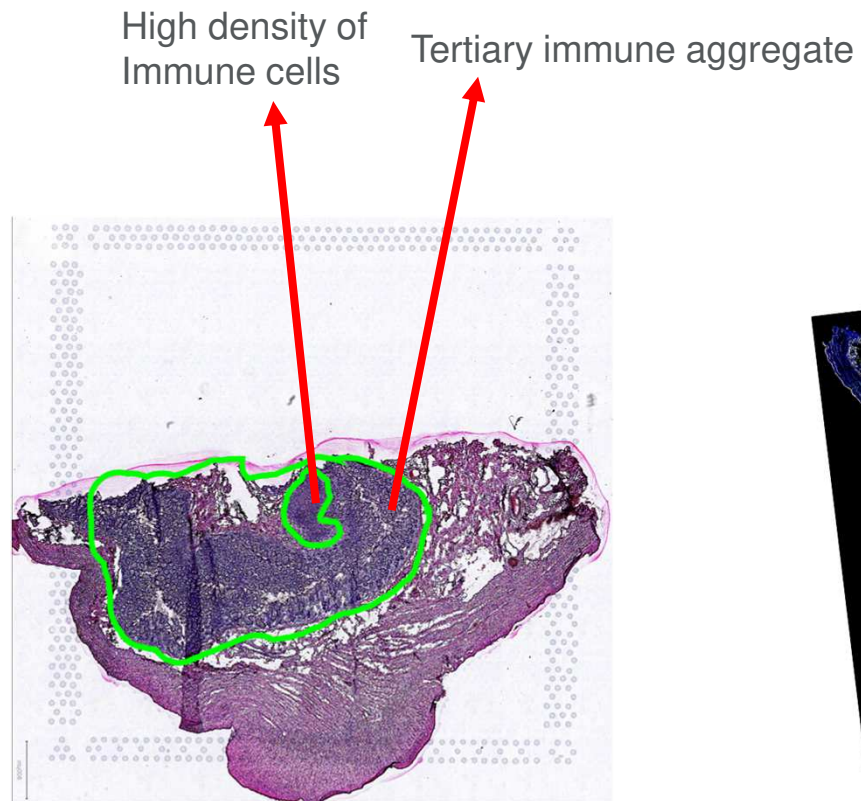
ISG15 was upregulated in tissue areas where infected cells are present



SIV gag+ infected cells

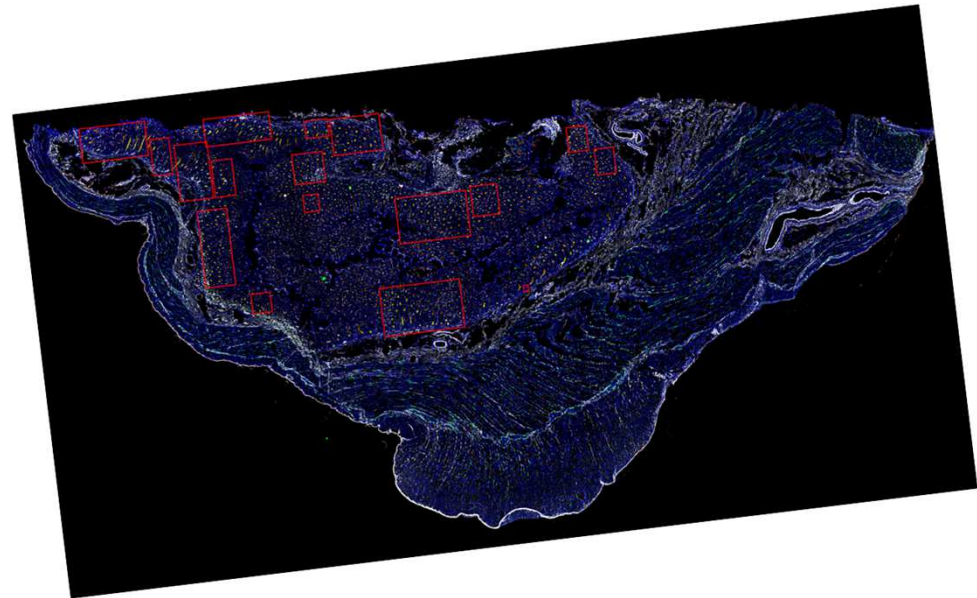


## Spatial transcriptomics for in-depth characterization of early ATI infected tissues

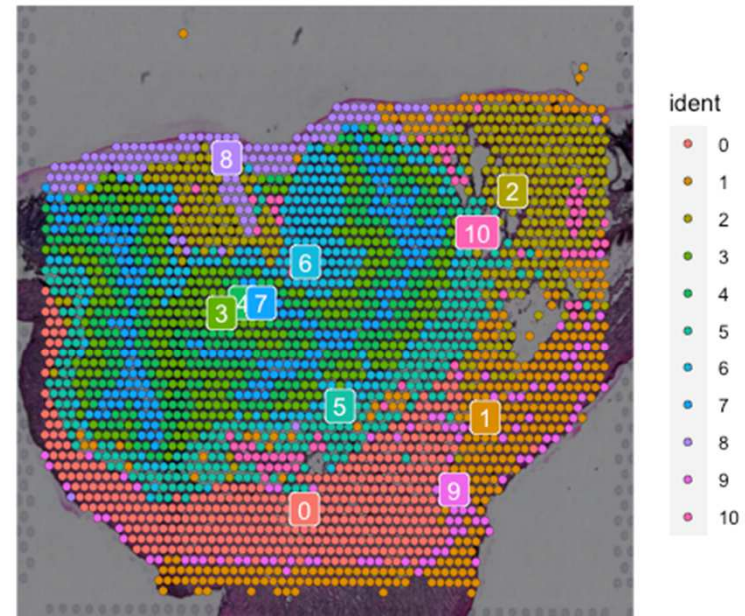
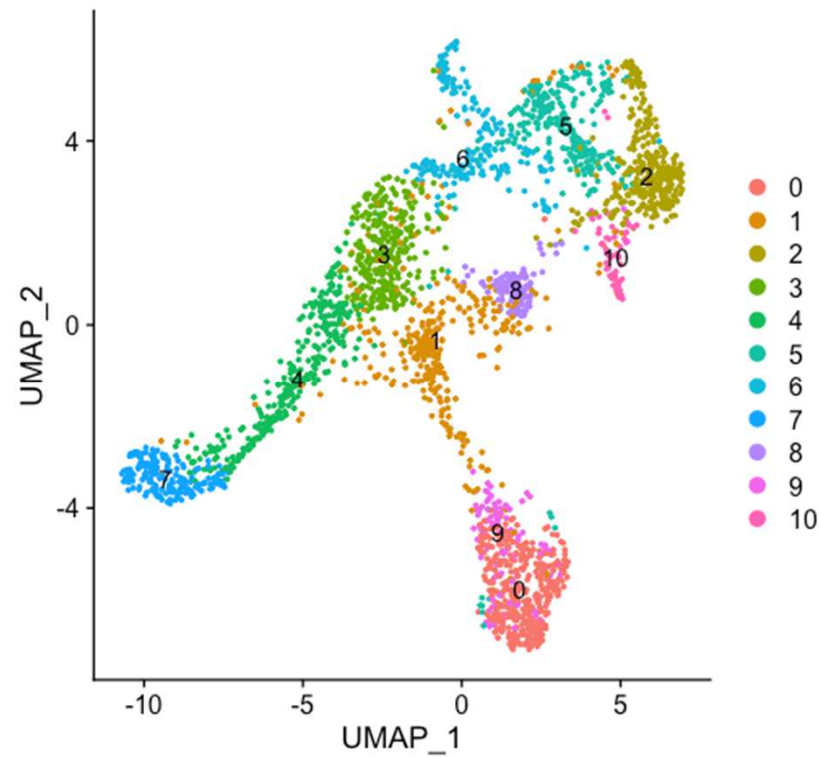


**4 days ATI  
T.Colon**

Areas of infected cells

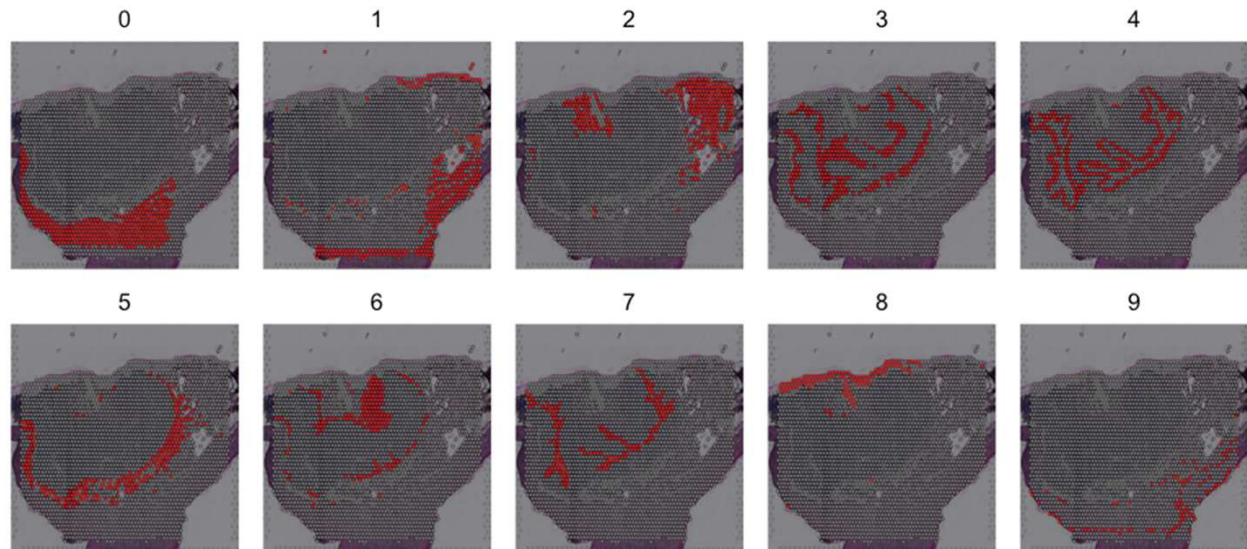
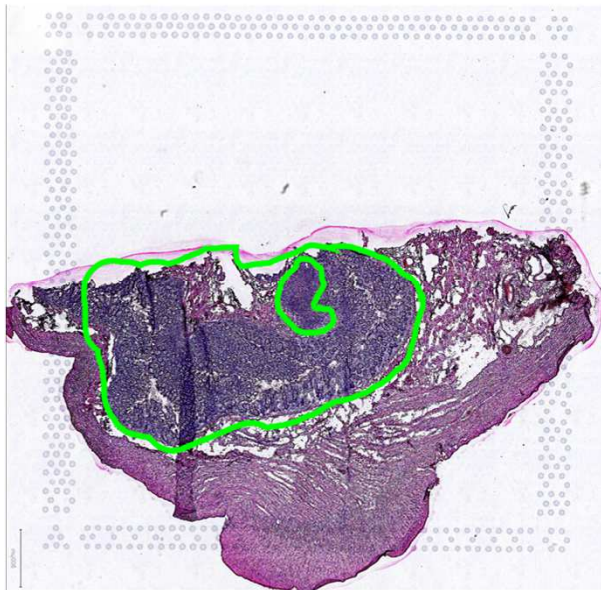


## Expression clustering and spatial distribution





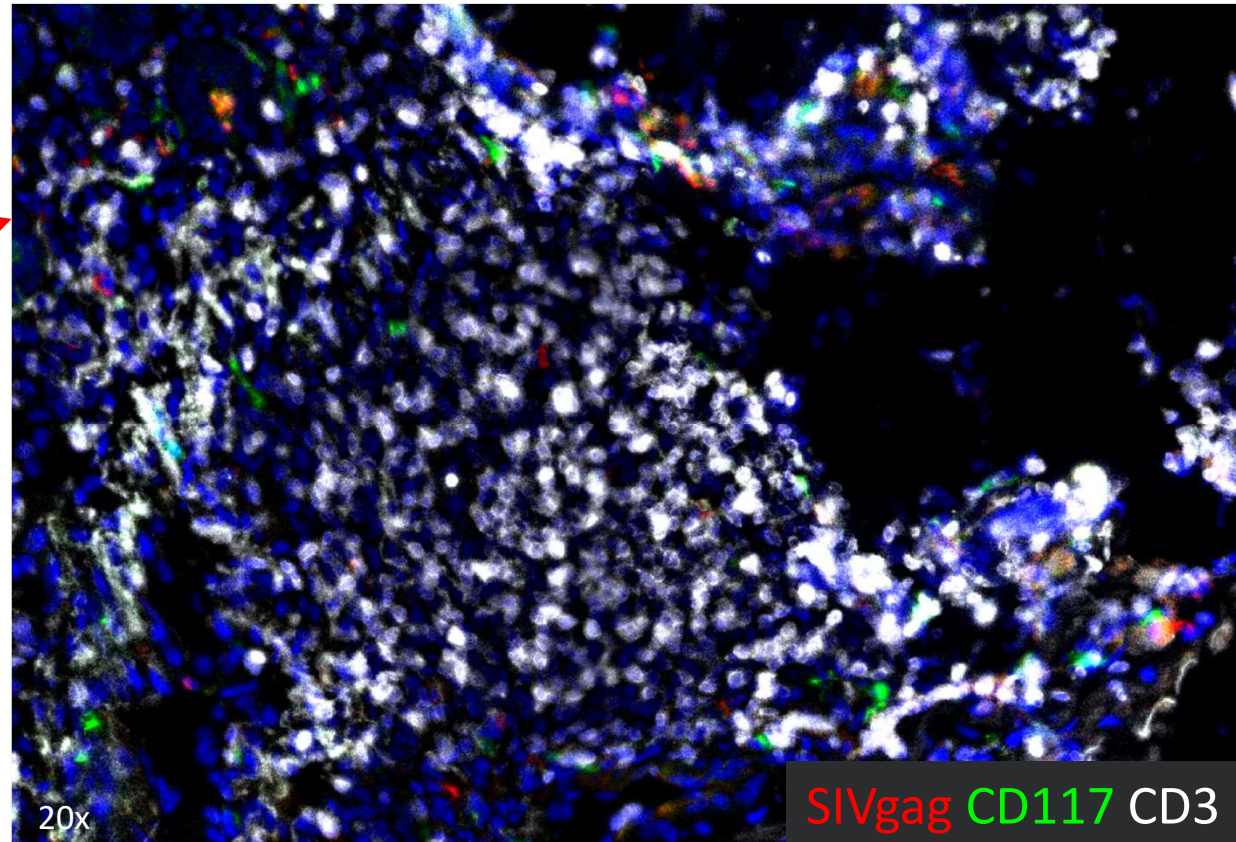
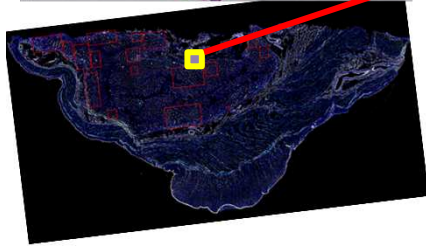
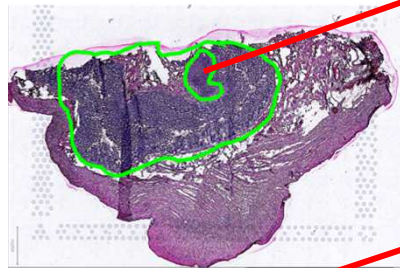
## Association of different expression cluster with tissue structures



# Rare virus detection in immune aggregates

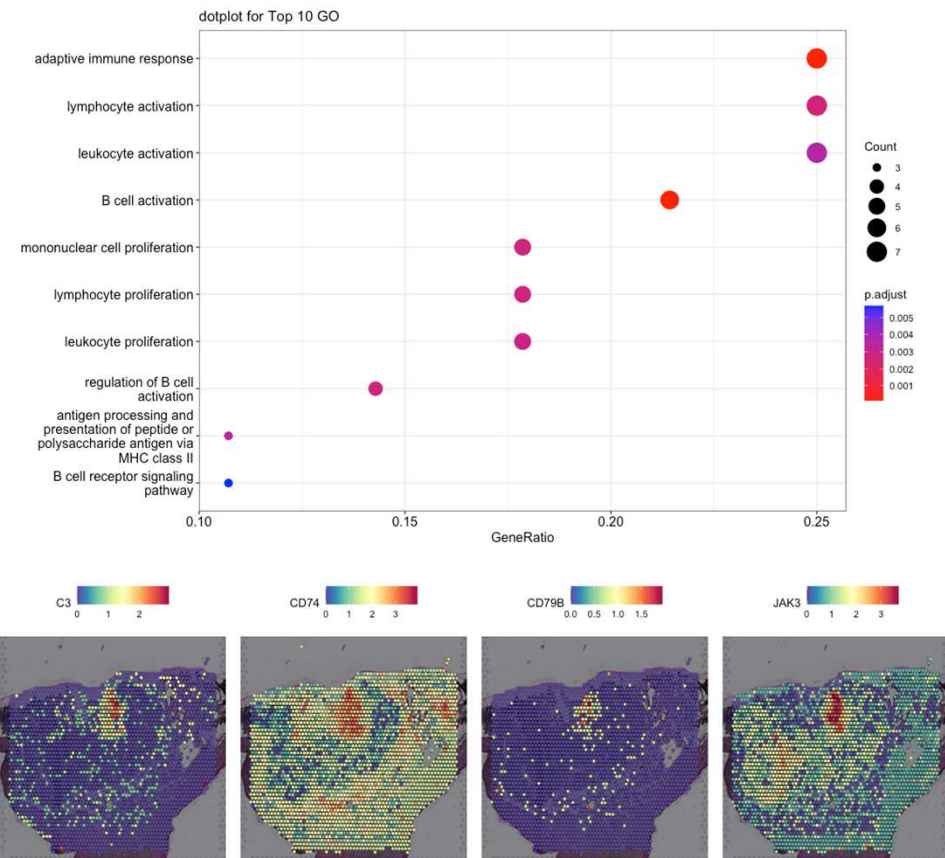
4 days ATI T.Colon

High density Immune cells



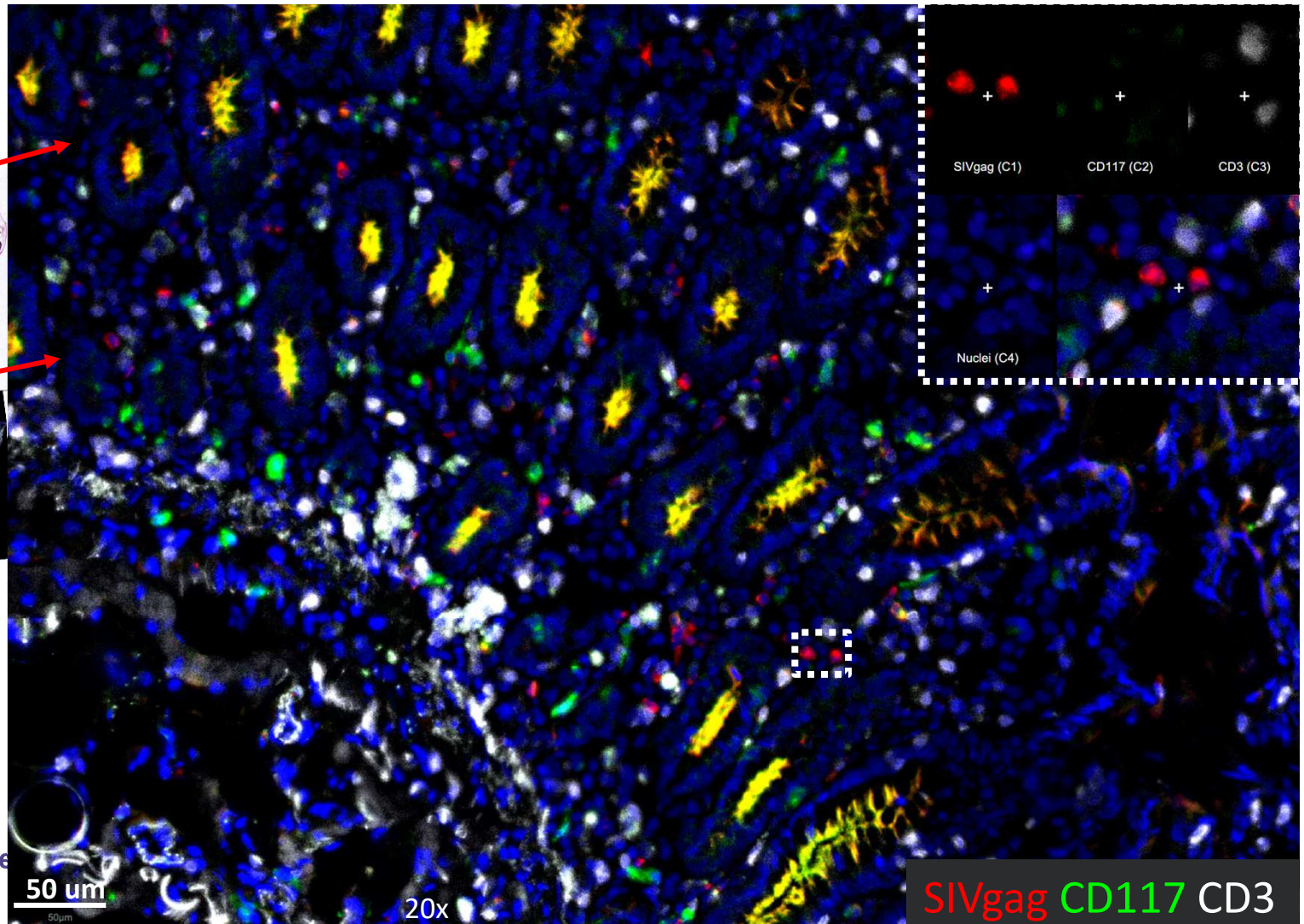
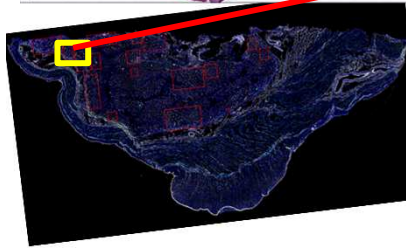
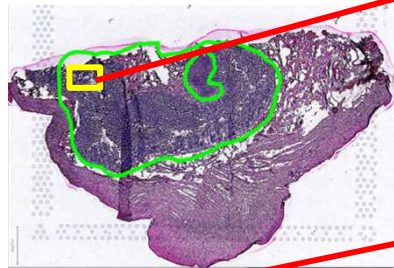


# Cluster associated with immune aggregates

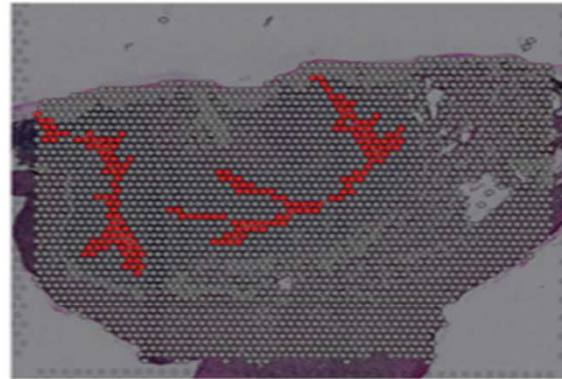
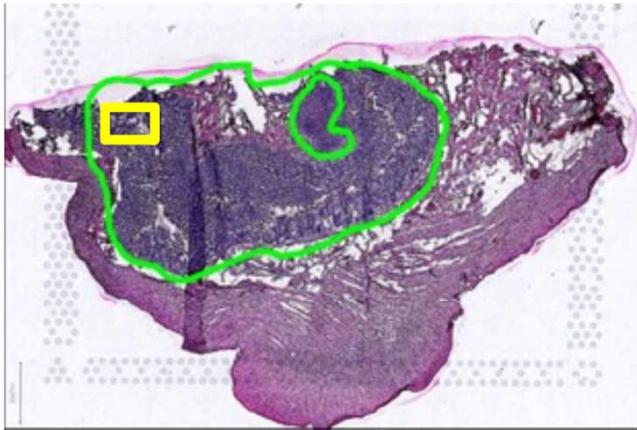




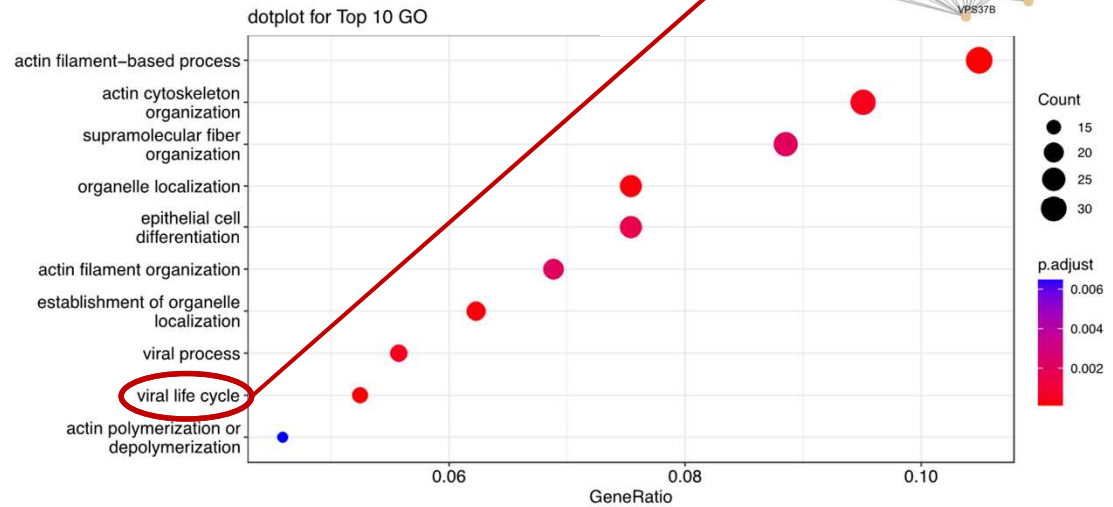
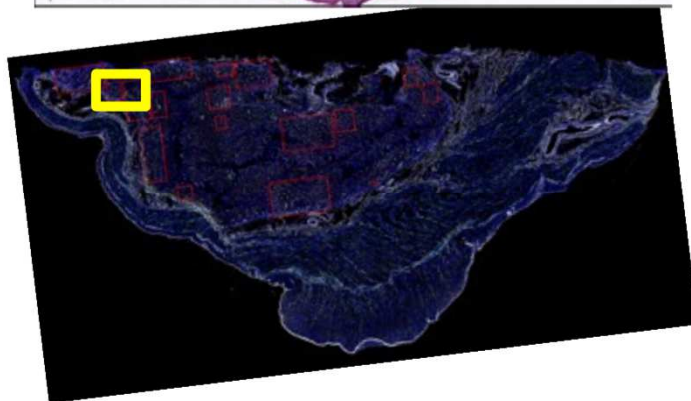
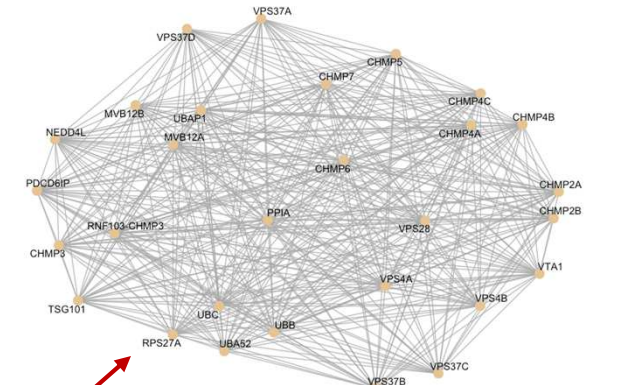
4 days ATI  
T.Colon



# Cluster associated with areas of infected cells



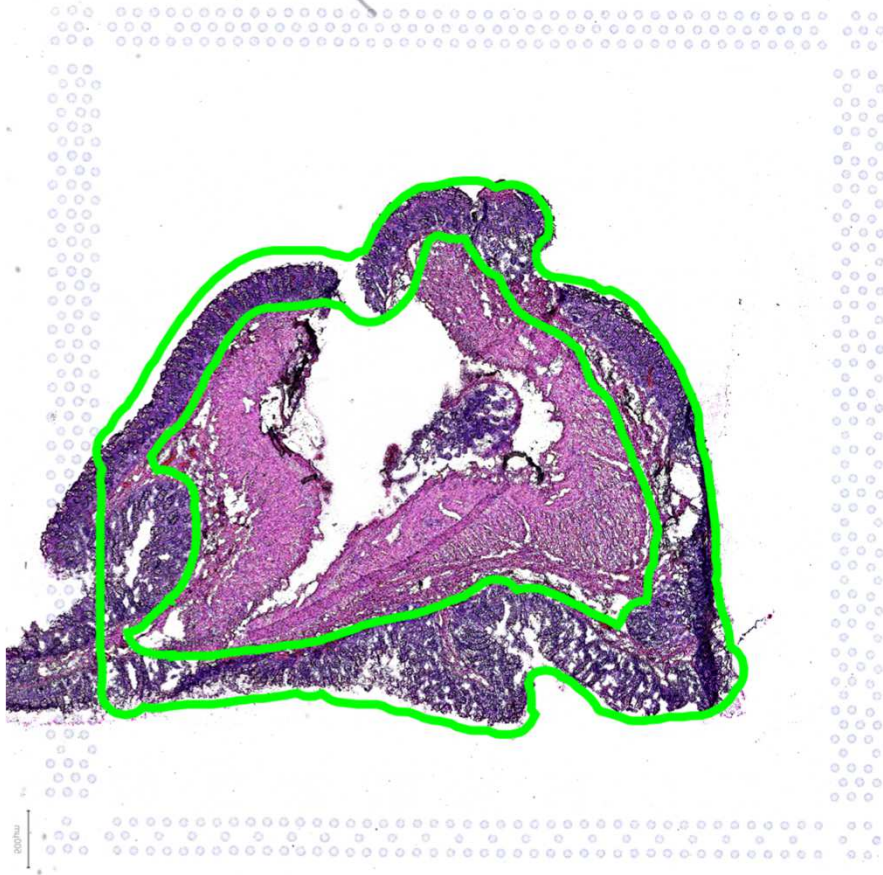
Budding and maturation of HIV virion



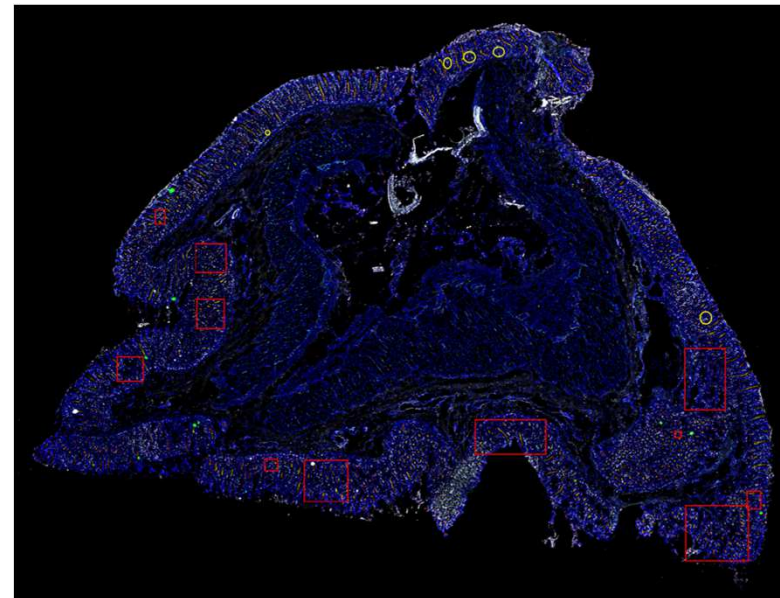


# Virus detection in tissues during ART

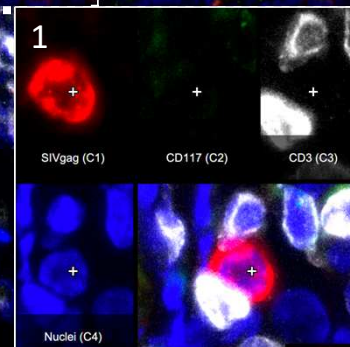
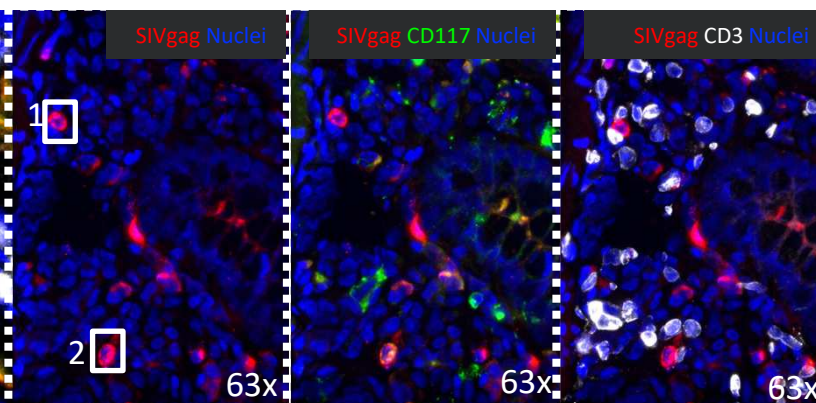
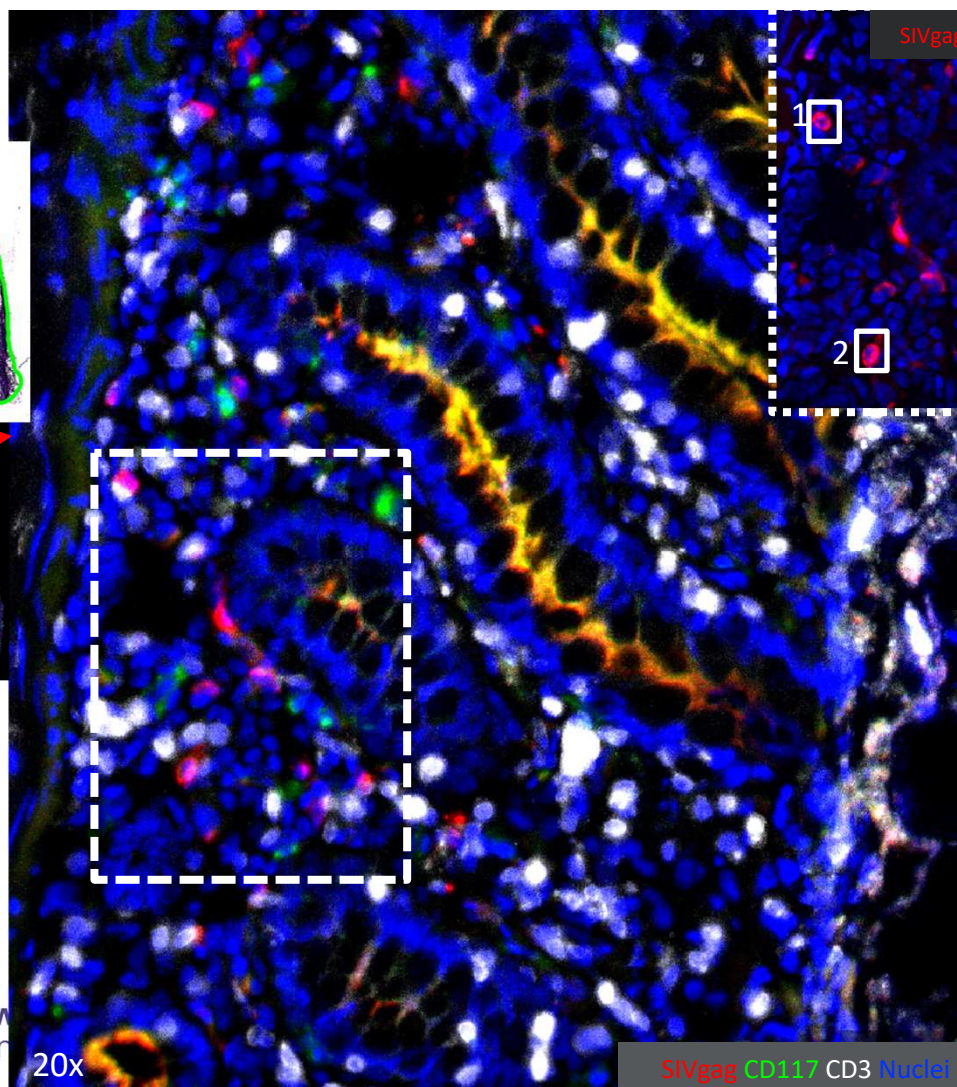
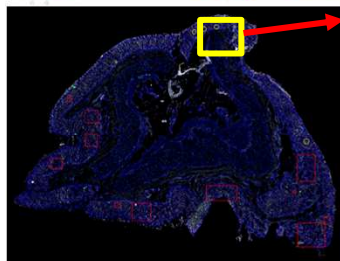
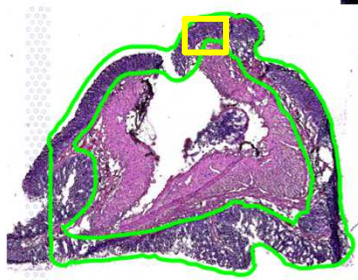
3 weeks ART T.Colon



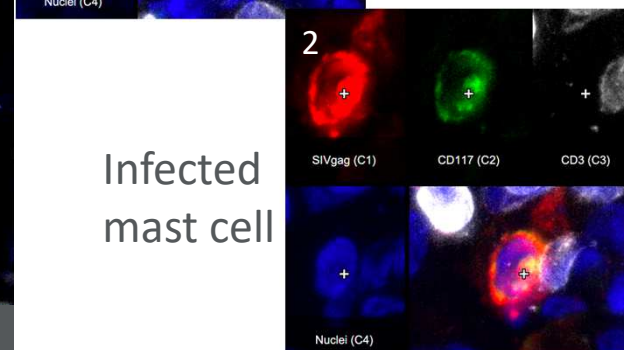
Areas of infected cells



3 weeks ART  
T.Colon



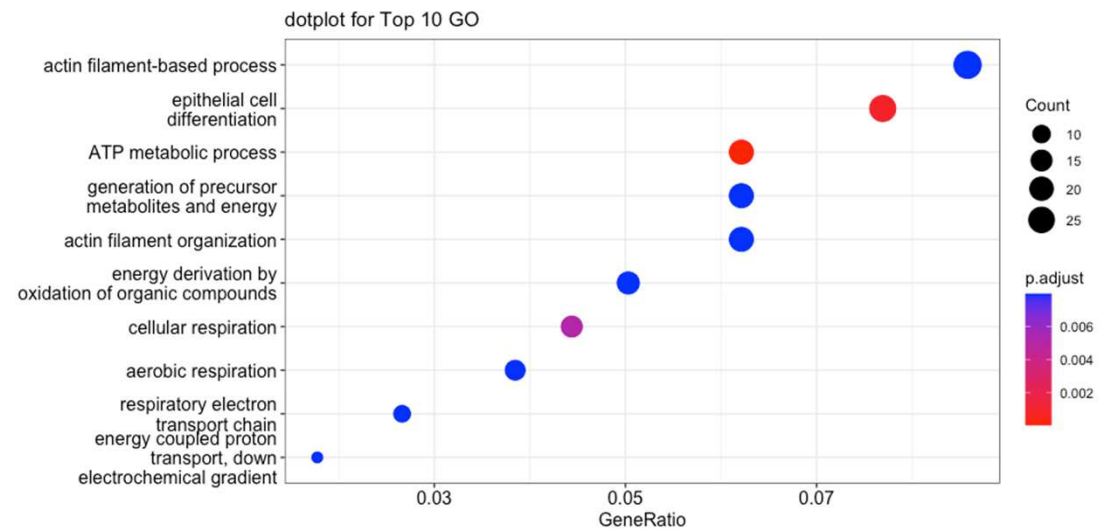
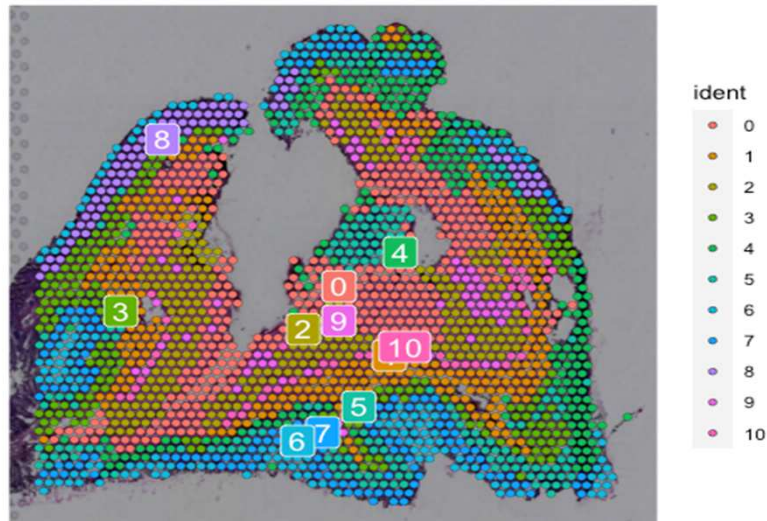
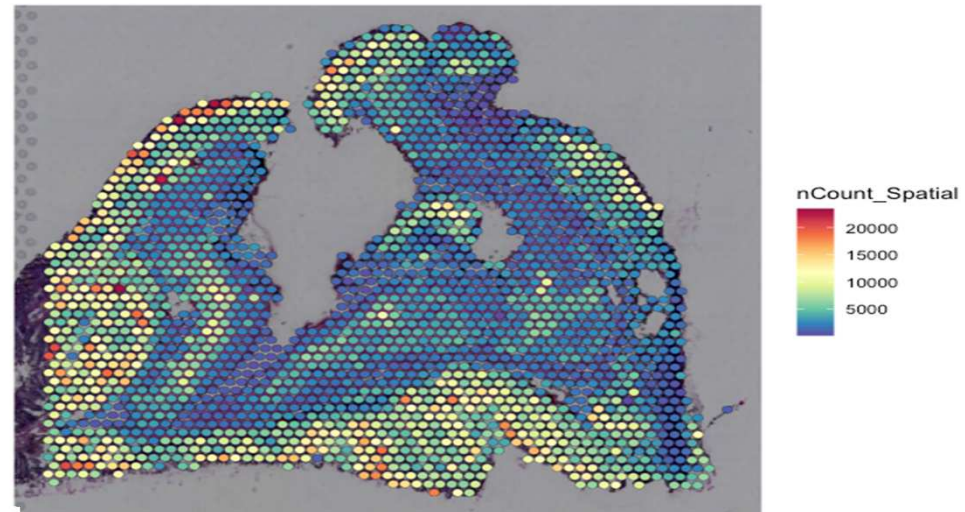
Infected  
myeloid  
cell



Infected  
mast cell

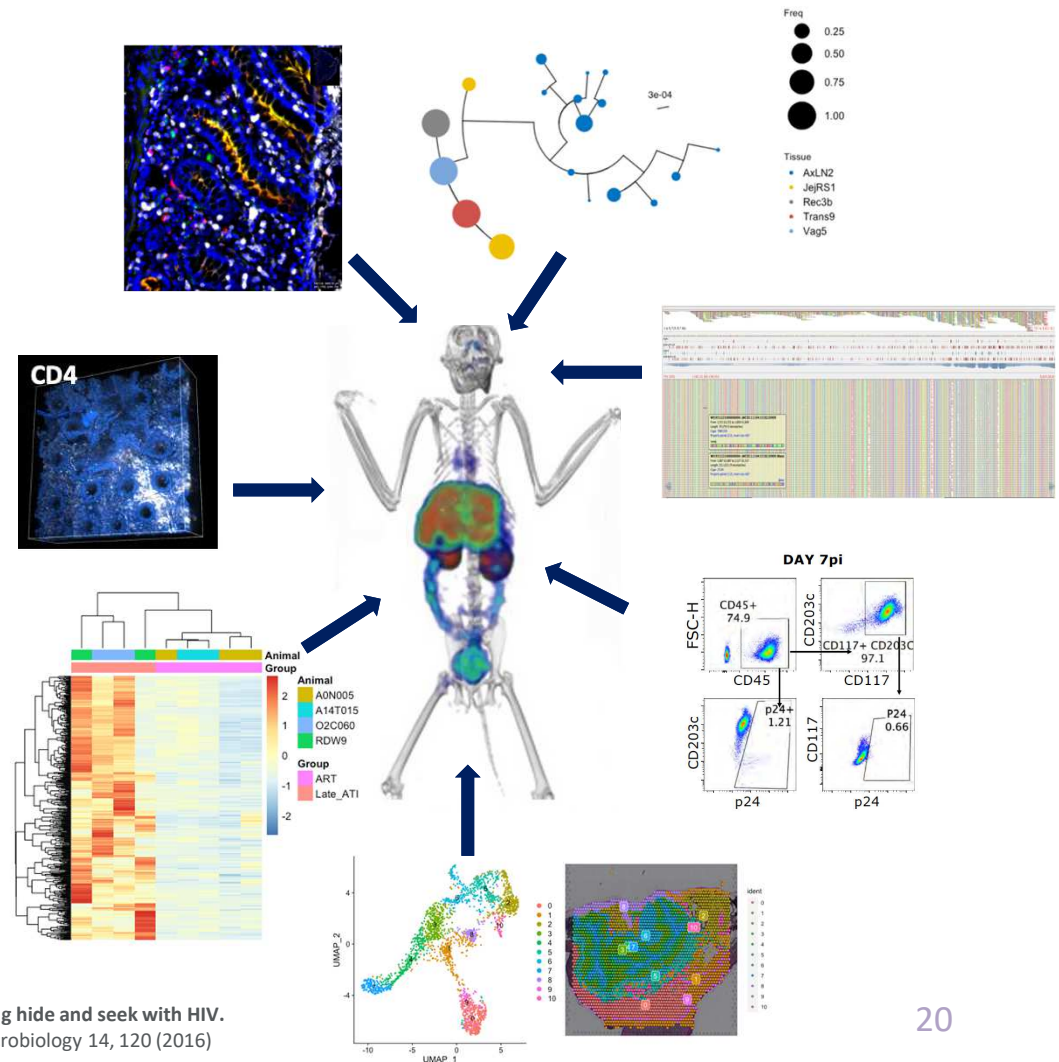
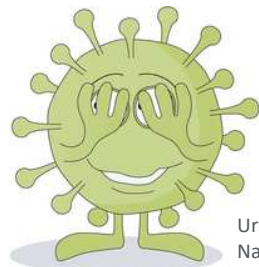


# Cell activation associated with areas of infected cells during ART



## Conclusions

- The addition of spatial transcriptomics to our set of tools has the potential to unveil key characteristics of the reservoir virus-host system at an unprecedented level.
- We detect very distinct transcriptional signatures after ART initiation, during the eclipse phase post-ATI, and late ATI.
- The toolbox is important, but the key is “to have the piece of tissue”. **Now we know where to look to find and characterize the reservoir.**





# Acknowledgements



Thomas J Hope



Muhammad Shoaib Arif



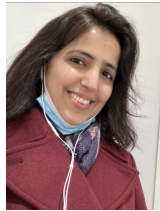
Elena Martinelli



Christopher Thuruthiyil



Francois Villinger



Sadia Samer

Mariluz Araínga



Mike McRaven



Richard T D'Aquila



Hope Lab

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of Health



NIAID



Thank You!

# Questions?