A Closer Look at HIV and Antibody-Based Interventions for Prevention, Treatment & Cure

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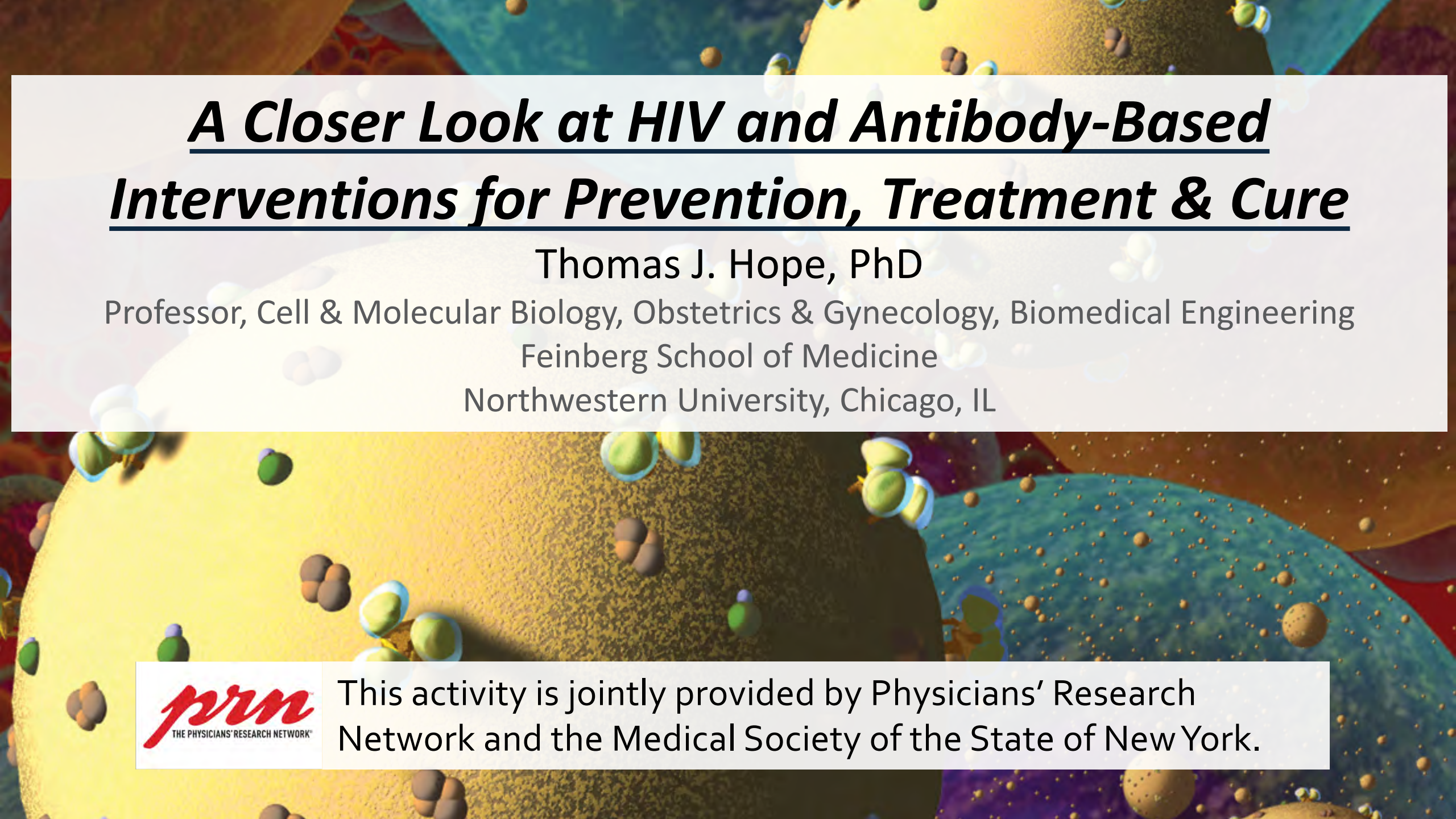


This activity is jointly provided by Physicians' Research Network and the Medical Society of the State of New York.

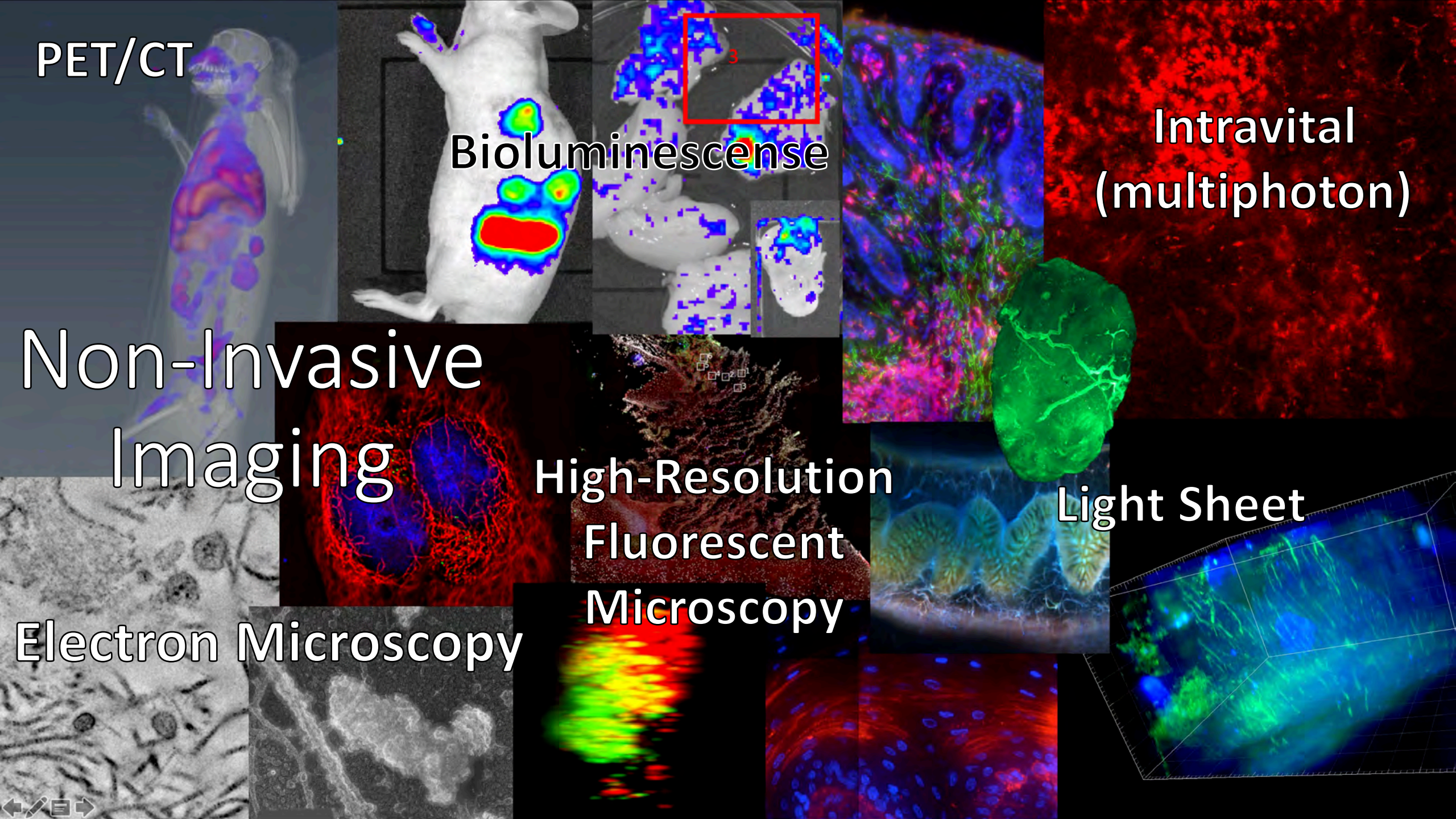
The Easy Questions Have Been Well Answered

Test tubes, tissue culture, sample analysis, epidemiology

- What is the HIV lifecycle?
- What are the drug targetable enzymes of HIV?
- Can we develop potent antiretrovirals that can treat HIV and prevent acquisition through prophylaxis?
- How is HIV transmitted?
- Where did HIV come from?
-

Understanding immune system function in humans and impact of HIV infection

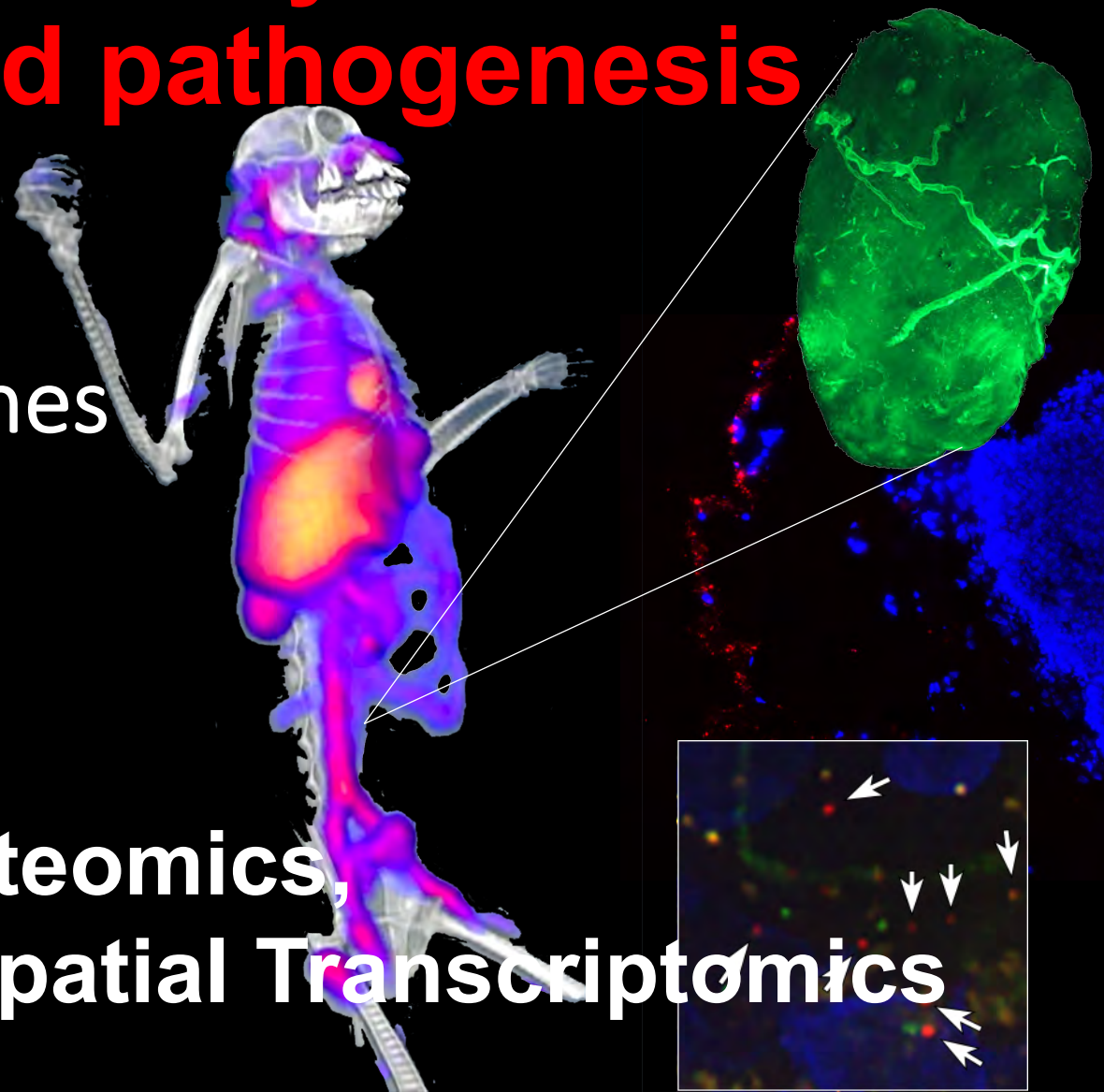
- How does HIV cause AIDS?
- Why does disease persist with successful viral suppression?
- What is the reservoir of persistence?
- Why can’t we make a vaccine?
- How do we incorporate the impact of anatomy and physiology to enlighten the questions above?


PET/CT
Bioluminescense
Intravital
(multiphoton)
Non-Invasive
Imaging
High-Resolution
Fluorescent
Microscopy
Light Sheet
Electron Microscopy

The impact of Anatomy and Physiology are needed to understand the Dynamics of HIV dissemination and pathogenesis

Imaging across all scales

We can utilize same approaches to study Transmission, Pathogenesis, Treatment, Persistence, Cure.

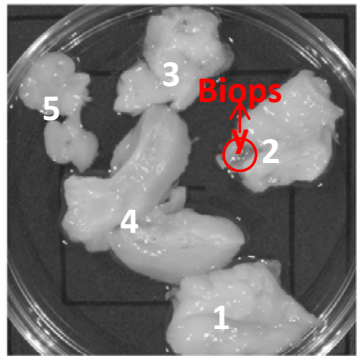


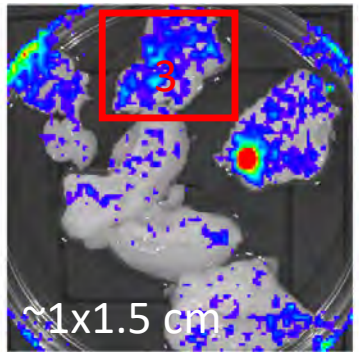
-omics **Metabolomics, Proteomics, Transcriptomics, Spatial Transcriptomics**

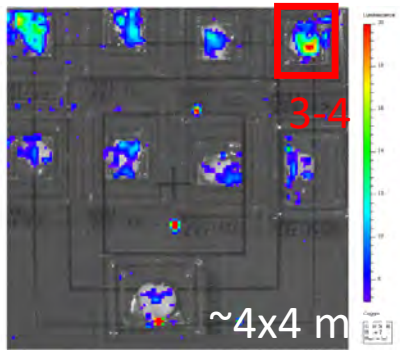
Beacon Guided Necropsy

How do you find rare focal events? -Need to know where to look.

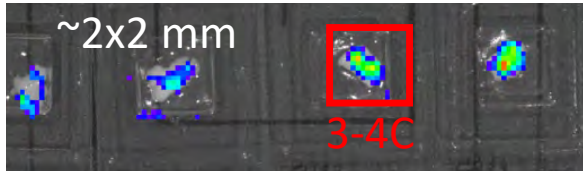
Correlative Bioluminescense

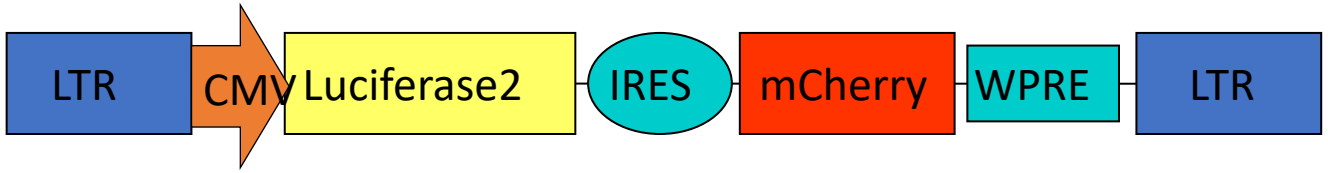






Subsection of the tissue and reimaging using IVIS

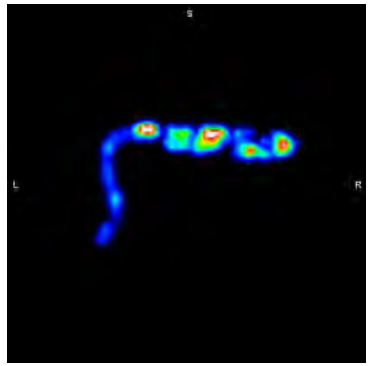


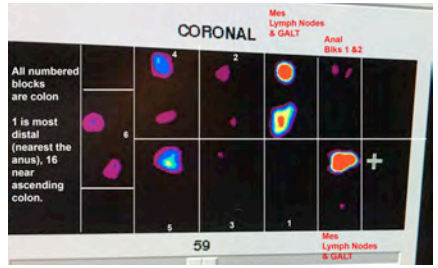


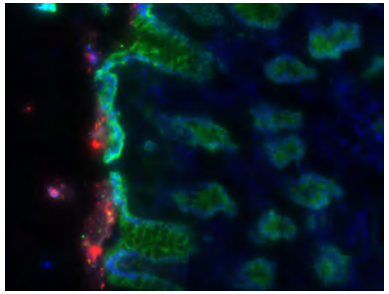
Reveals areas of barrier disfunction revealing site of SIV infection

Correlative PET

Combine fluorescent labeling (100%) with ^{64}Cu labeling (~5%)





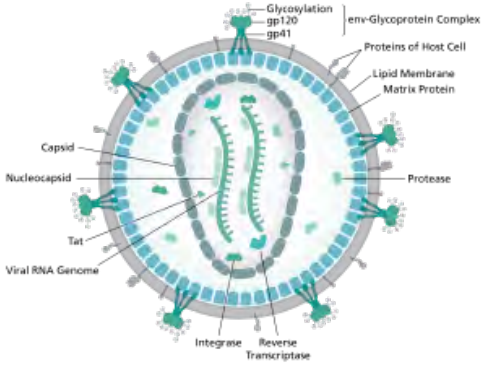


Dissect out the piece of tissue that contains the correlative signal

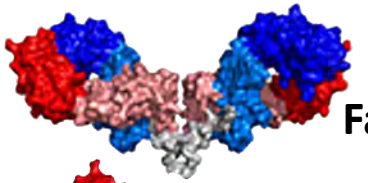
Use PET/CT with 3 kinds of probes (3-ways so far)

Combine PET/CT with fluorescence methods. Powerful enrichment to our fluorescent methods.

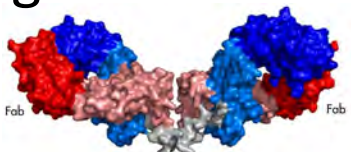
1) labeling of SIV/HIV particle to monitor particle distribution.



2) labeling of probe to detect viral envelope. Currently Fab2 anti-SIV envelope antibody. NEW: J3 cameloid nanobody. Detection of Viral envelope protein in vivo (envelope expressing cells)



3) labeling antibodies (intact) to monitor kinetics and extent of distribution (Fc influence).



Logistics

- **CT scan takes 90 seconds**
- **PET scan takes 15-20 minutes**

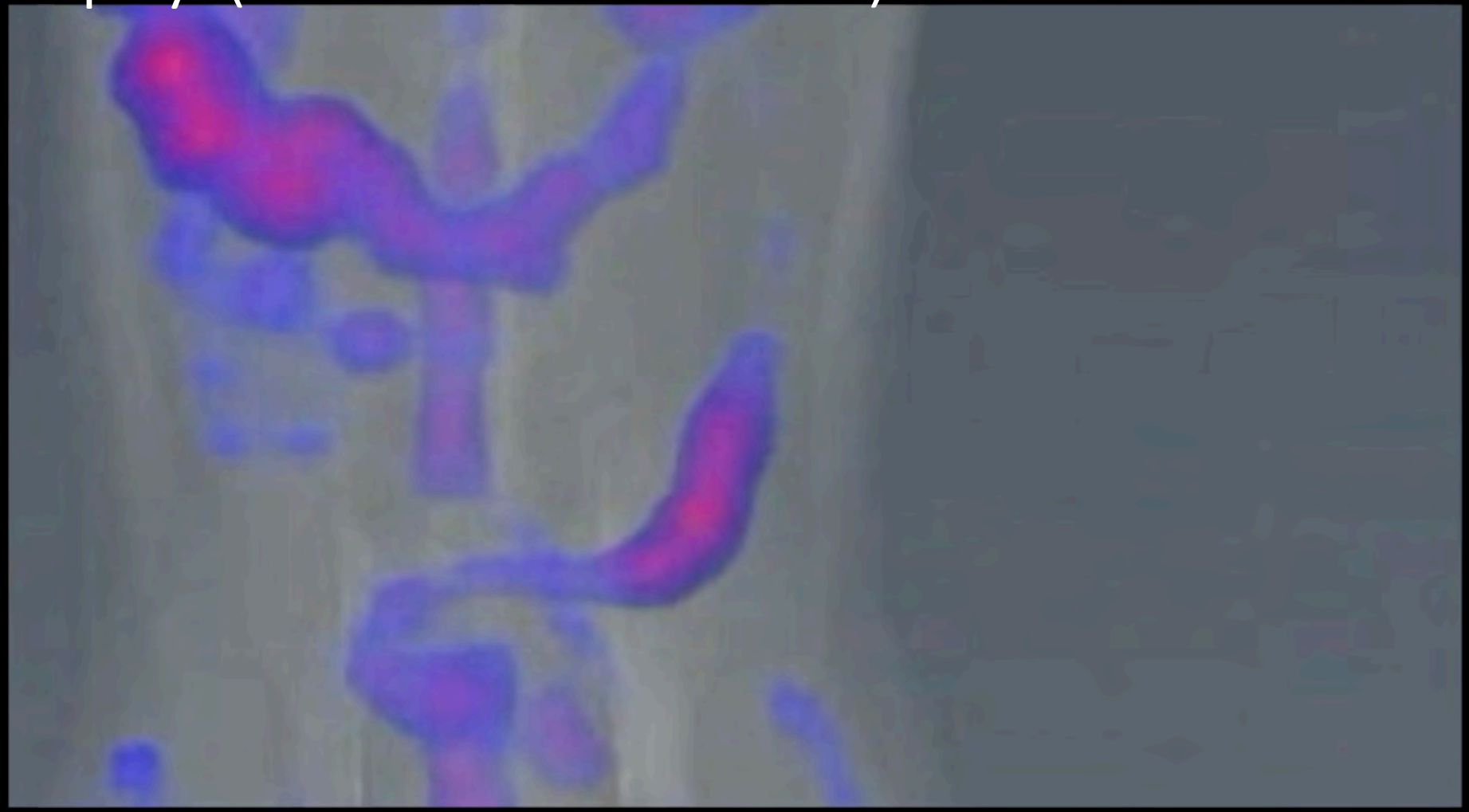
- **Half-life of radioactive probe**
  - longer half-life's can allow longer kinetics

^{64}Cu 12 hours

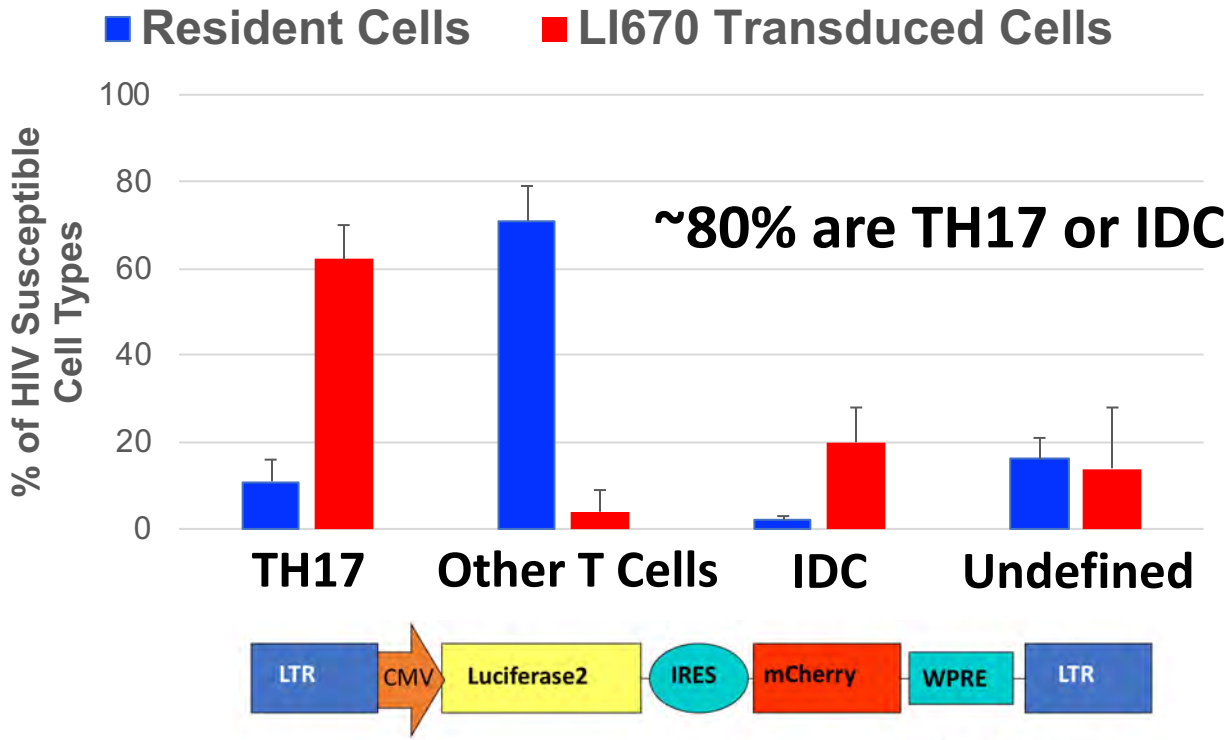
^{89}Zr 3.3 days

"Hot" monkeys and excrement

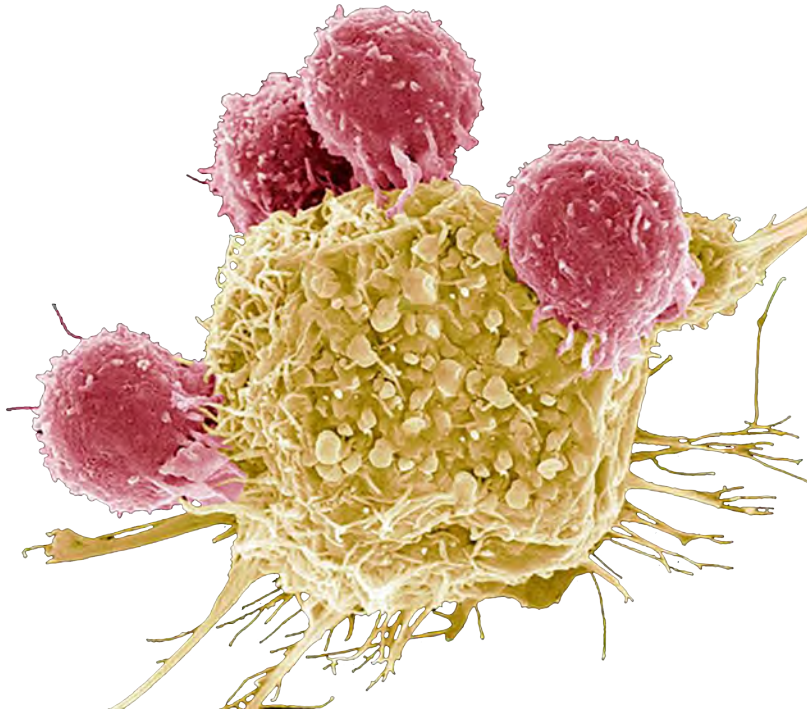
Finding infected cells – Beacon guided necropsy (Bioluminescense) – LICh



Th17 lineage and immature dendritic cells (IDCs) are the primary initial targets of vaginal and rectal transmission.



IDCs and Th17 cells monitor local tissue environment via antigen surveillance.



- Th17 lineage cells are known to be depleted during acute infection of HIV/ SIV (McKinnon et al 2015, JAIDS, Xu et al. 2012 Muc Imm)
- Immature dendritic cells are localized in mucosal sites.
- Both cell types are metabolically active and provide an excellent cellular environment to facilitate HIV replication.

PET/CT provides transformational insights into the impact of anatomy and physiology on HIV

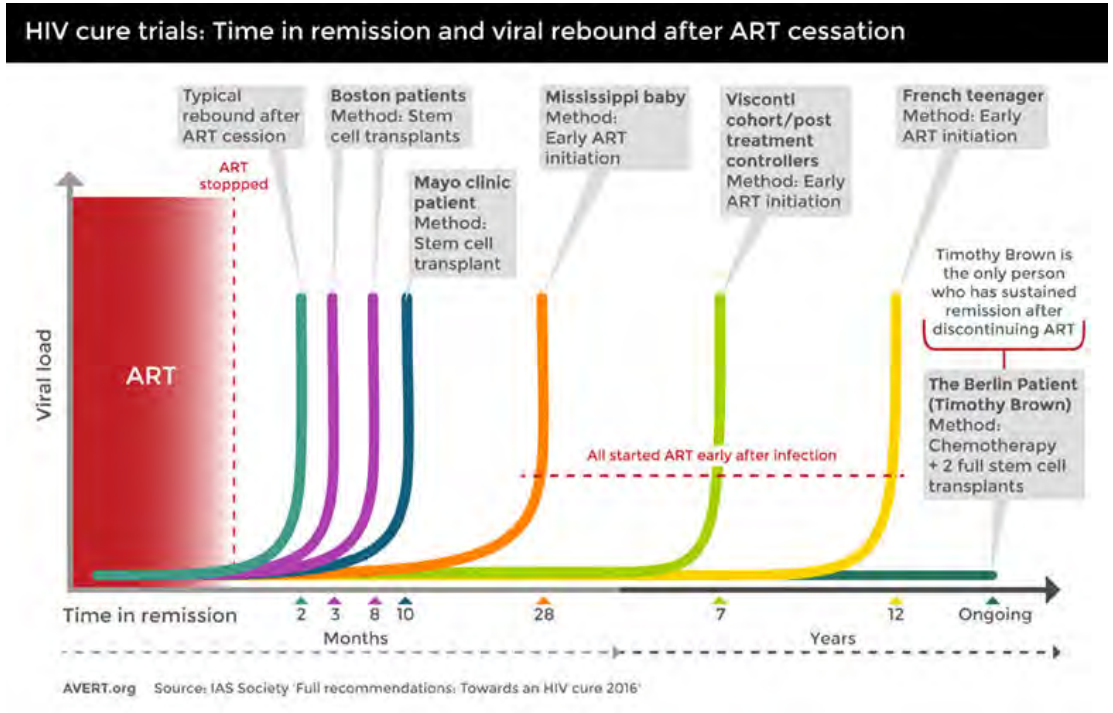
- The radioactive signal not influenced by tissue.
- PET/CT provides unbiased localization of radiolabeled probe in context of anatomy (CT or MRI)
- Repeated scanning provides information about dynamics.
- Different probes can ask different questions.
- Can directly visualize the impact of anatomy and physiology on biomolecular dynamics.

Reservoir of persistence with successful treatment

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?



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What is the source of persistence of replication competent HIV during ART suppression?

- Immediate rebound after ATI. (Our premise is that source is continuously producing virus)
- Established immediately (early reservoir)
  - can start as early as starting ART 1 day post challenge
  - the longer the time delay before ART start, the longer the reservoir persists.
- Need to wait 6-7 days to initiate ART before persists reliably for 1 year. 4 days doesn't last a year.
- This persistence can survive in humans after decade(s) of suppressive ART. Source of renewal?
- **Can we adapt technologies that we developed to study the earliest events mucosal transmission (eclipse phase before viremia) in non-human Primate models to study rebound and reservoirs?**

Recent insights into the HIV reservoir

- Rebounding virus is impacted by antibody and CTL responses (filtered).
- Rebounding virus is highly resistant to interferon indicating filtering by innate responses.
- Reservoir in blood primarily represented by virus populations dominant at time of cART initiation.

Are these consistent with latent T cell origin of rapid rebound after ATI? –NO!

Using PET a as tool for studying the viral reservoir.

Monkeys were infected vaginally and rectally.

Four days post infection, the monkeys were started on ART

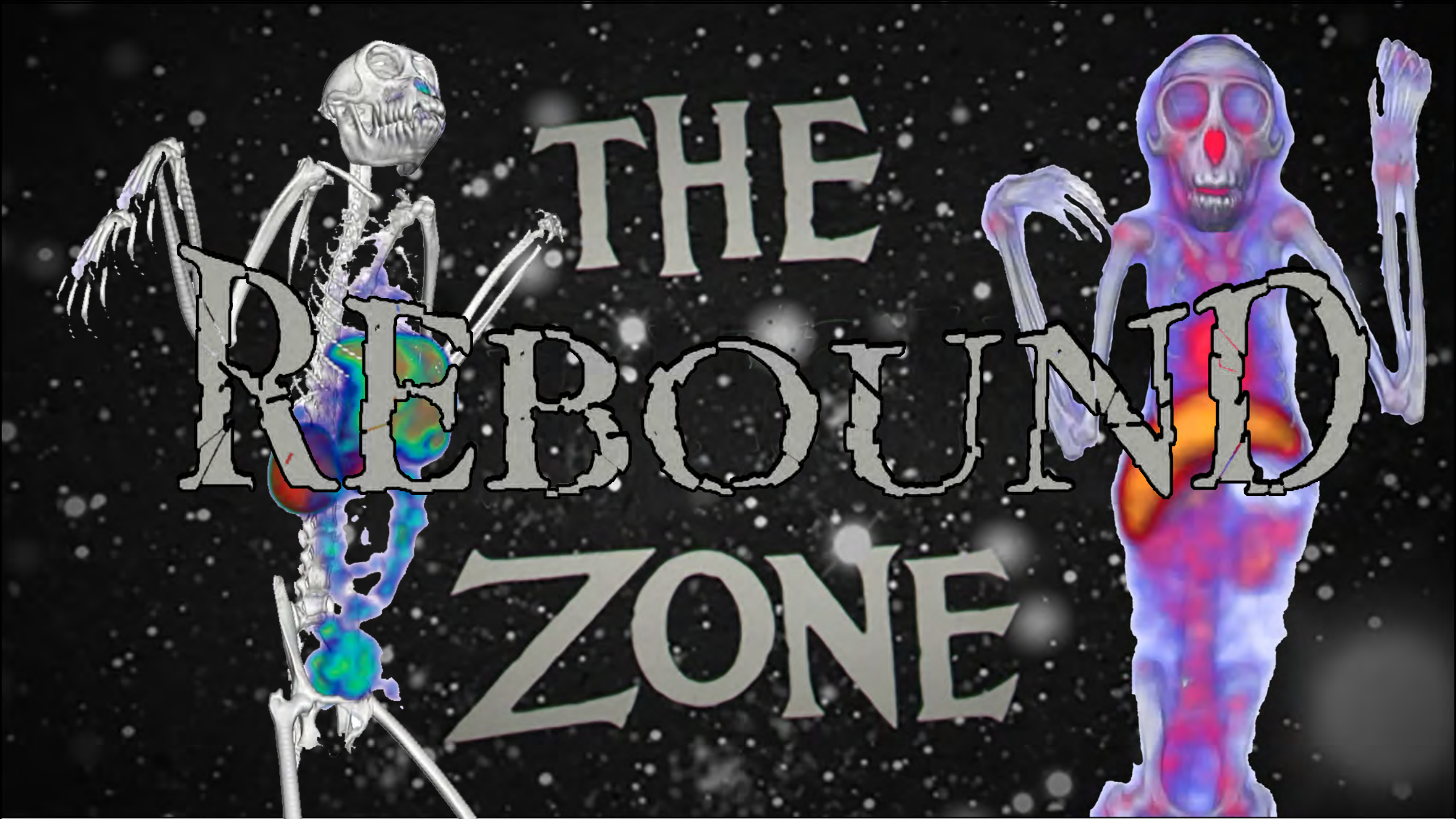
6 MONTHS!!!

ART concluded

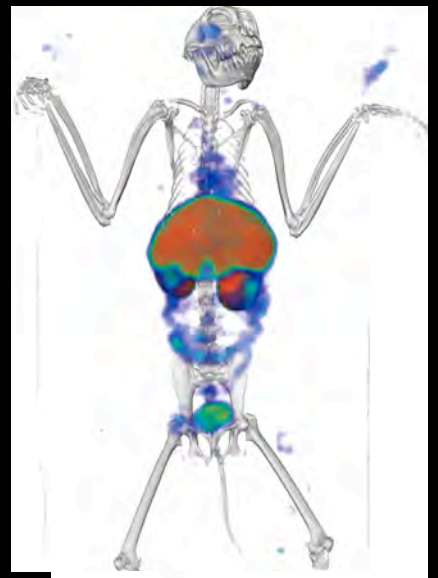
Tissues harvested for microscopy and PCR

PET/CT scans were taken periodically while on ART and after ART was concluded

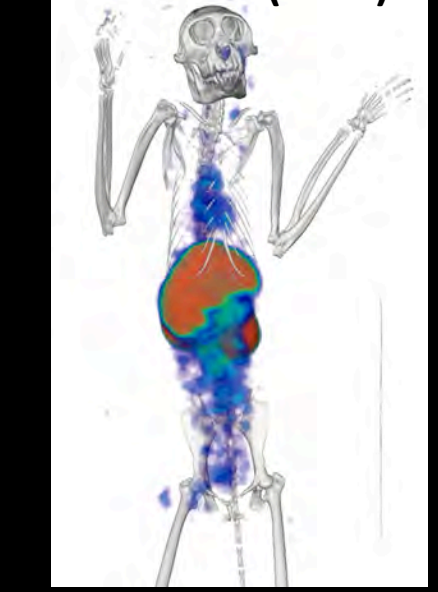
4 ,5,7, or 10 days

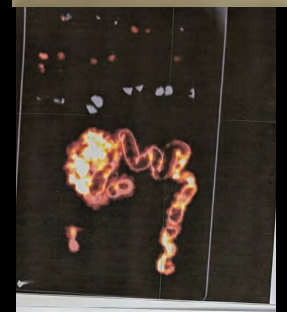

THE
REBOUND
ZONE

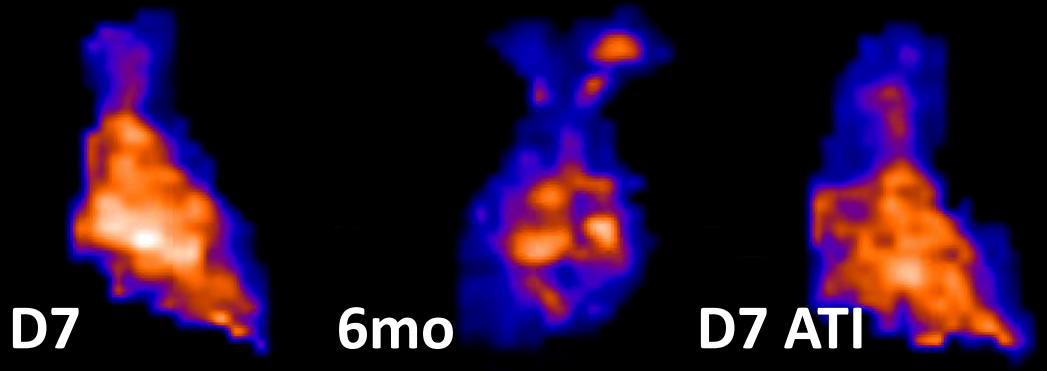
Rebound Zone – Defining foci of Rebounding SIV



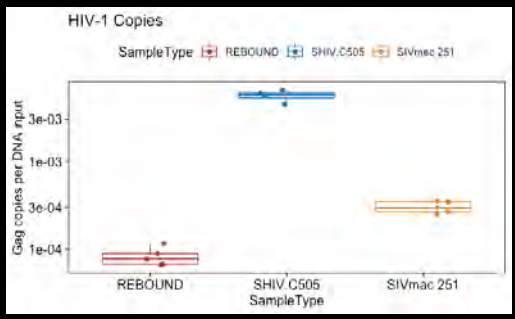
First Scan (1 wk)





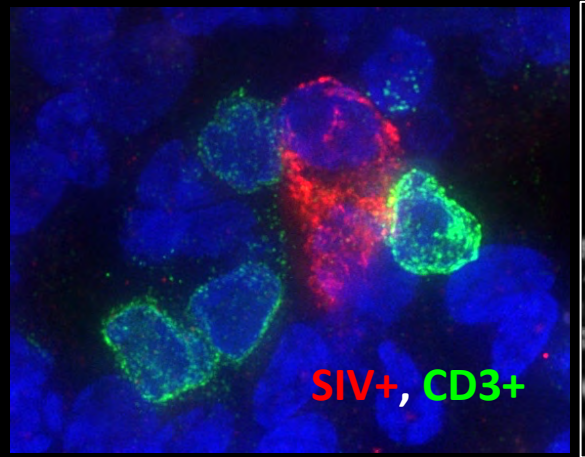


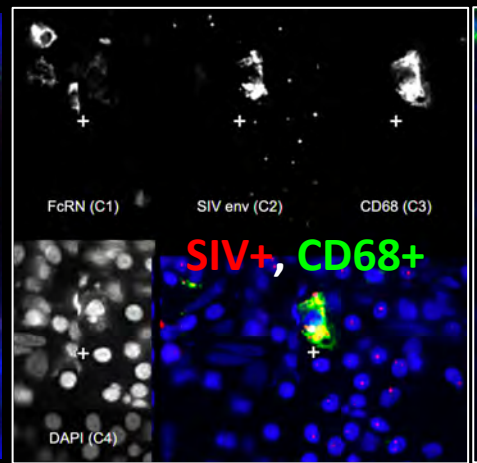
D7 6mo D7 ATI

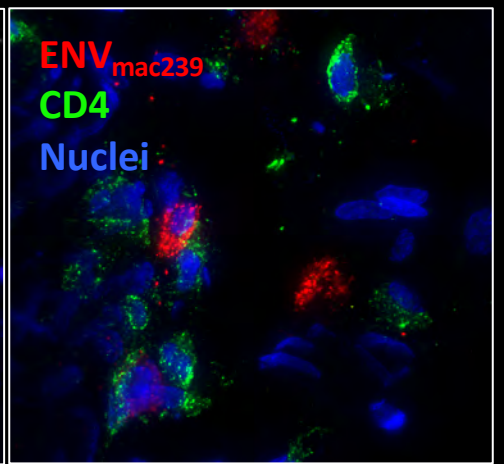


PCR of heart tissue

- Rebound signal detected in heart of multiple animals
- No infected T cells are detected in any rebound animals. CD11b-, MHCII-, SIV+ population confound definition







REBOUND REFLECTS RESERVOIR

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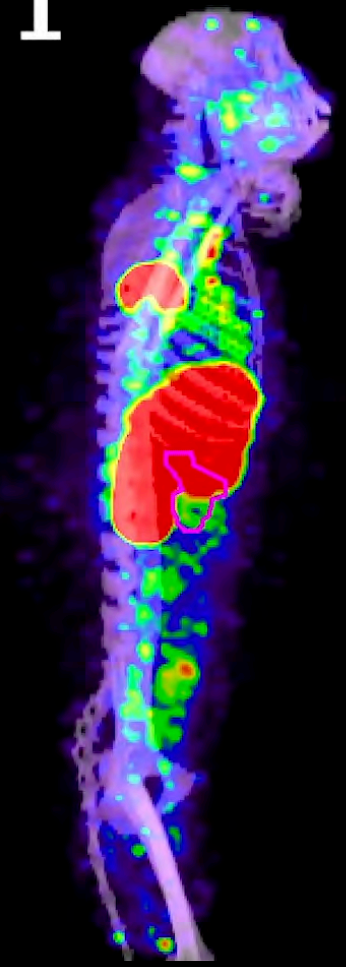
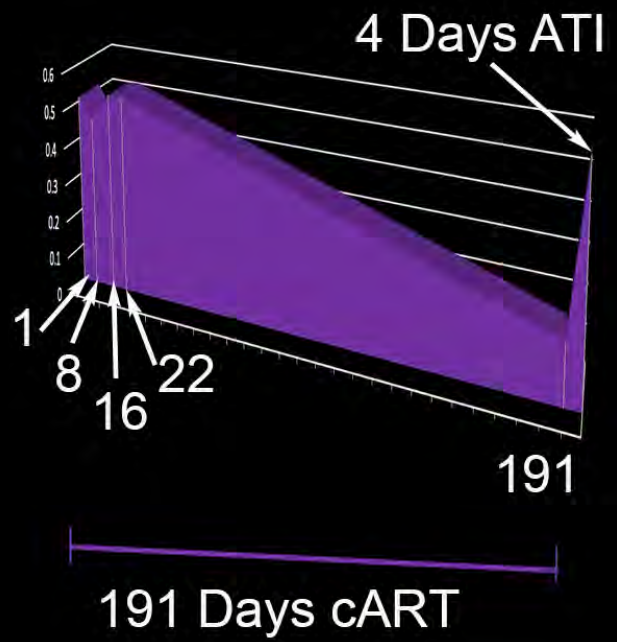
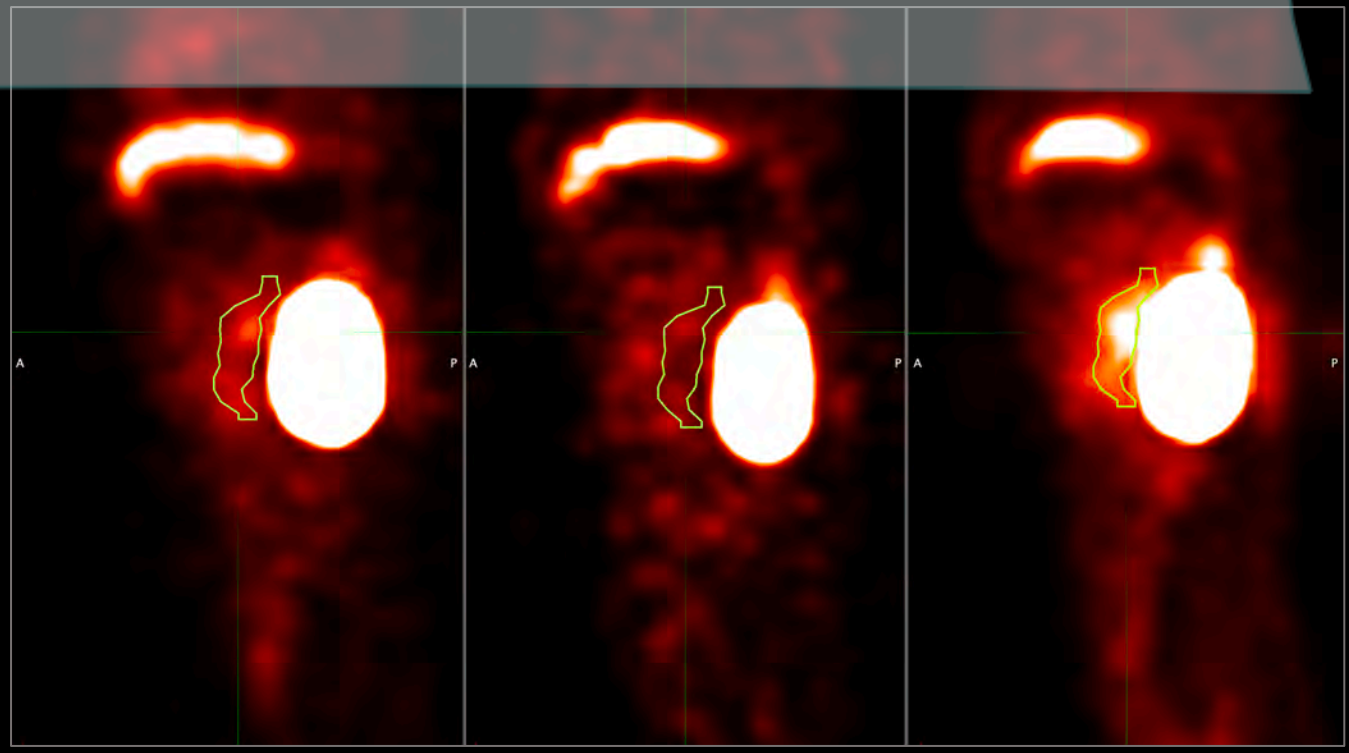
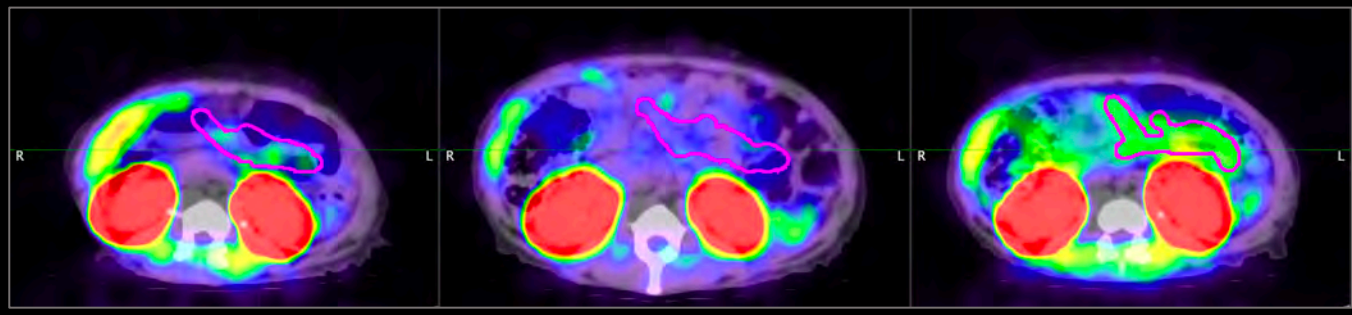


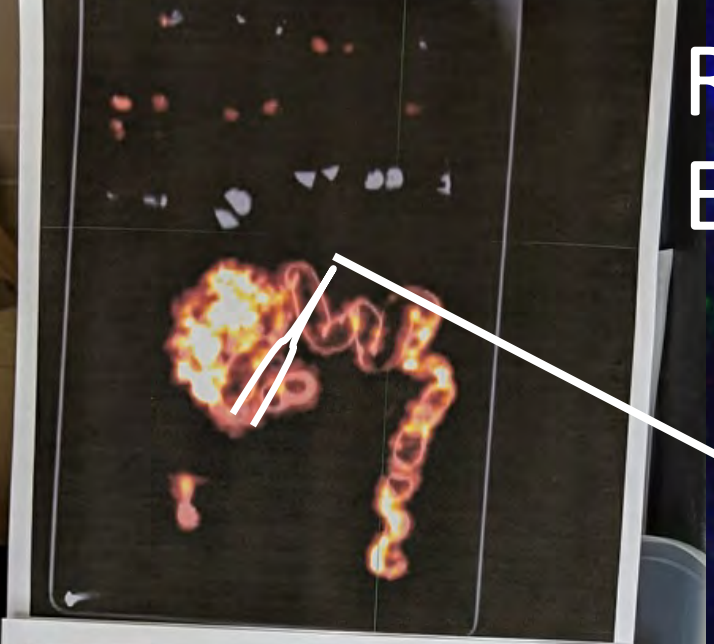


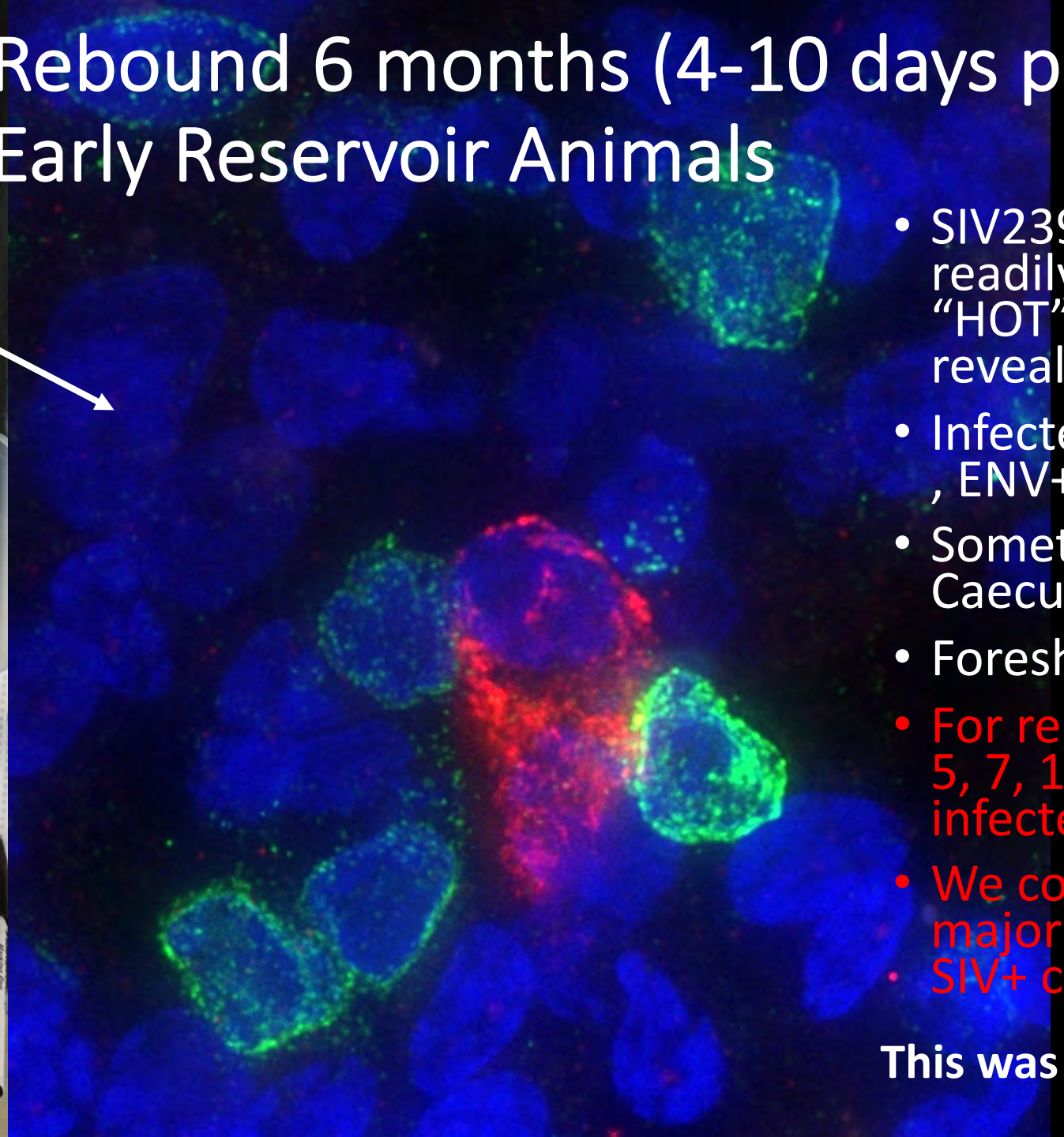




Rebound can return at sites of active signal when cART initiated. An immobile reservoir.

Rebound 6 months (4-10 days post ATI) in Early Reservoir Animals

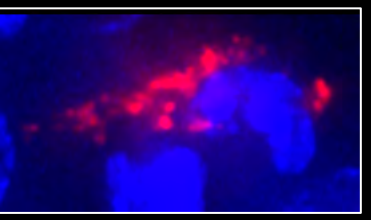




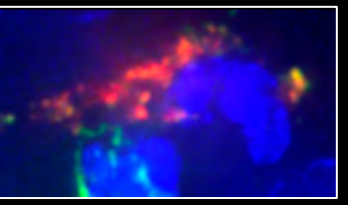
- SIV239 infected cells readily identified in “HOT” tissues revealed by PET/CT
- Infected cells are CD3-, ENV+
- Something about Caecum (????)
- Foreshadowing?
- For rebound after 4, 5, 7, 10 days no SIV infected T cells.
- We could not identify major population of SIV+ cells

This was making me crazy!

MAST CELLS



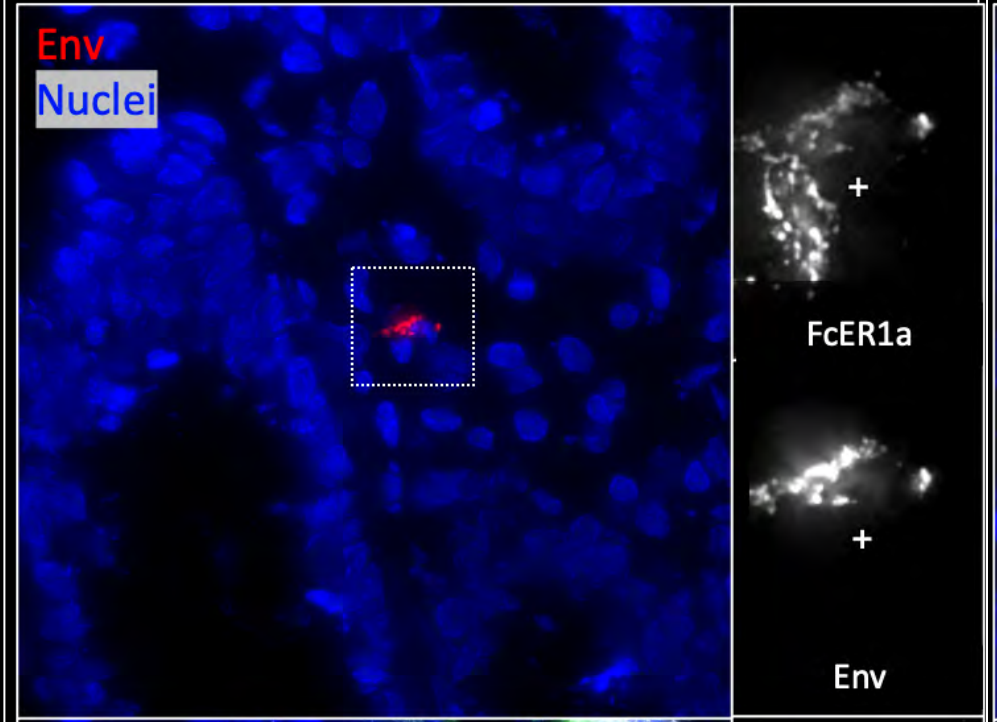
Env
Nuclei

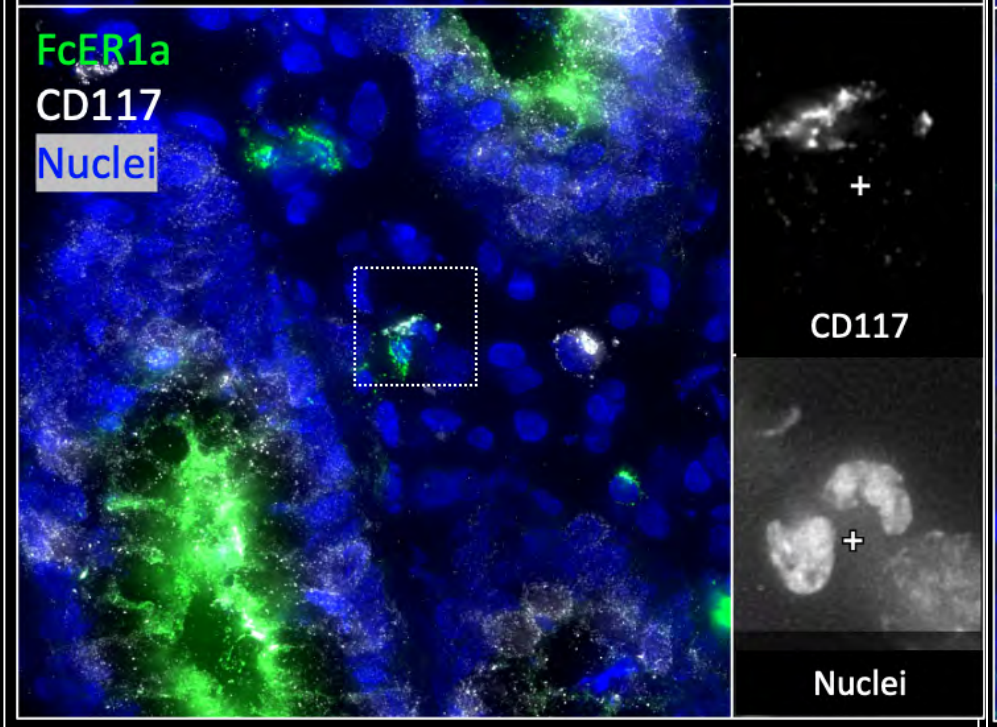


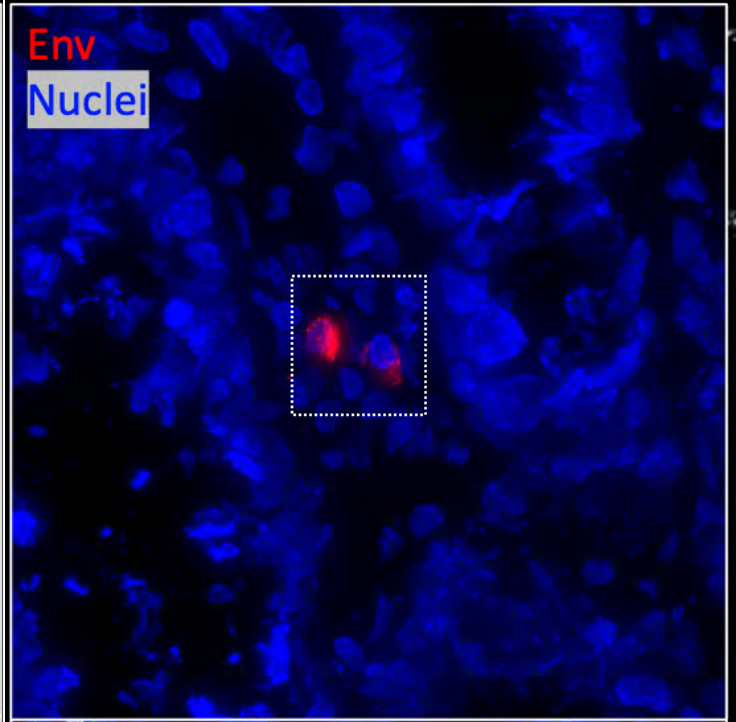
FcER1a
Env
Nuclei

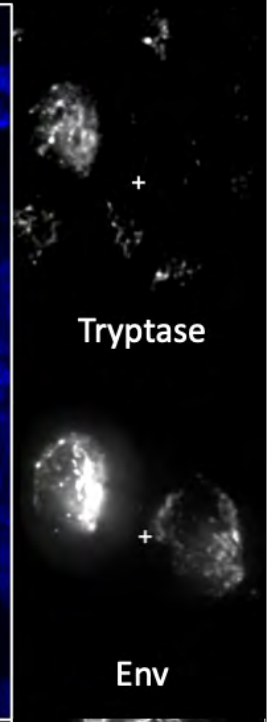
Env
CD117
Nuclei

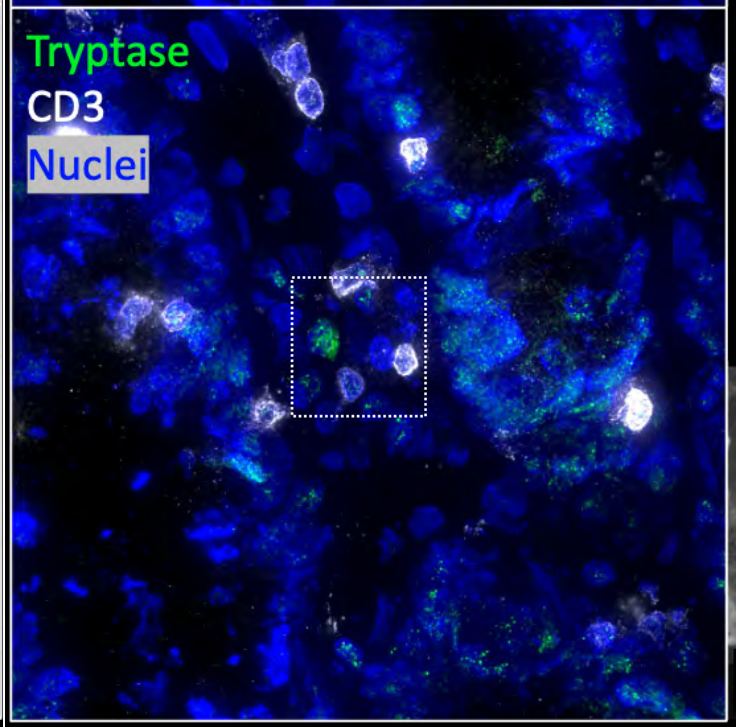
FcER1a
Env
CD117
Nuclei

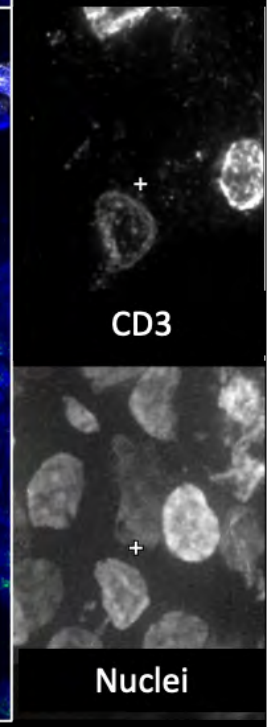






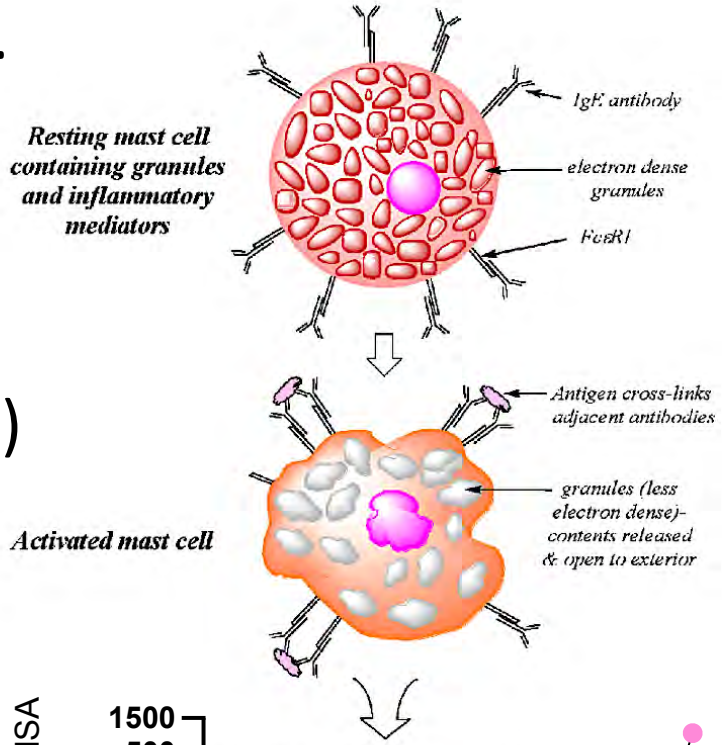


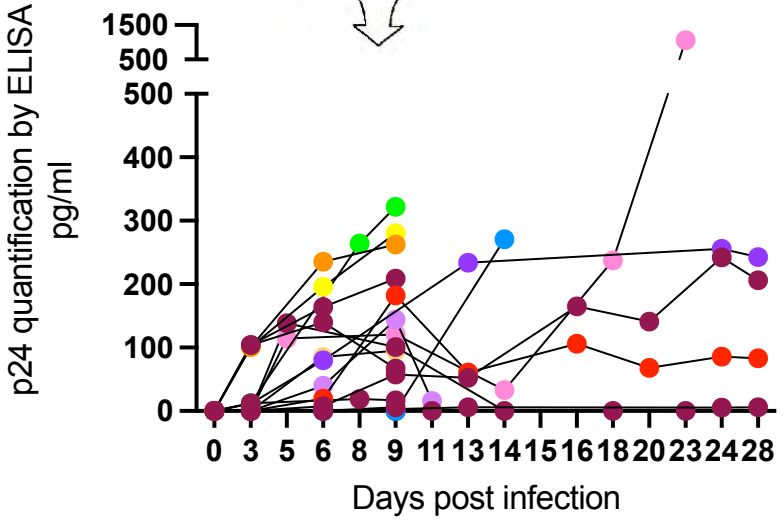


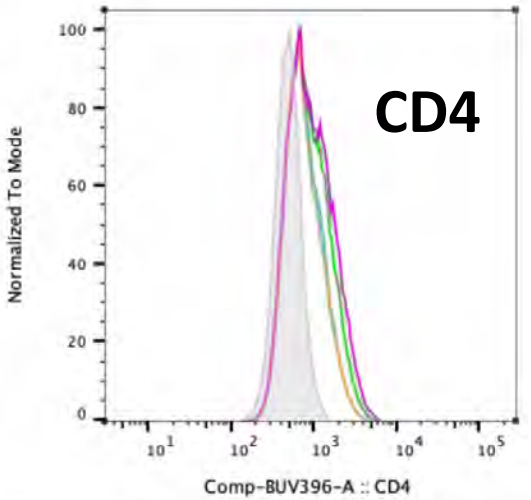


SIV/HIV infection of Mast Cells

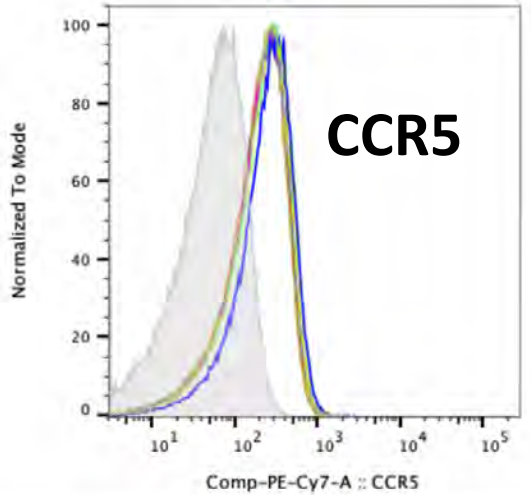
- Mast Cells express CD4 and CCR5.
- 4 groups reported (9 papers) the infection of blood precursor derived mast cells (2000-2009) with HIV.
- Northwestern Expertise (Bochner) has provided access to **two models of primary human MC cultures.** Culture of skin derived mast cells in SCF (6-8 wks) stimulates MC proliferation and survival generating homogenous primary mature human skin MC population. Second model is humanized mouse model (peritoneal MC).







Sample Name	Geometric Mean : Comp-BUV396-A
MC SKIN MICE STIM JULY 14_CTRL_ISOT_001.fcs	476
MC SKIN MICE STIM JULY 14_SKIN_PBS_005.fcs	882
MC SKIN MICE STIM JULY 14_SKIN_FCER1_006.fcs	736
MC SKIN MICE STIM JULY 14_SKIN_TGFB_008.fcs	744
MC SKIN MICE STIM JULY 14_SKIN_TNFA_007.fcs	828



Sample Name	Geometric Mean : Comp-PE-Cy7-A
MC SKIN MICE STIM JULY 14_CTRL_ISOT_001.fcs	15.3
MC SKIN MICE STIM JULY 14_SKIN_TNFA_007.fcs	103
MC SKIN MICE STIM JULY 14_SKIN_TGFB_008.fcs	147
MC SKIN MICE STIM JULY 14_SKIN_PBS_005.fcs	989
MC SKIN MICE STIM JULY 14_SKIN_FCER1_006.fcs	90.1

Using PET to study the convential ”late” viral reservoir.

Typical immune response!

Monkeys were infected IV.

6-8 weeks post infection, the monkeys were started on ART

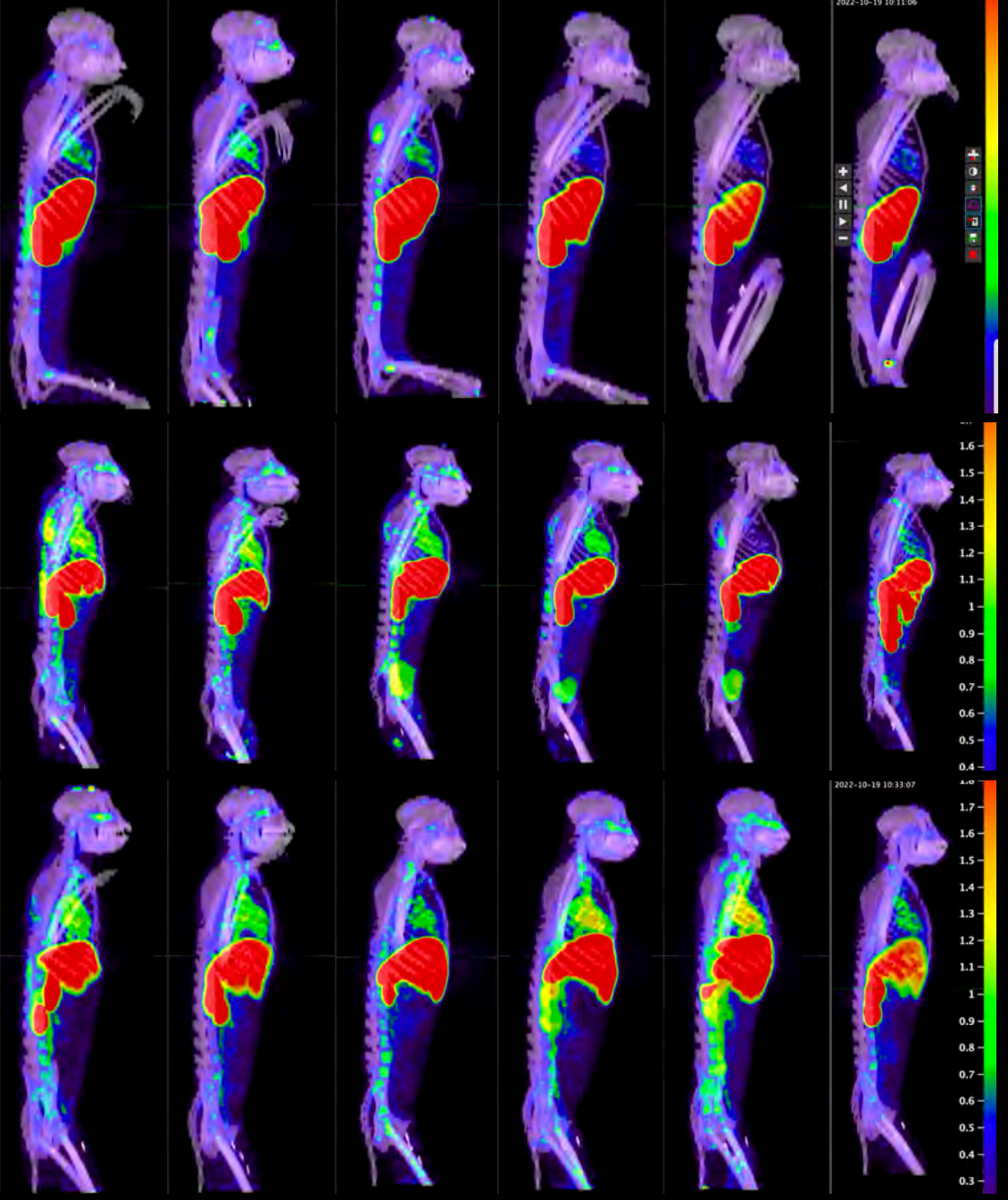
At least 1 year

ART concluded

Tissues harvested for microscopy and PCR

PET/CT scans were taken periodically while on ART and after ART was concluded

4 or 5 days



PET/CT down – Change of plans, repeated ATI

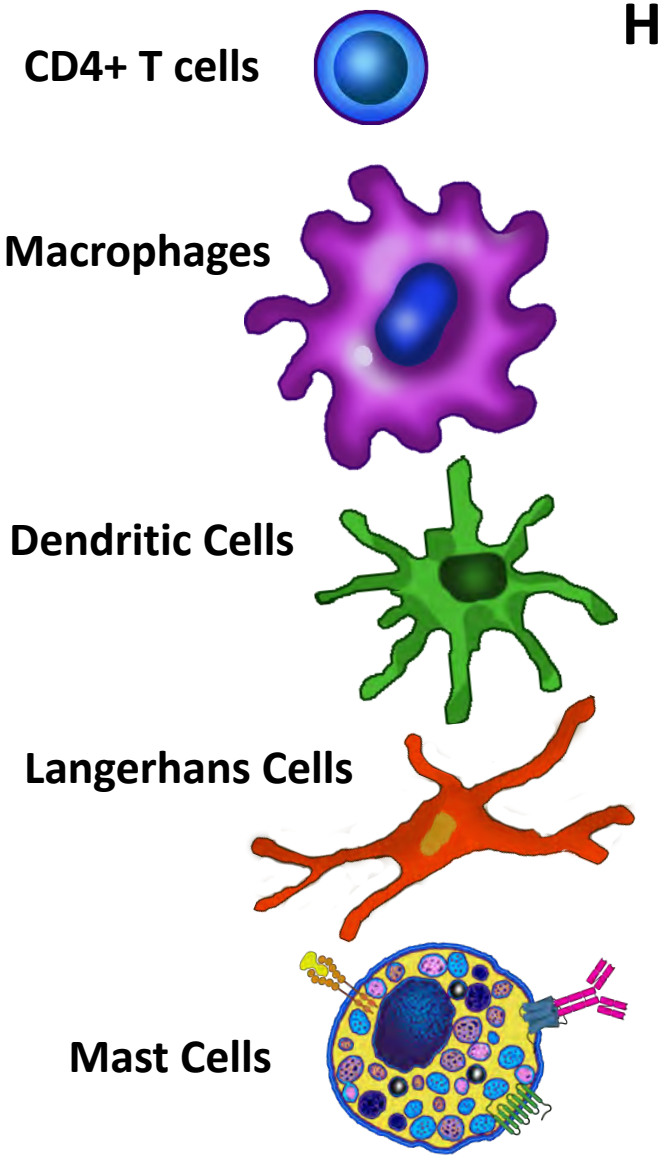
- IV challenge
- Delayed ART initiation
- PET scanner down for extended period
- Tried to evaluate reservoir with repeated short (4 day) ATI.
- No Rebound observed.
- Found multiple foci in all animals with PET/CT guided necropsy

Difference between early and late Reservoirs

- No explosion of PET signal after ATI as seen for early reservoir.
- Some infected T cells observed in eclipse phase rebound.
  - Memory CD4+ T cells?
- Mast cells overrepresented and infected in early rebound foci.
- Difference between IV vs mucosal challenge?
  - Unlikely (and will be tested)
- Impact of active humoral and cell mediated immune response?
  - Current favored hypothesis -Testing in early 2024 with early reservoir and infused antibodies from late reservoir animals.

Which cell type holds the reservoir of persistence

HIV Target Cells (CD4+CCR5+)



- Cells must be long-lived because reservoir is long lived. –"Must be memory T cells"
  - Proliferation of T cells harboring HIV can impact the reservoir (clonal expansion)
- Other CD4+CCR5+ Immune cells are long-lived and self renewing.
- Yolk sac macrophages are self renewing in a variety of tissues (T. Roszer, 2018, Cells. PMC6115919).
- Mast cells can be self renewing (SCF dependent)

Rebound is a Process Not an Event

Current, But Rapidly Evolving Model

Unique features of Rebound Virus.

Rebound virus different from blood "Reservoir"

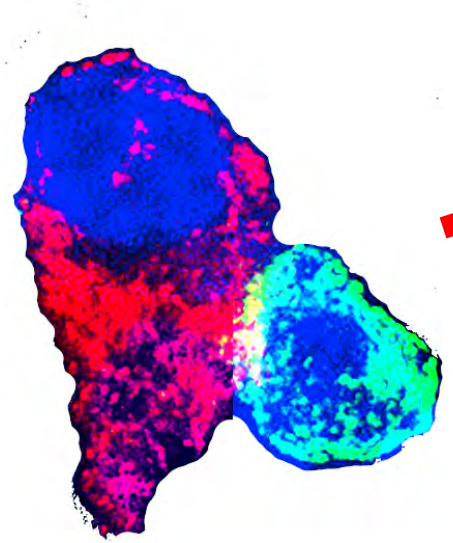
Interferon resistance of TF and Rebound virus.

Myeloid phenotype of rebound virus.

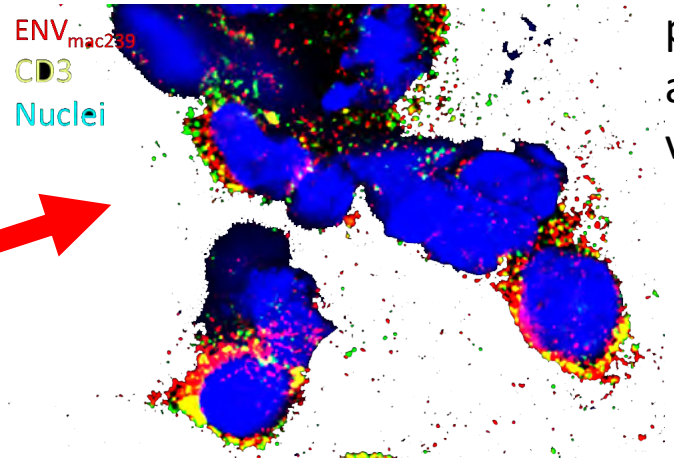
Local (systemic) antiviral environment (Interferon, etc), T cells protected, Myeloid cells are not.

Reservoir
?

Myeloid Cells infected but somehow invisible to Immune system. But T cells infiltrating and curious



Naïve CD4 T cells protected by restriction factor SAMHD1



Can overcome restriction factors with more virus?

Virus overcomes restriction, Virus production amplifies leading to viremia

Viremia

Caveats:
SIV early reservoir
Macaque Model
Limited N

How do we advance the goal of a cure for HIV?

If reservoir is everywhere in different cell types and dynamic.

- Elimination of reservoir of persistence may not be possible (without ablation, i.e. bone marrow transplant).

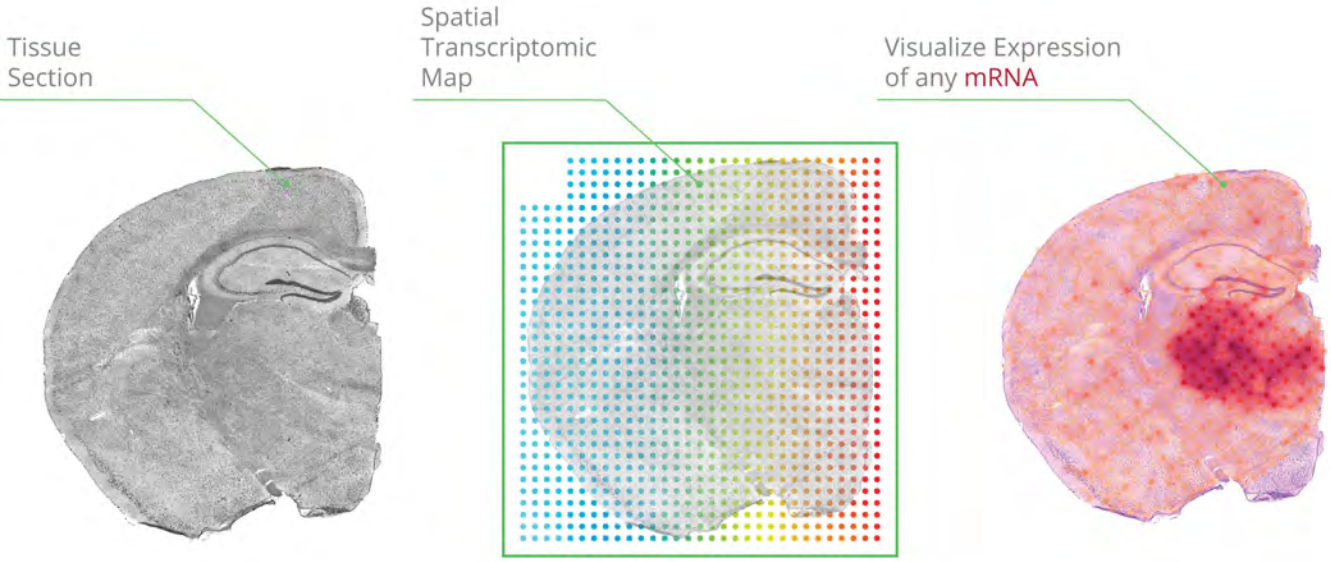
We need alternative approaches/strategies for functional cure.

- Fortify innate and adaptive immunity which currently limits rebounding virus making it look like a single latent cell reactivation seeds rebound.
- How does HIV fly under immune system radar in early reservoir?
- What is mechanism of immune activation/accelerated aging during efficient suppression with ART?

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

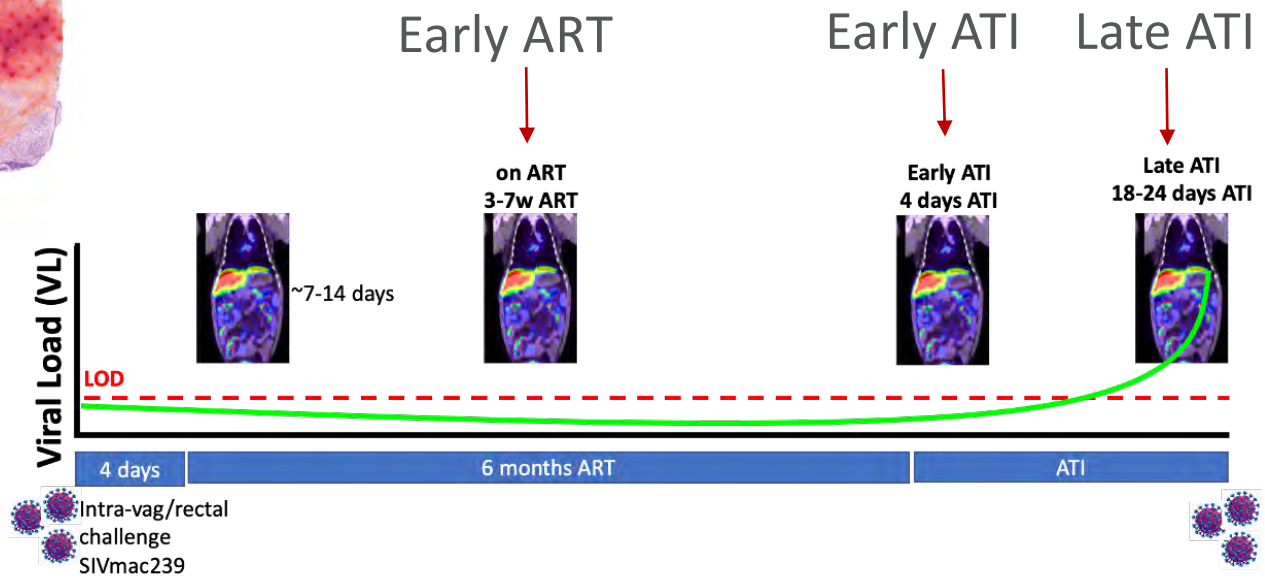
10x GENOMICS®

Visium Spatial Gene Expression



It's not the method. It's the piece of tissue you interrogate.

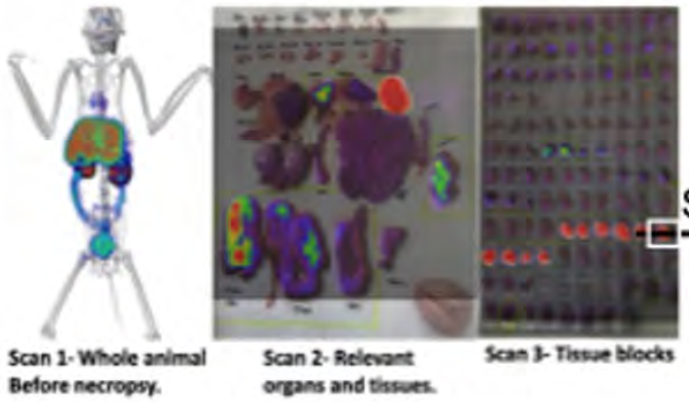
High dose (mac239) challenge vaginal and rectal with biopsies.
Early reservoir: ART initiation 4 days post-challenge.
ATI: 6-8 months post ART initiation.



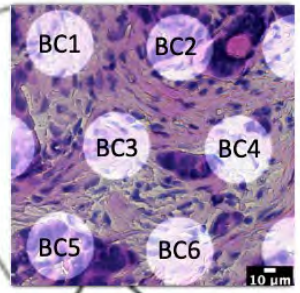
PET-CT-Guided Spatial Transcriptomics of SIV Tissue Reservoirs

Steps optimized

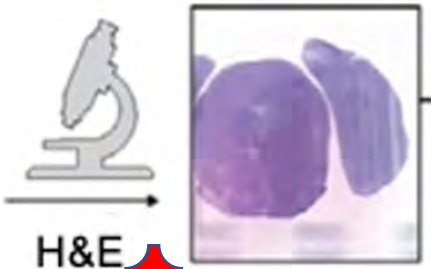
Screening of tissues
- PET/CT
- Infected vs Non-infected
- RNA Quality
- Tissue Quality

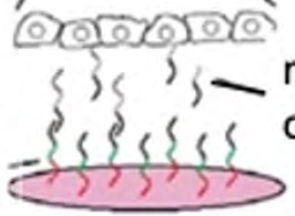


Sectioning



Permeabilization



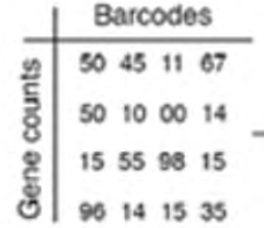


10X Visium Slide
- 4x quadrants
- 5000 Spots/quadrant
- **Average 1-10 cells/spot**

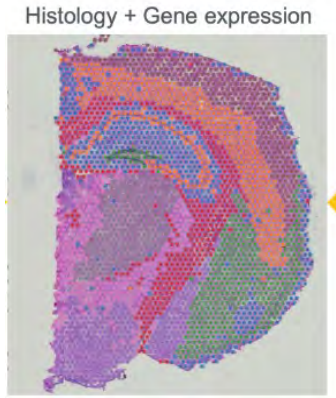
NGS library

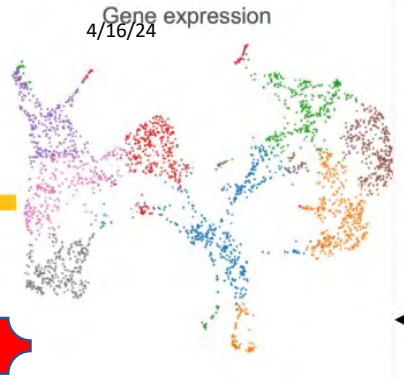
Sequencing

Mapping



Input

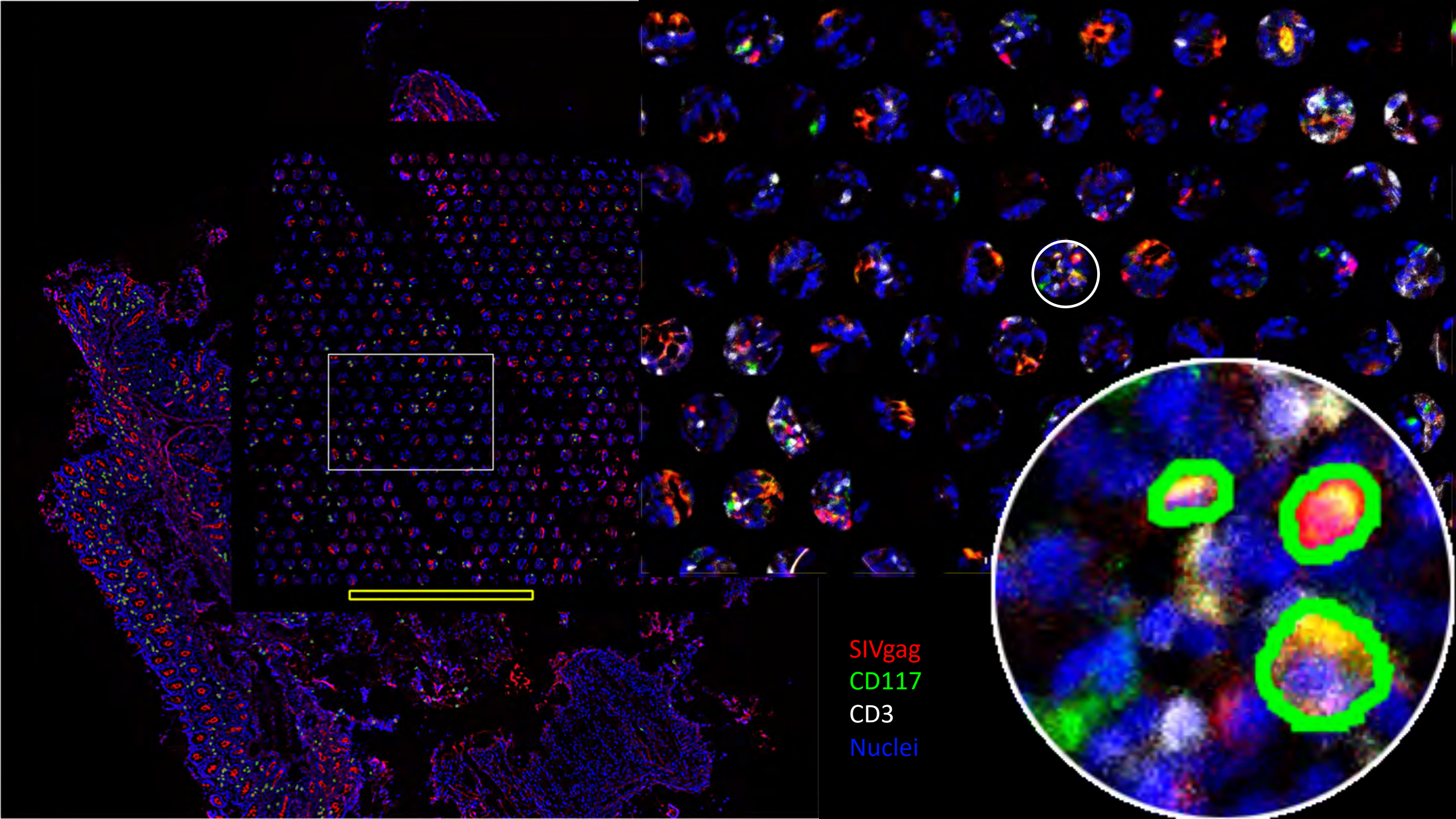




Muhammad Shoaib Arif, PhD

Ramon Lorenzo Redondo, PhD

Christopher Thuruthiyil


SIVgag
CD117
CD3
Nuclei

Moving Forward: Evaluation of a More Conventional Reservoir (Start ART 6 weeks after IV challenge)

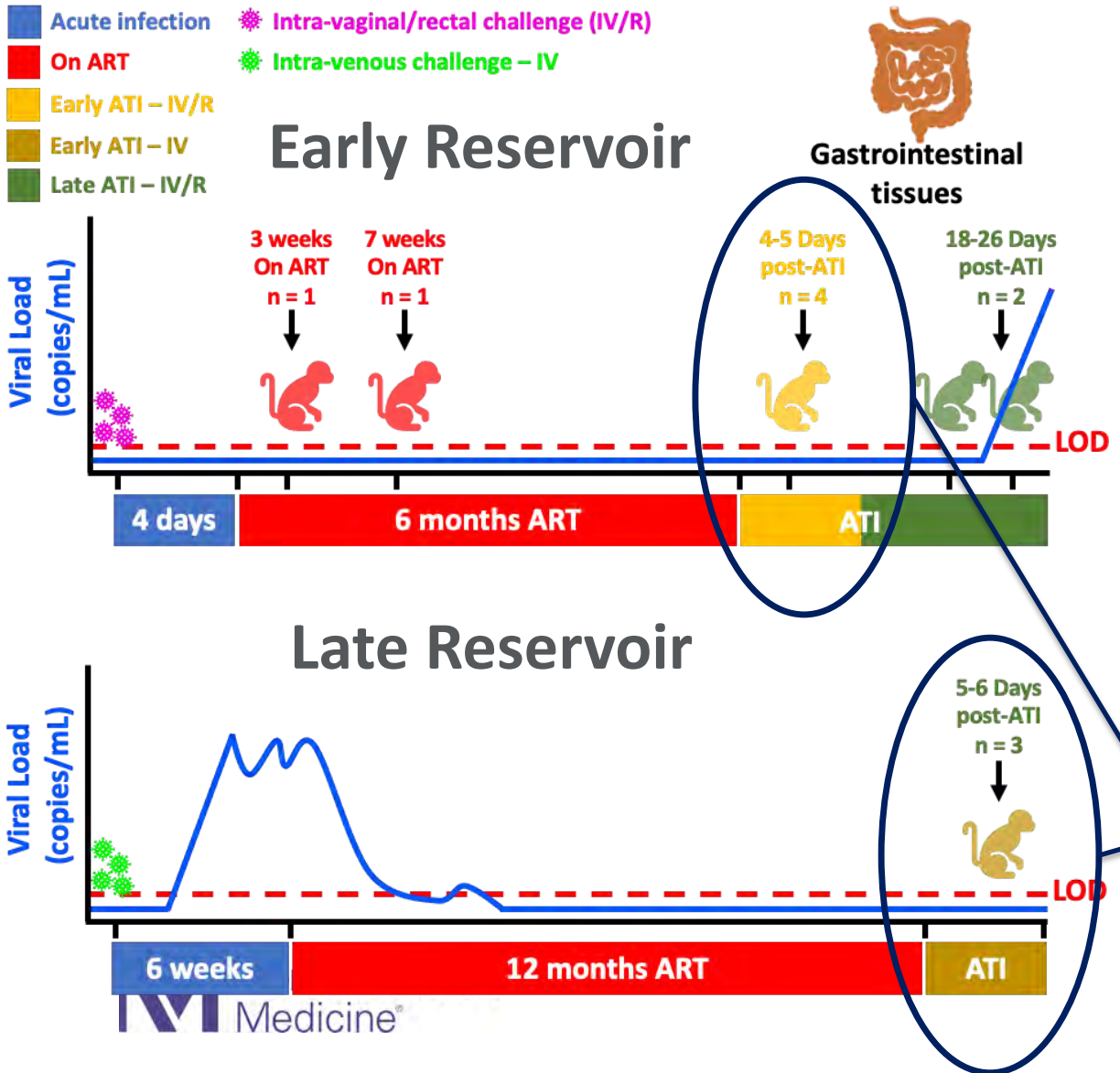
Early ART Reservoir (4 days)

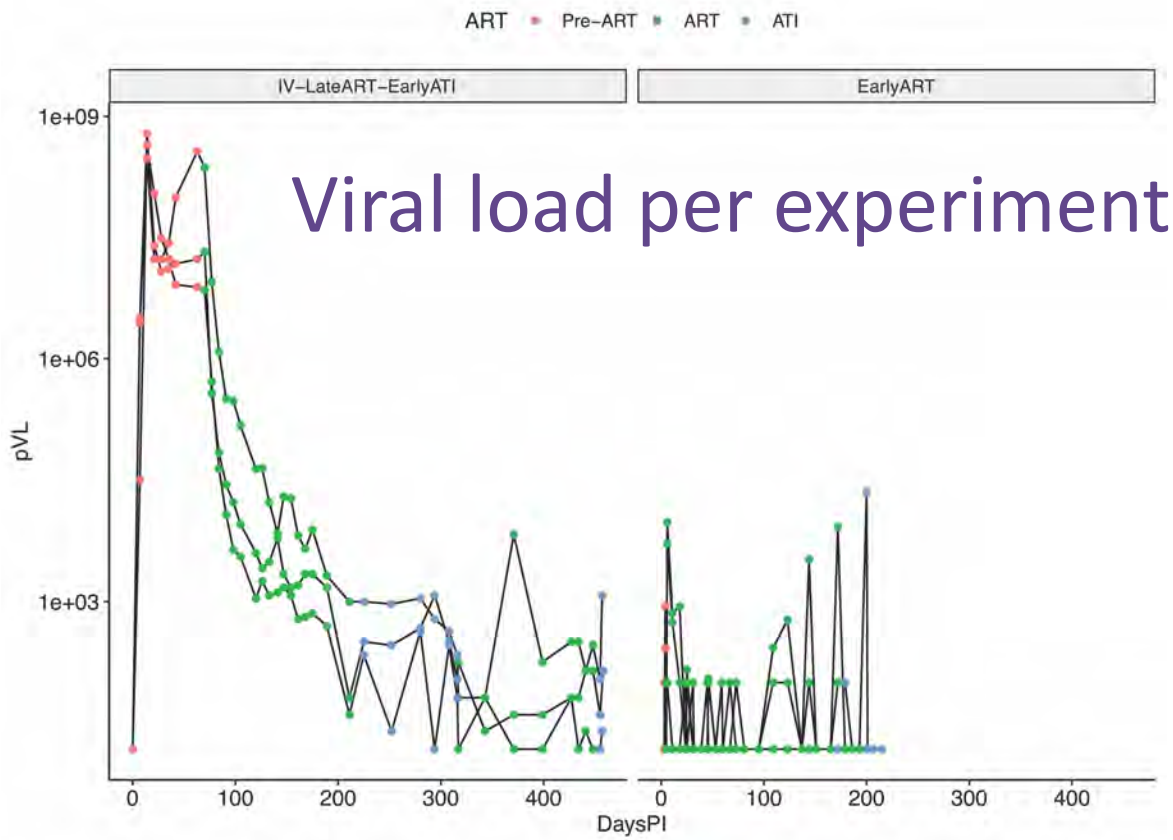
- Mucosal challenge (rectal/vaginal) with biopsies
- No Humoral Response
- No Cell mediated Response
- Minimal Innate Response(?)
- No infected T cells in eclipse phase foci.
- Infected Mast Cells
- PET/CT detected explosion at day 4/5 post ATI

Late ART Reservoir (6 weeks)

- Intravenous (IV) challenge
- Humoral Response
- Cell mediated Response
- Innate Response
- Few infected T cells in eclipse phase foci.
- Infected Mast Cells
- No PET/CT detected explosion at day 4/5/6 post ATI (immune response)

Understanding SIV reservoirs and rebound in two different experiments





Viral load per experiment

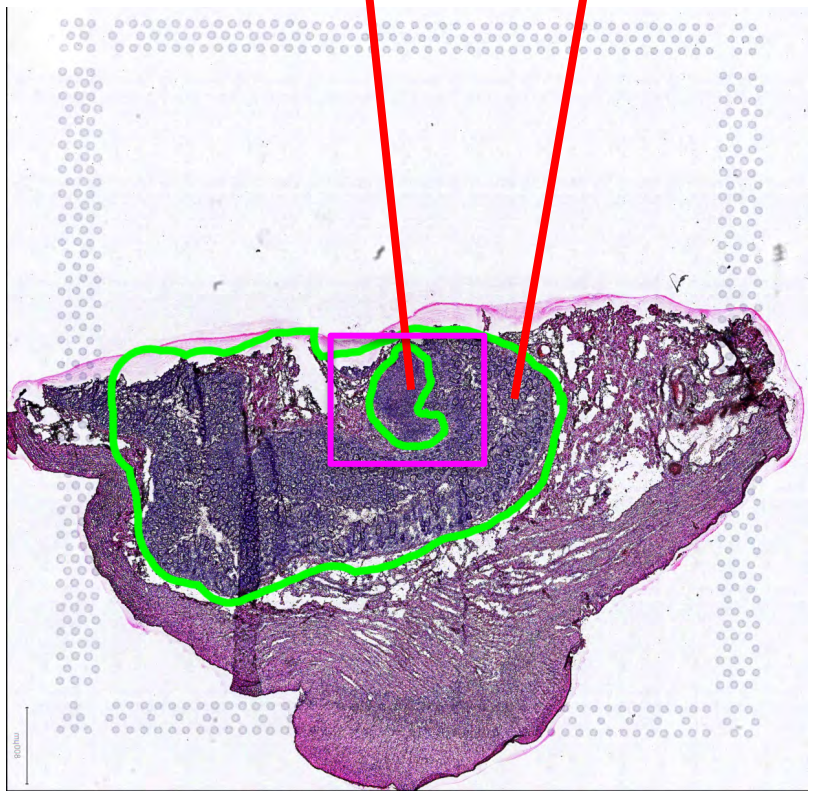
For this presentation we will compare mucosal tissue eclipse phase foci 4-6 days after ATI in early and late reservoir animals

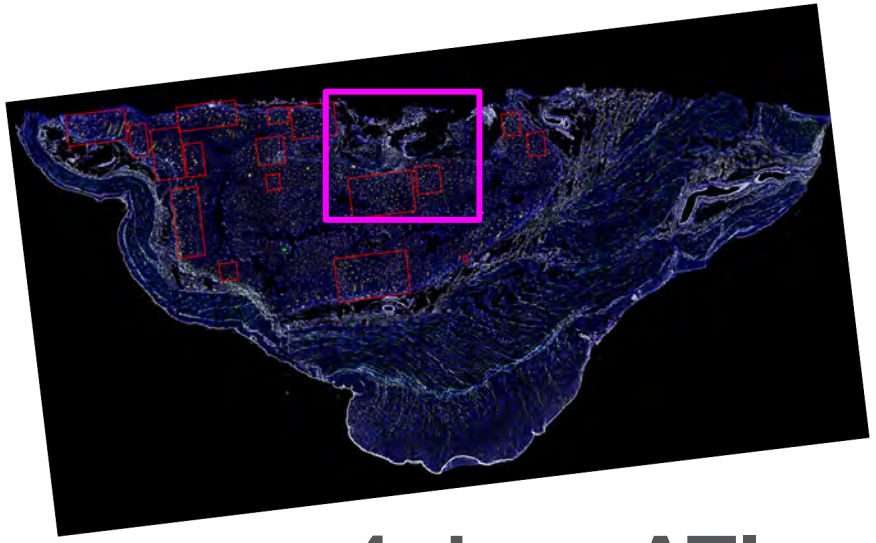
32 Slides from 11 Animals

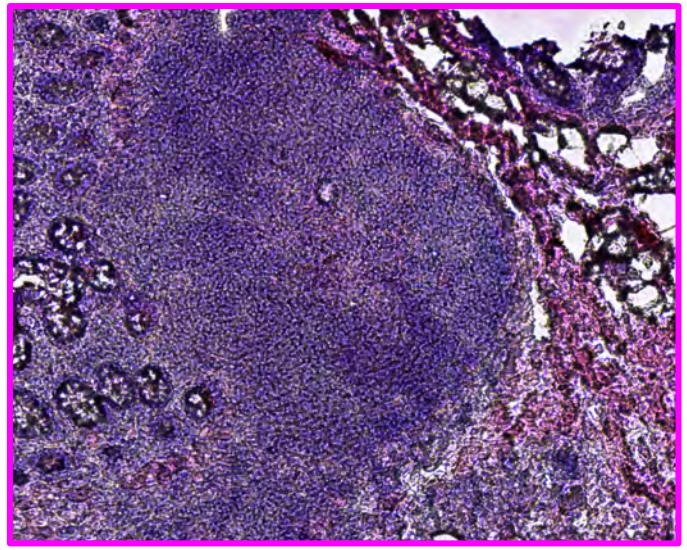
H&E images from 10x spatial slide aligns well with IF images from serial sections

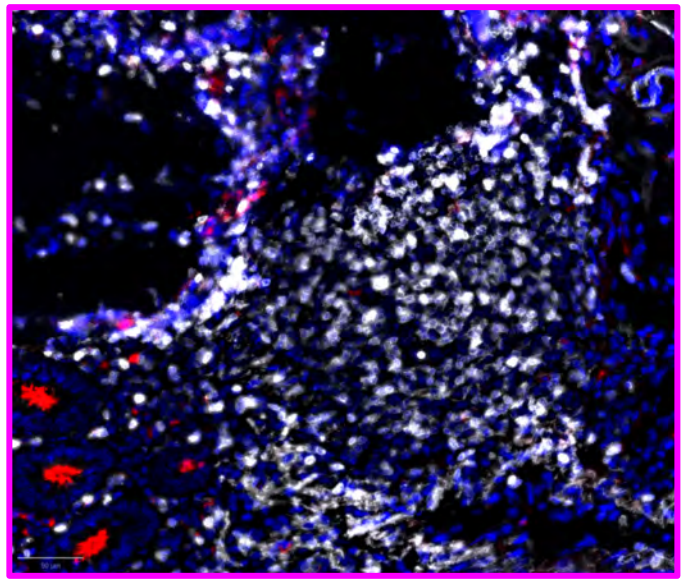
High density of
Immune cells

Tertiary immune aggregate









4 days ATI
T.Colon

CD3 Nuclei

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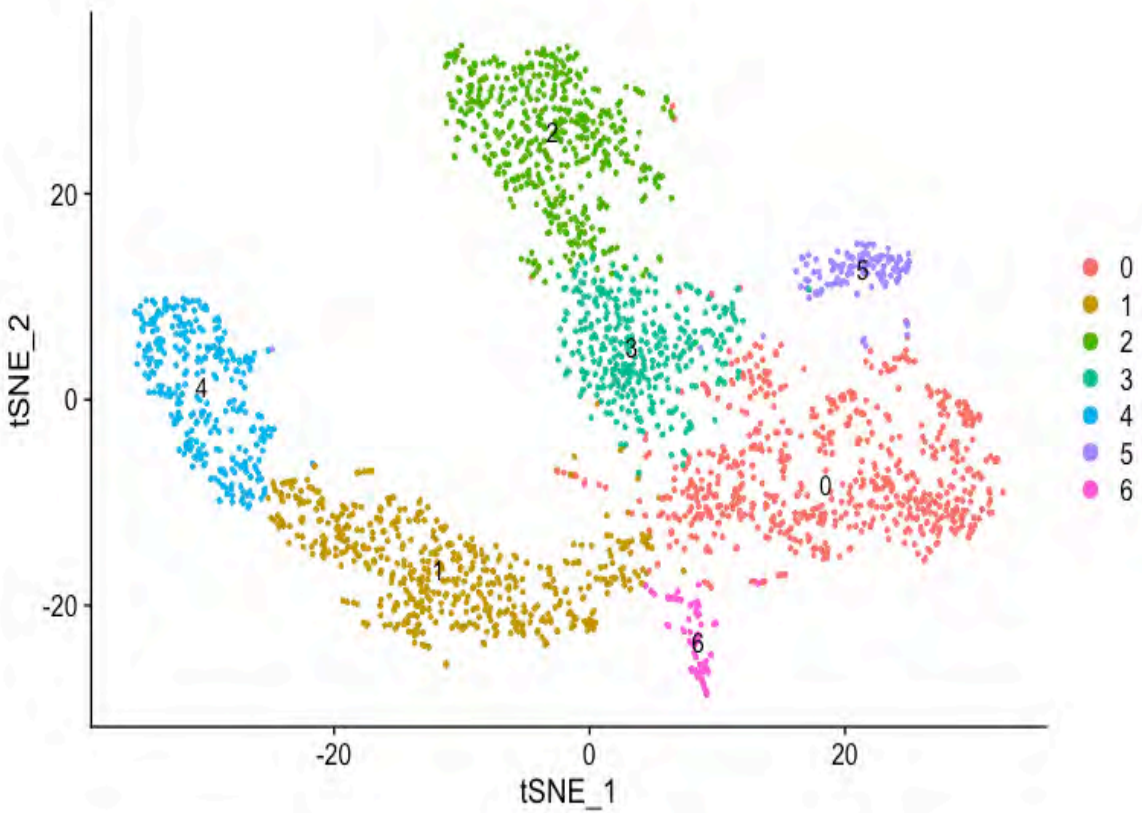
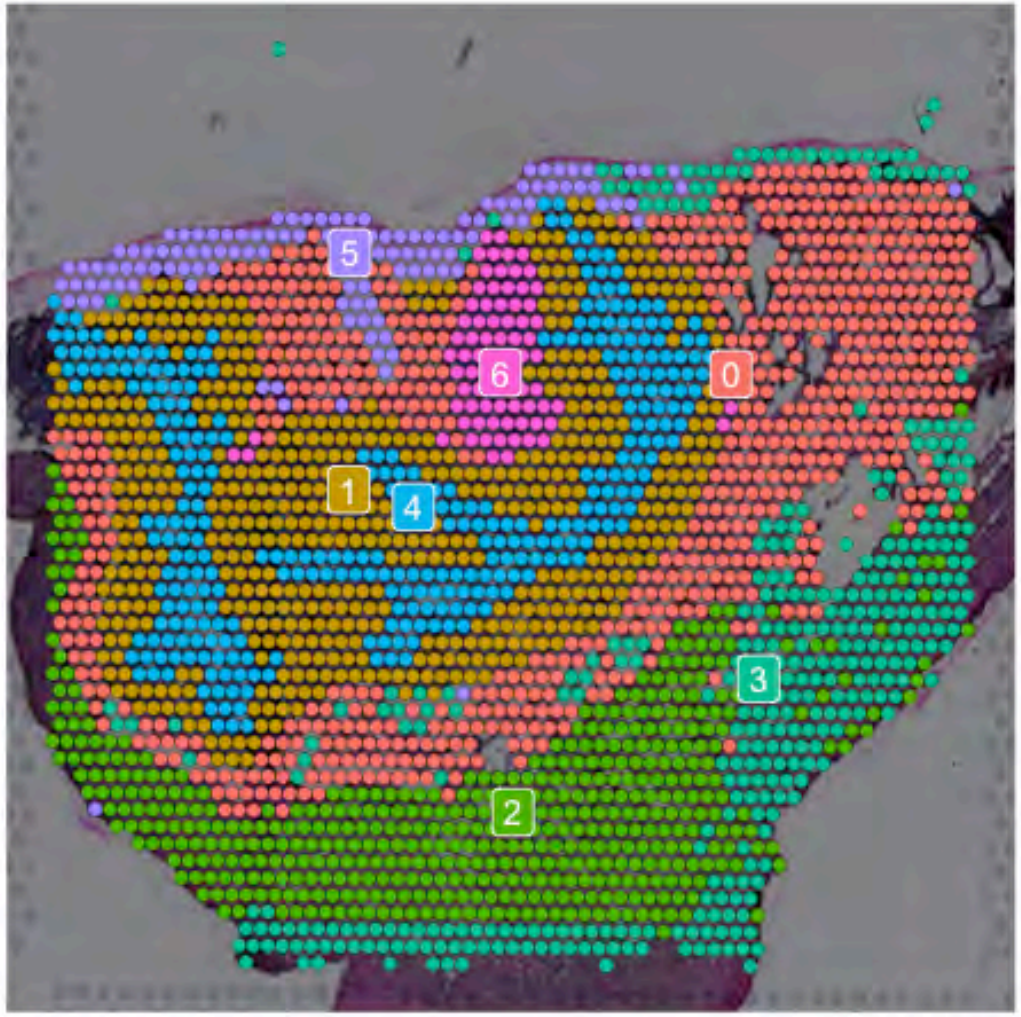
Expression clustering and spatial distribution





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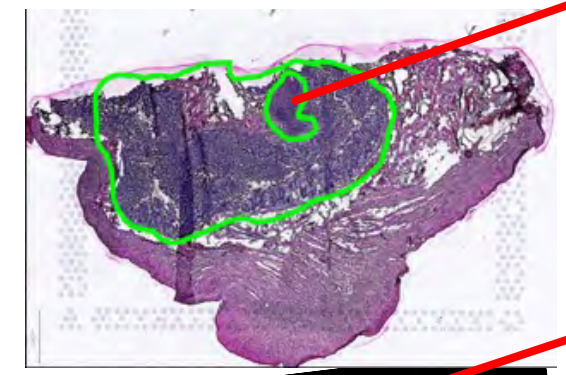
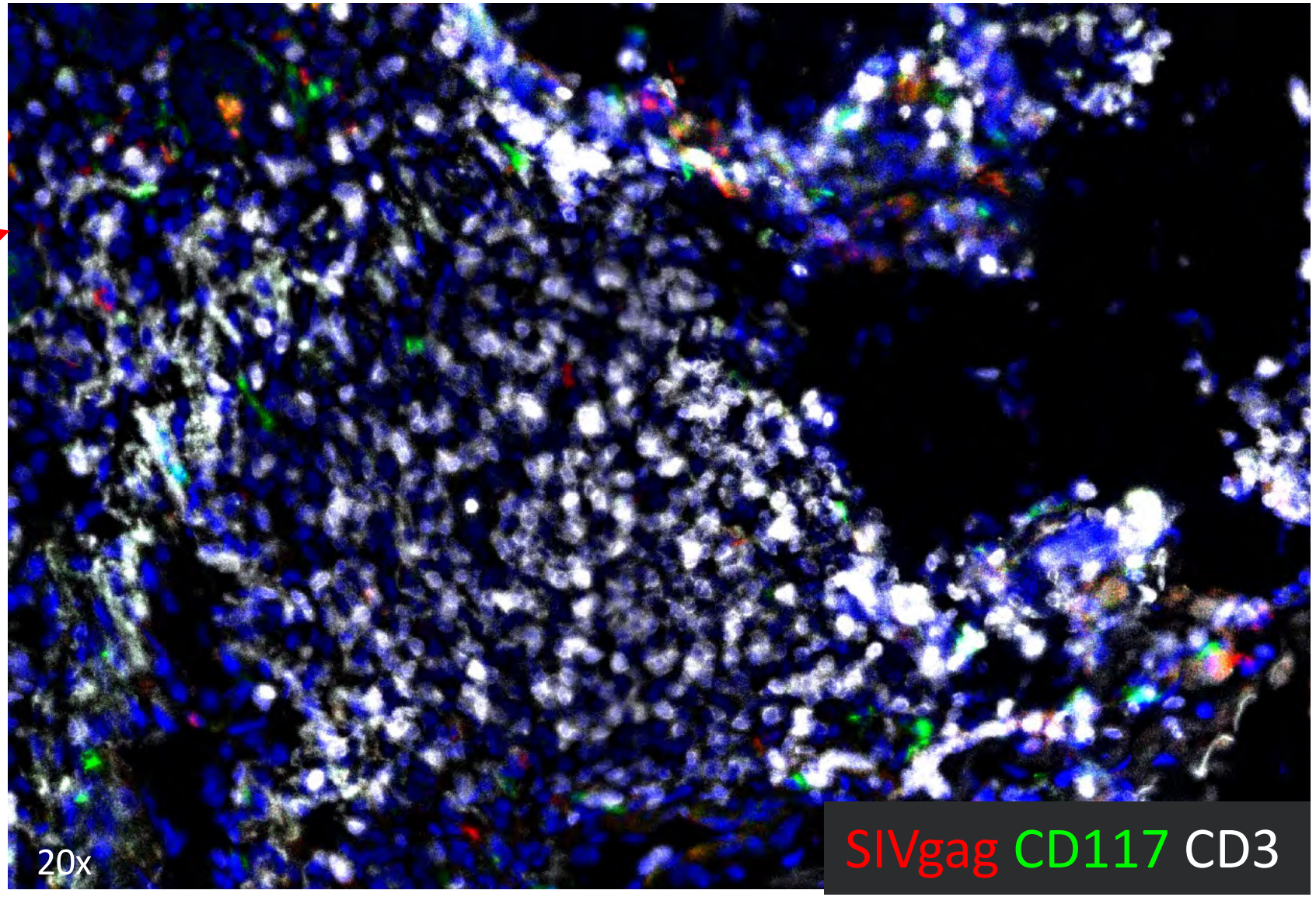
Rare virus detection in immune aggregates

High density Immune cells



4 days ATI T.Colon



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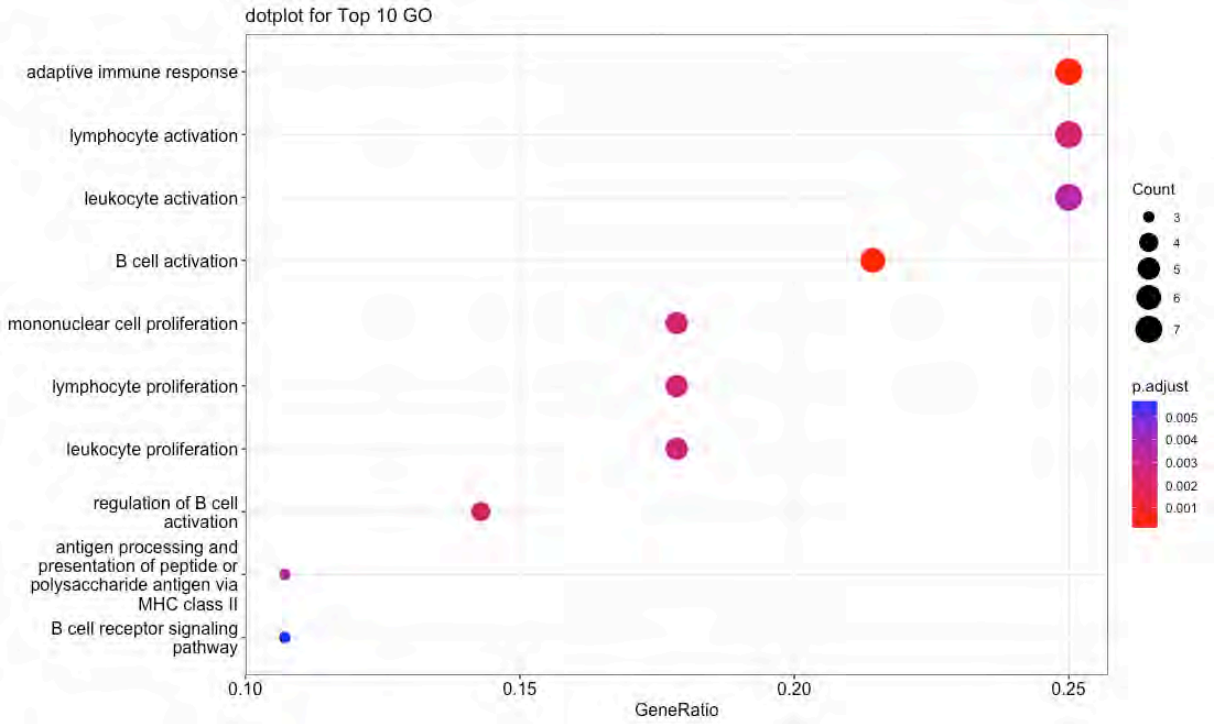
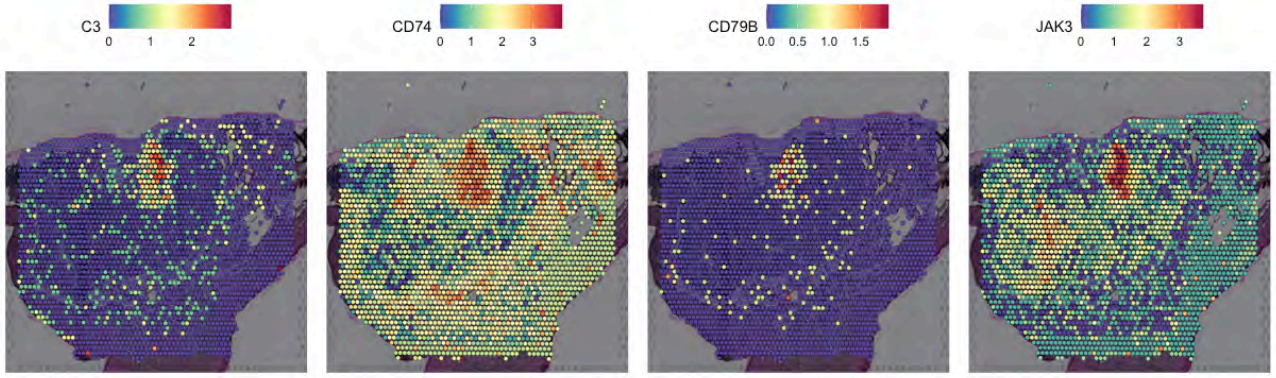
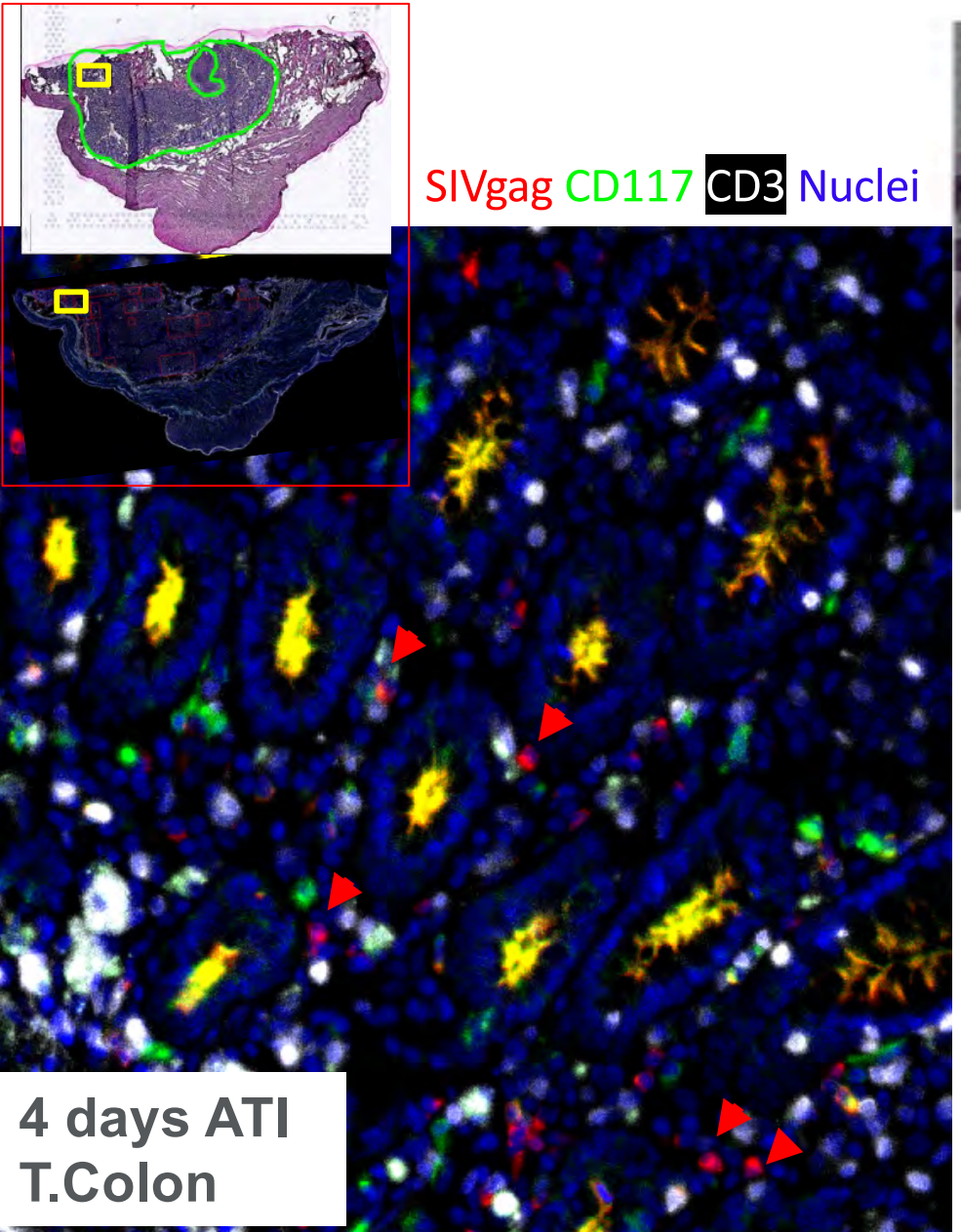
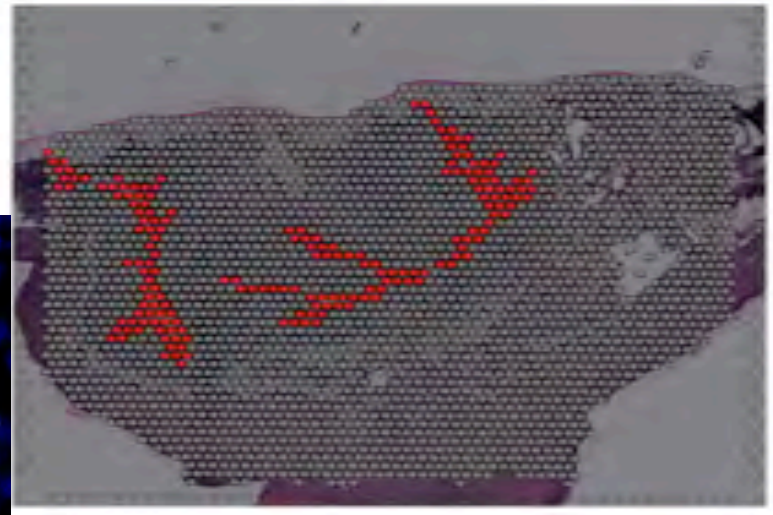
Cluster associated with immune aggregates



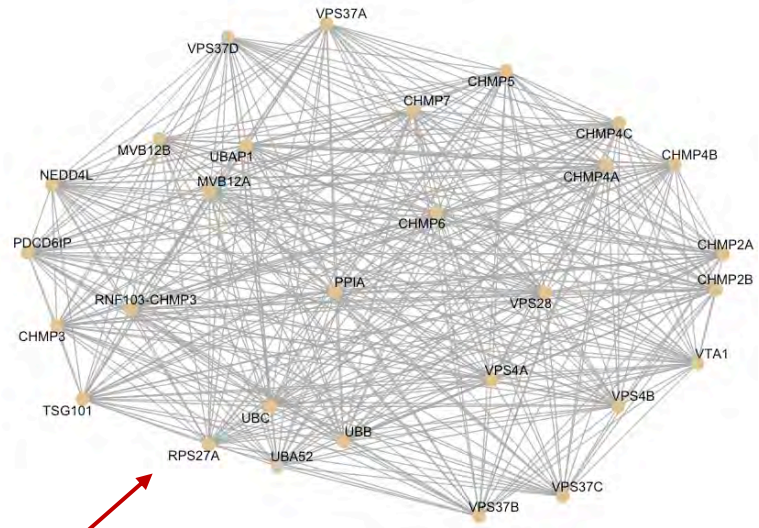


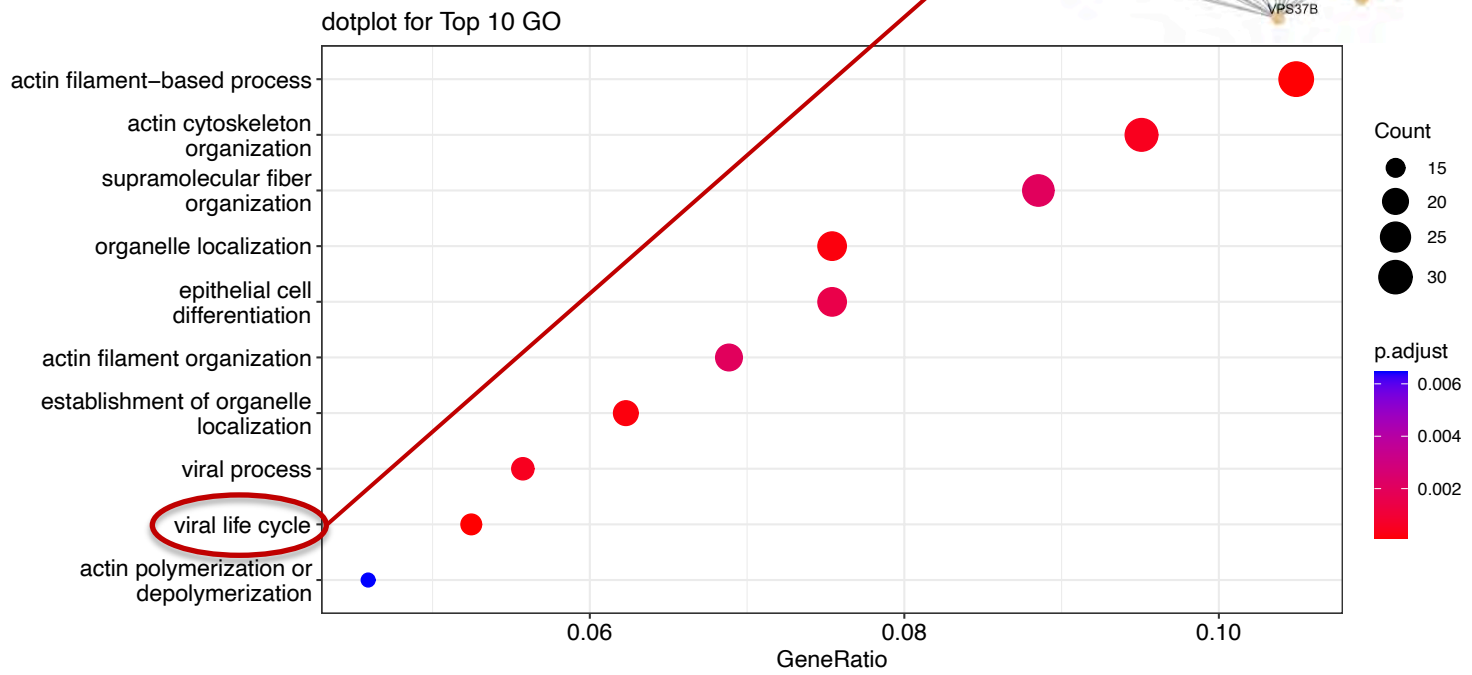
Cluster associated with areas of infected cells





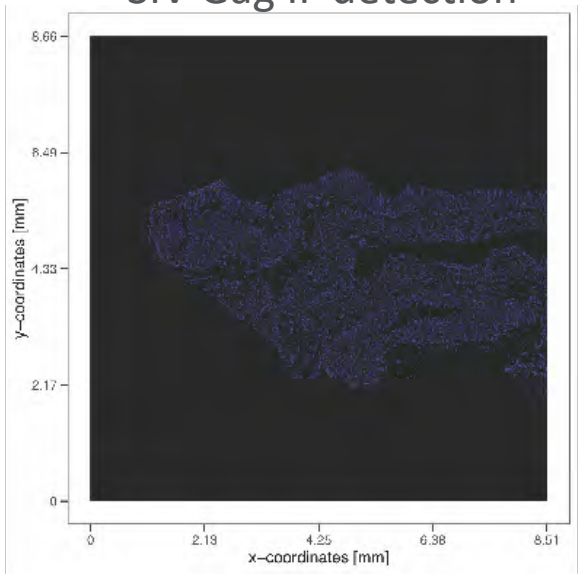
Budding and maturation of HIV virion



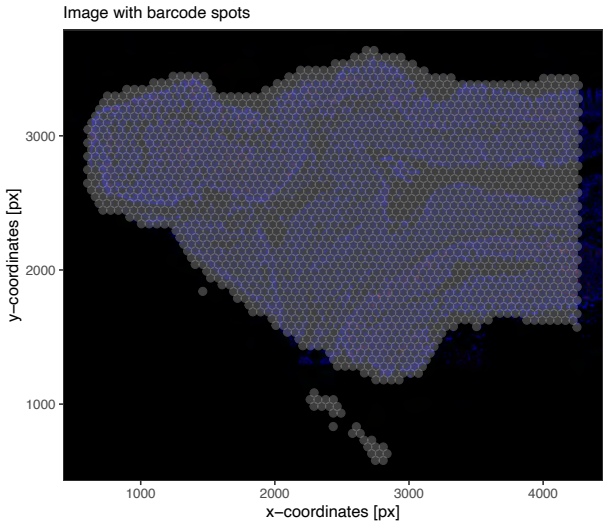


Spatial Transcriptomics associated to virus detection

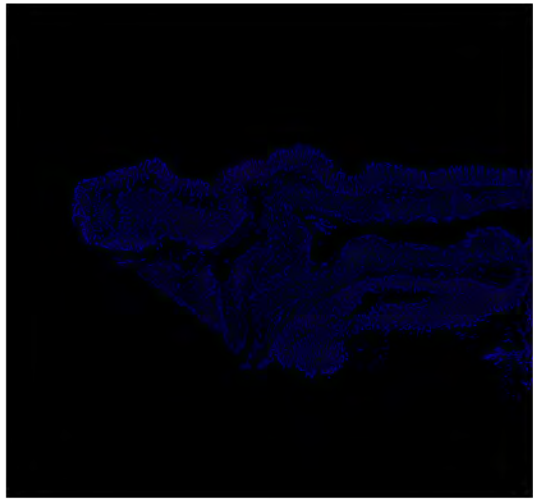
SIV Gag IF detection



Visium overlay



A)



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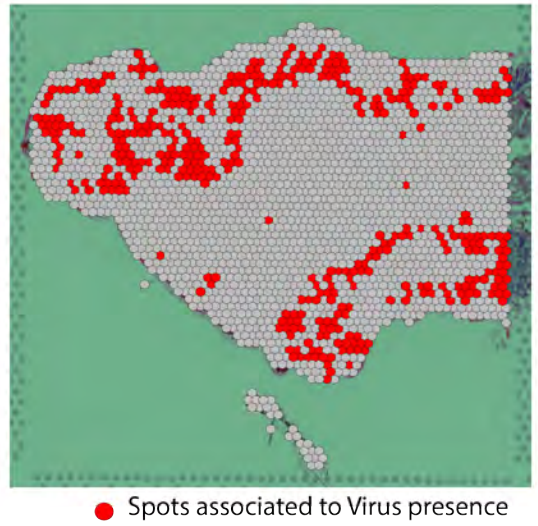
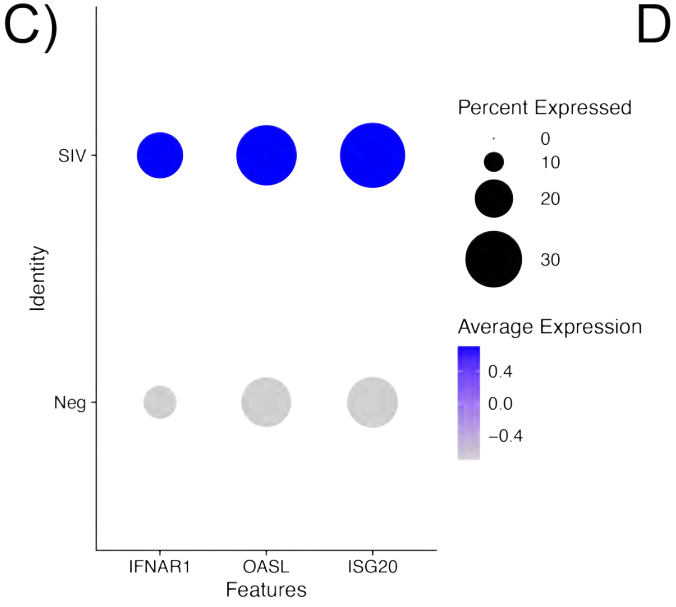
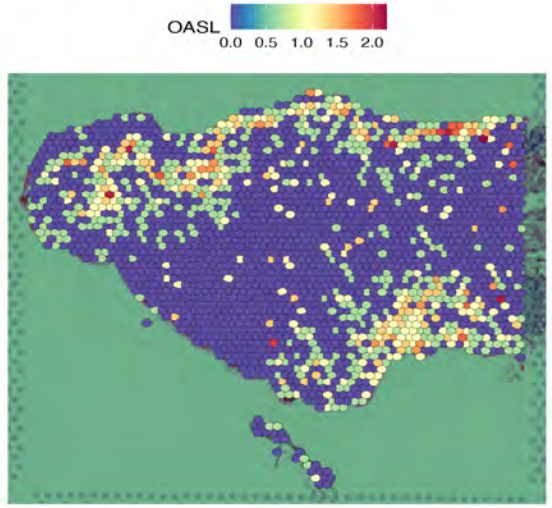


C)



D)



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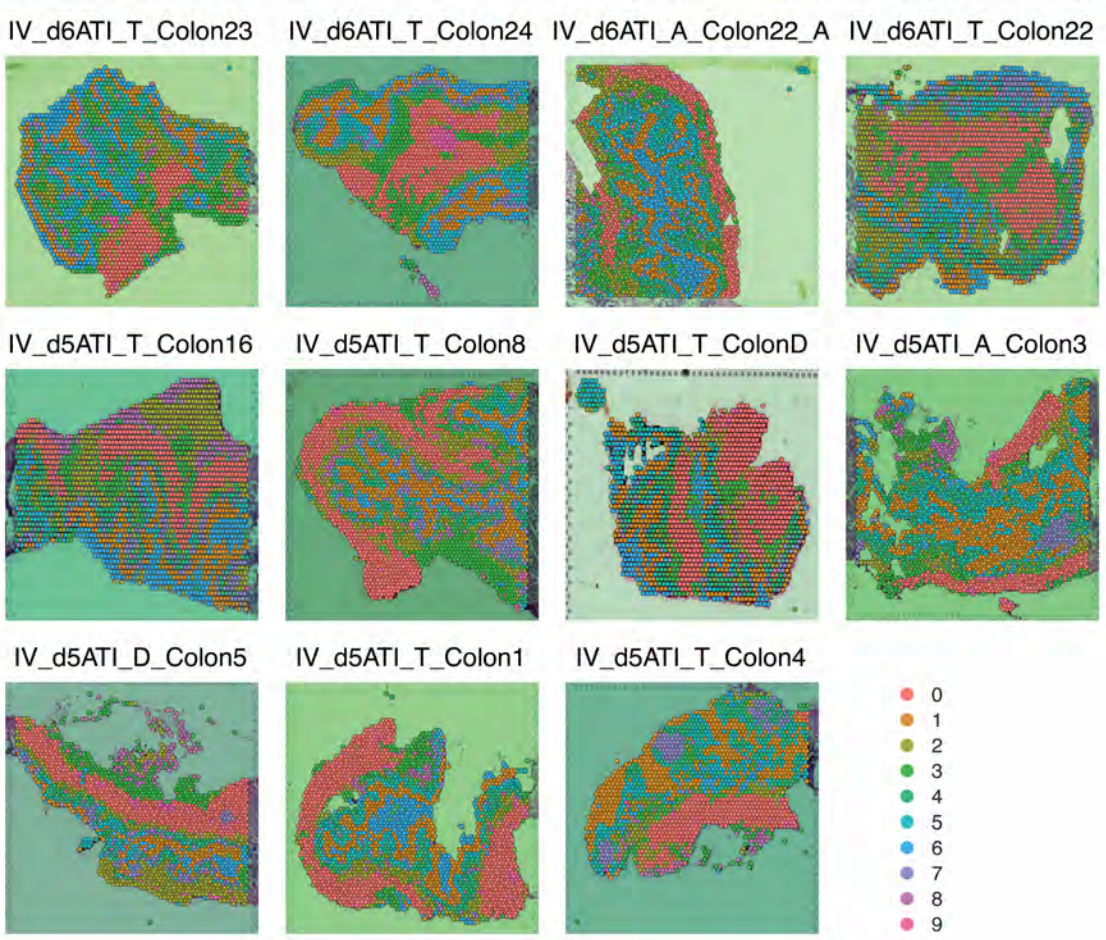
Expression Clustering among all tissues

Early ATI 6 weeks ART Initiation (IV challenged)

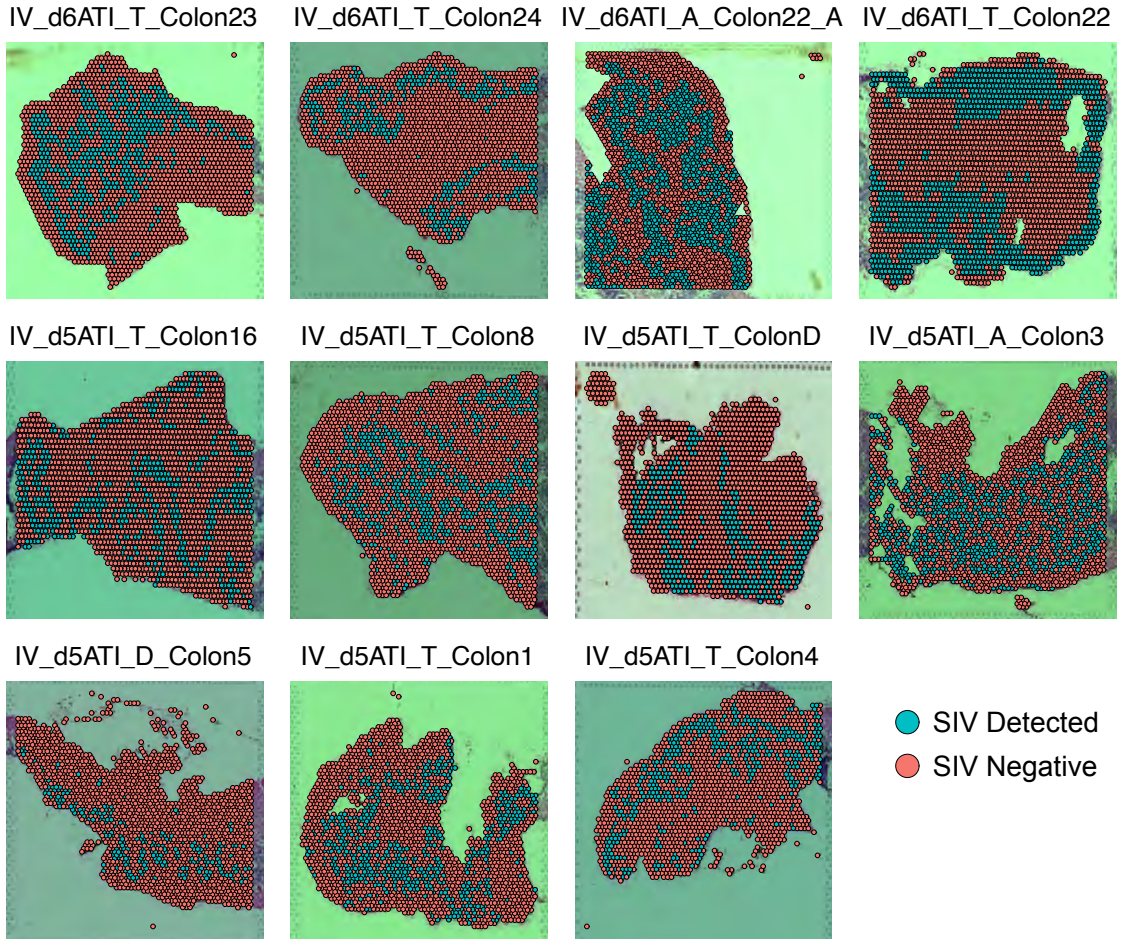


Animal 1

Animal 2

Animal 3

SIV associated spots



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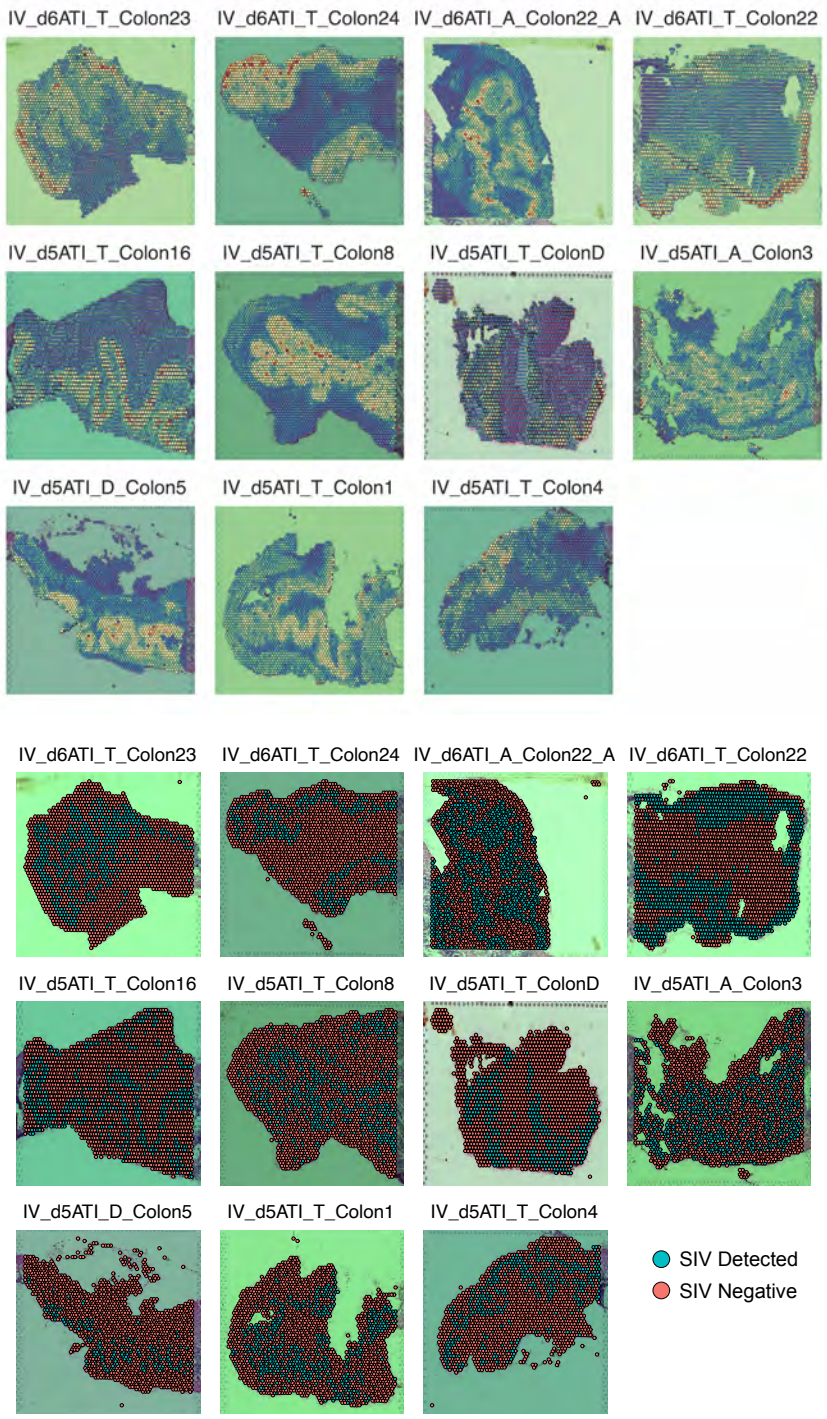
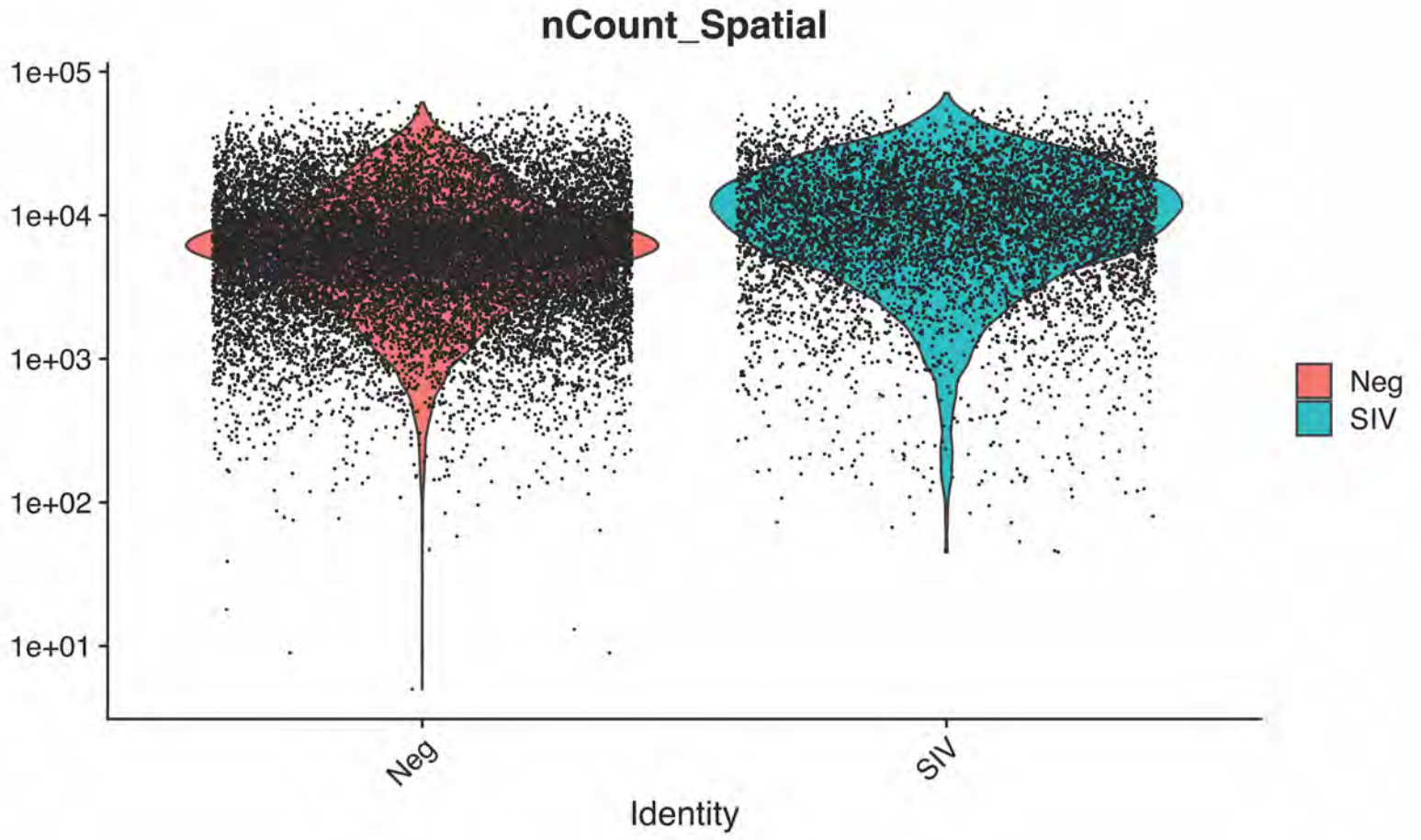
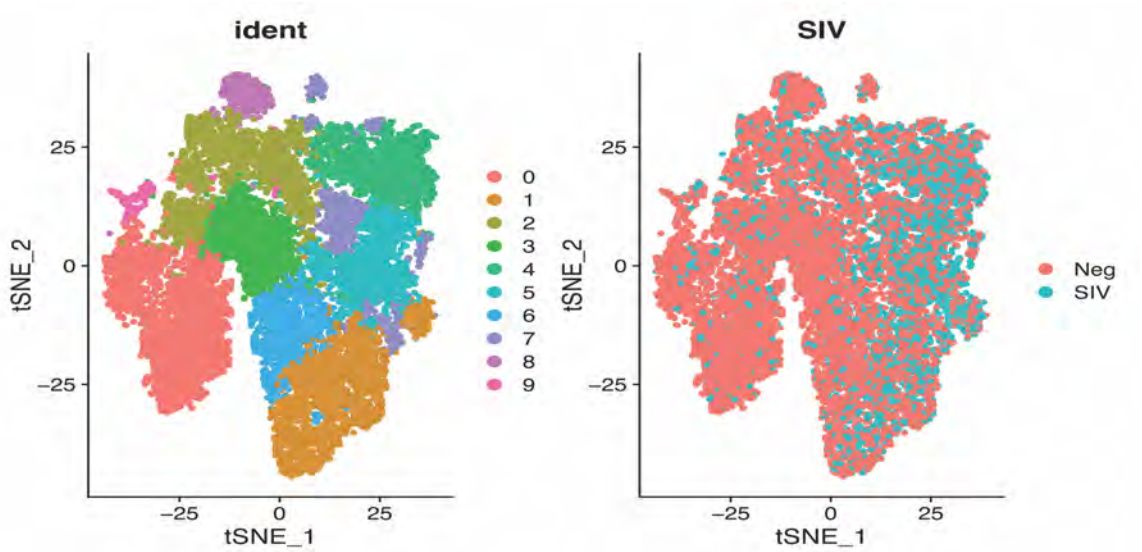
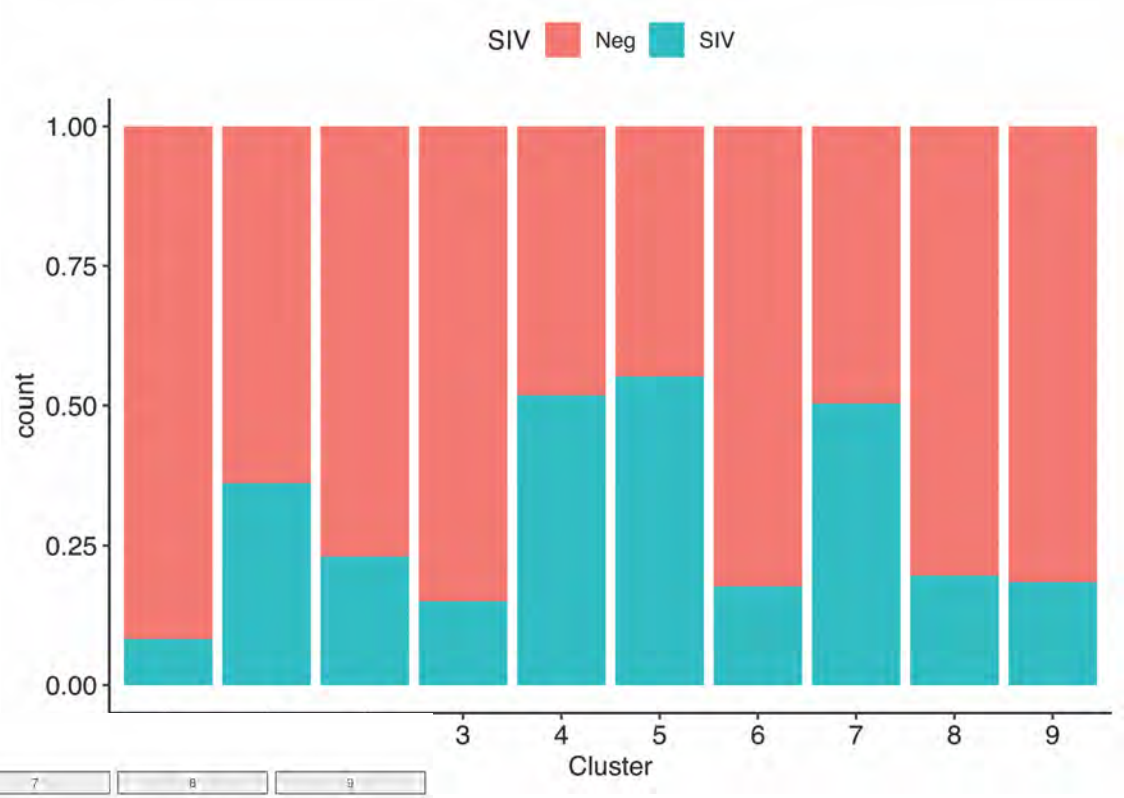
SIV Presence associated with higher transcriptional levels

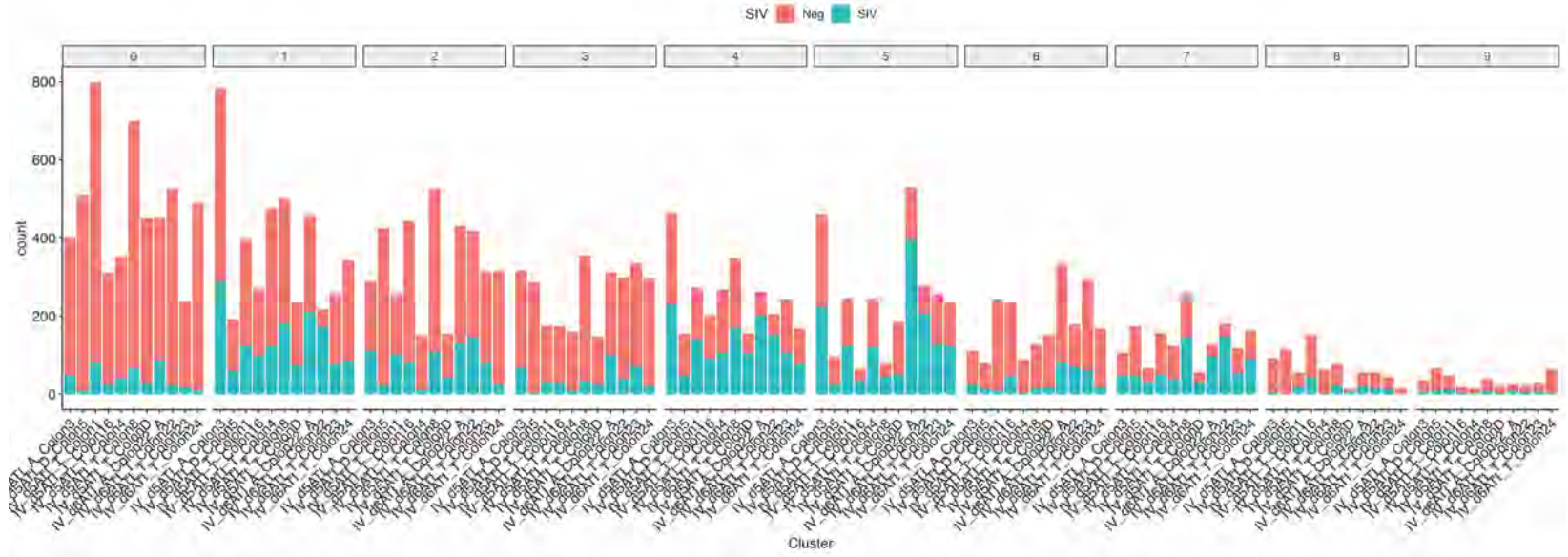




Infection Per Cluster

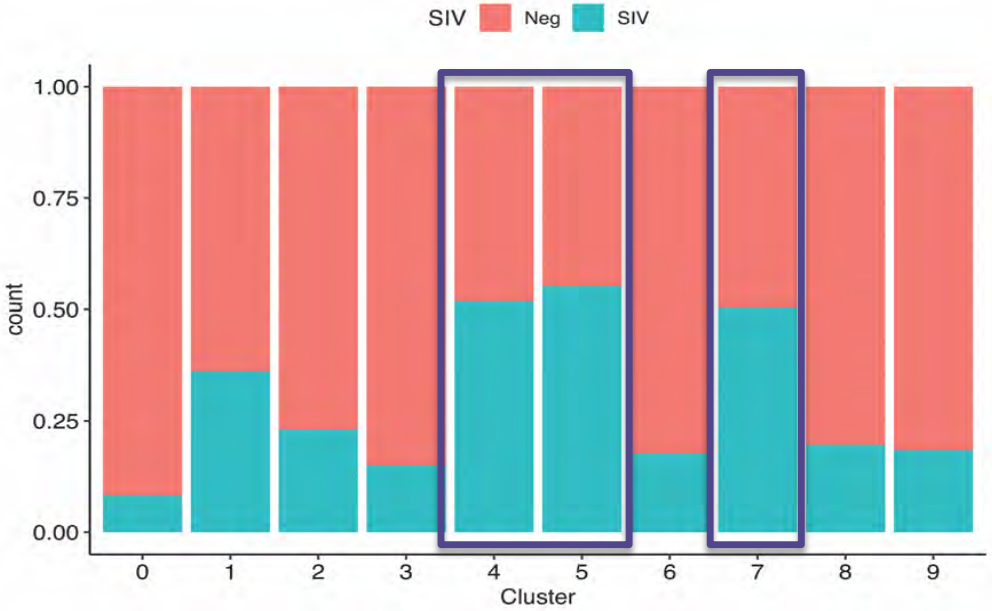


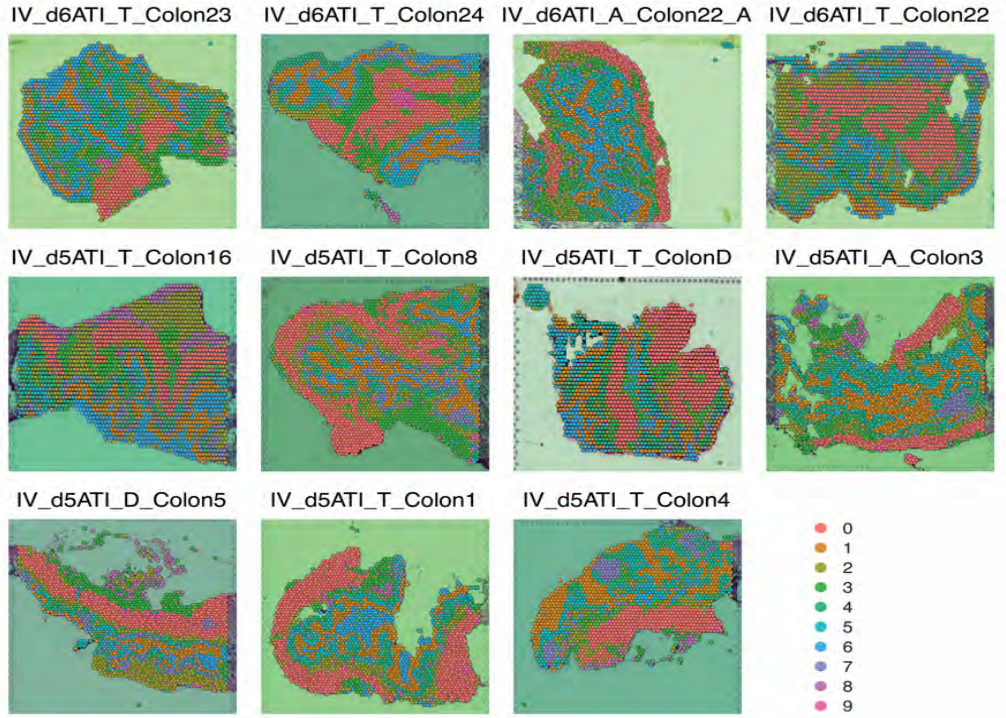


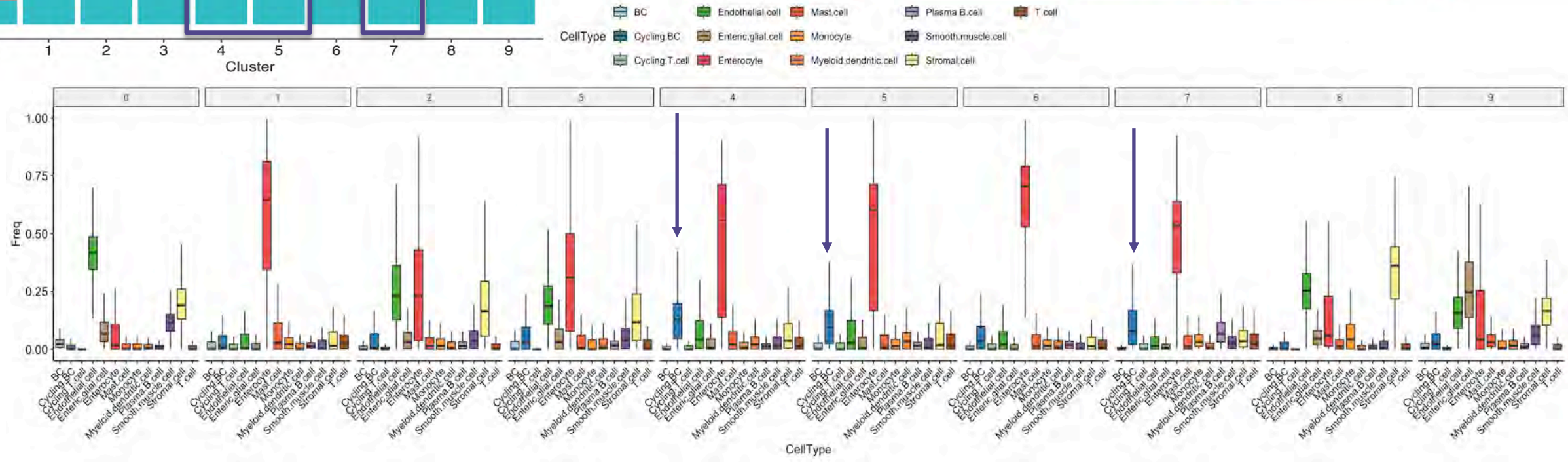


Cell Type Deconvolution

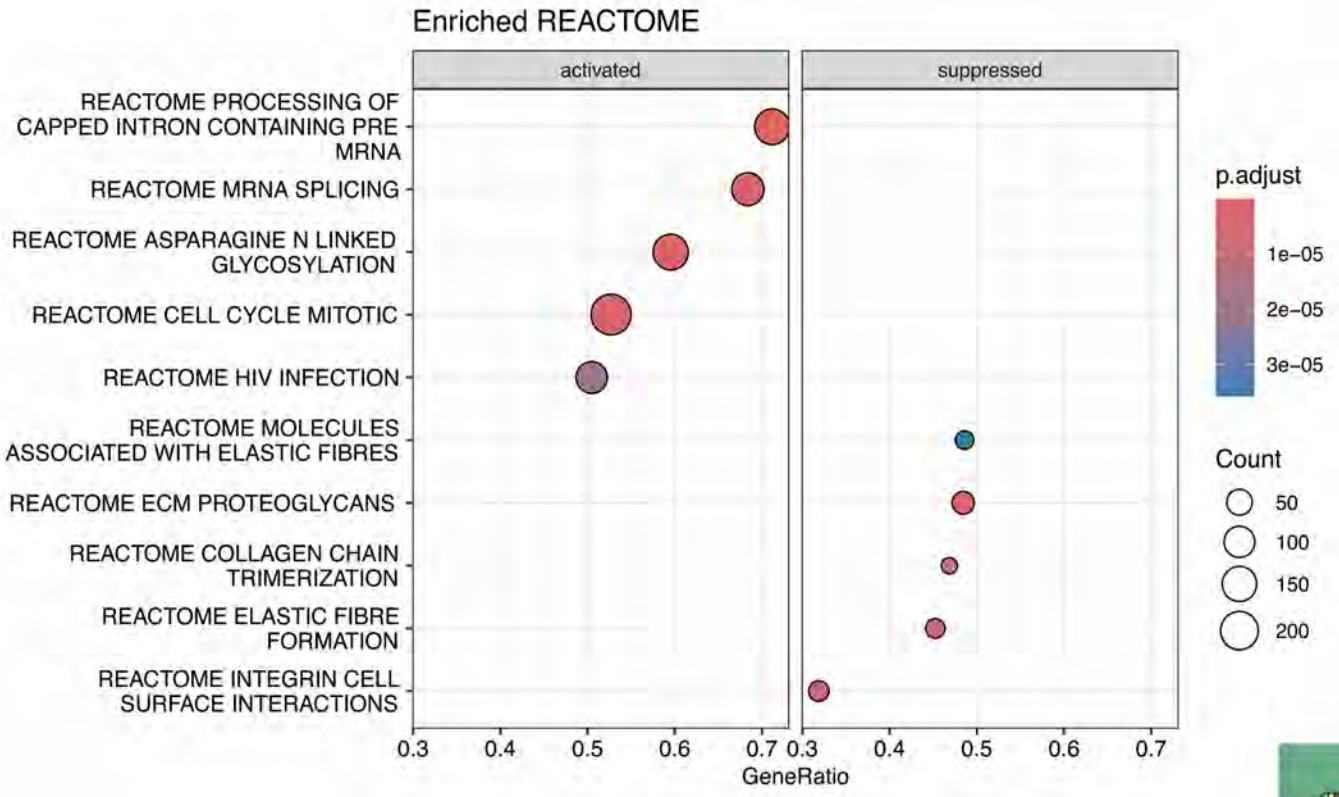
Sean Pascoe

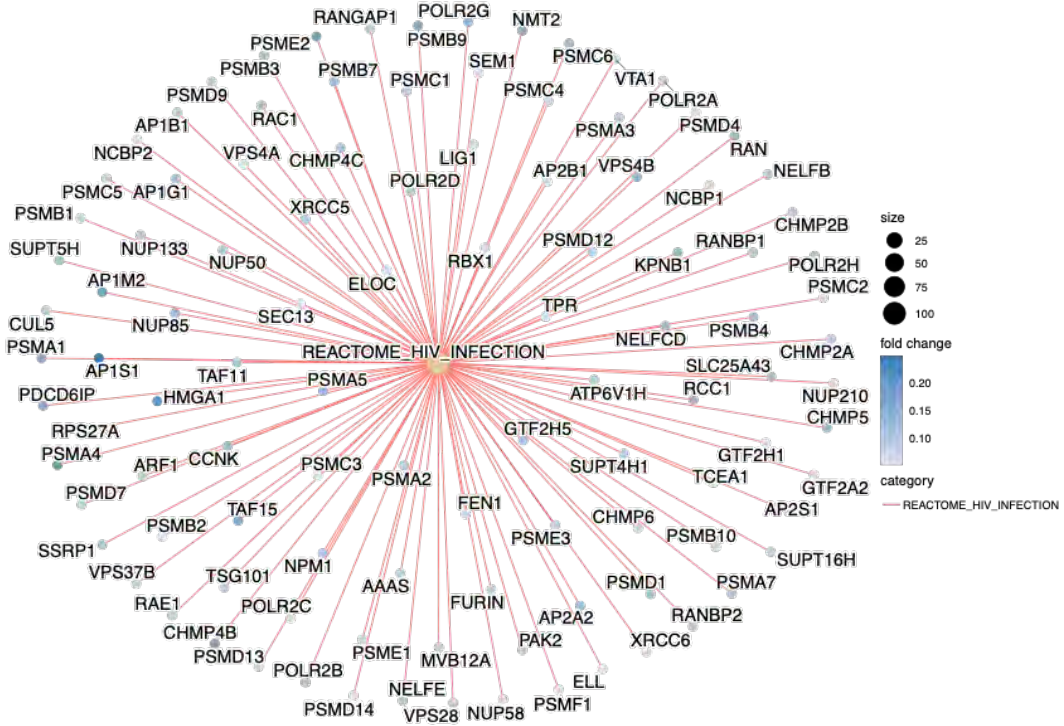


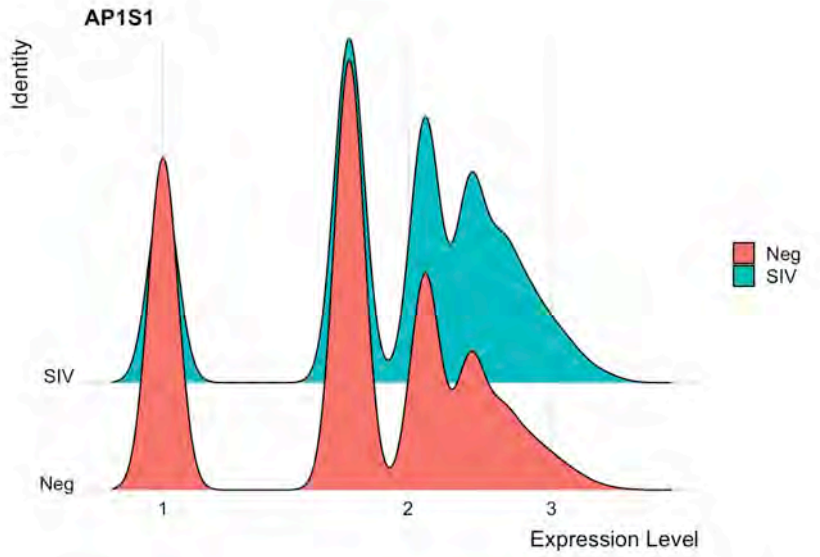




SIV Associated DEGs







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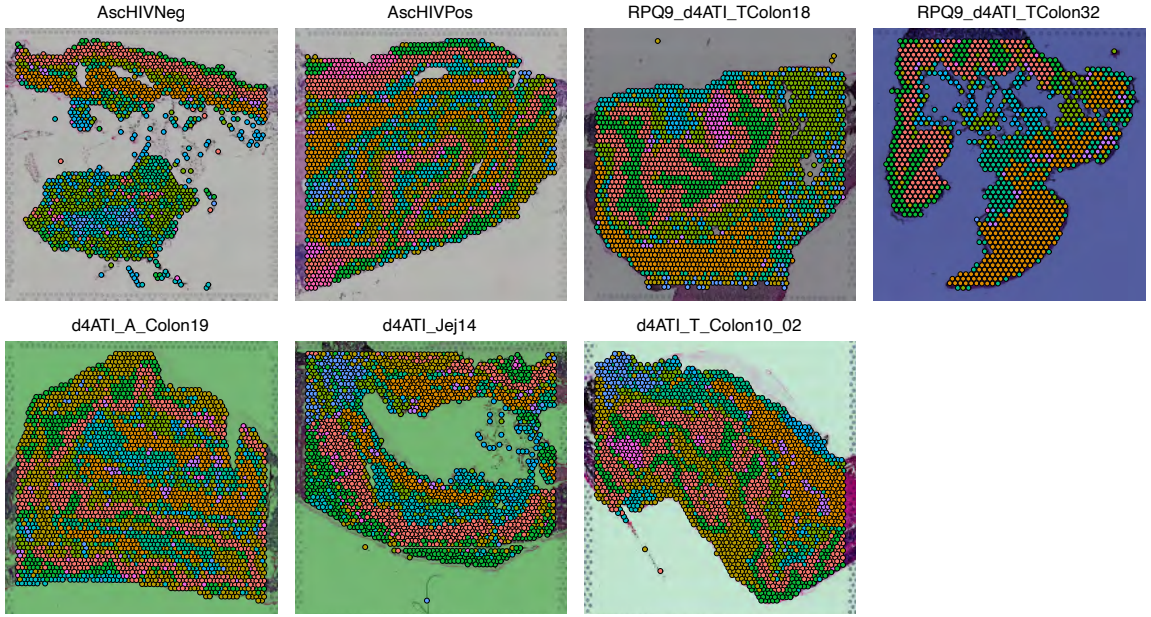
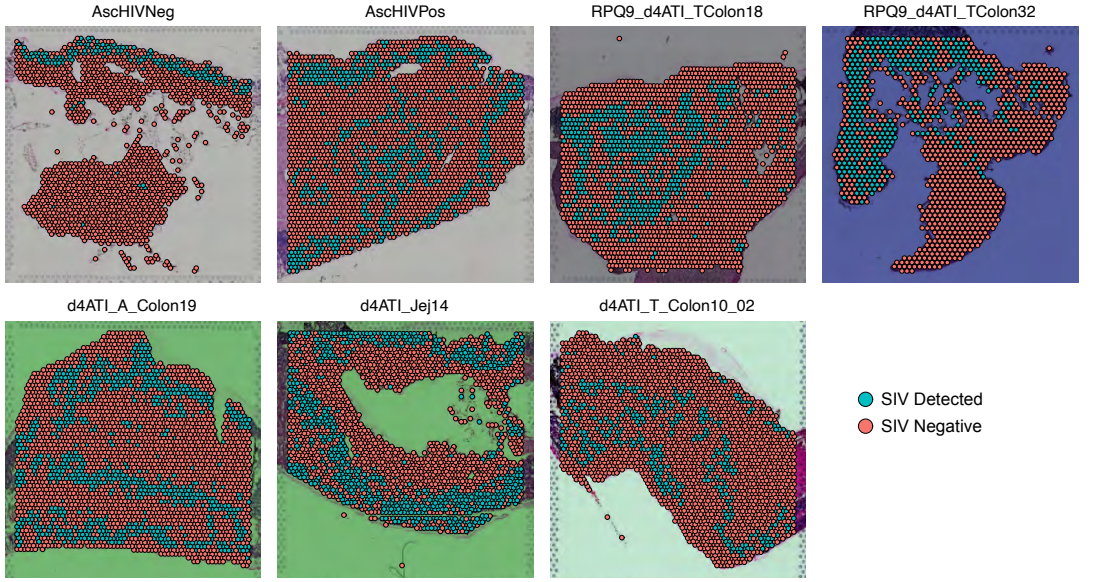
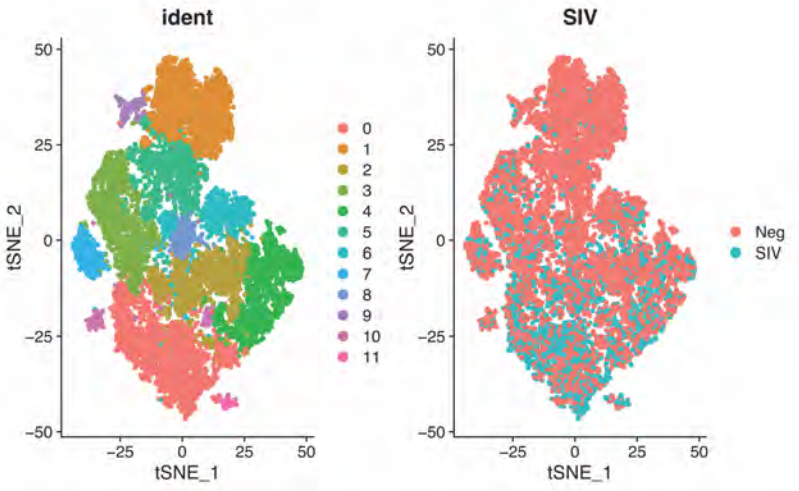
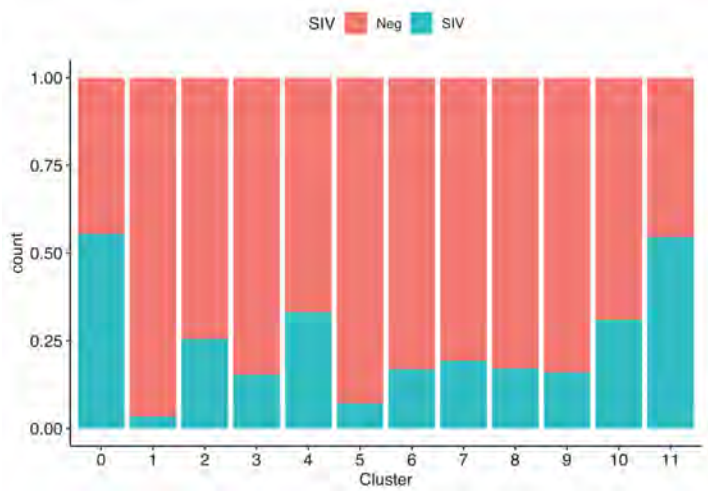
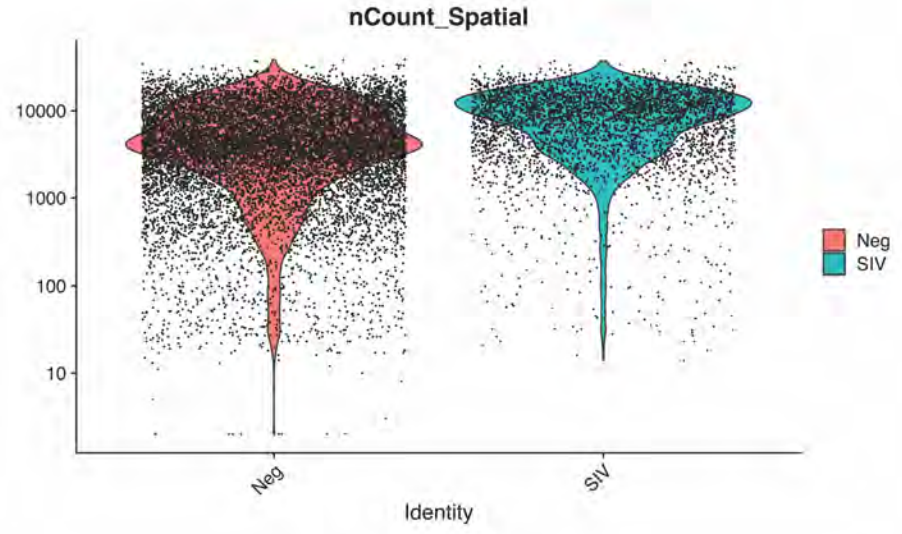
Early ART Animals After Early ATI



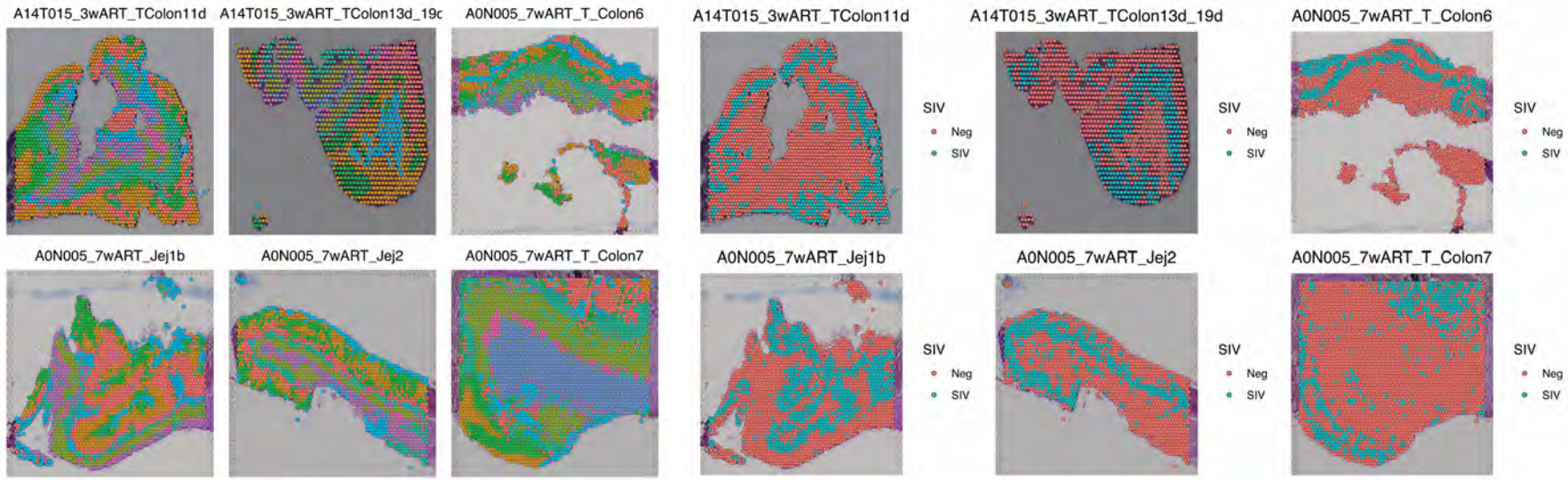


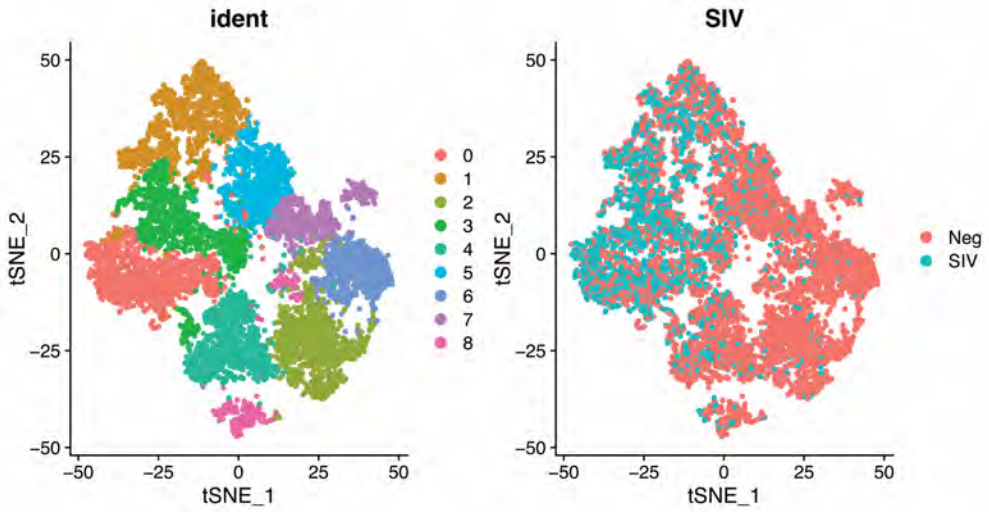


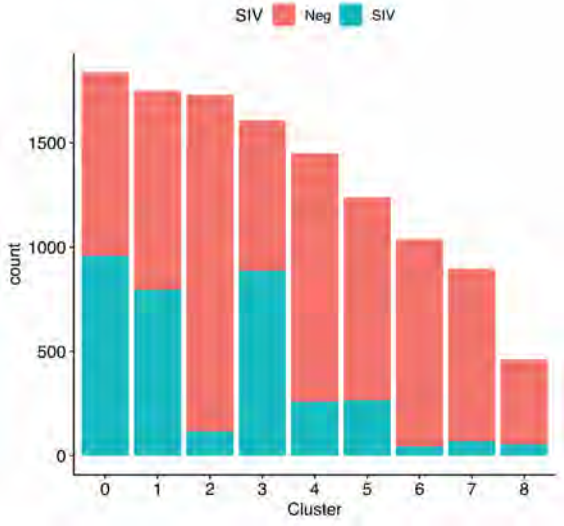


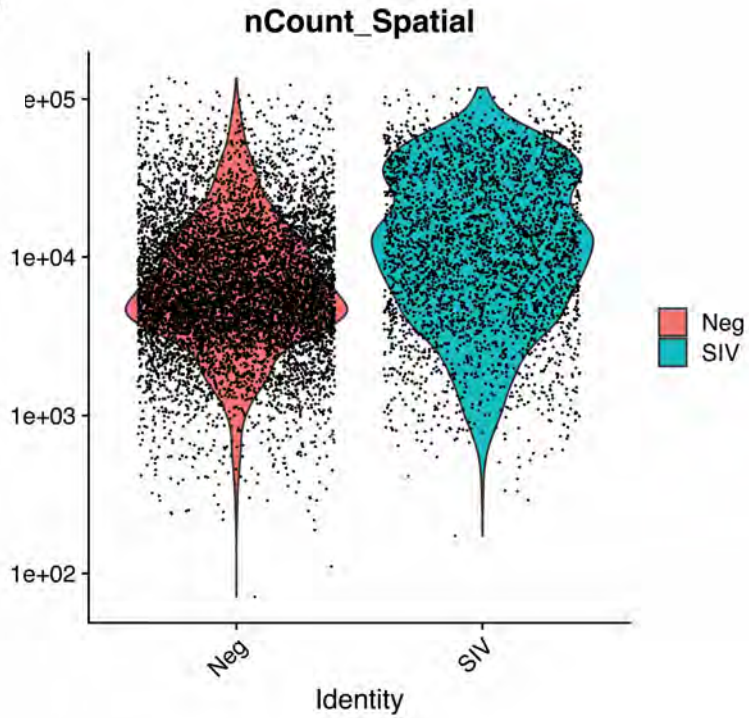


Early ART Animals During ART



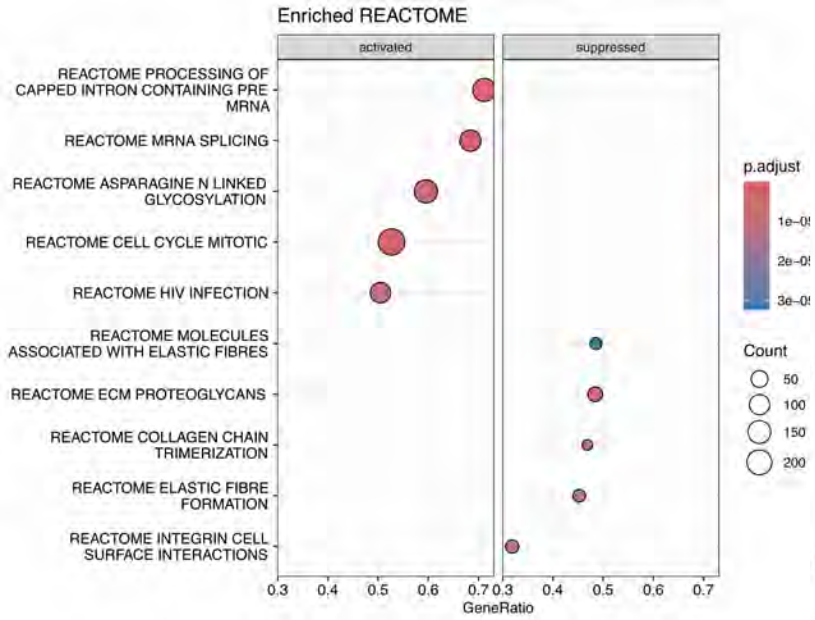




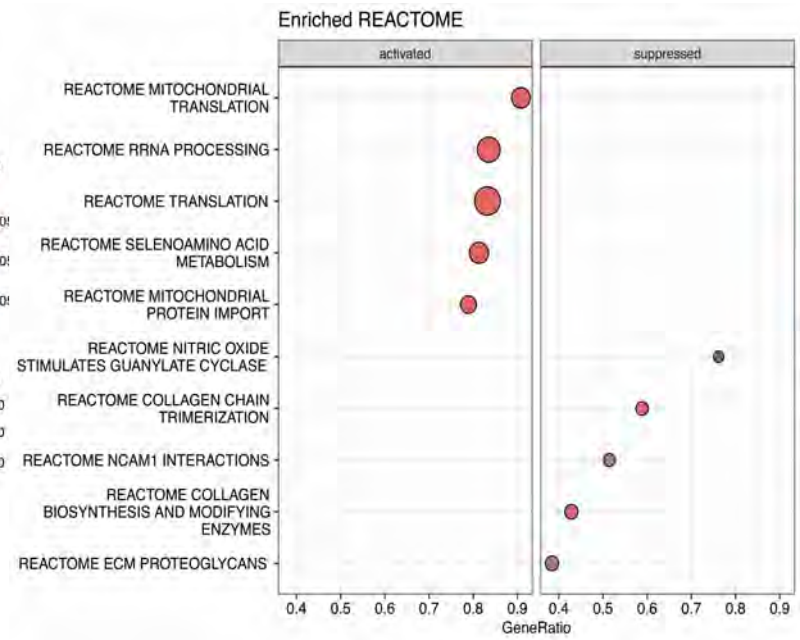


Enrichment for the 3 experiments

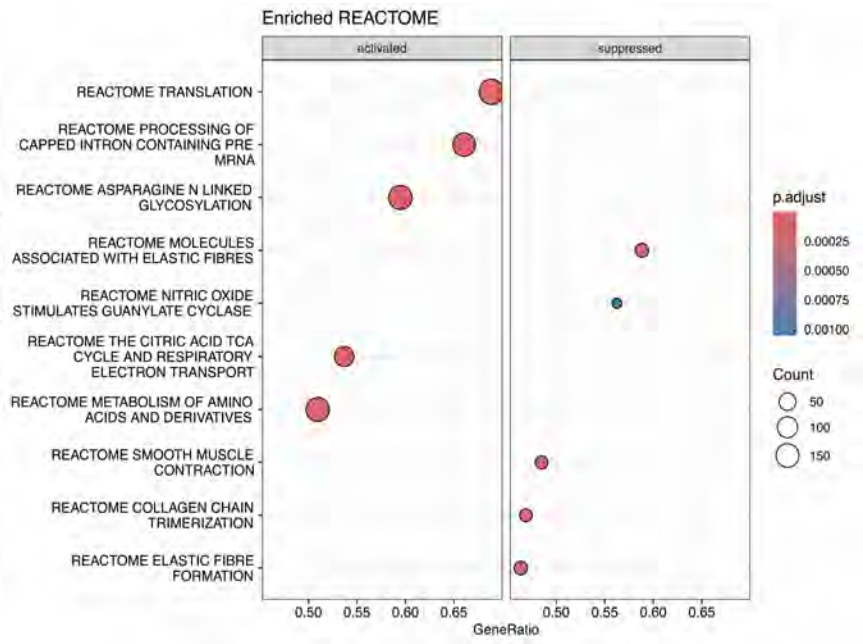
Early ATI 6 weeks ART



Early ATI 4 days ART



ART 4 days ART



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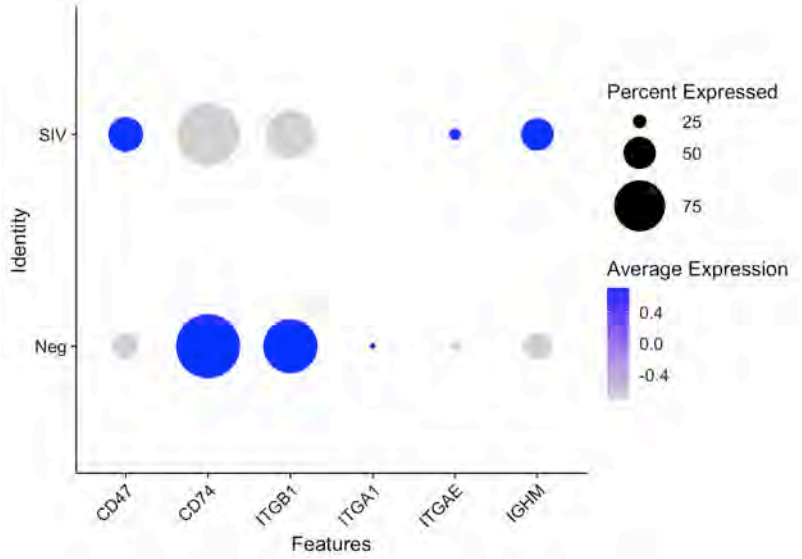
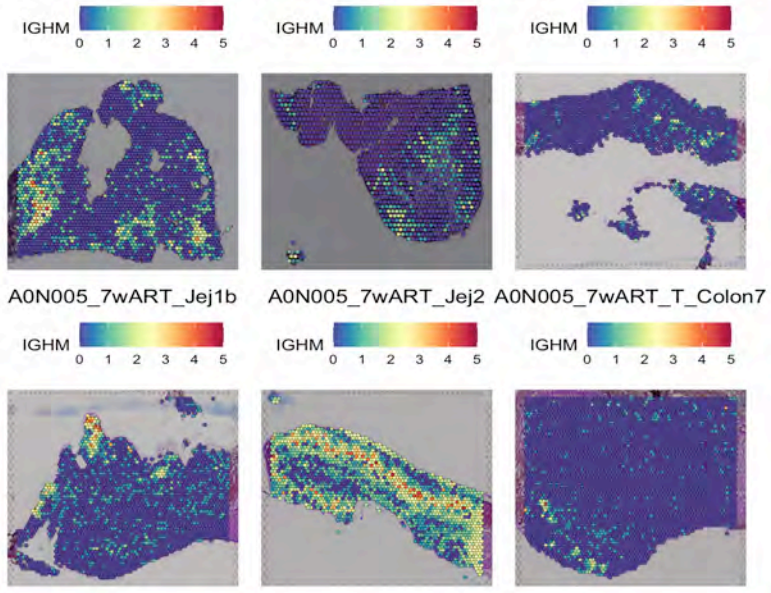
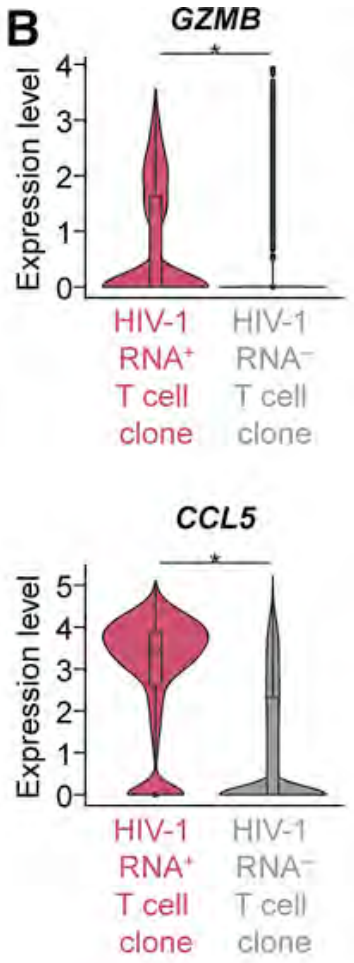
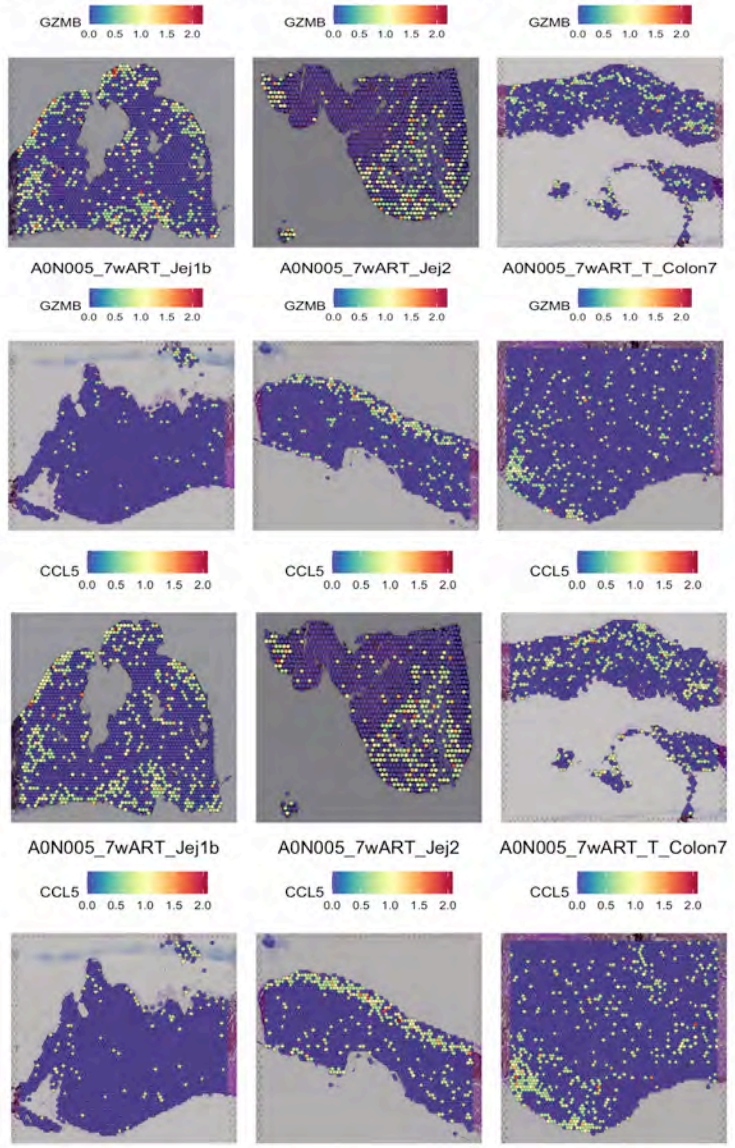
Possible candidates for SIV associated markers







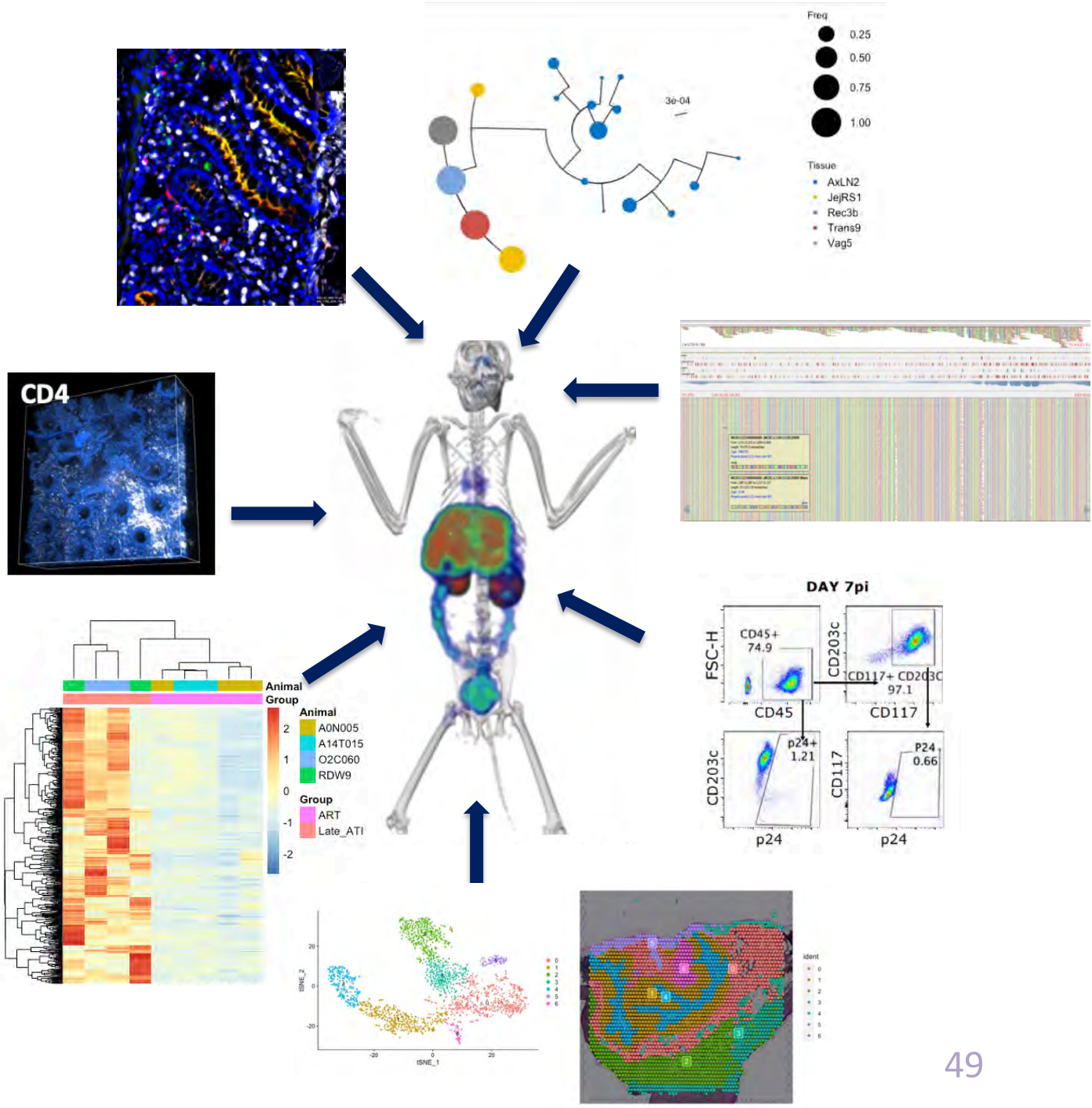
Collora JA et al. Immunity. 2022



Conclusions

- Viral protein production in tissue reservoirs is highly associated with higher transcriptional levels.
- There are clear spatial and transcriptional patterns associated with viral presence.
- Well-seeded reservoir shows earlier activation of virus-specific genes.
- Viral presence is associated with higher transcriptional levels of adaptive immunity responses.

- Combining an immunoPET/CT-guided system with genomics, spatial transcriptomics, and viral long-read deep sequencing allows us to study with unprecedented detail the nature and characteristics of SIV the tissue reservoirs involved in the establishment of the viral rebounding population immediately after ART cessation.
- 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.**



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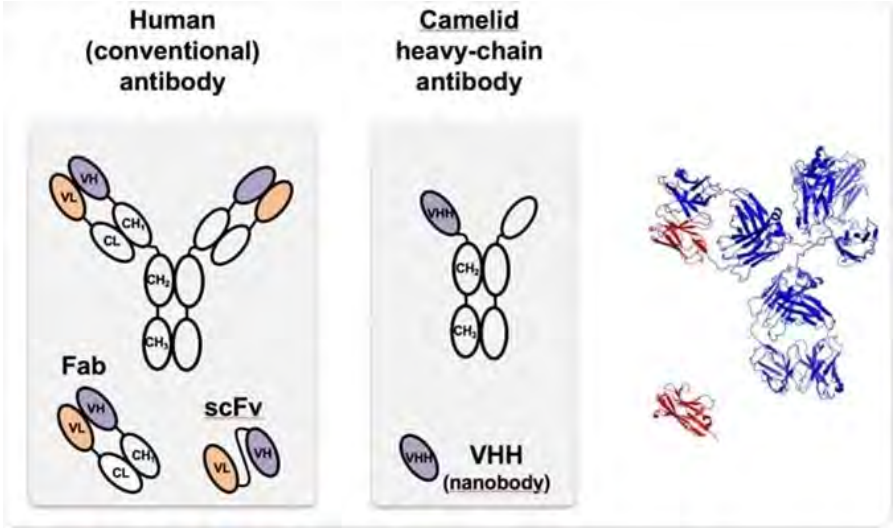
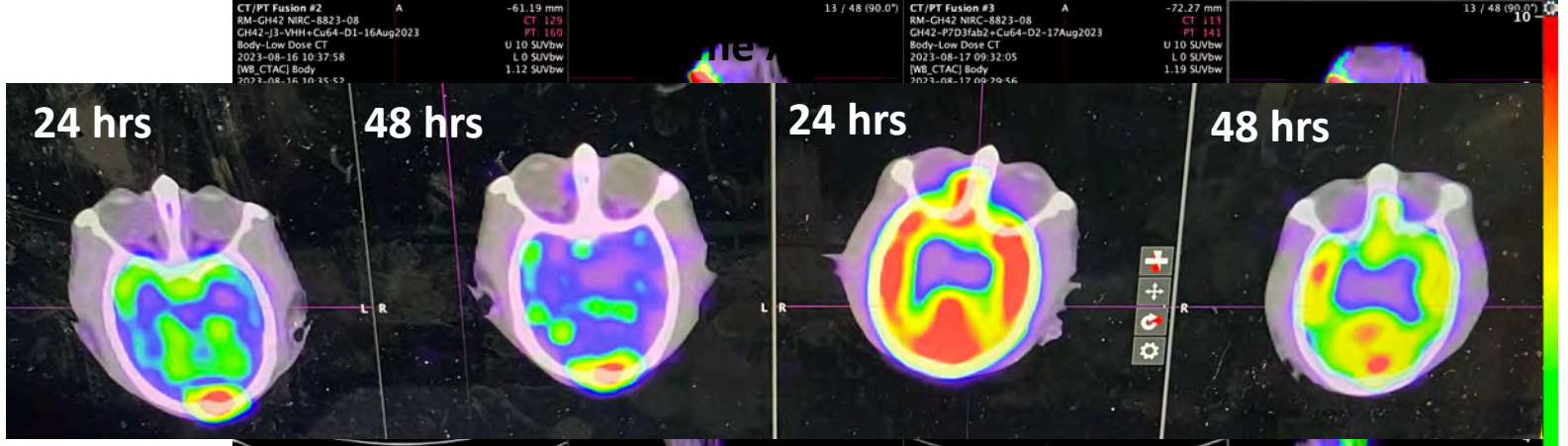
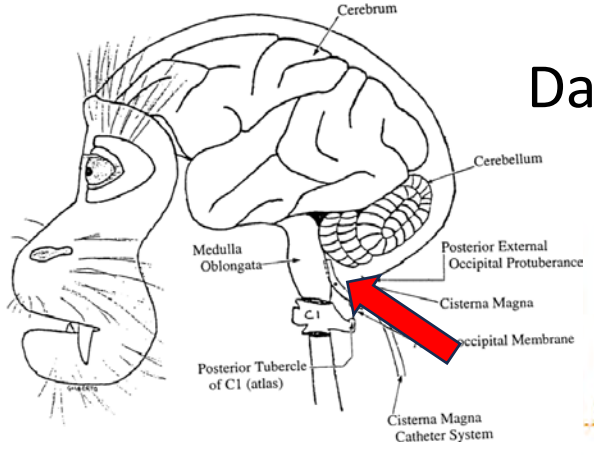
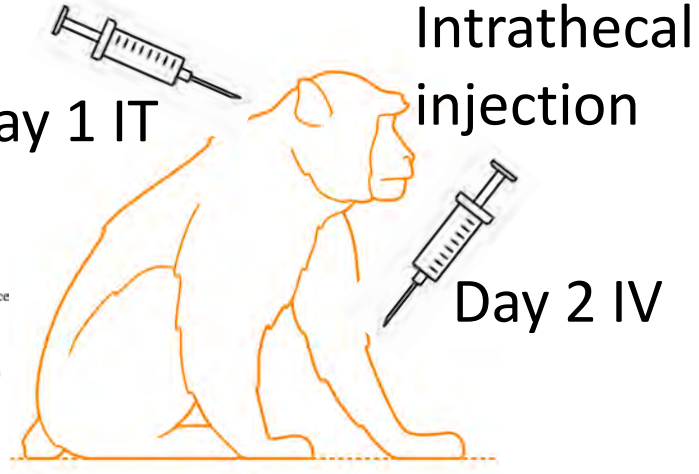
Detection of SIV/SHIV in Brain

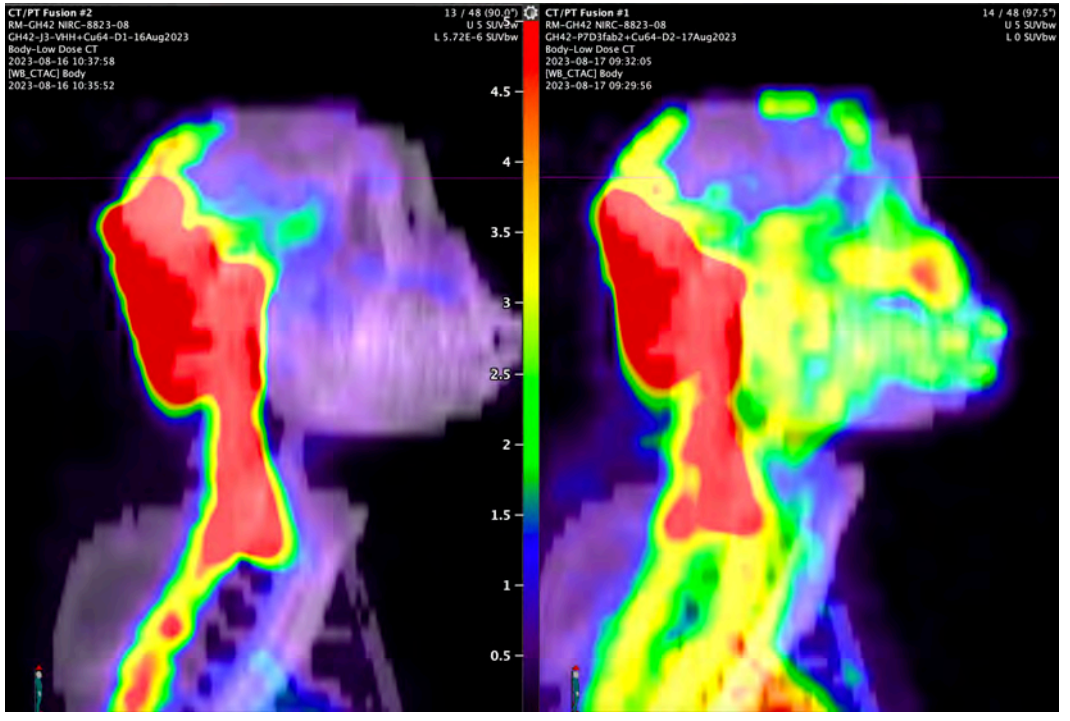


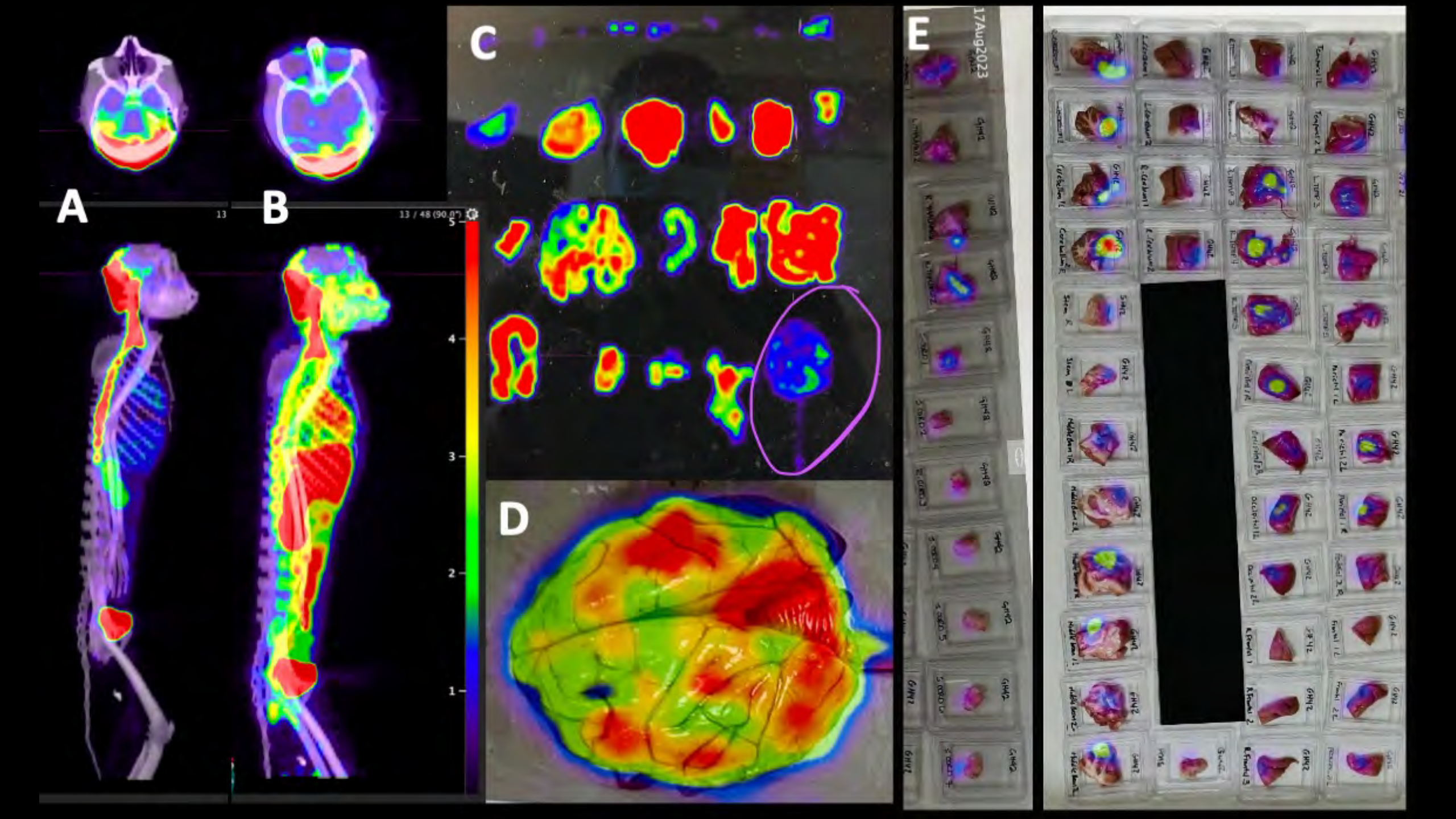


J3-NOTA VHH to CD4bs (Ploegh, Clayton, Weiss)





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Acknowledgements

Hope Lab


Thomas J Hope


Muhammad Shoaib Arif


Christopher Thuruthiyil


Sean Pascoe


Mike McRaven


Sadia Samer


Flora Engelmann


Yanique Thomas

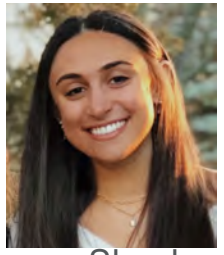
Villinger Lab


Francois Villinger


Mariluz Araínga


Richard T D'Aquila


Elena Martinelli


Maryam Shaaban


Lacy Simons

Lorenzo-Redondo Lab

Hope Lab

Funding Sources:

- NIH/NIAID
  - 1P01AI169600-01
  - 1R01MH125778
- Third Coast Center for AIDS Research

Third Coast CFAR
CENTER FOR AIDS RESEARCH IN CHICAGO

Center for Pathogen Genomics and Microbial Evolution
Robert J Havey, MD Institute for Global Health
Northwestern University Feinberg School of Medicine

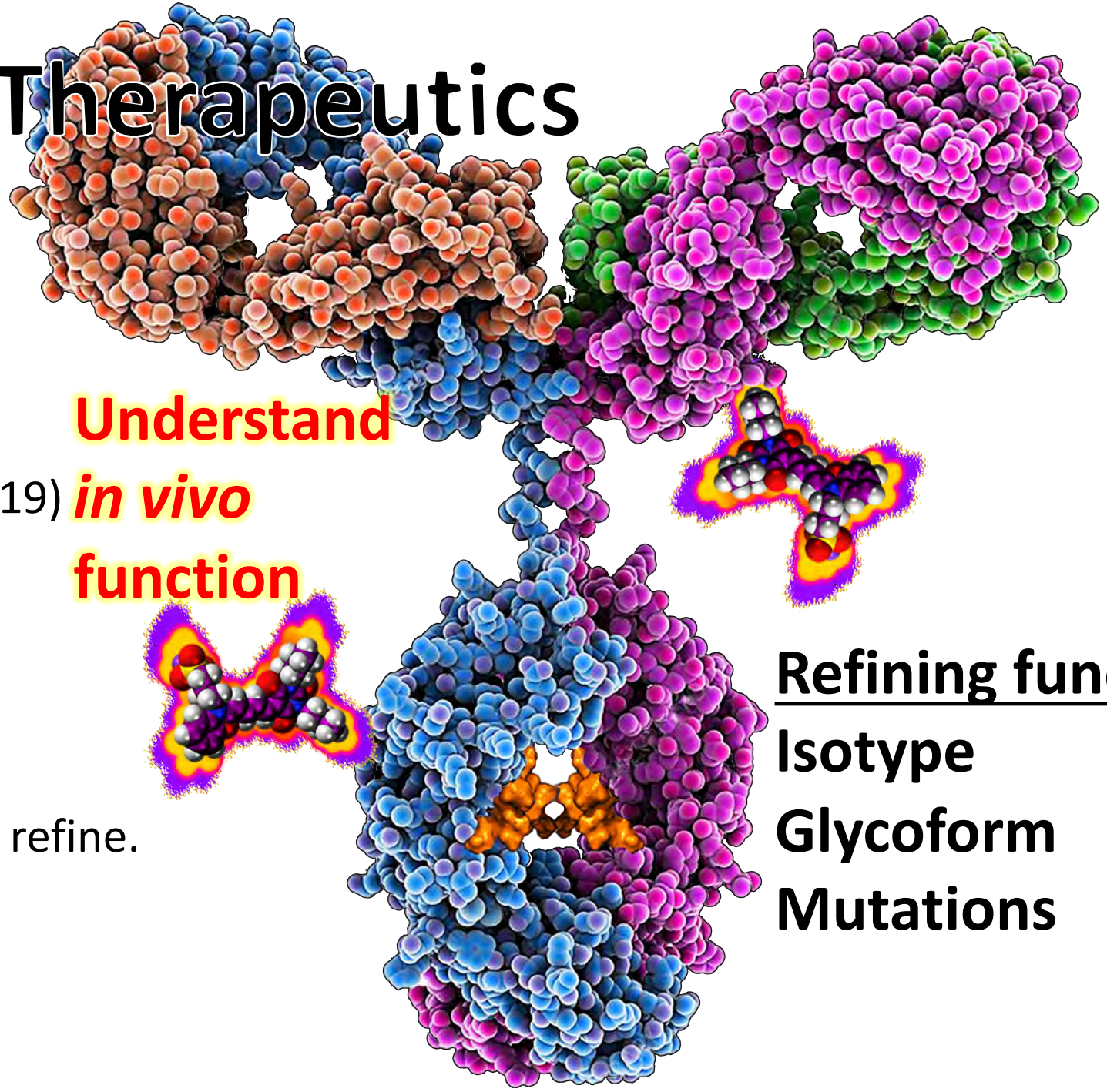
NIH NIAID
National Institutes of Health

UNIVERSITY OF LOUISIANA AT LAFAYETTE
NEW IBERIA RESEARCH CENTER
University Research Division

Northwestern Medicine
Feinberg School of Medicine

Antibodies as Therapeutics

- 100+ approved
- Multiple targets
  - Cancer
  - Autoimmune diseases
  - Infectious diseases (COVID-19)
- Multiple approaches
  - Targeted killing
  - Receptor blocking
  - Soluble antigen capture.
- Great potential to improve and refine.
  - Stability
  - Targeting
  - Function



Understand *in vivo* function

<u>Refining function</u>

Isotype

Glycoform

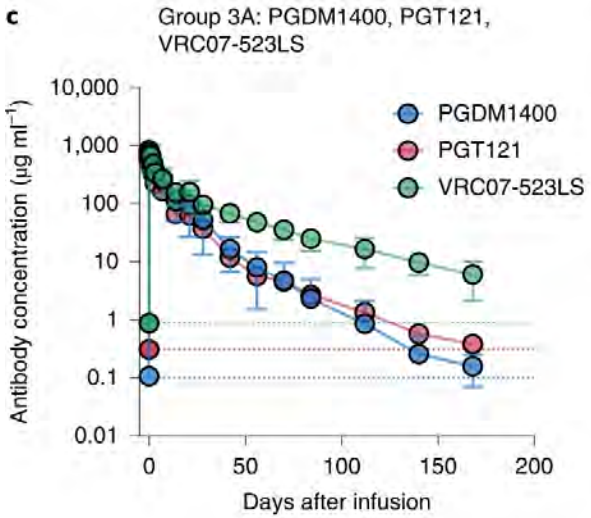
Mutations

Development of Monoclonal antibodies for HIV Prevention, Treatment, and Cure.

Clinical Trial: T002 (NCT03205917)

A Phase 1 study that evaluated the safety, tolerability, pharmacokinetics, and antiviral activity of the bNAbs PGDM1400, PGT121, and VRC07-523LS in adults without HIV and adults with HIV who were not on ART

Julg et al. (2022) Nat Med



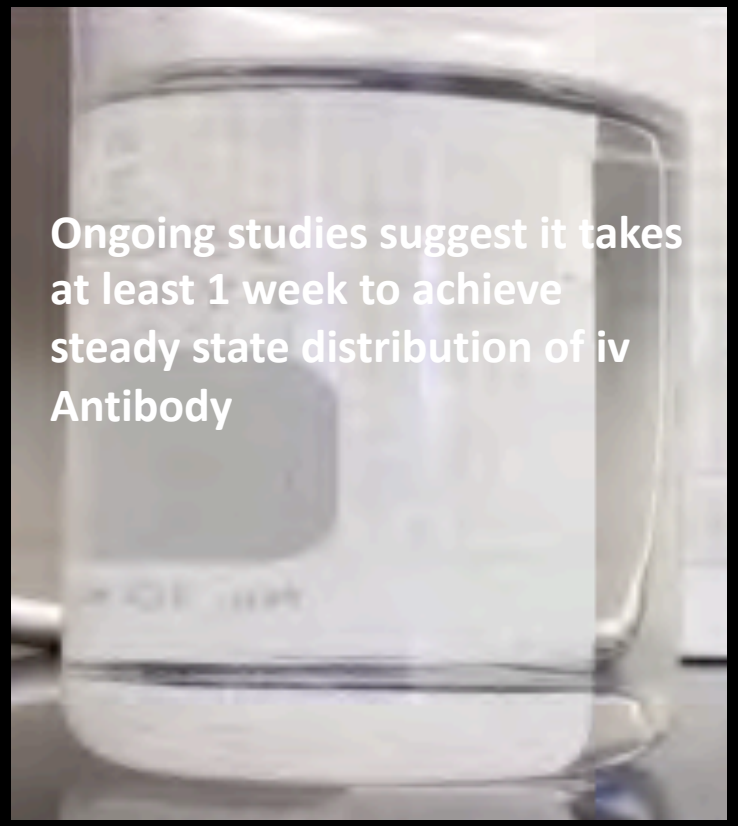
Clinical Trial T003 (NCT03721510)

A Phase 1/2a study that evaluated a triple bNAb regimen consisting of PGT121, VRC07-523LS, and PGDM1400. Initially, the safety, tolerability, and pharmacokinetics of the triple bNAb regimen was assessed in adults with and without HIV. Thereafter, the antiviral activity of the combination bNAb regimen was **evaluated in adults with HIV undergoing an analytical treatment interruption of ART.**

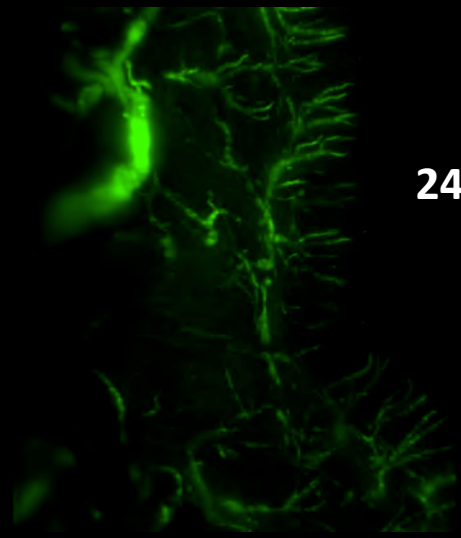
Julg et al CROI 2024

- All viremic participants responded
- Selection of partially or completely resistant variants to PDGM1400 and PGT121 emerged.
- Rebound Virus was still susceptible to VRC07-523LS (~90ug/ml)
- Something strange happening. Not all antibodies are the same.

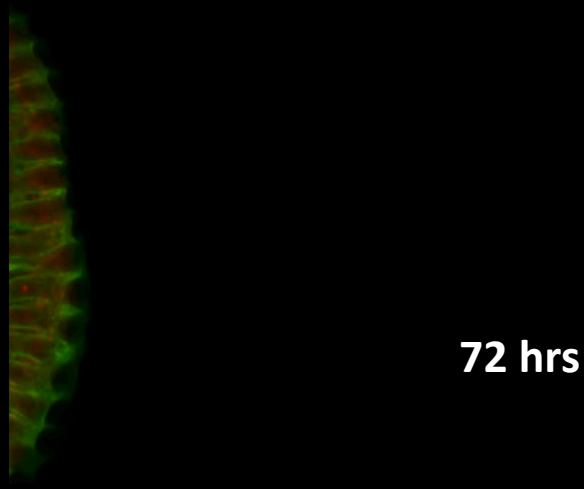
Antibody Distribution after IV Instillation (How does it work?)



0 min 2 sec



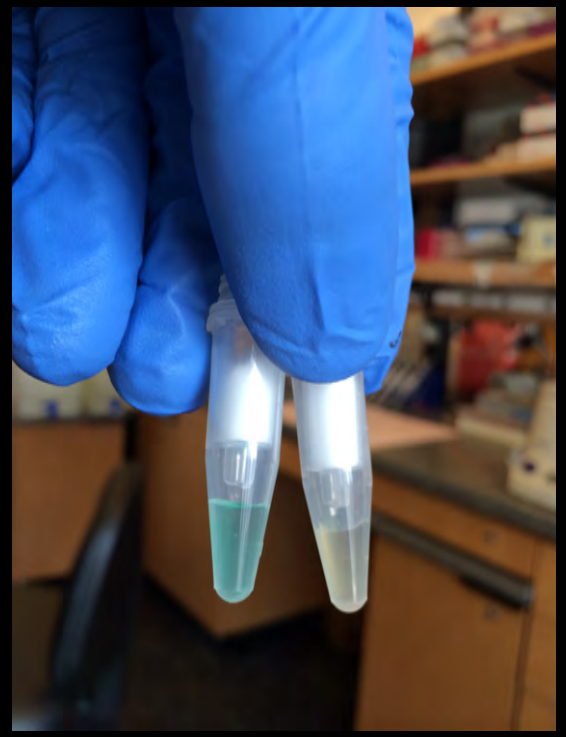
24 hrs

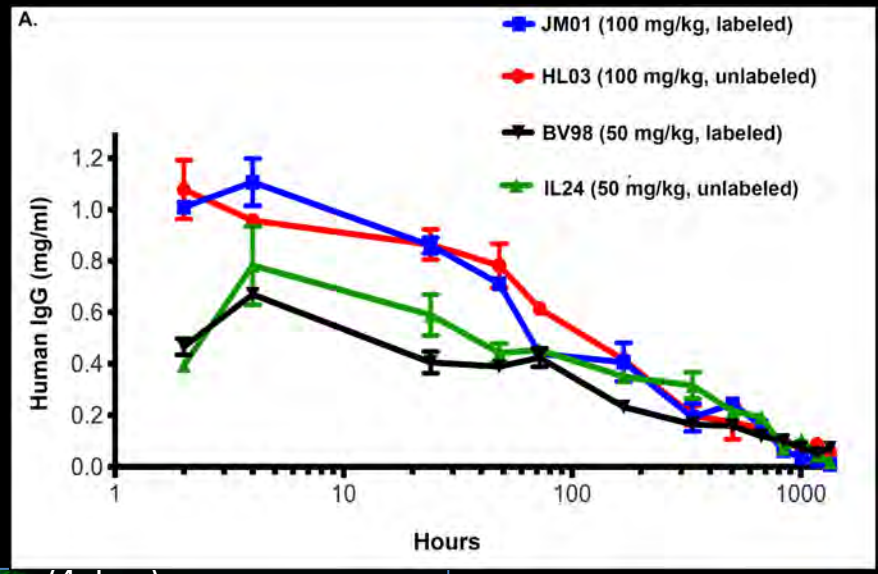


72 hrs

UC Davis SuperPET
18FDG

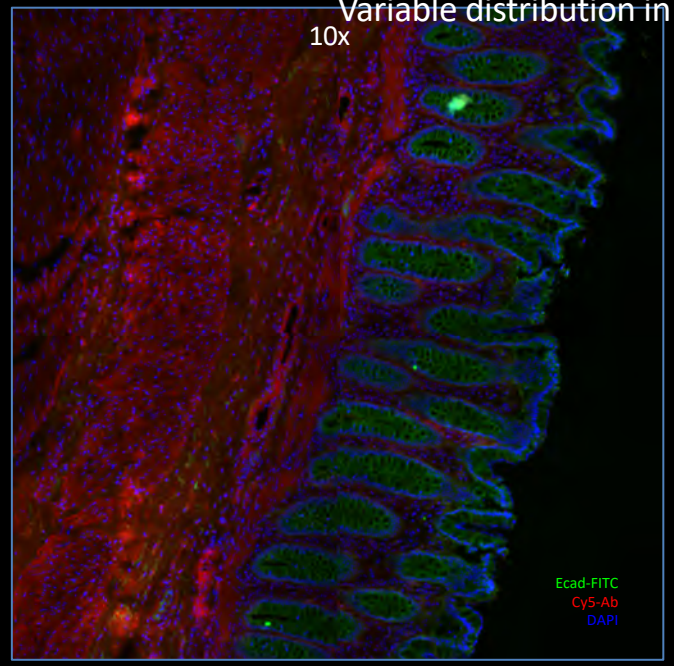
Visualizing Antibodies *In Vivo*

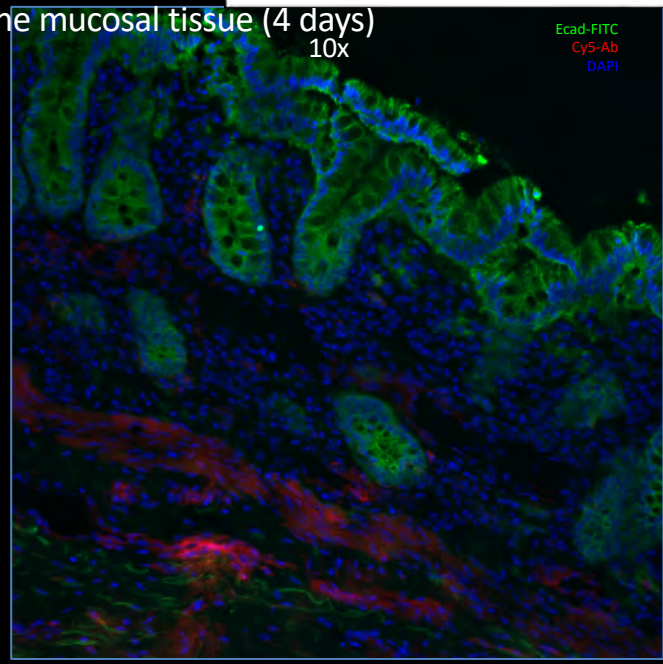




Variable distribution in the mucosal tissue (4 days)

IV injected antibodies take time
To distribute into the tissue

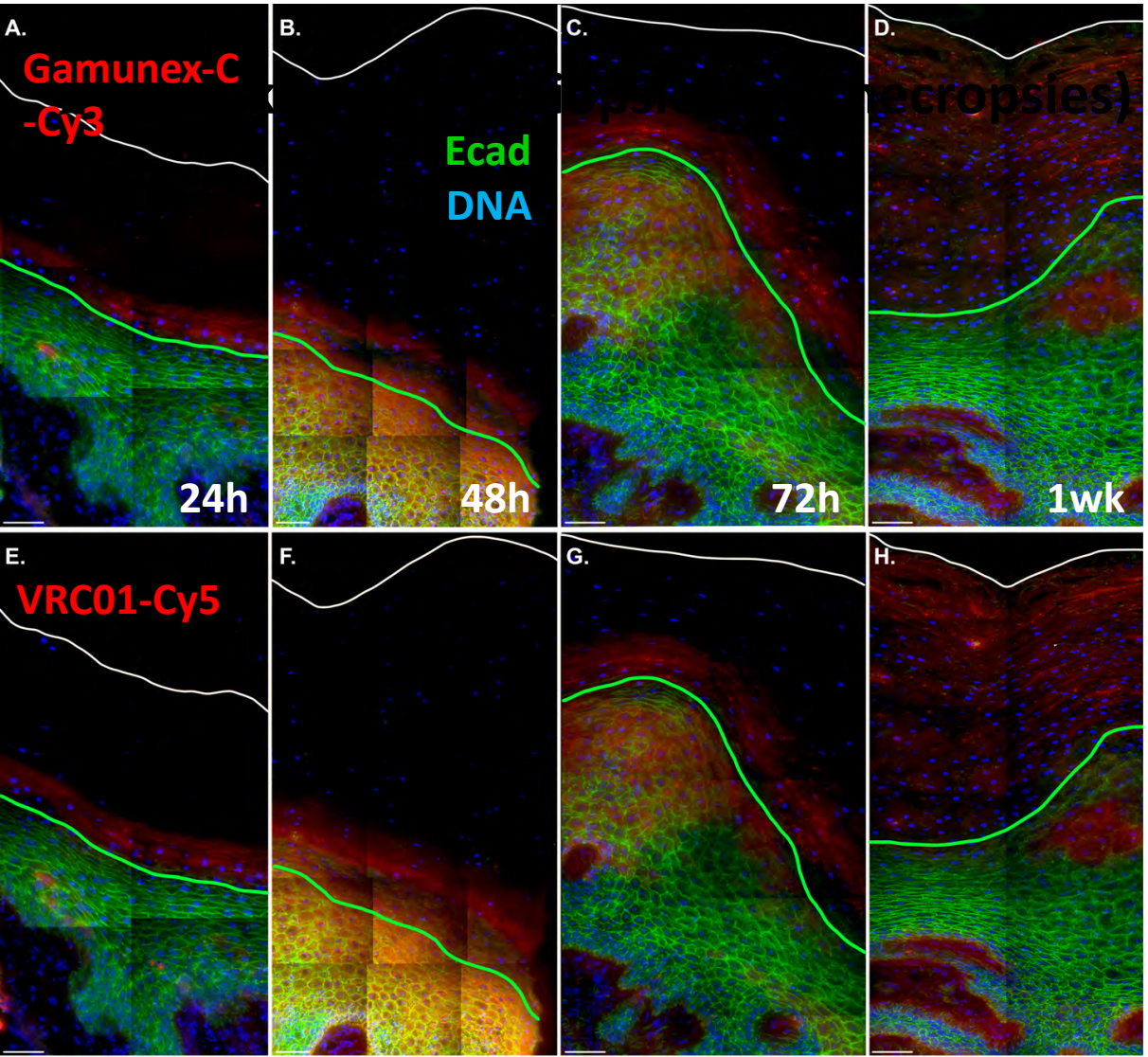


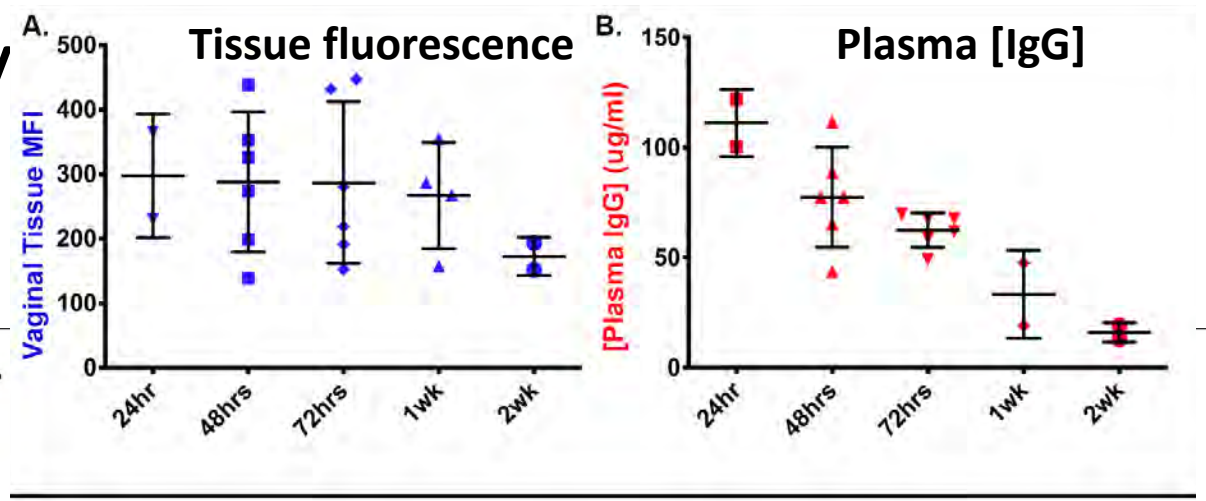


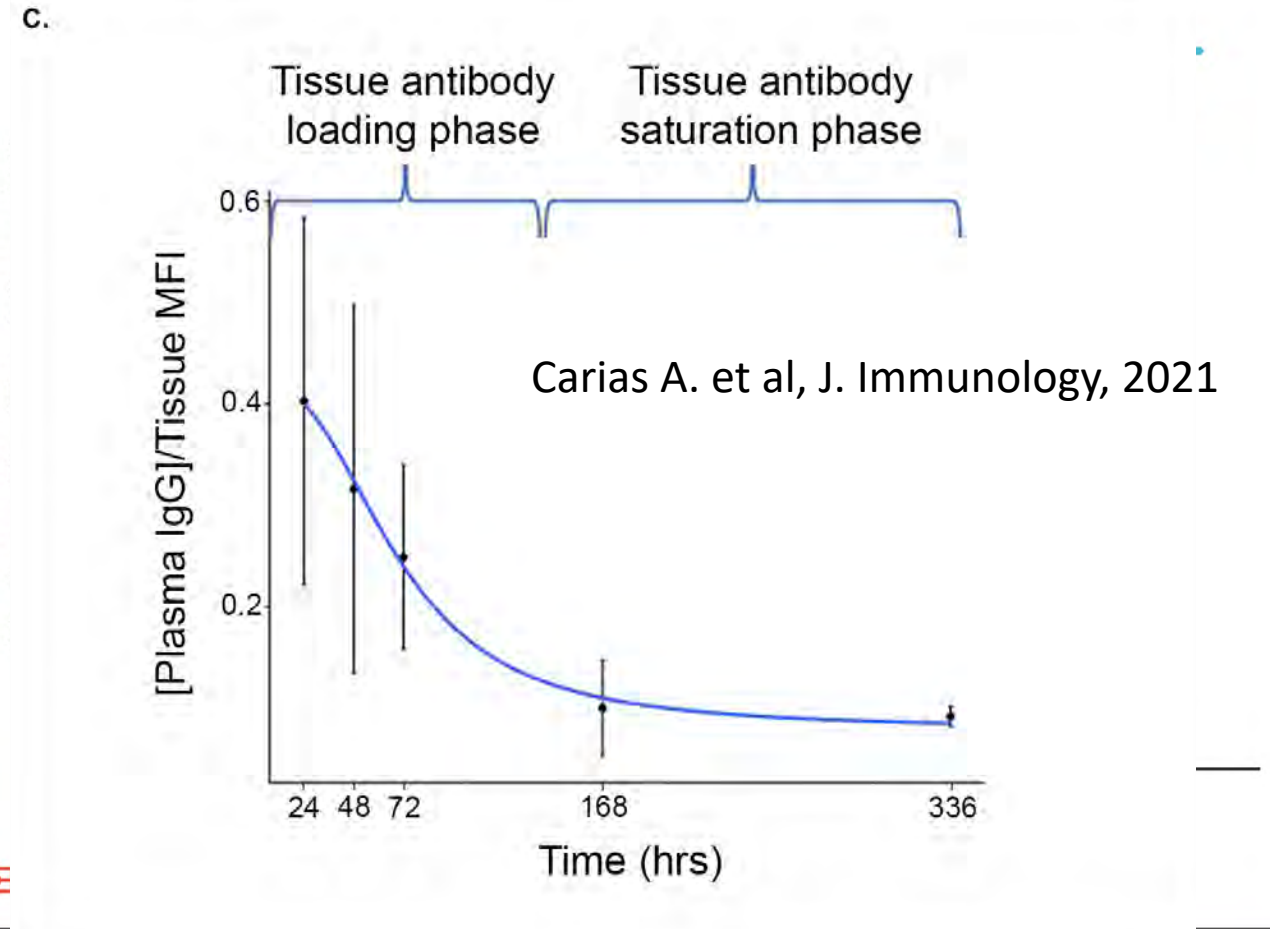
Wait 1 week (?) to get complete systemic distributio n of injected antibody

Schneider et al. Journal of immunological Methods, 2017

~1 wk needed to achieve steady state distribution of Ab in vaginal epithelium

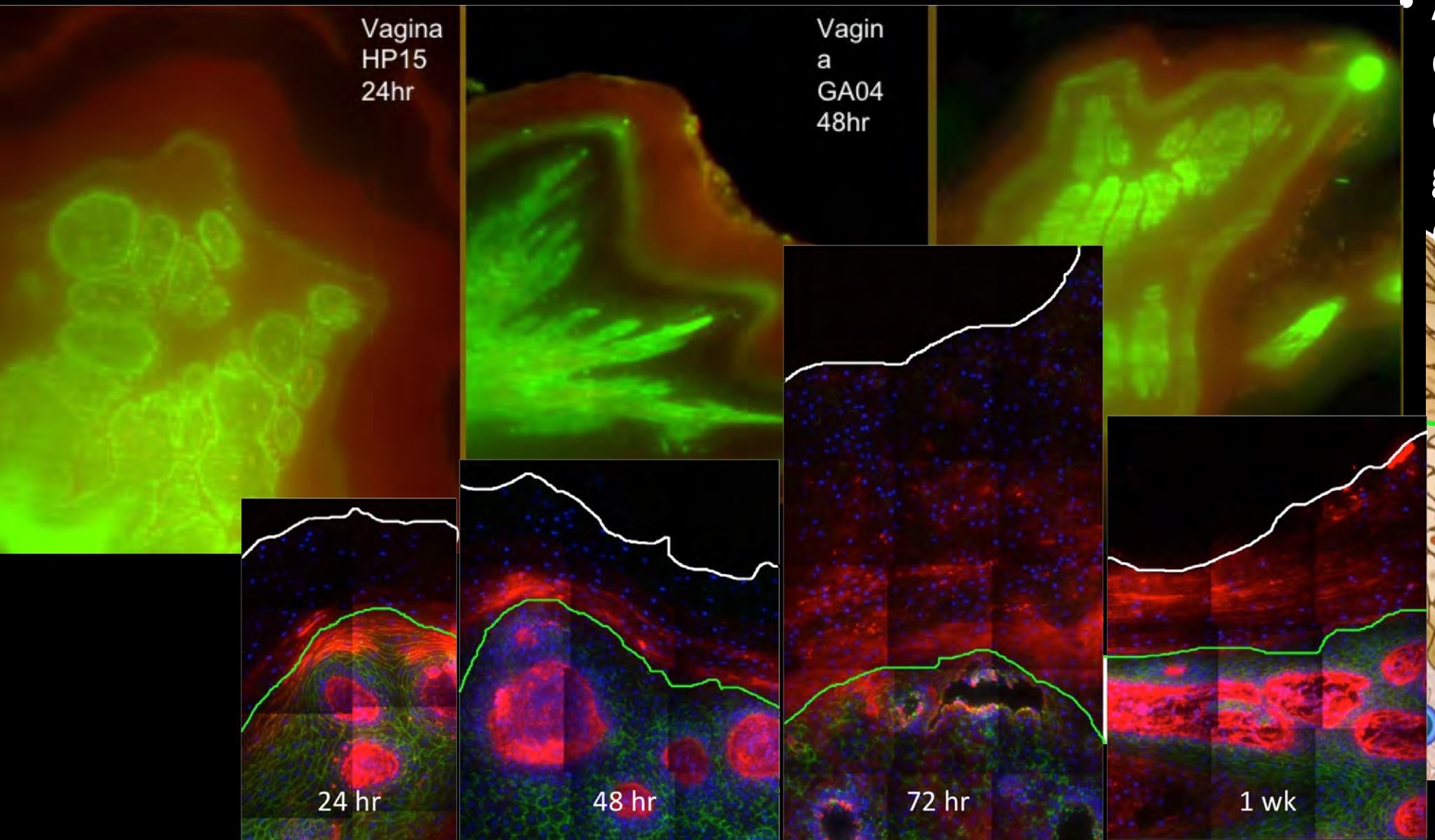




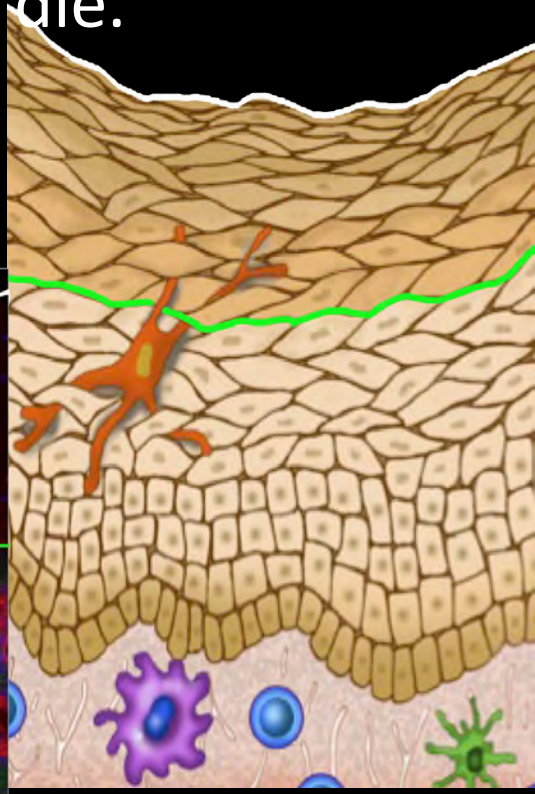


Carias A. et al, J. Immunology, 2021

Antibodies enter squamous epithelium from below.



- Antibody containing basal cells migrate to granulosum and die.

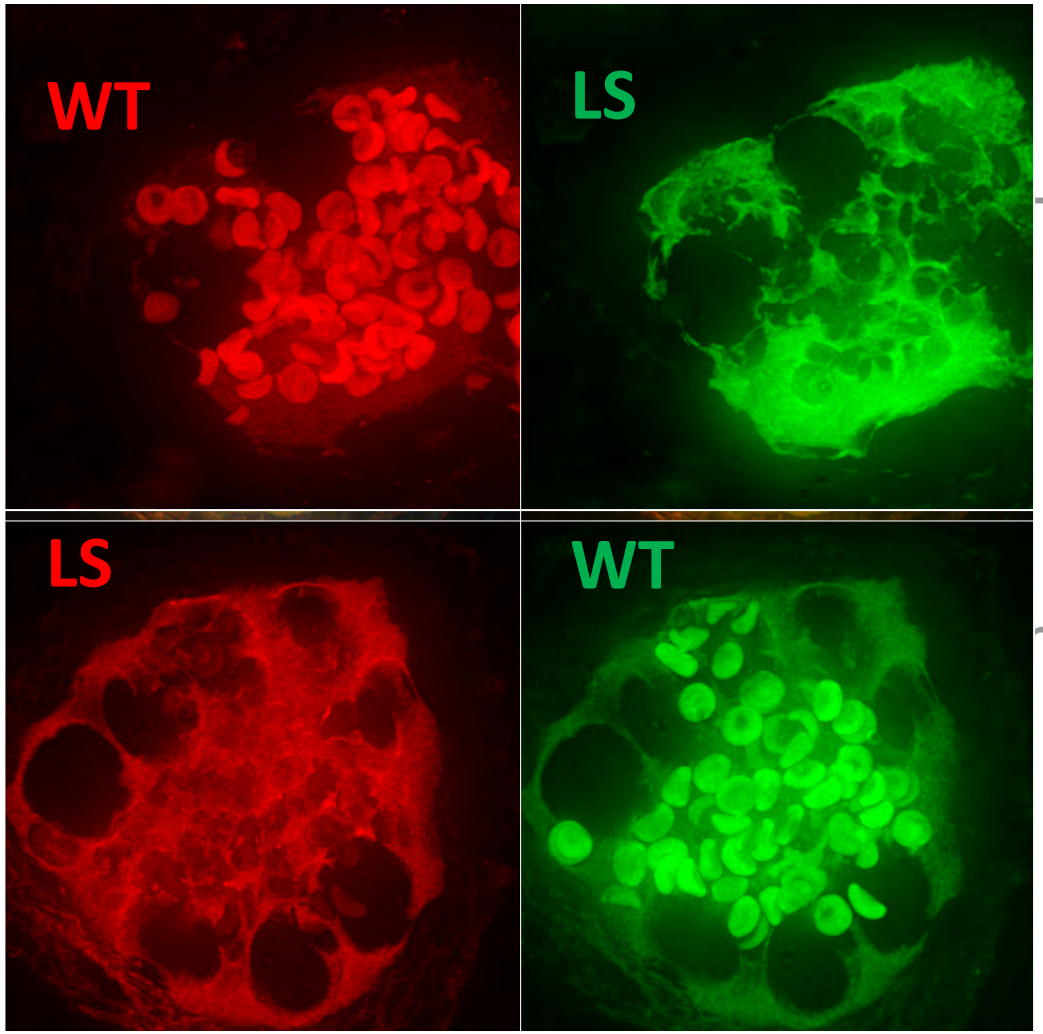


Comparing two labeling modalities for robust data set

Microscopy: Fluorescence

- Detailed analysis of antibody localization at tissue and cell level
- **Light sheet imaging** of clarified tissues: tissue level (~10 um^3).
- **Fluorescence microscopy** reveals: cellular level (~0.1 um^2).
- Co-injection of differentially-labeled antibodies (Fluor-Swap) reveals antibody specific interactions and differences.
- Can be quantified in relevant luminal fluids.

Fluor-Swap



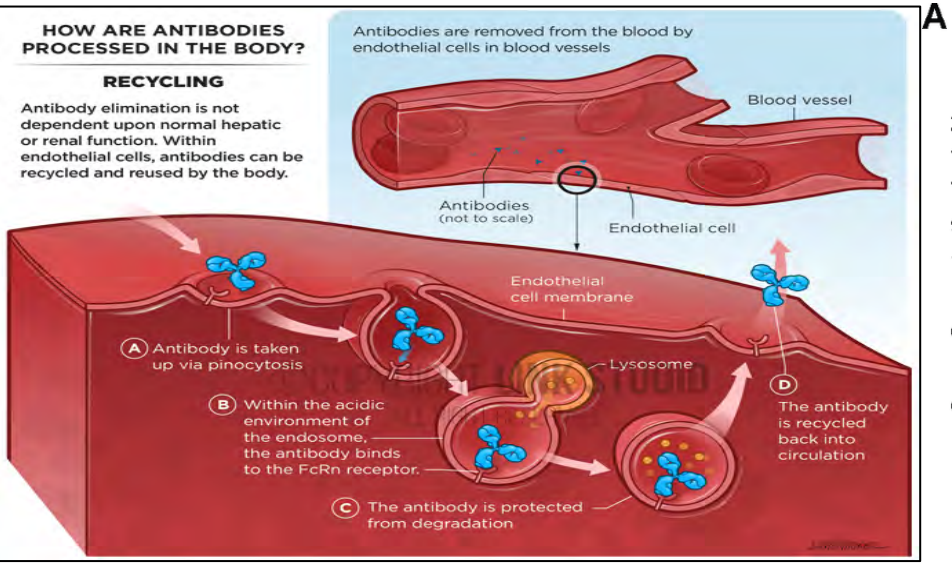
Paraffin embedded tissues from 2 different animals with the fluorescent tags switched.

Antibody Modification to Improve Function and PK

Specific mutations in Fc-region are known to increase or decrease specific interactions with specific receptors.

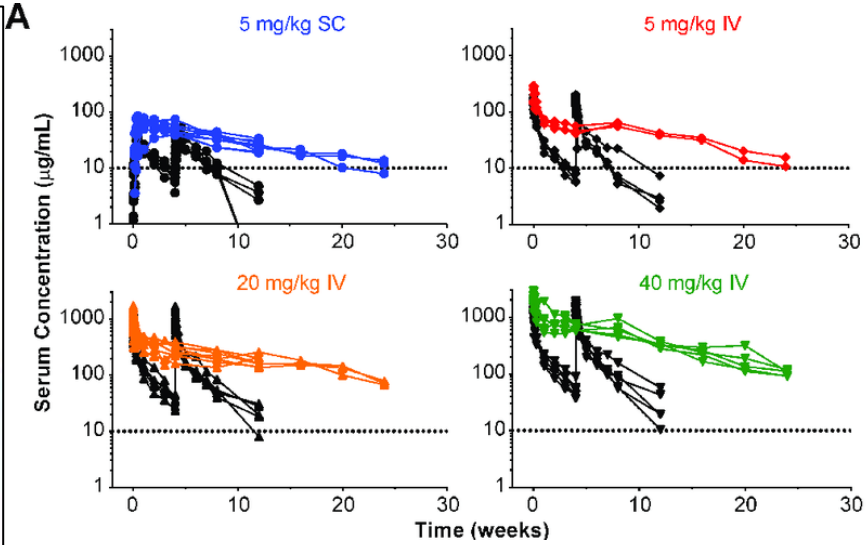
Neonatal Fc Receptor (FcRn). Delivers IgG across placenta and from mothers milk to child's circulation.

FcRn Mediates IgG Homeostasis

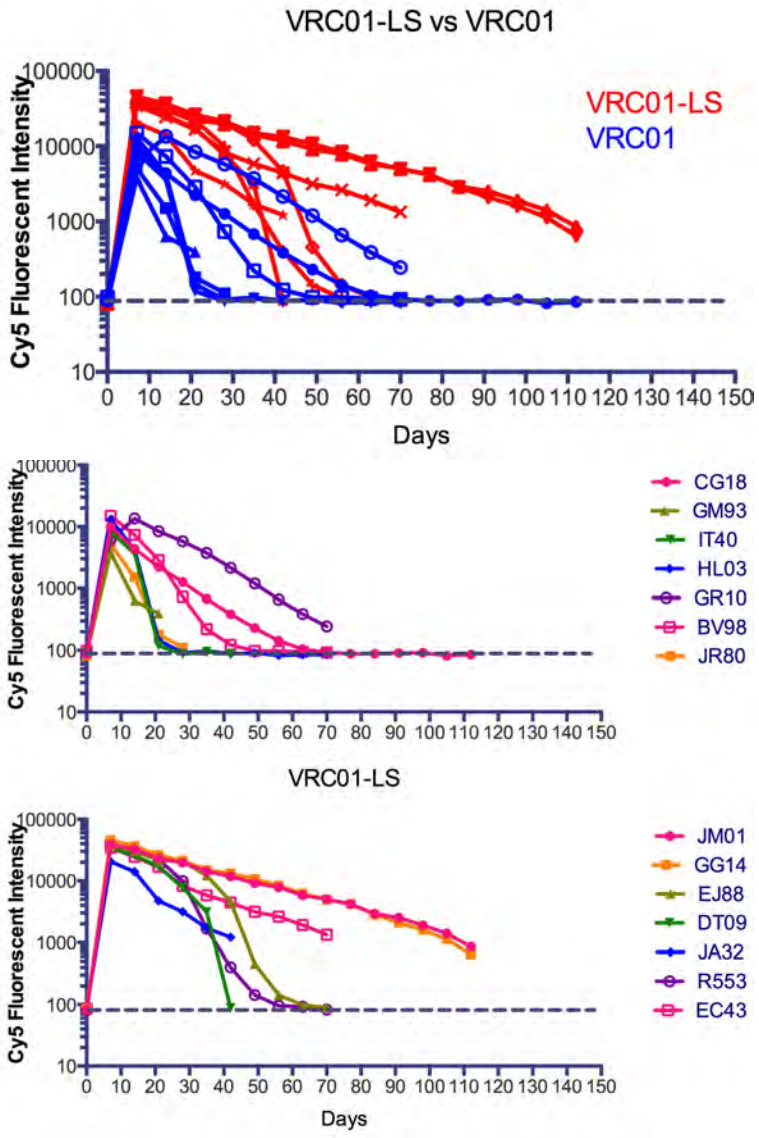


LS mutation increases interactions with Neonatal Fc Receptor (FcRn).

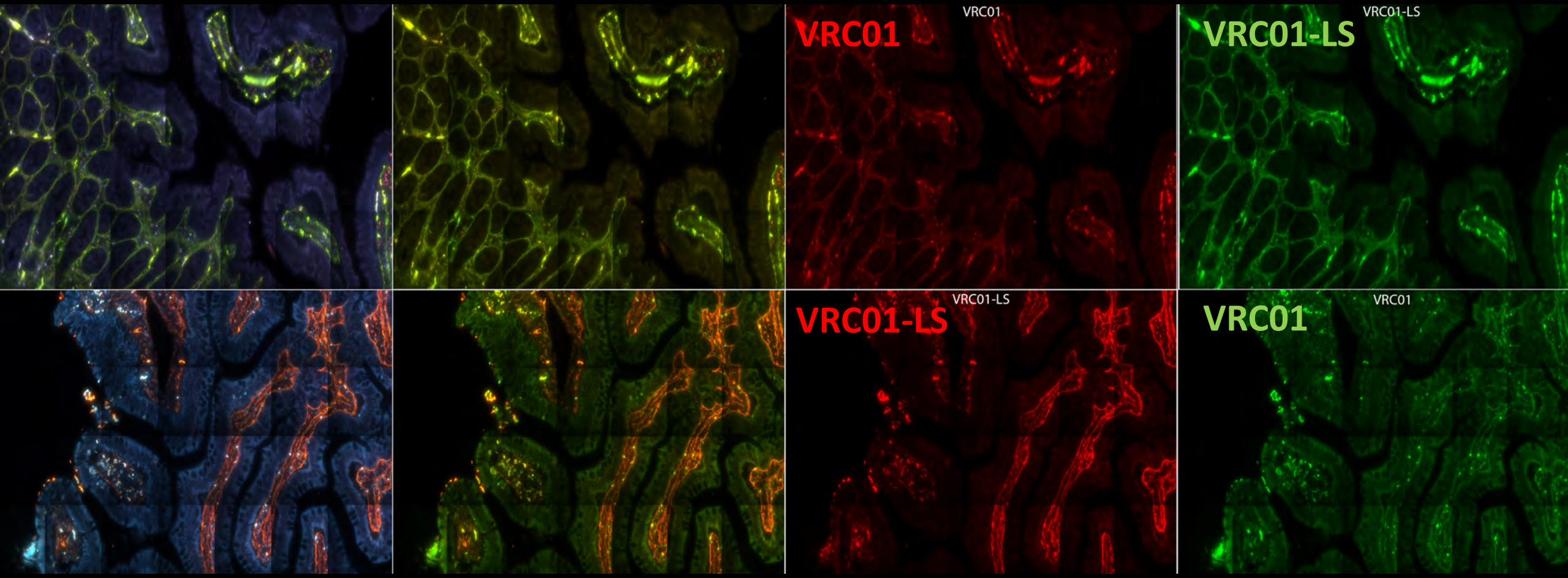
Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults



Gaudinhski et al, 2018, PLoS Medicine



72 hours mixture of VRC01 and VRC01-LS

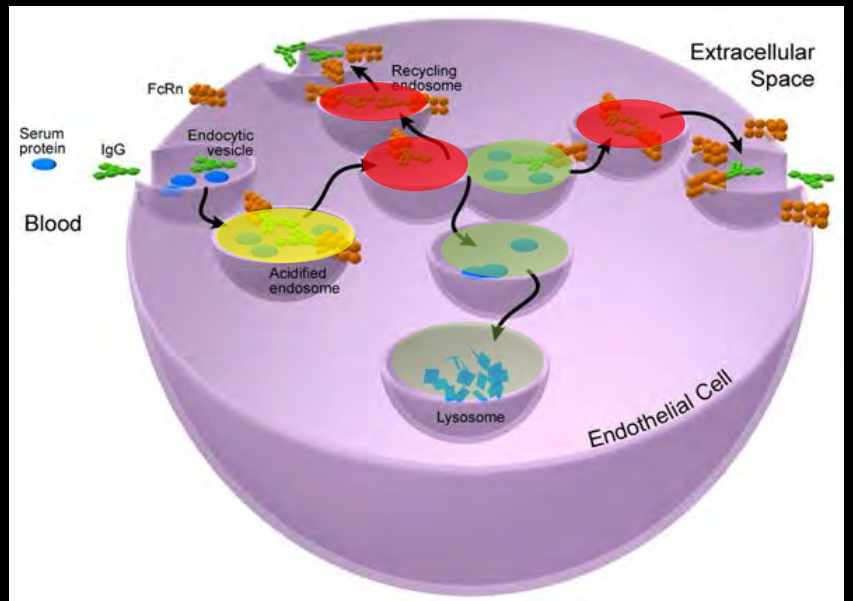


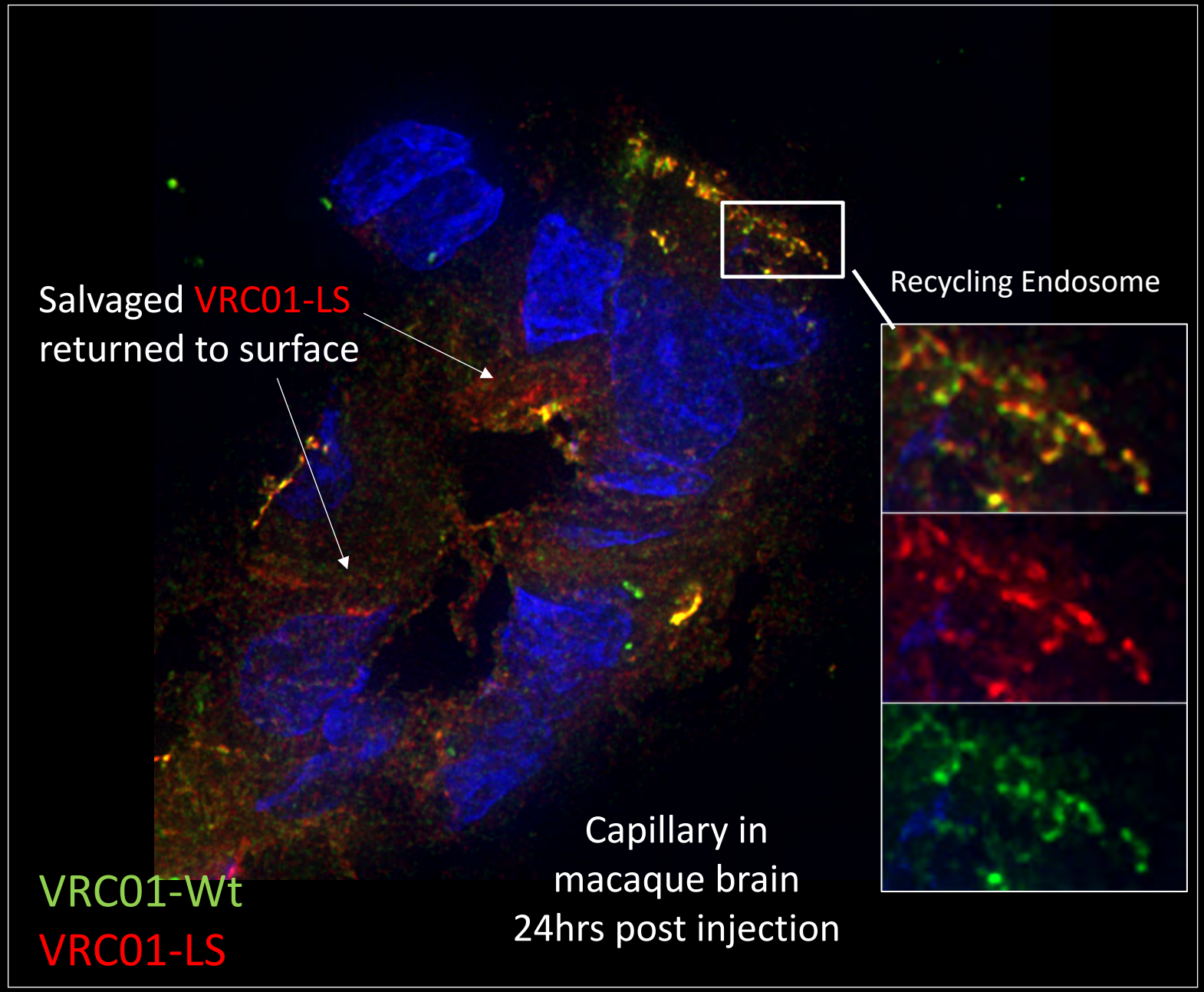
Concentration in Capillaries = Less Ab in Tissue?

Visualization of a mechanism of LS function (FcRn in action?)

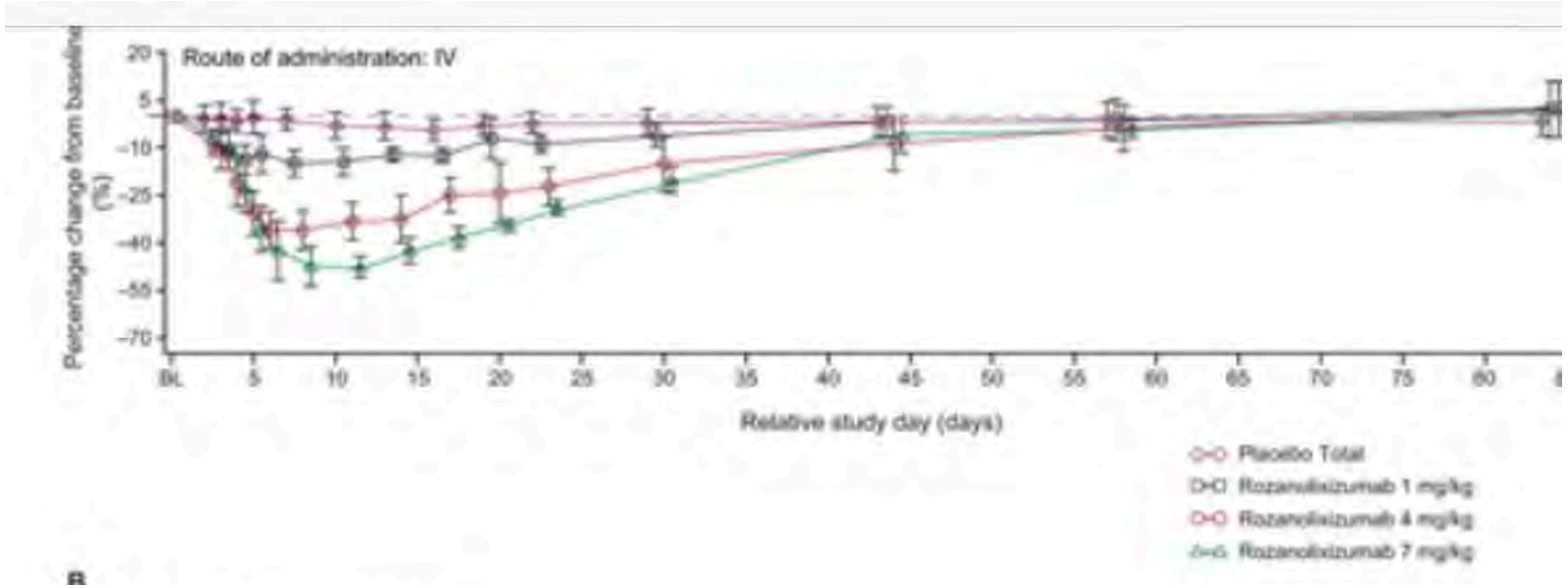
Will VRC01-LS be separated from VRC01-Wt in the recycling endosome?

1) VRC01-Wt (green) and VRC01-LS (red) endocytosed together (yellow).
2) VRC01-Wt and VRC01-LS sorted in sorting/recycling endosome. LS will preferentially bind to FcRn.
3) VRC01-LS returned to circulation or distributed to tissue. VRC01 is degraded.

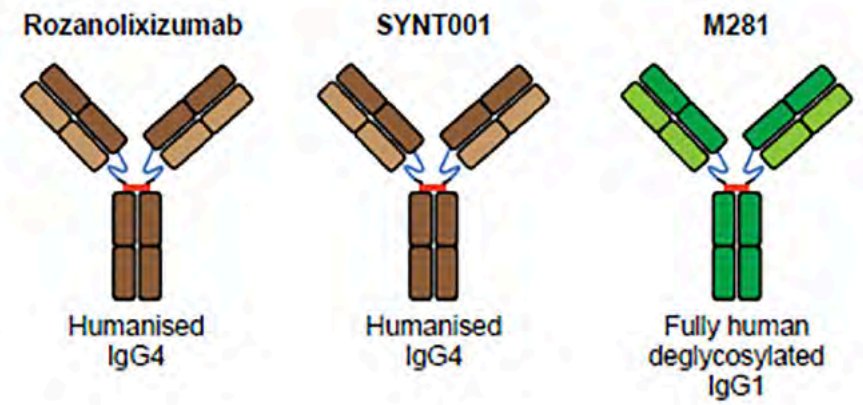




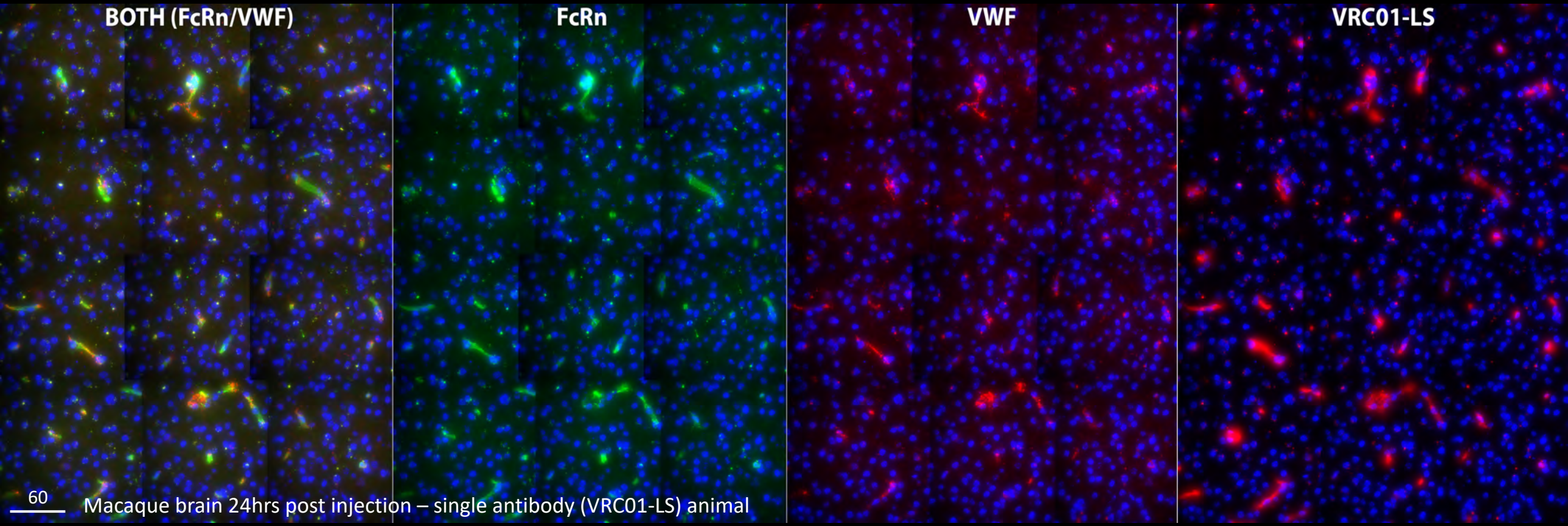
To advance this work, a “good” Antibody to FcRn is required



- Rozanolixizumab – Therapeutic Ab to IgG binding site of FcRn
- “Underground Ab World”
- Success!

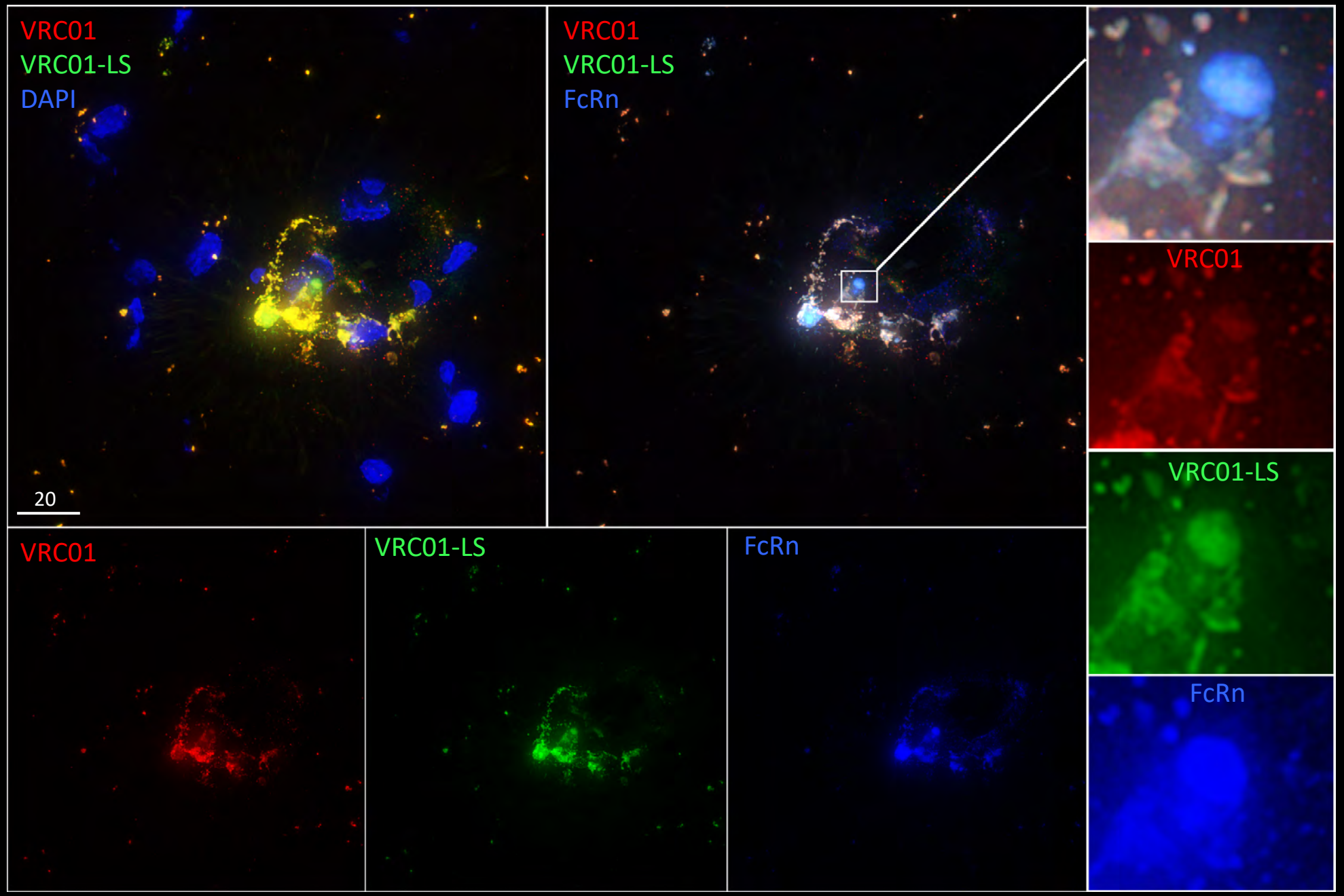


FcRn is expressed in capillaries



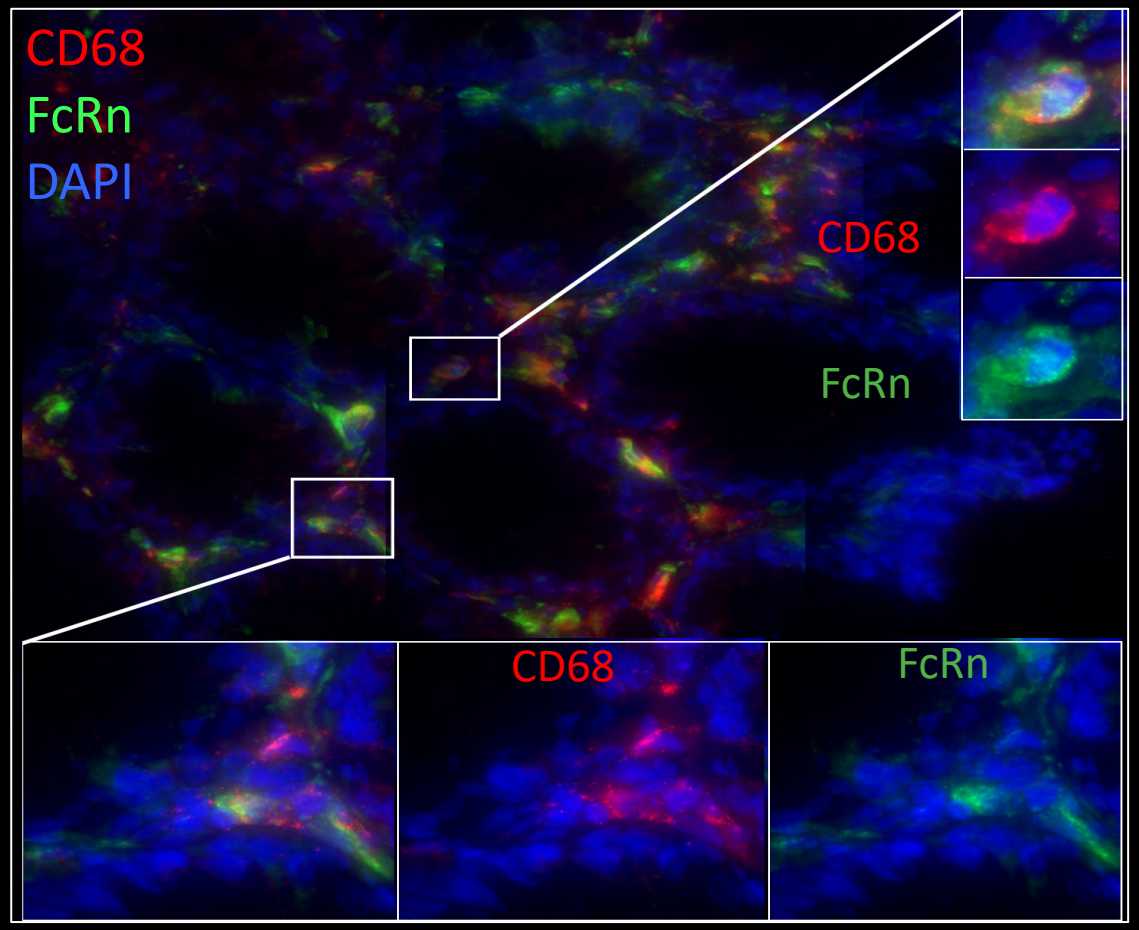
We find VRC01-LS in the same capillaries!

In capillaries, we can visualize VRC01-LS, and not VRC01-Wt, preferentially trafficking with FcRn



Capillary in Macaque brain 24hrs post injection

In rectal mucosal tissues, FcRn is found associated with macrophages!



MHC Class I-Related Neonatal Fc Receptor for IgG Is Functionally Expressed in Monocytes, Intestinal Macrophages, and Dendritic Cells[1]

Xiaoping Zhu*, Gang Meng§, Bonny L. Dickinson‡, Xiaotong Li†, Emiko Mizoguchi†, Lili Miao*, Yuansheng Wang†, Caroline Robert[2,†], Benyan Wu*, Phillip D. Smith§, Wayne I. Lencer‡, and Richard S. Blumberg[3,*]

- FcRn is functionally expressed in immune cells

Neonatal Fc receptor expression in macrophages is indispensable for IgG homeostasis

Dilip K. Challa, Xiaoli Wang, Héctor Pérez Montoyo, Ramraj Velmurugan, Raimund J. Ober & E. Sally Ward

- FcRn activity in macrophages is essential for regulating IgG homeostasis

However, when staining for FcRn, it is difficult to determine if FcRn is expressed in mucus-producing simple columnar tissues!

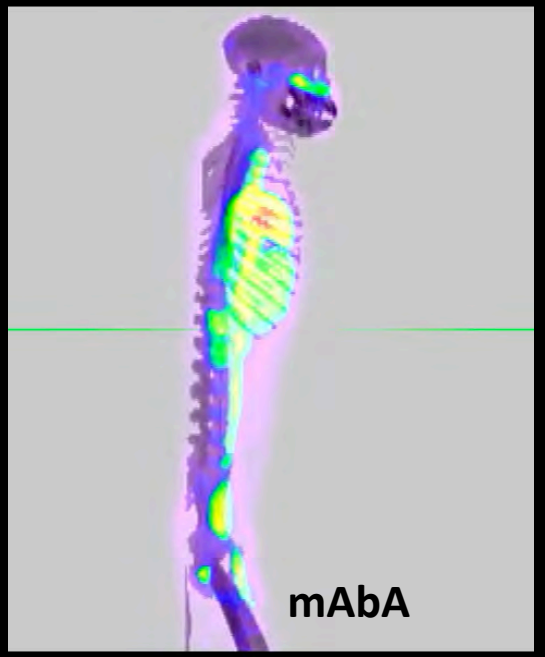
NON-SPECIFIC BINDING IS A PROBLEM!

- 2 macaques IV-injected with fluorescently-tagged anti-FcRn (8mg/kg).
- Necropsy 72hours later.

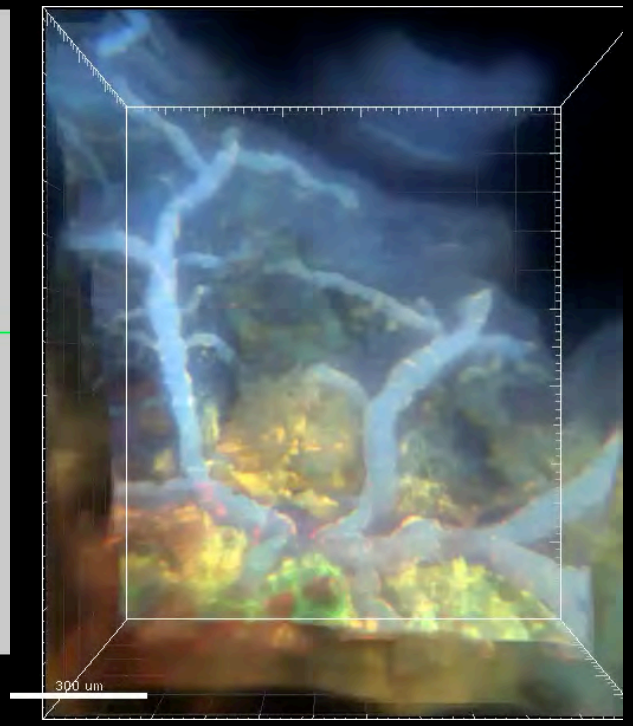
Approaches and Insights from fluorescently and radioactively labelled antibodies

Light Sheet

PET/CT



KL05 Left Upper Lung 3

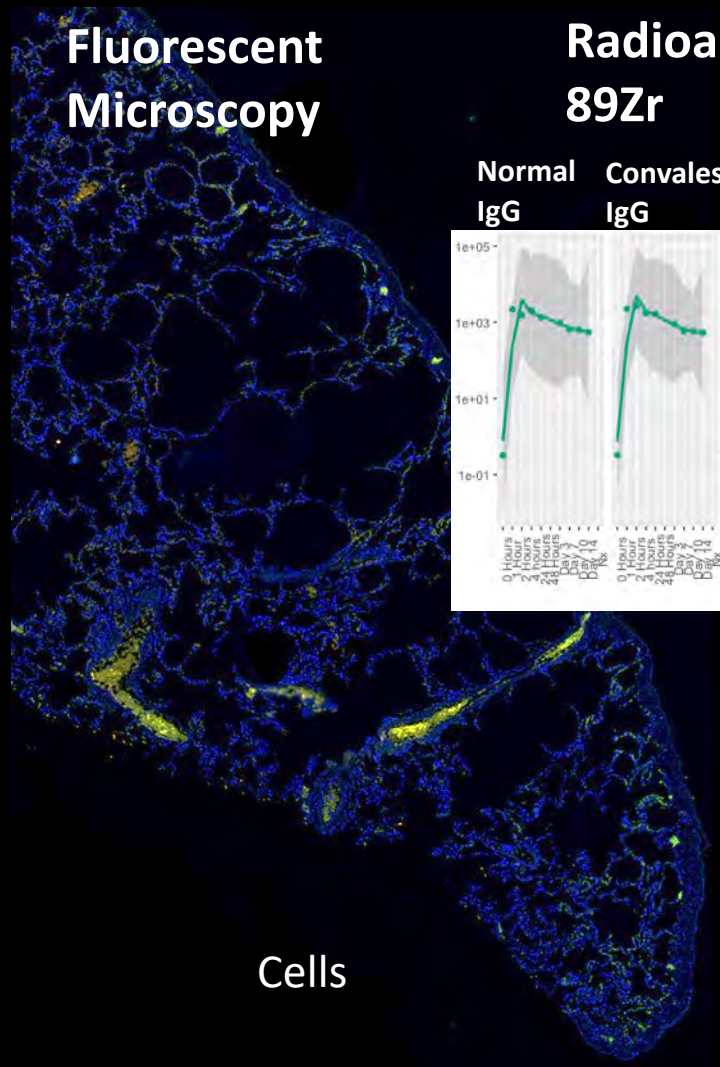


Cy3
Cy5
FITC background

Organ/Tissue Structure

Whole animal (kinetics)

Fluorescent Microscopy



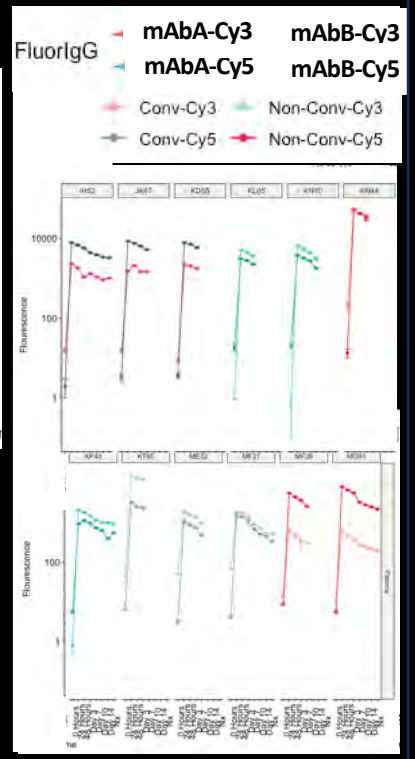
Cells

Radioactivity 89Zr

Fluids

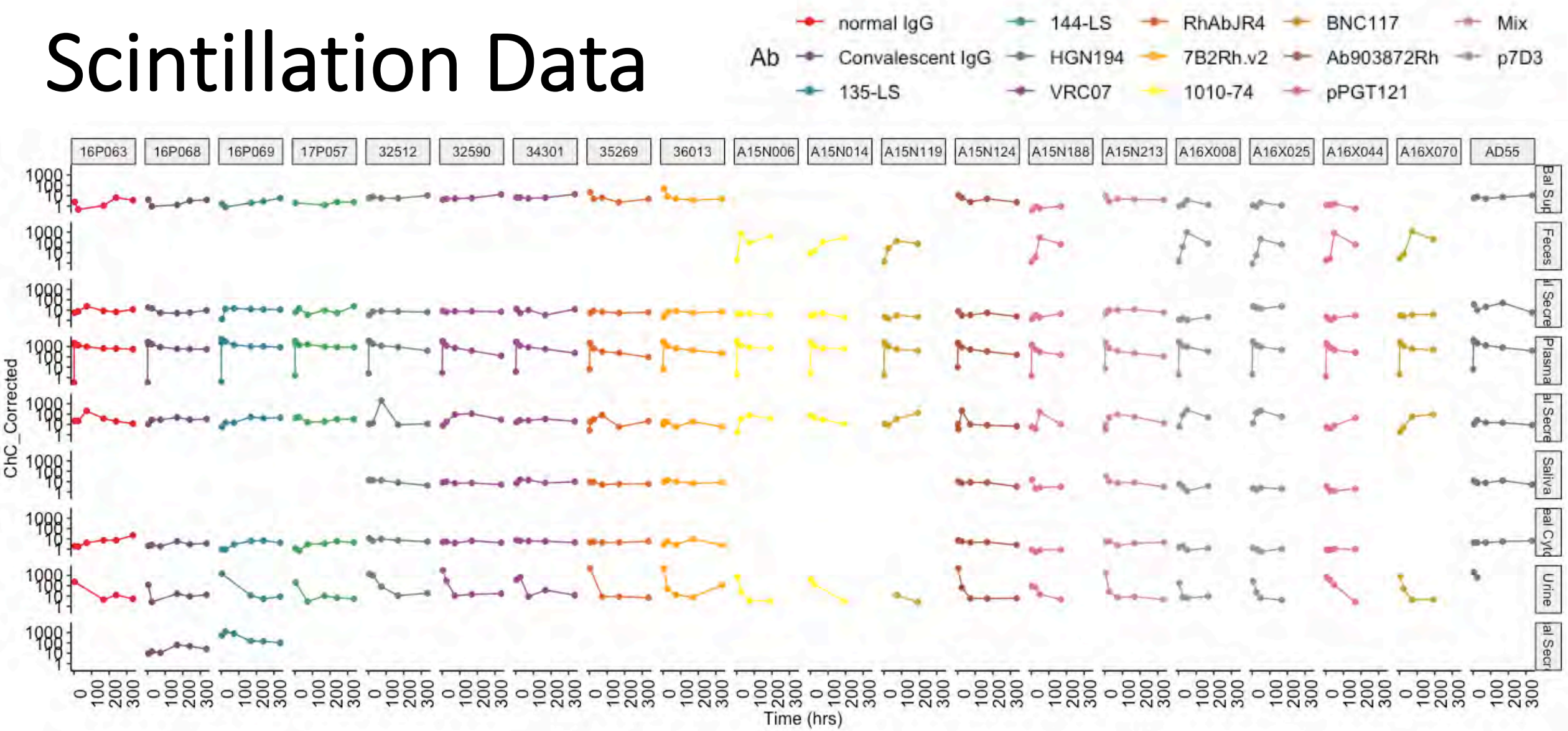
Normal IgG | Convalescent IgG | mAbA | mAbB

Fluorescense (Cy3, Cy5)

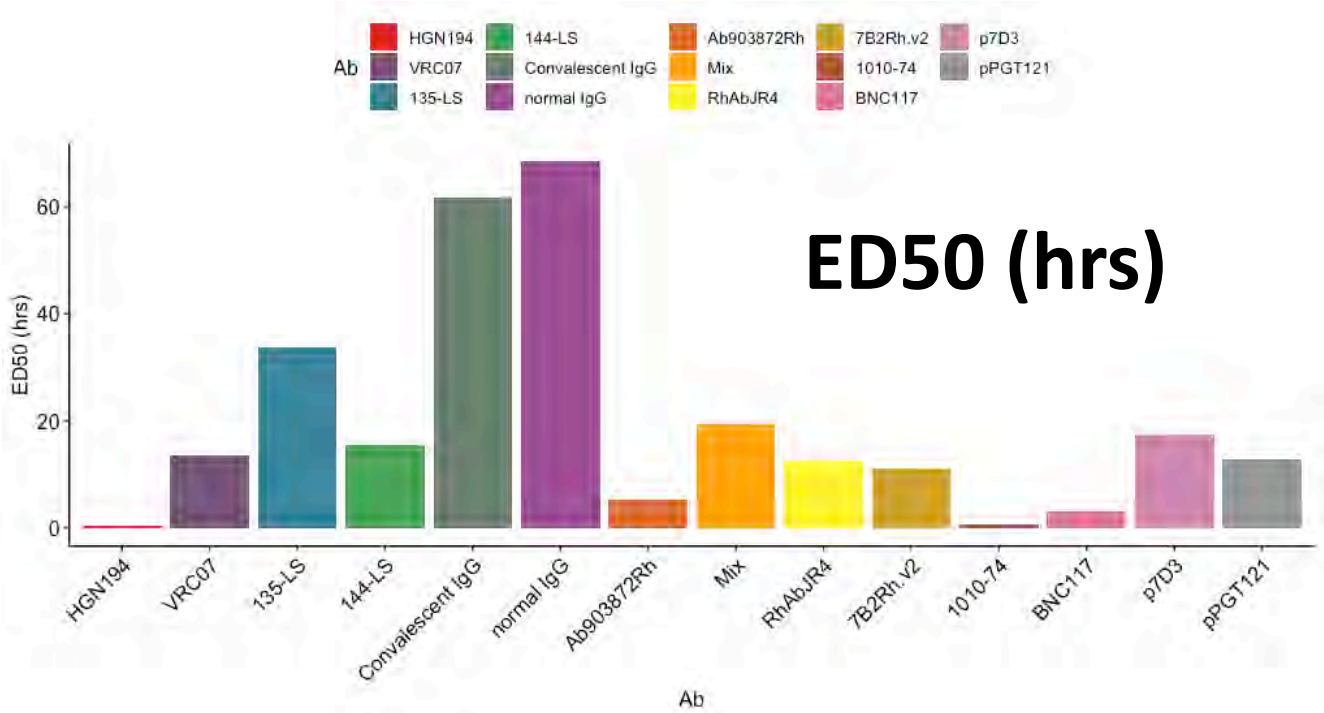


Compartments (kinetics)

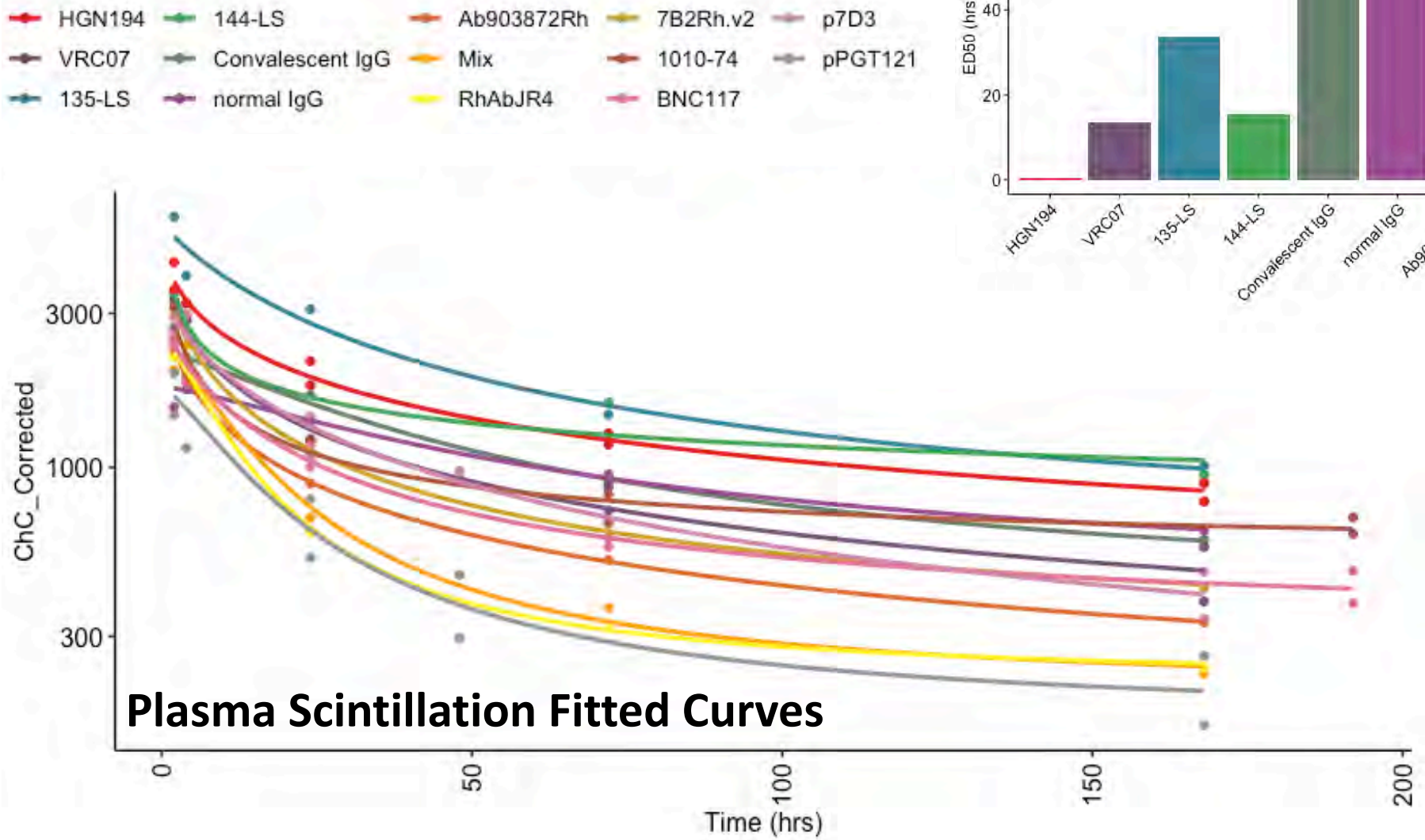
Scintillation Data



PK evaluation



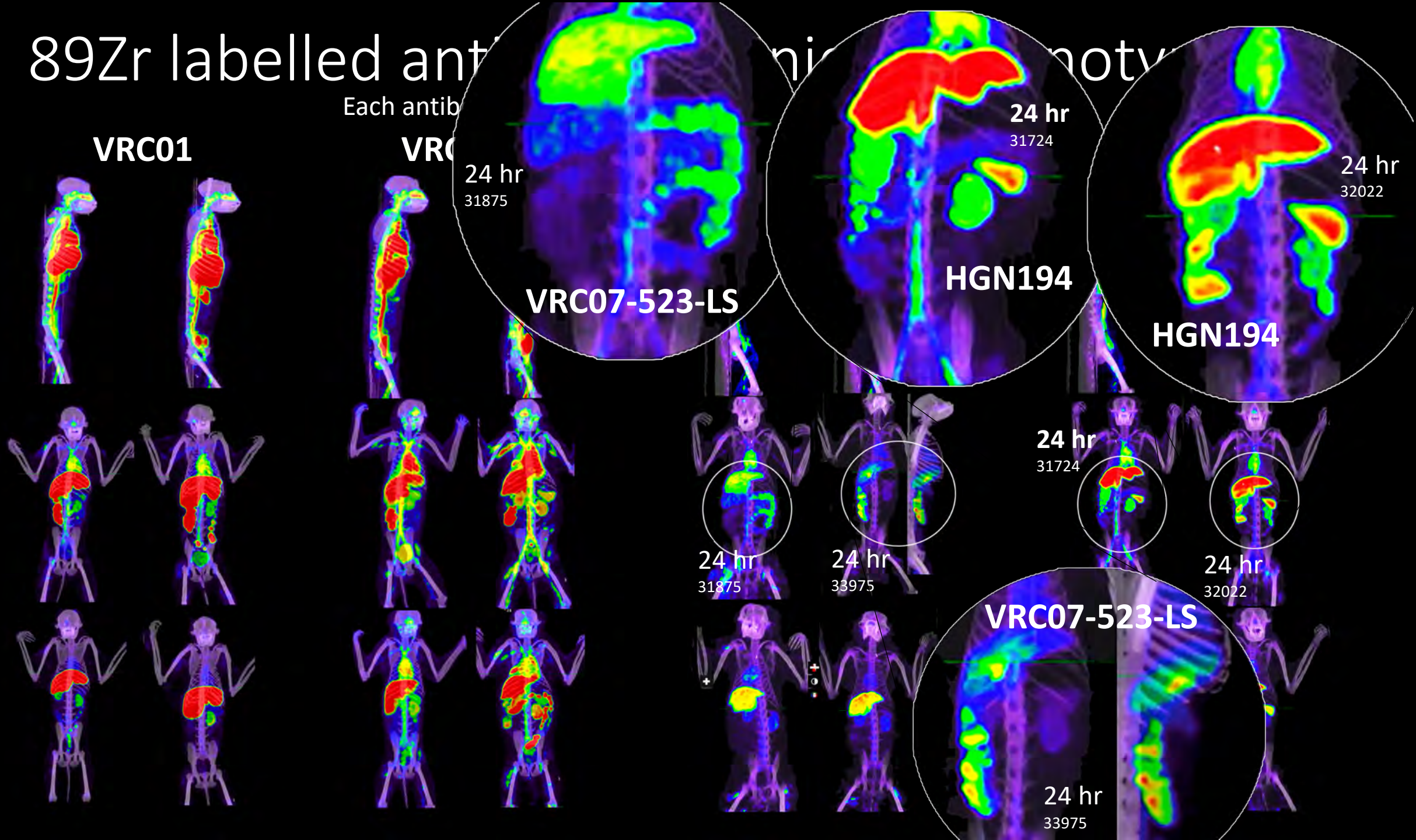
ED50 (hrs)



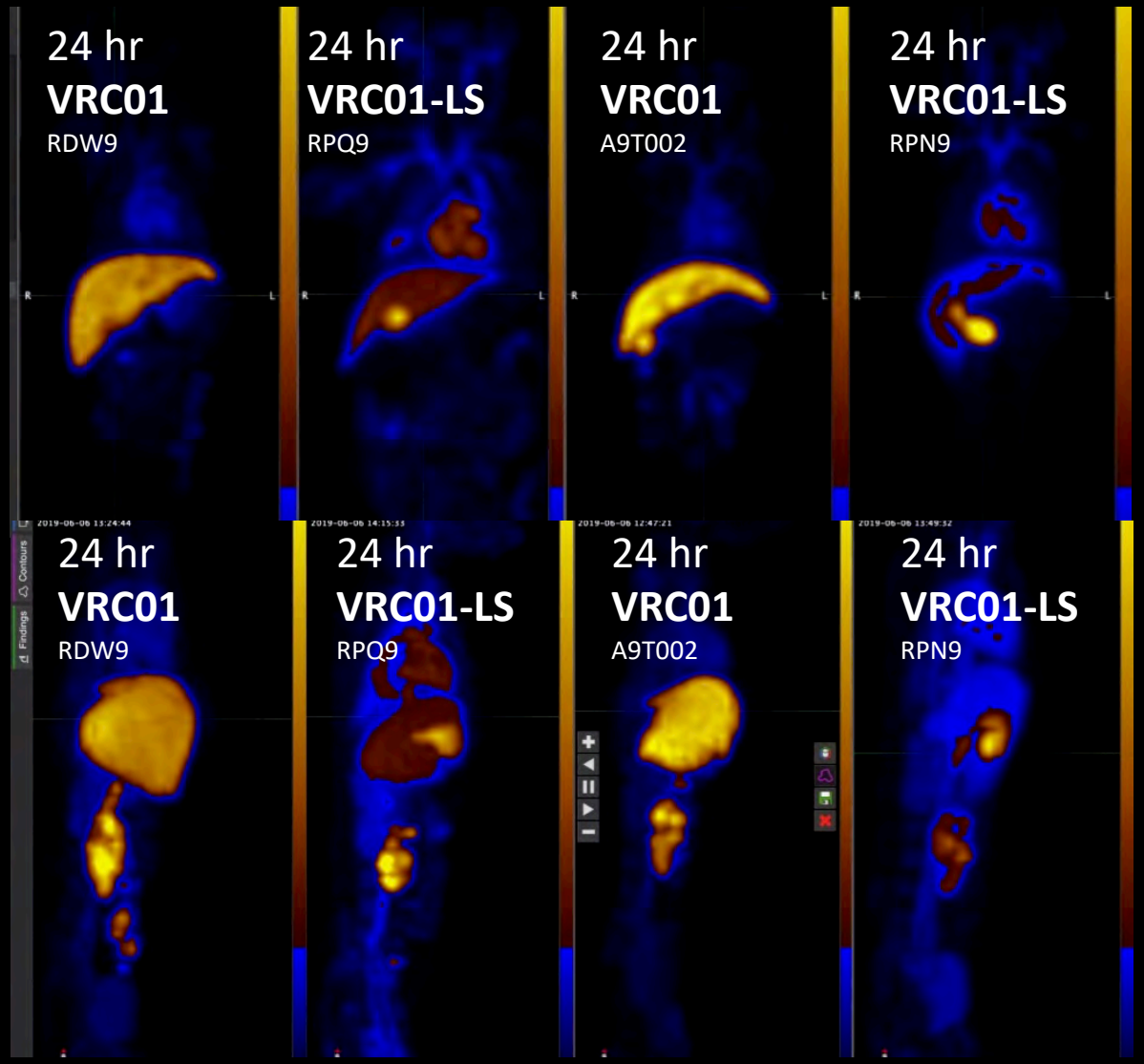
Plasma Scintillation Fitted Curves

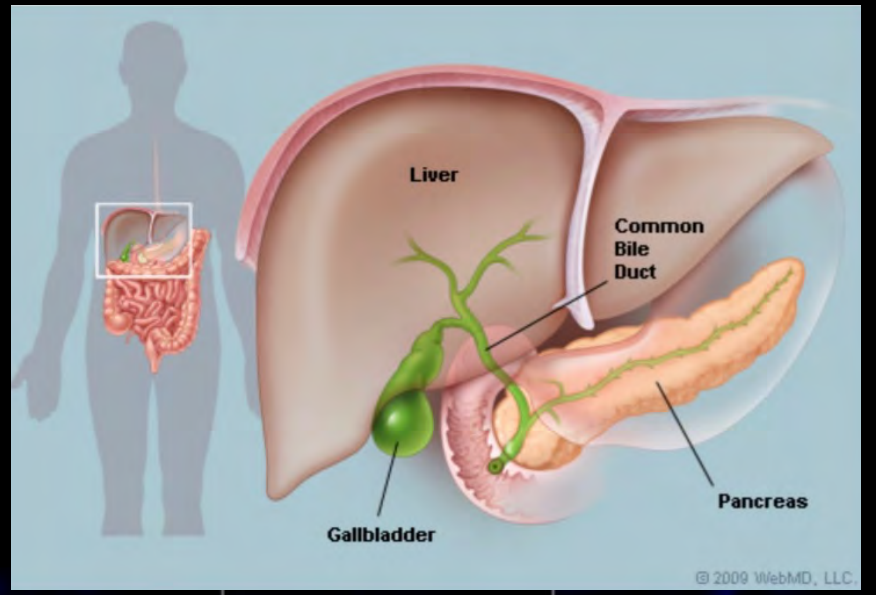
89Zr labelled ant ni notv

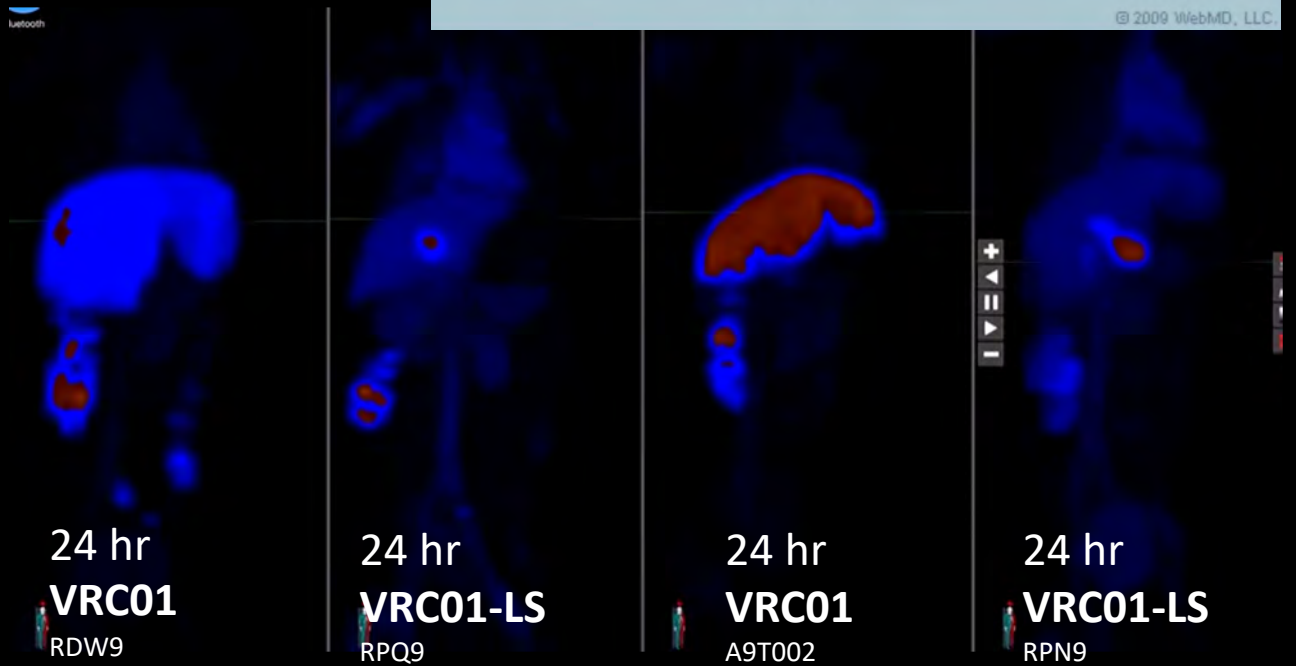
Each antib



IV injected IgG can enter the biliary system





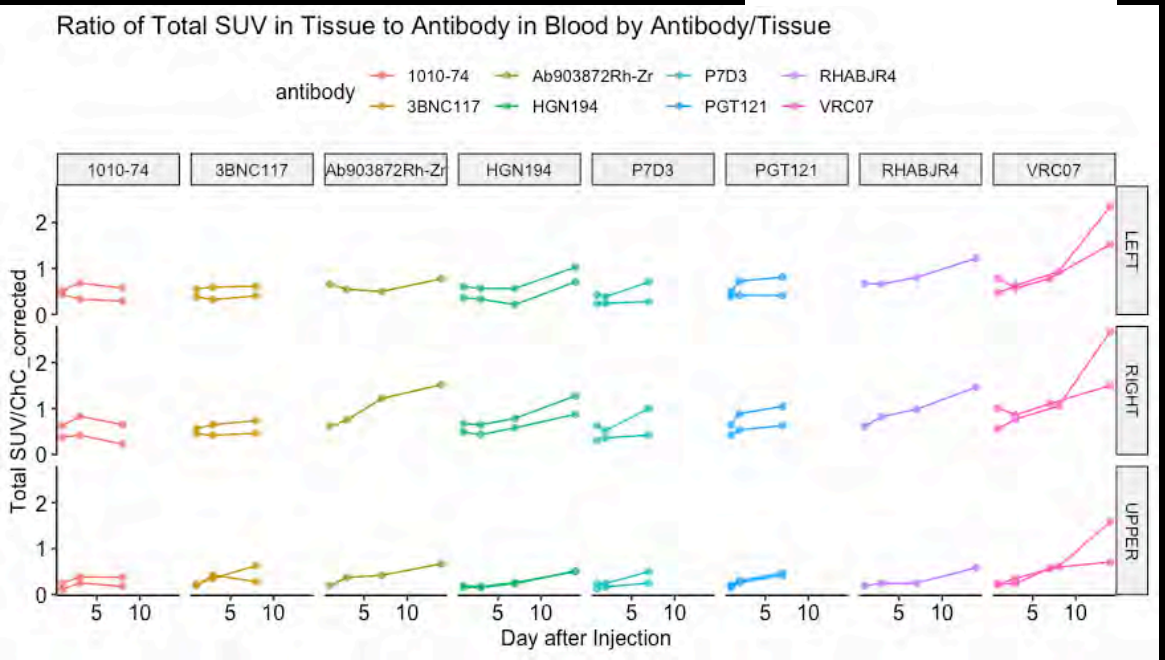


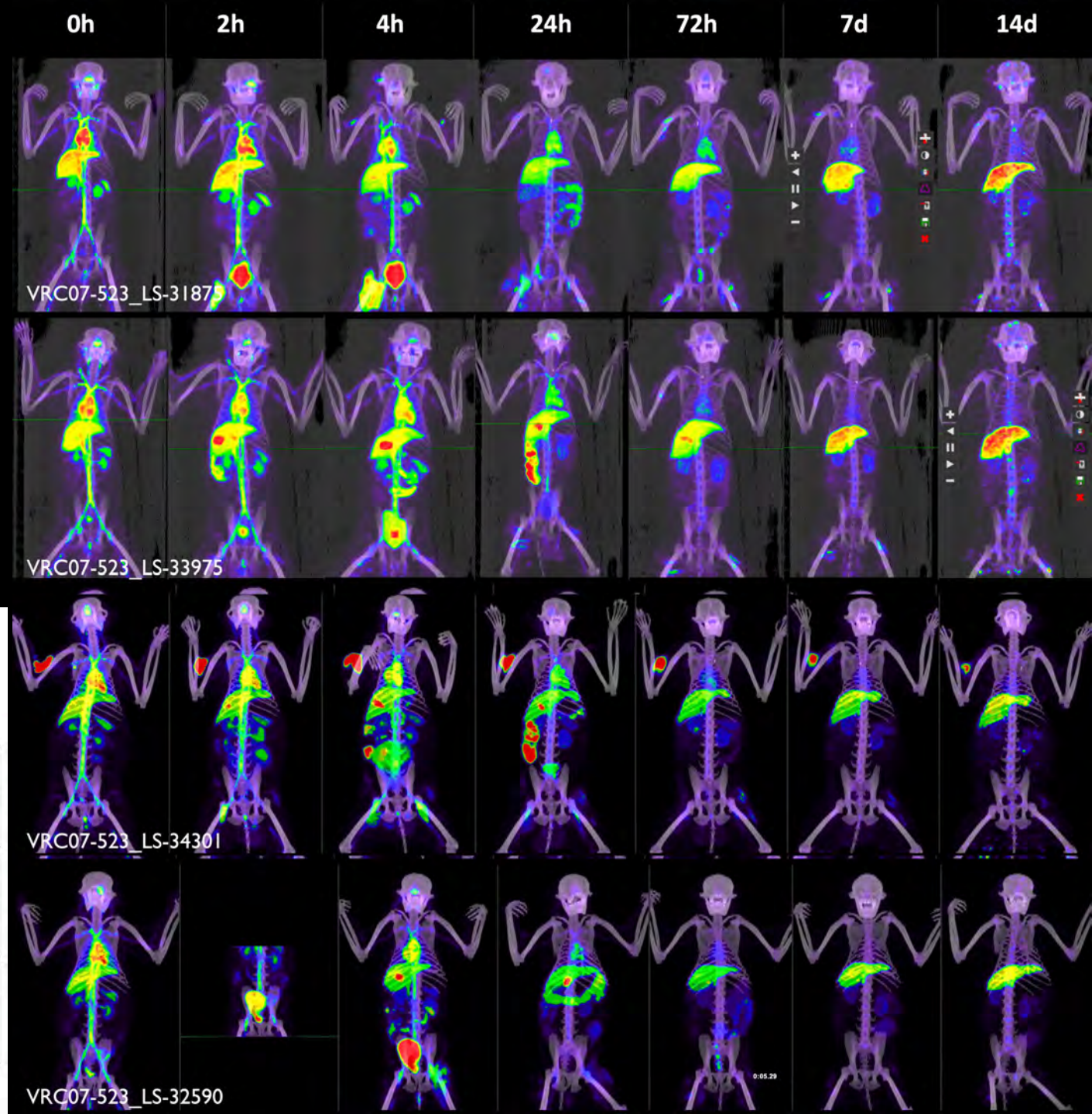
- Blood > Liver > GB > GIT > Circulation?

The Mysterious VRC07-523-LS

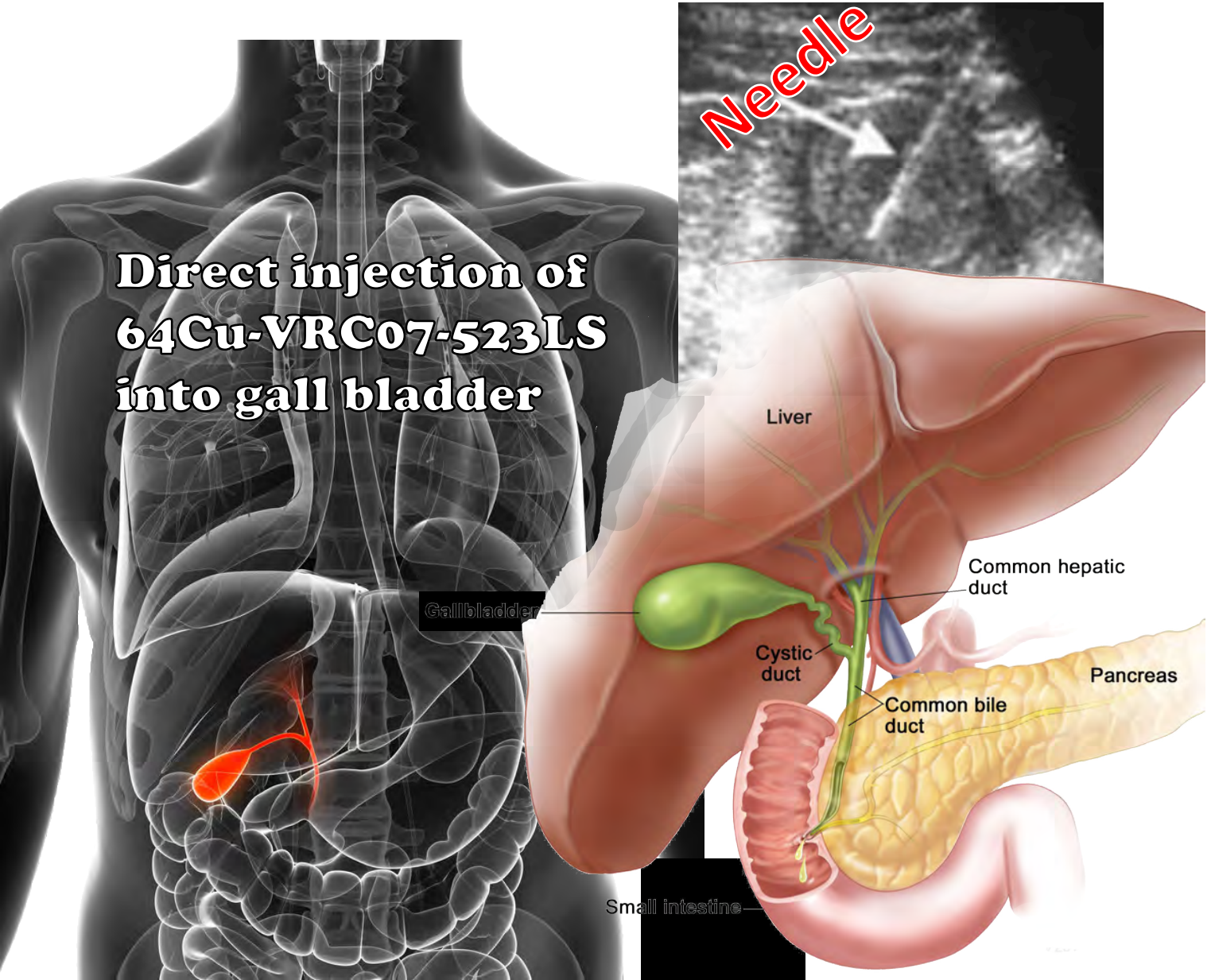
- Disappointing plasma PK for LS
- But shows *in vivo* potency
- Compared to other Abs, VRC07 persists in tissues longer

$$\frac{Total\ SUV_{upper\ or\ lung}}{Antibody\ amount_{in\ plasma}}$$



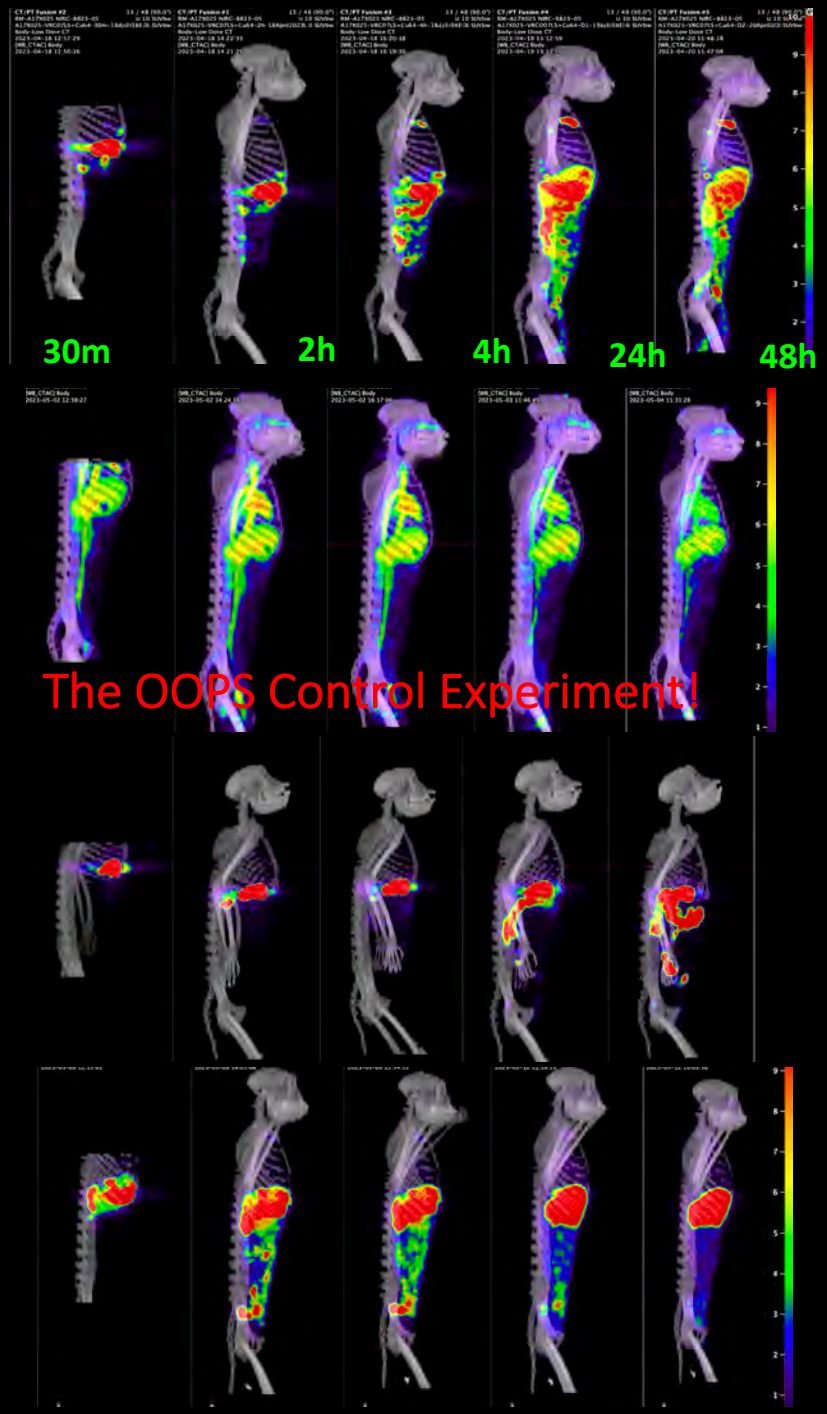


Hypothesis: Antibody dumped into duodenum with bile will be recovered from the gut as observed in neonates.

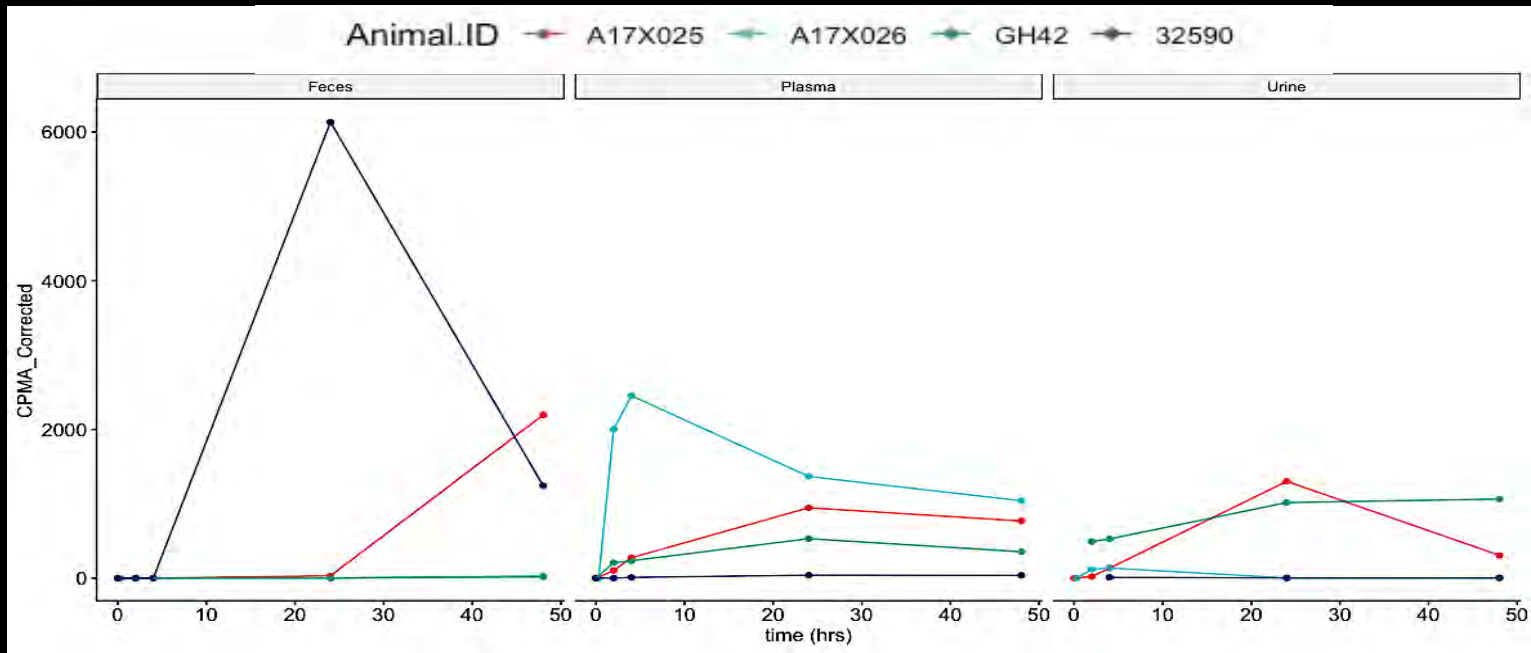


- Bile is produced in the liver and stored in the gall bladder.
- Bile plays a key role in digestion of fats.
- The introduction of bile into the duodenum is stimulated by food, especially high fat foods.
- dIgA in the blood is routed by the liver into bile where it is introduced into the duodenum and gut.

After bile injection, Cu^{64}-VRC07-523LS is dumped into the GIT

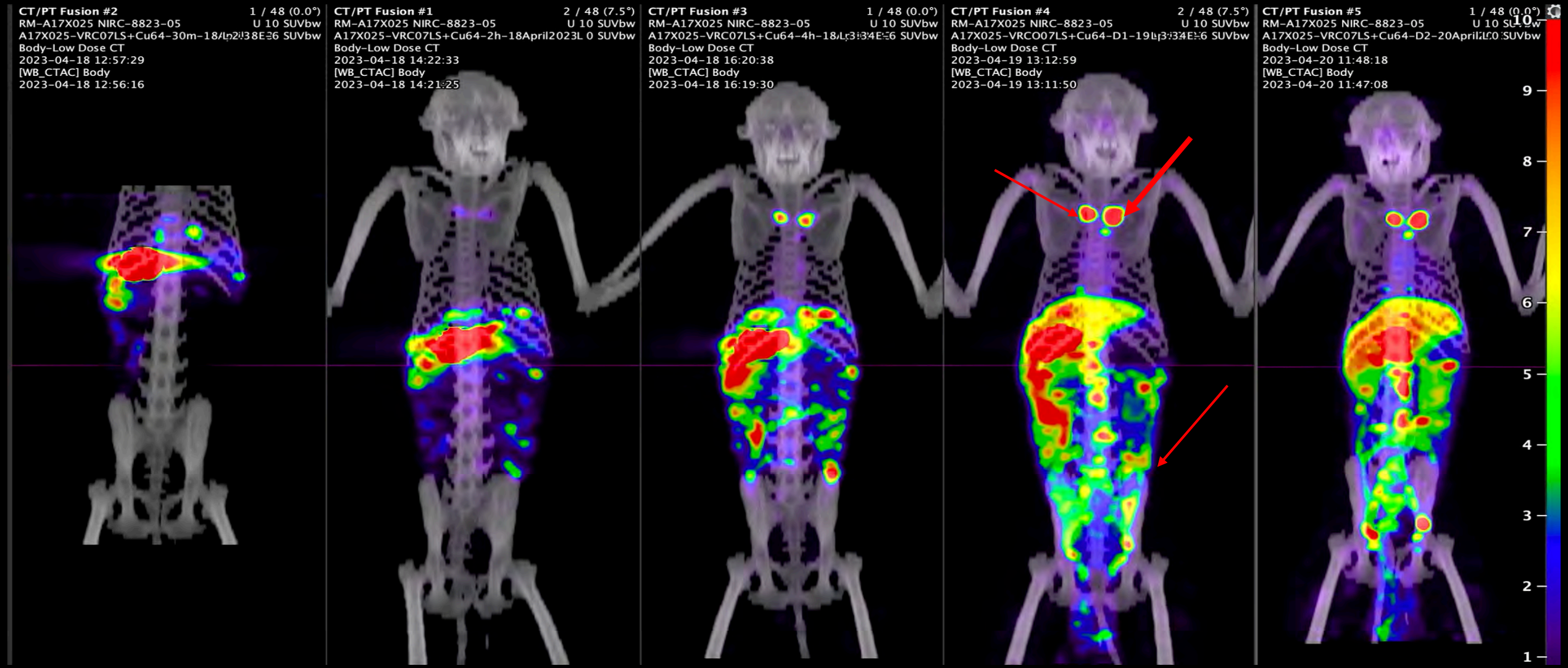


- Antibody is found in the plasma at early time points (2-4 hours).
- Only small amount in feces.
- Goes back into circulation at different rates.
- Suggests potential Ab recycling mechanism!



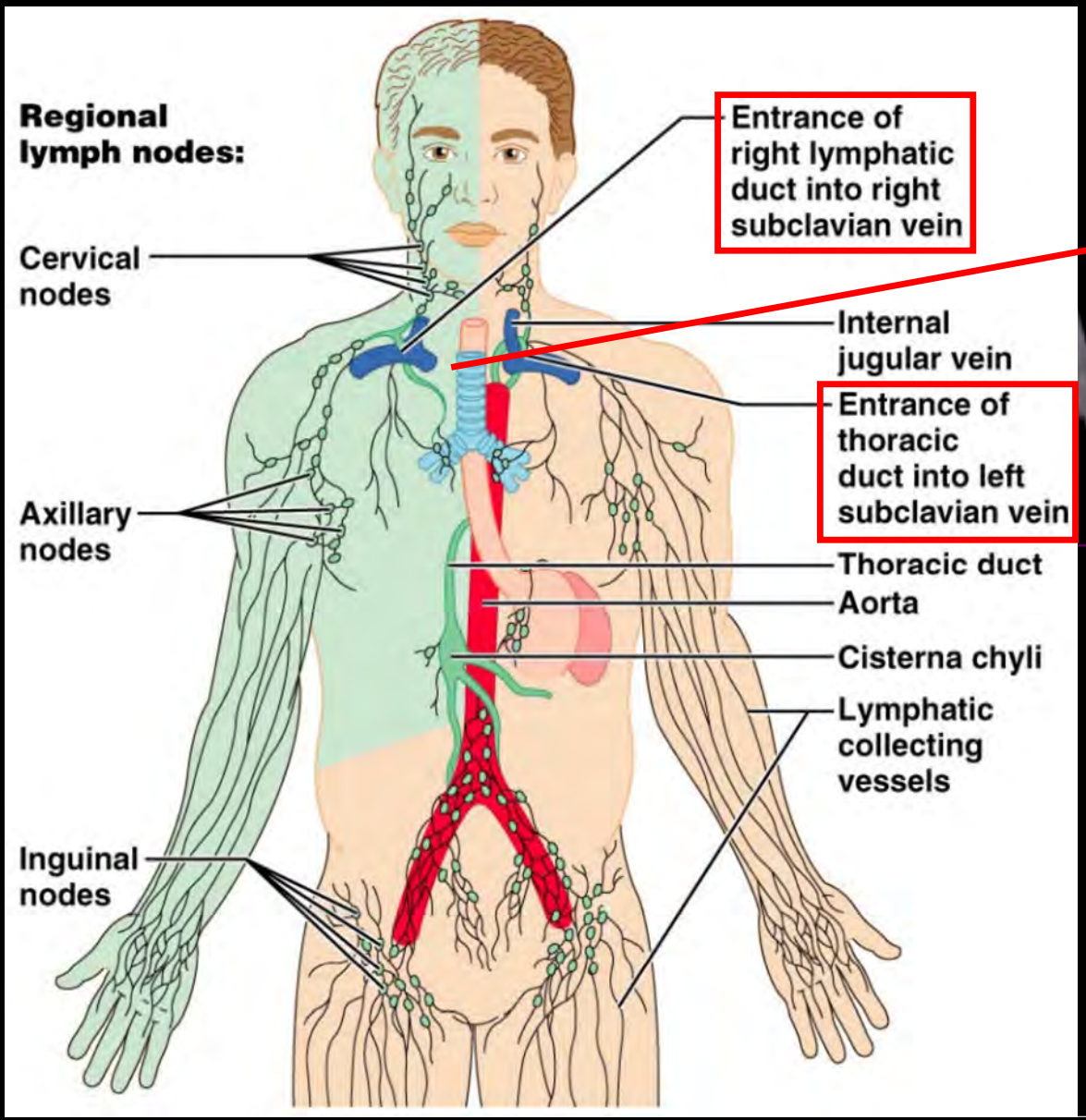
VRC07 is recycled back to the system to site-specific areas

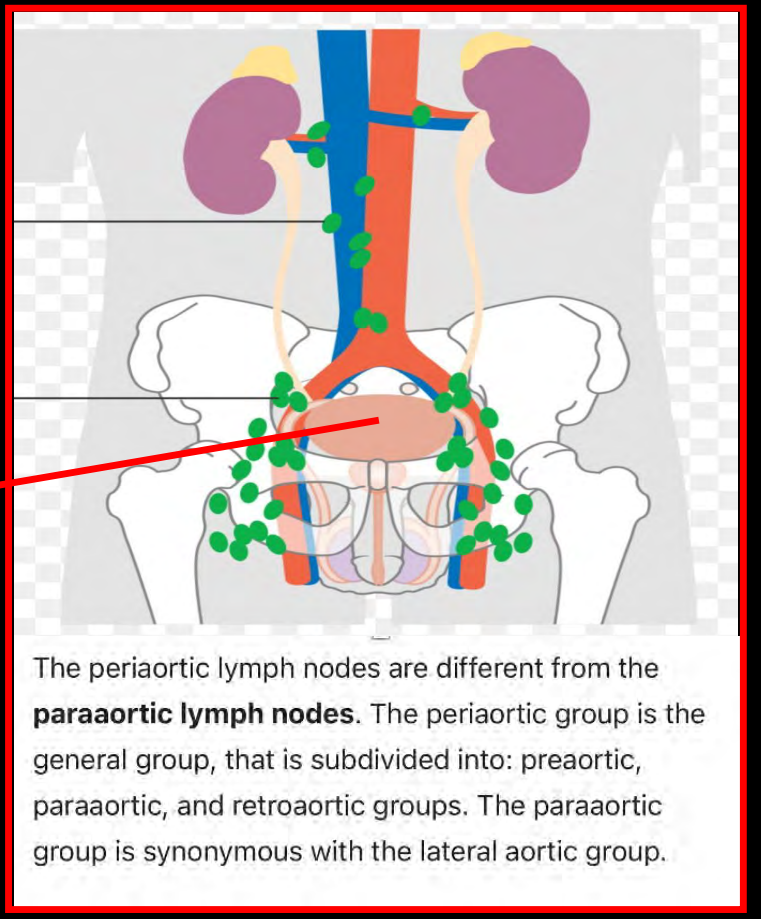
Bile Injection VRC07-523LS (0-10 SUV)



30 min **2 hr** **4 hr** **24 hr** **48 hr**

VRC07-523LS leaving gut and entering Lymphatics

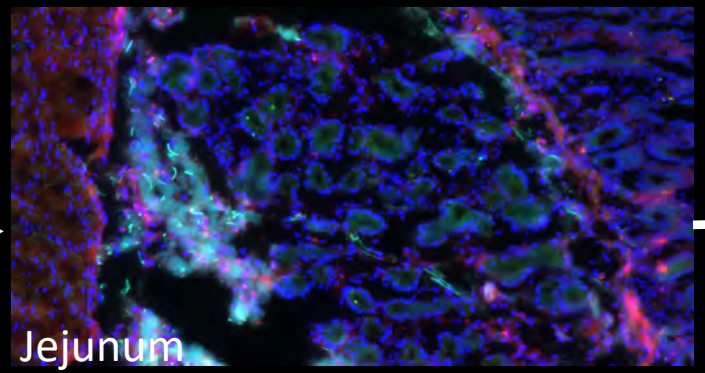


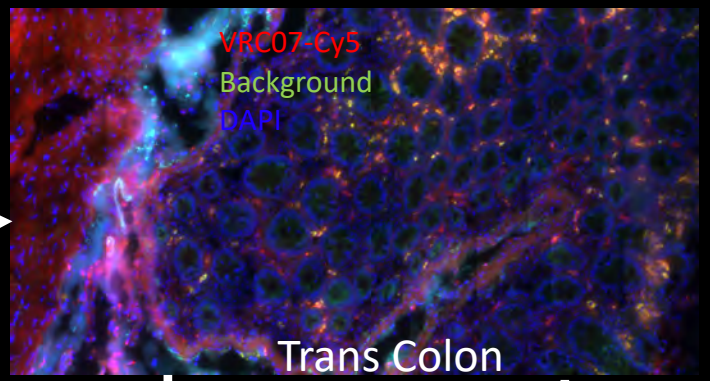


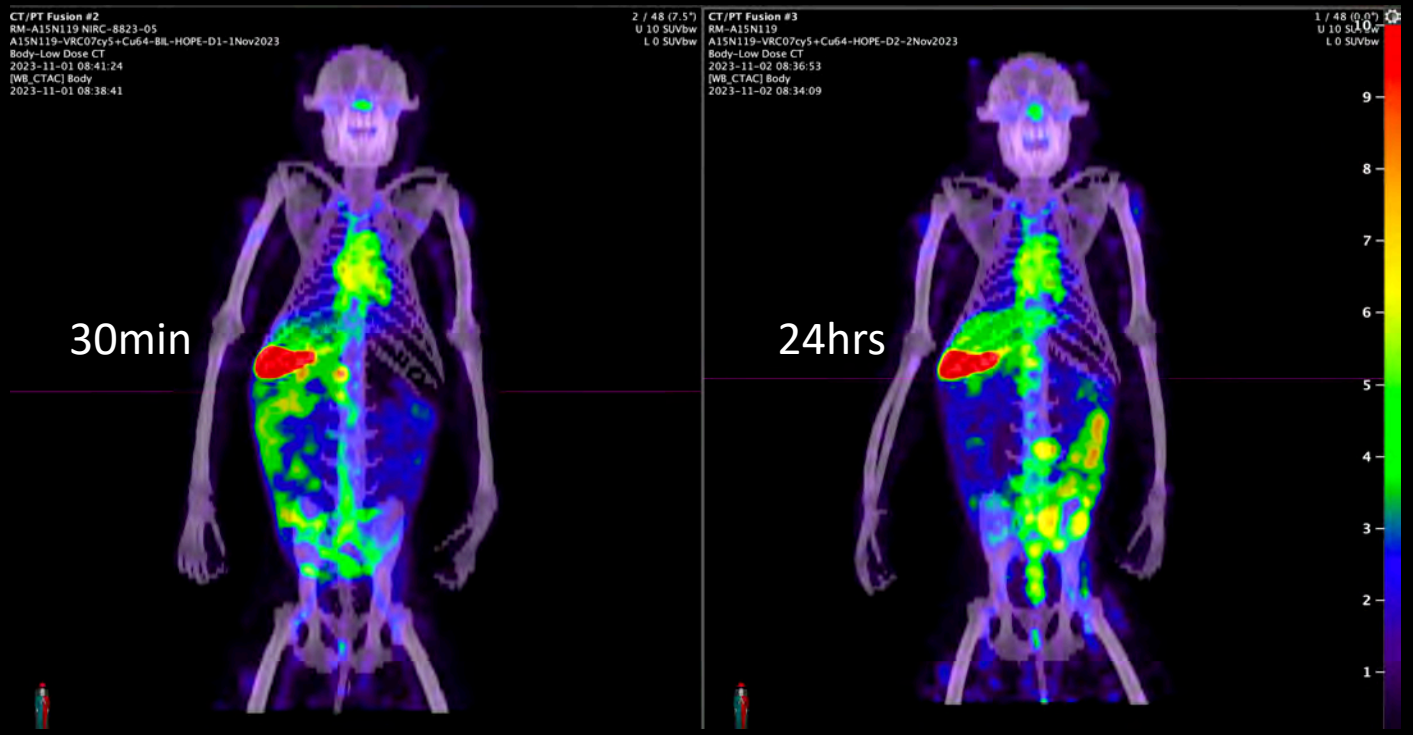
The periaortic lymph nodes are different from the **paraaortic lymph nodes**. The periaortic group is the general group, that is subdivided into: preaortic, paraaortic, and retroaortic groups. The paraaortic group is synonymous with the lateral aortic group.

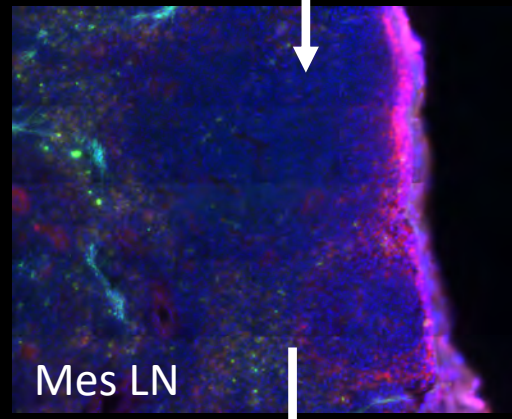
GB > GIT > Lymphatics > Blood

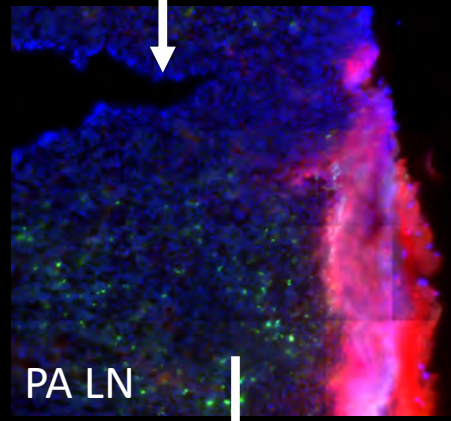
By combining VRC07-Cy5 + VRC07-Cu64 and injecting into the gallbladder, we can see where the antibody is distributed

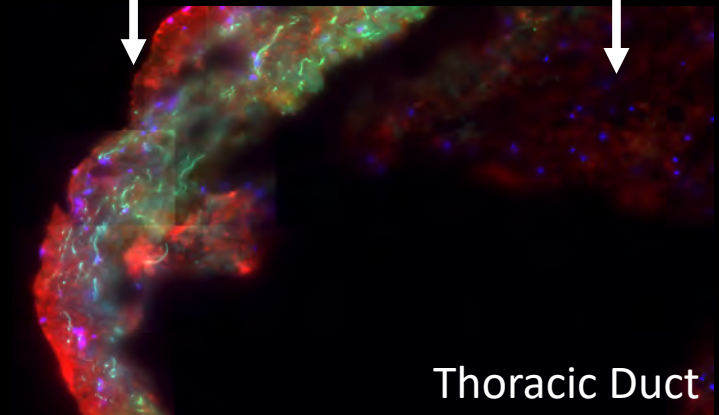






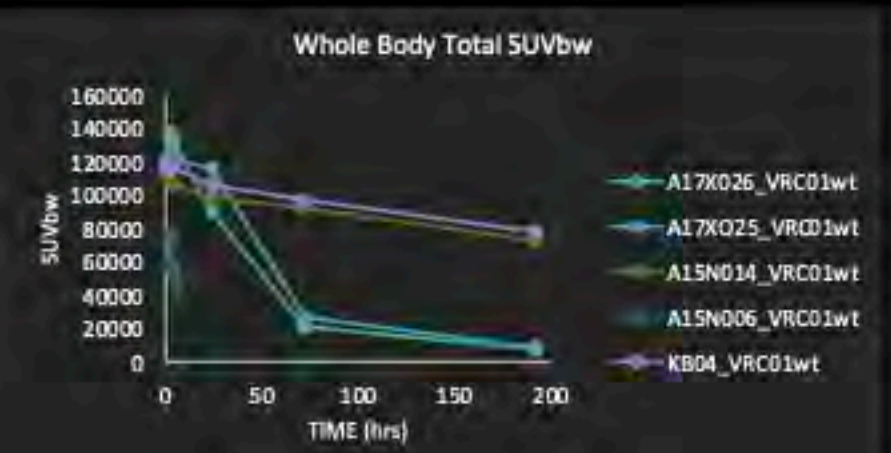


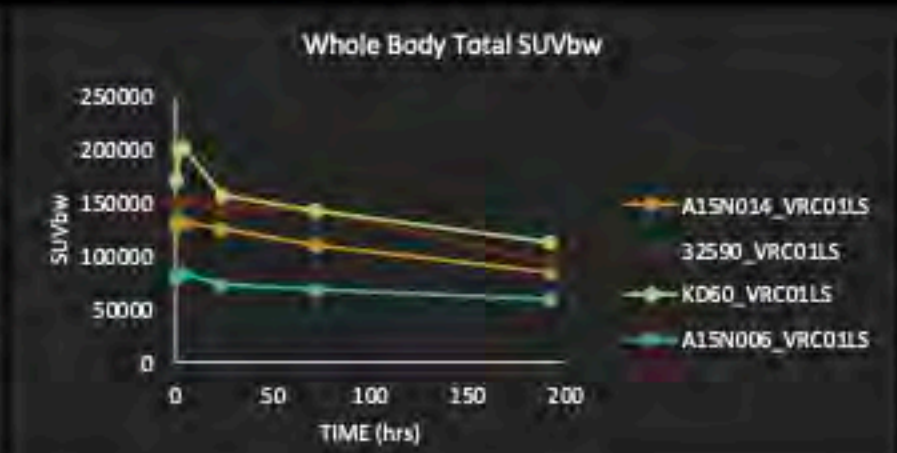


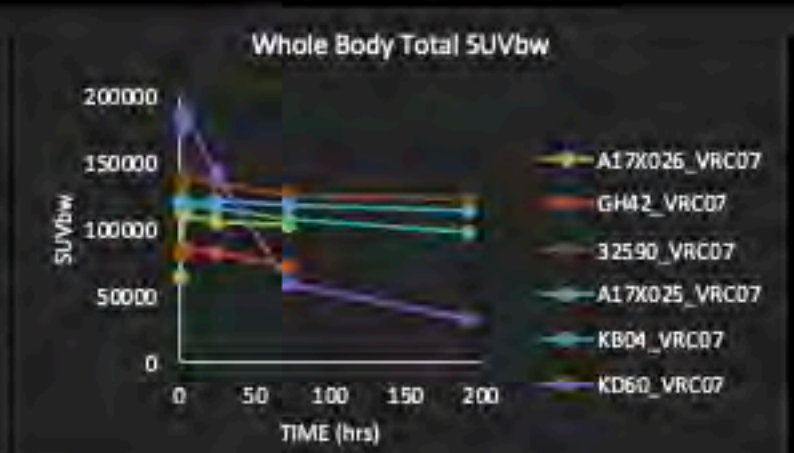


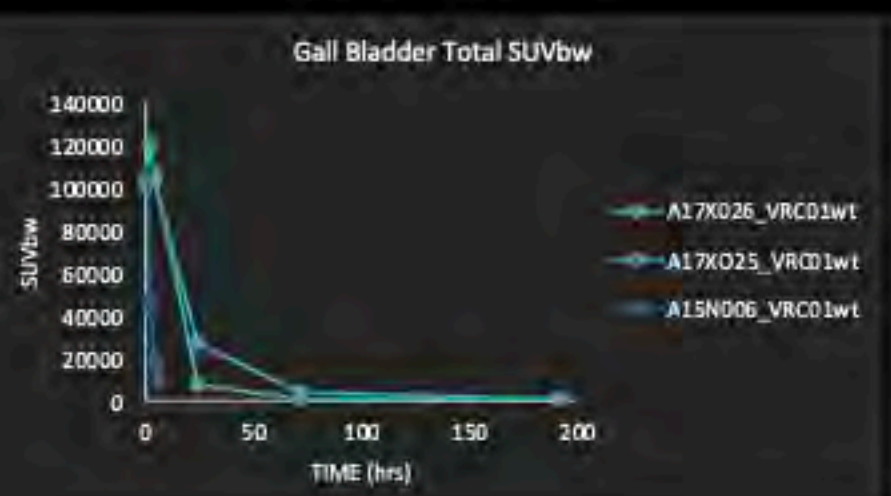
- GB > GIT > Lymphatics > Blood

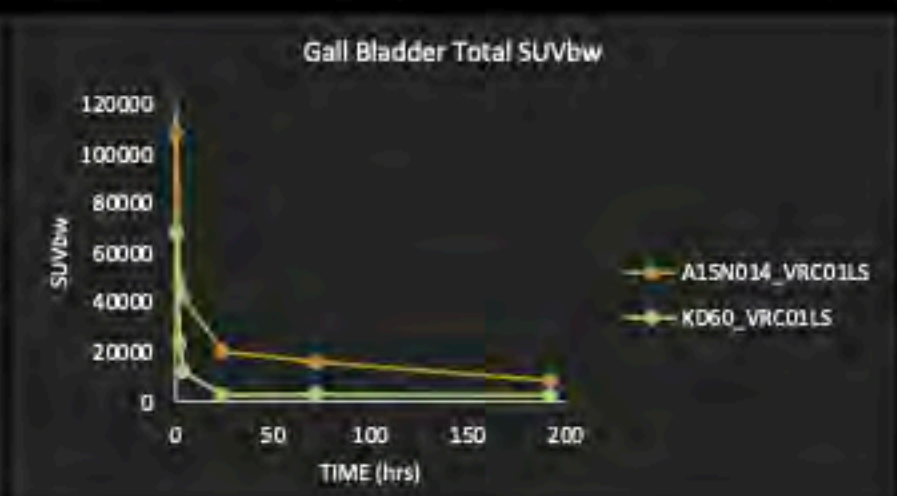
Antibodies remain in circulation!

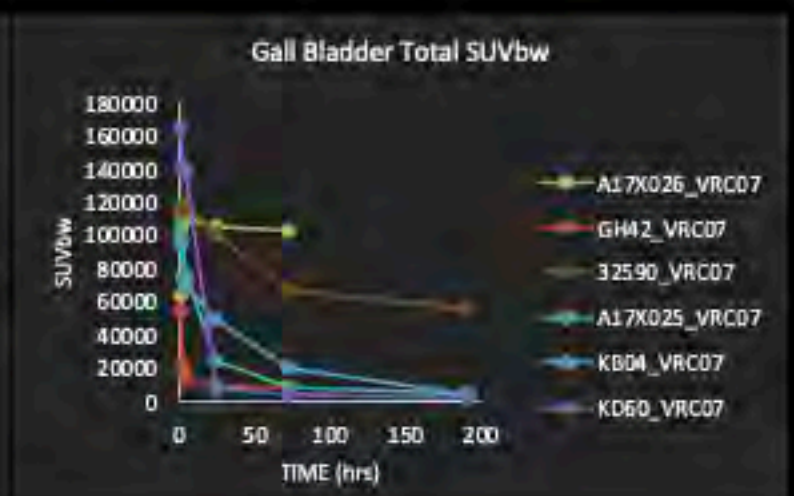












Therapeutic Antibody Biodistribution

- We have developed a multiscale imaging and evaluation toolbox to study antibody biodistribution.
- Antibody biodistribution is complex and highly regulated.
- Antibody biodistribution is influenced by isotype, glycoform, variable region glycosolation, PI, ? (DNS).
- Antibodies can be recovered from the gut in neonates **and adult mammals**.
- FcRn likely plays a role in antibody circulation from blood – bile – gut – lymphatics – blood.
- Antibody circulation through gut may be a “car wash” to disrupt immune and effector protein complexes.
- Antibody engineering should allow specific targeting and optimal physiology for clinical antibody functions.

ACKNOWLEDGEMENTS

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Pat Madden
Jeff Schneider
Gianguido Cianci
Meegan Anderson
Mike McRaven
Elena Martinelli
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Christopher Thuruthiyil
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