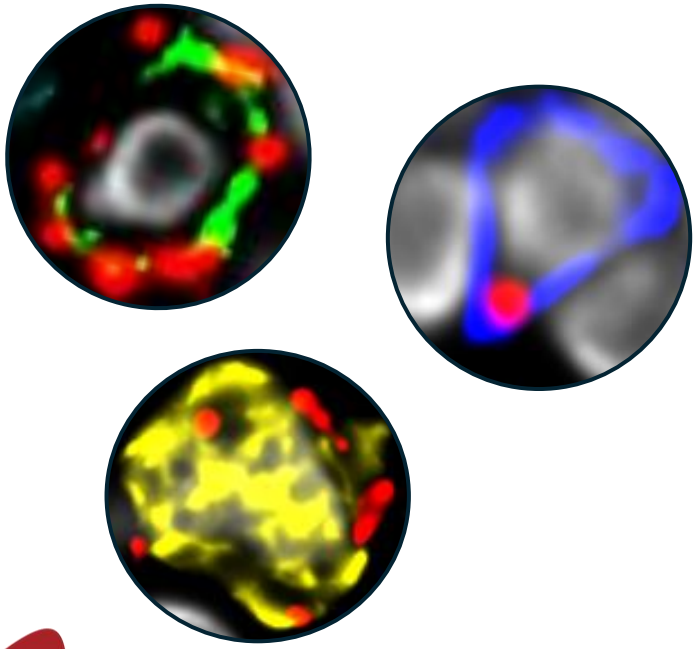
Early events in HIV transmission across human anogenital mucosa

An in situ *quantitative map of immune cell interactions with HIV*



Thomas O'Neil

Westmead Institute for Medical Research

Human Immunodeficiency Virus

A better understanding of the **early HIV transmission events** is vital for development of **a vaccine** and more **targeted therapies**

No vaccine or cure

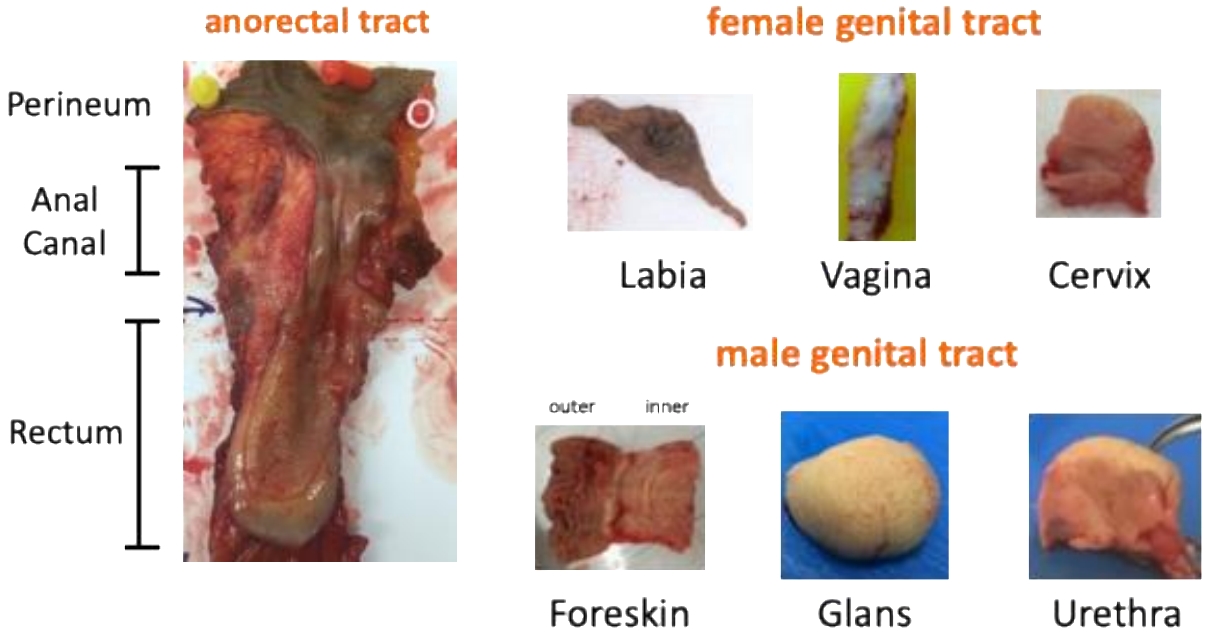
The Westmead Institute FOR MEDICAL RESEARCH

What are the early determinants in HIV infection?

Aim to explore dynamics between HIV and immune cells
***in tissue** during transmission*

Collaborations with Clinicians

Access to every tissue exposed to HIV



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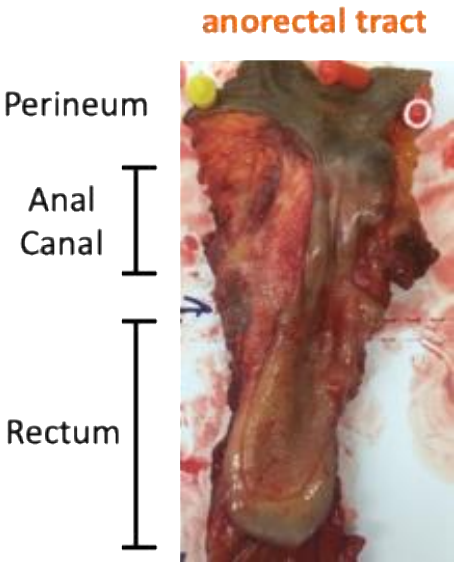

Human *in situ* transmission model

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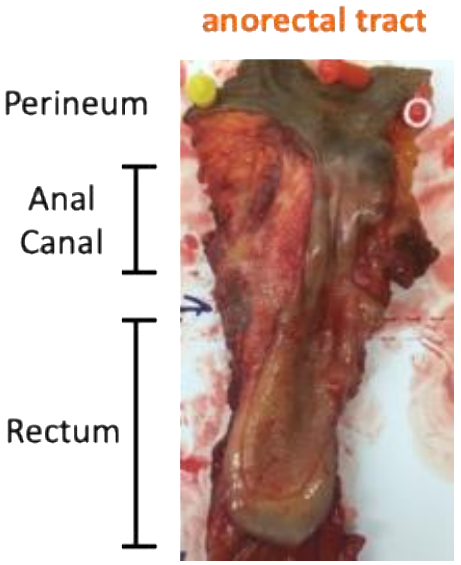
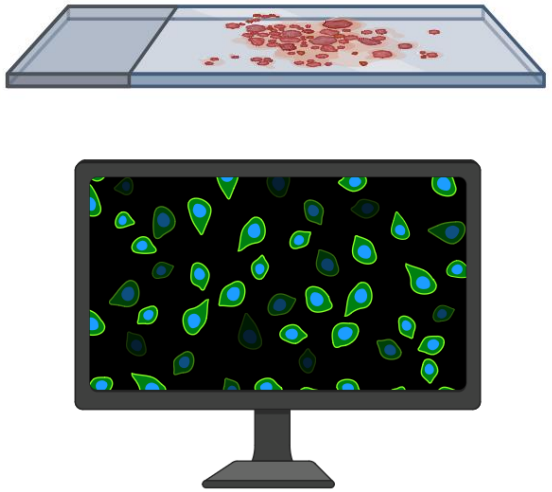
What are the early determinants in HIV infection?

Aim to explore dynamics between HIV and immune cells
***in tissue** during transmission*

Collaborations with Clinicians





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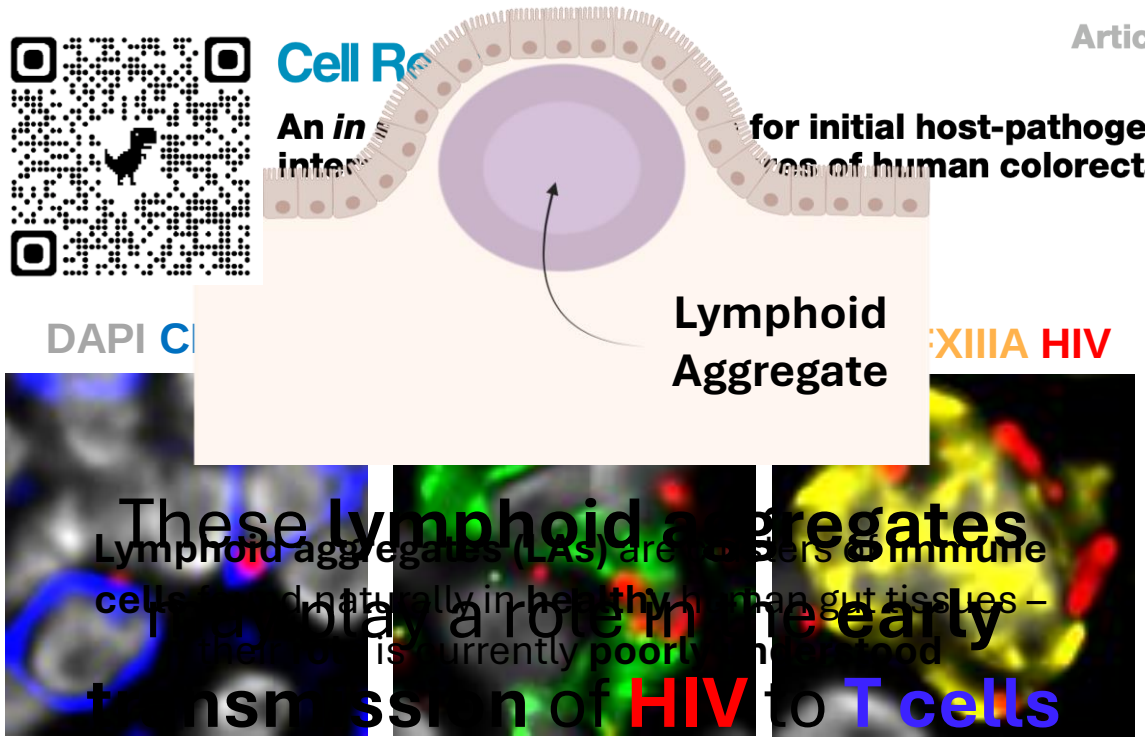
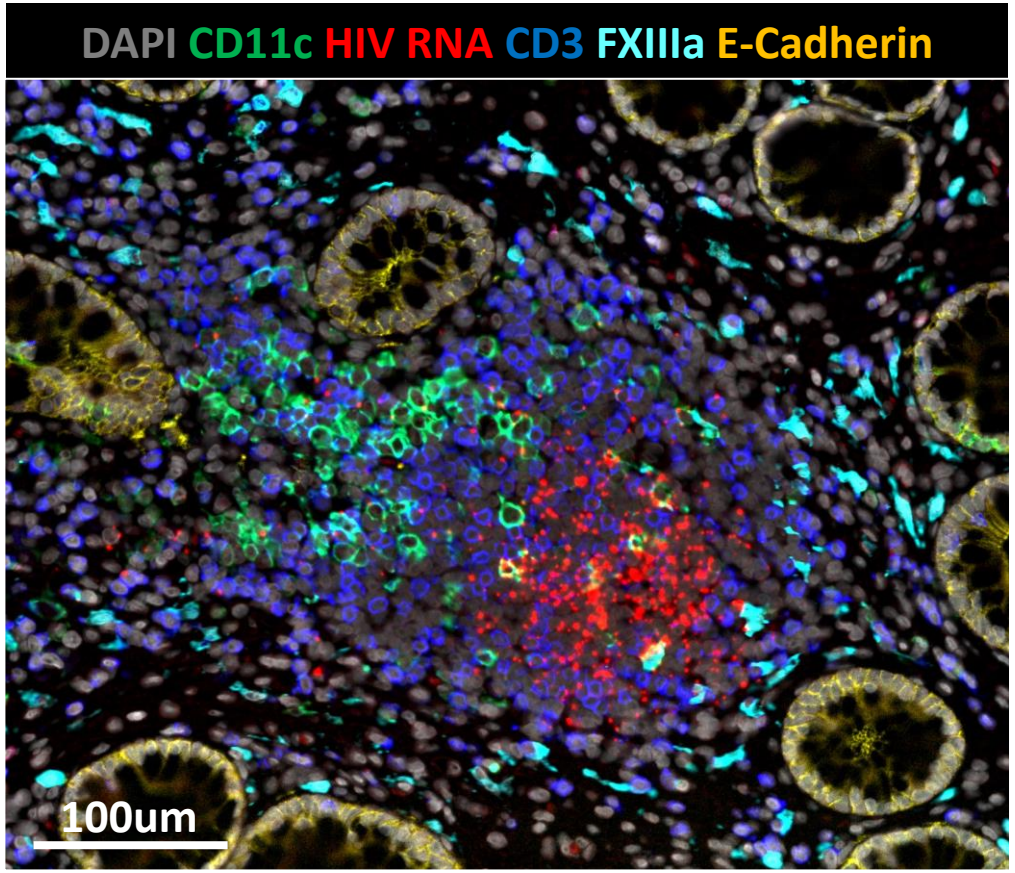
Detecting HIV interacting with target cells *in situ*






Kevin Hu


Heeva Baharlou


Nicolas Canete

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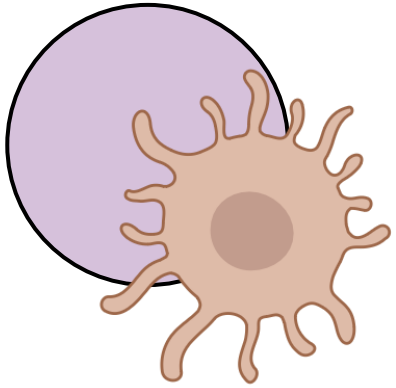
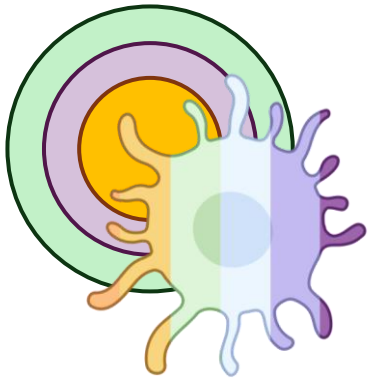
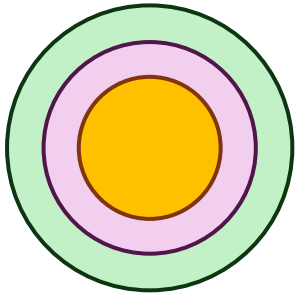
Detecting HIV interacting with target cells *in situ*

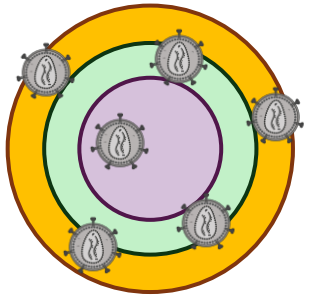


We wanted to explore the **diversity** and **distribution** of immune cells in **lymphoid aggregates**





We wanted to investigate how the **composition** of the lymphoid aggregate **changes** during **HIV** transmission



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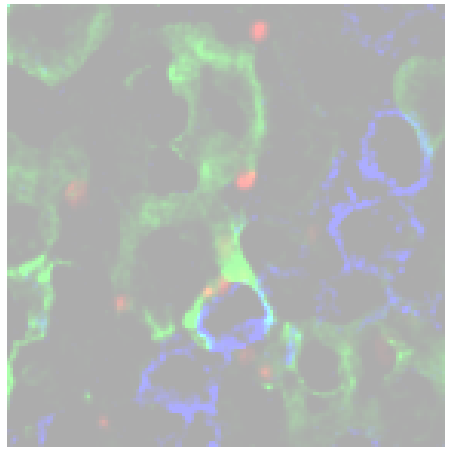
Increasing our parameters!!!

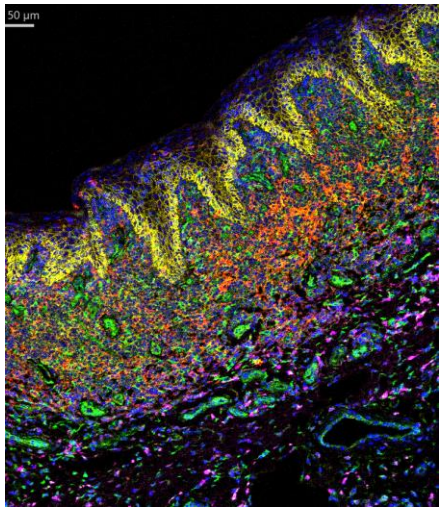
Iterative fluorescence microscopy

6 parameters + HIV RNA



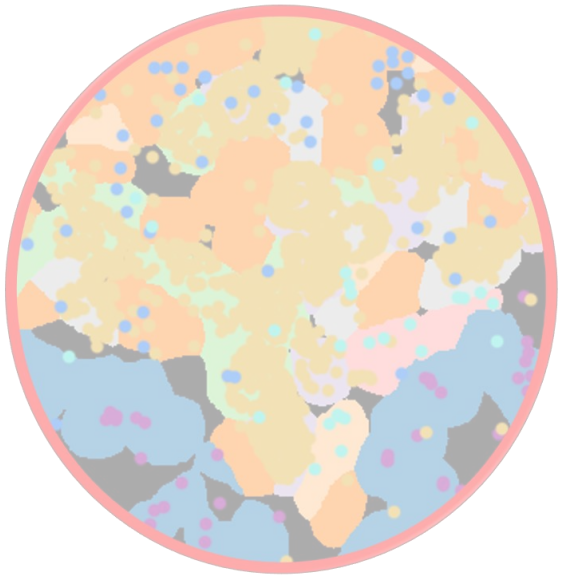
Imaging mass cytometry

37 parameters + HIV RNA



Spatial transcriptomics

1000 genes + HIV RNA



Kevin Hu

Heeva Baharlou

Thomas O'Neil

Oscar Dong

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User-friendly & end-to-end analysis pipeline

Part 1: Cellular Segmentation

Outline cell borders in the image

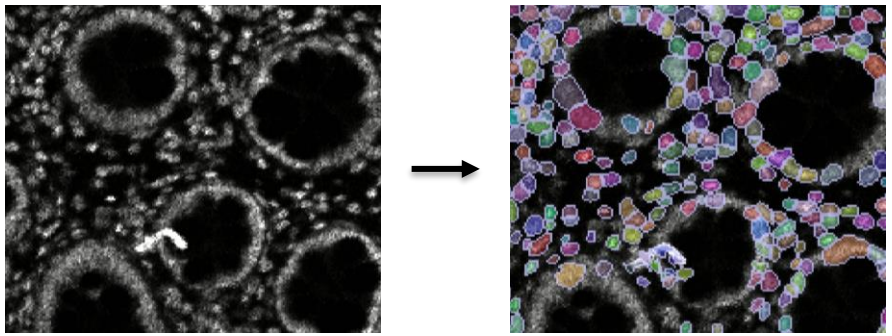
Part 2: Extract Cell Features

Determine each cell's characteristics

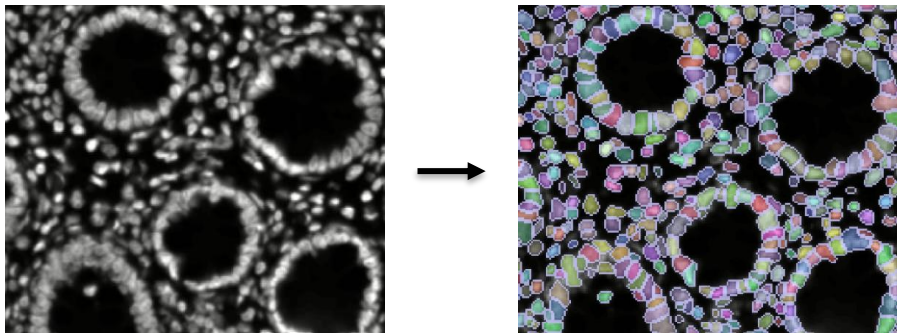
Part 3: Cell Annotation

Classify the types of cells present

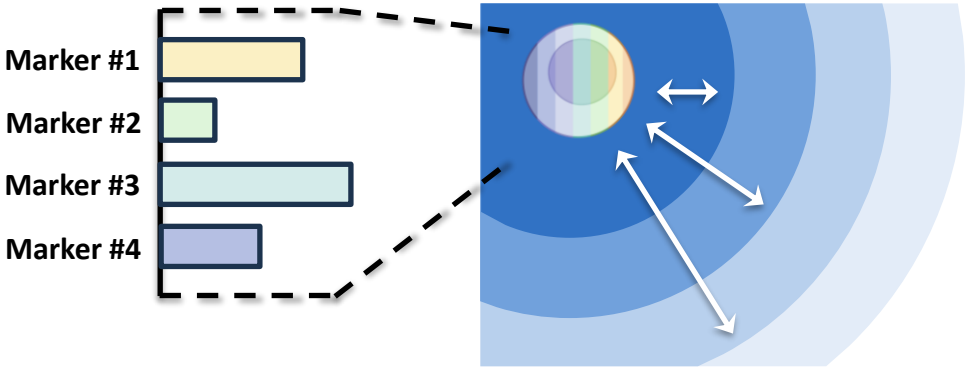
Raw Image: Poor Segmentation



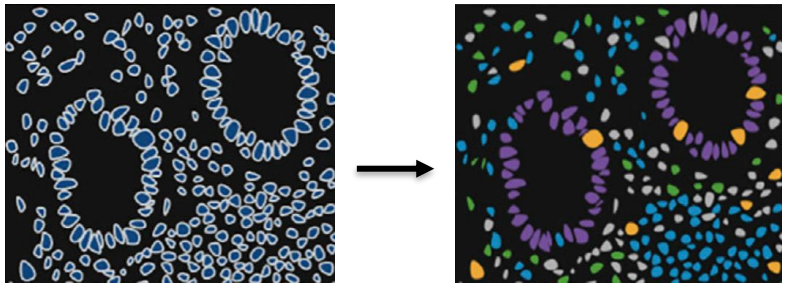
Registered Image: Better Segmentation



Measuring **marker expression** in each cell



Using **spatial maps** to calculate **distance** metrics



Cell type #1
Cell type #2
Cell type #3

Using **user-friendly** software to **classify** and **visualise** cell populations

Workflow:

Oscar Dong

https://github.com/CVR-MucosalImmunology/IMC

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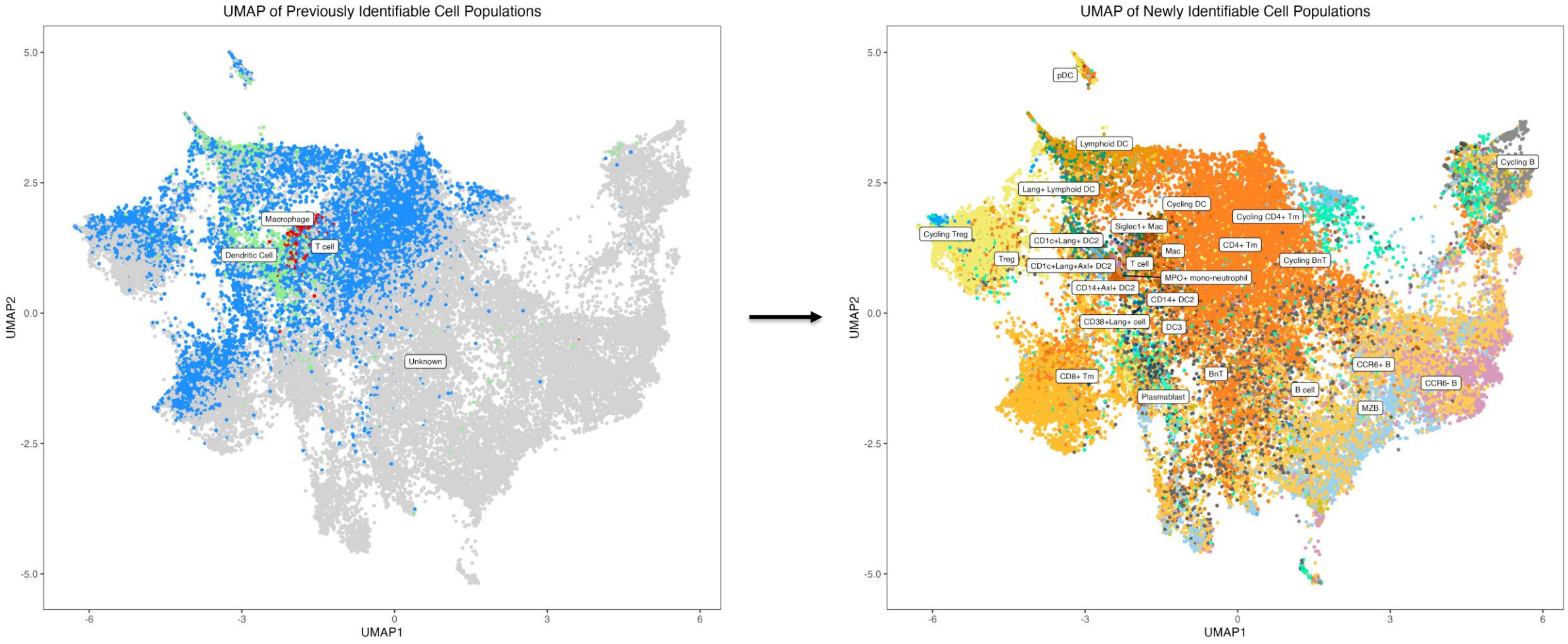
20 immune populations identified in the LA



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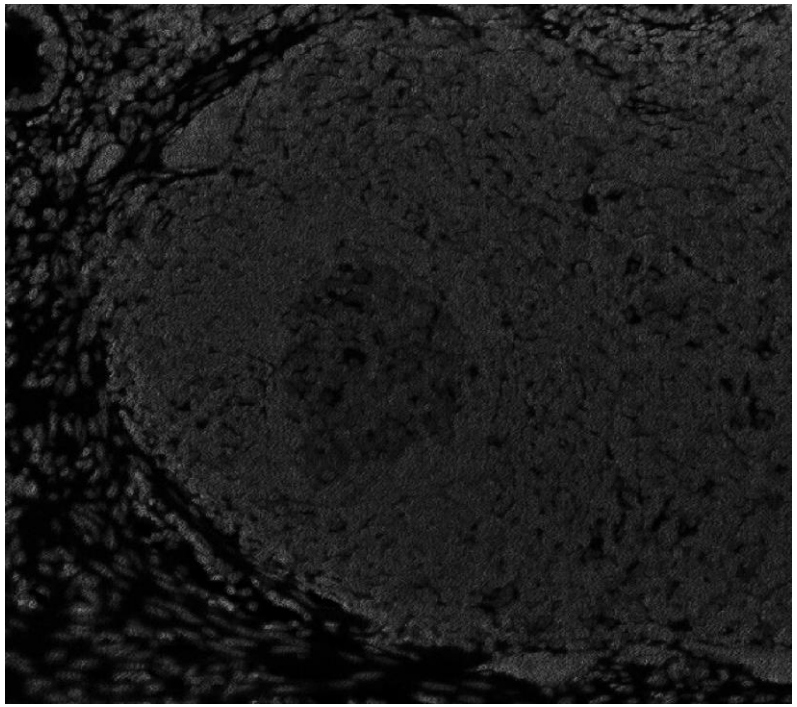
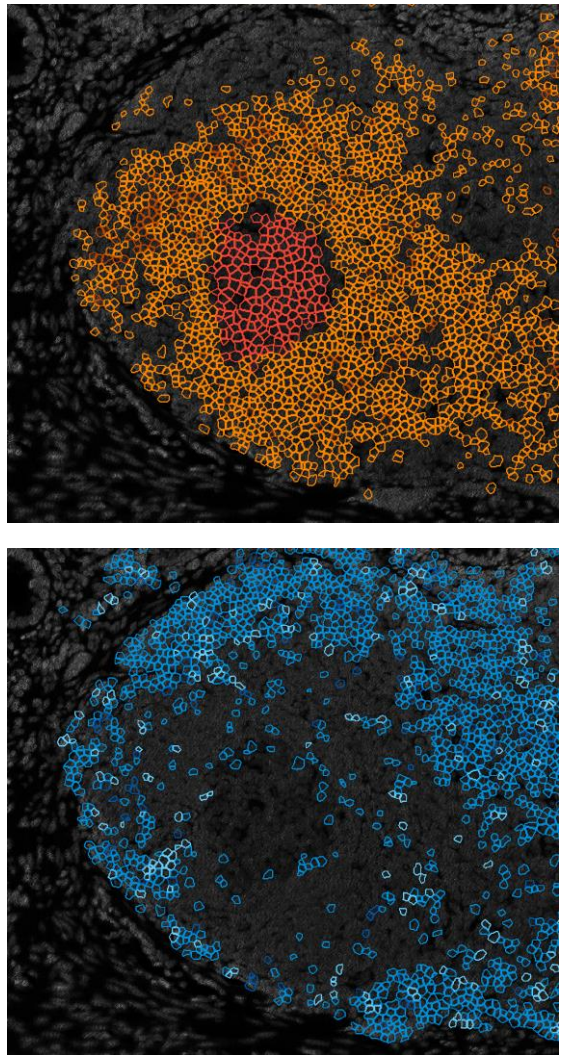
Lymphoid Aggregates are organised

Unlabeled microscope image

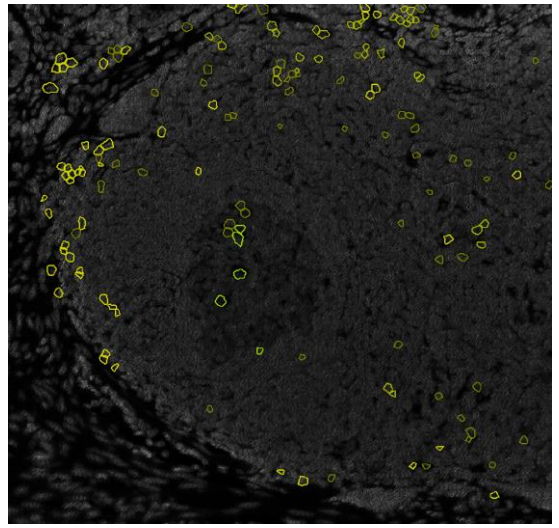


B cell subsets (located **centrally**)

T cell subsets (located **peripherally**)



Myeloid cell subsets, including **dendritic cells** (dispersed **throughout**)



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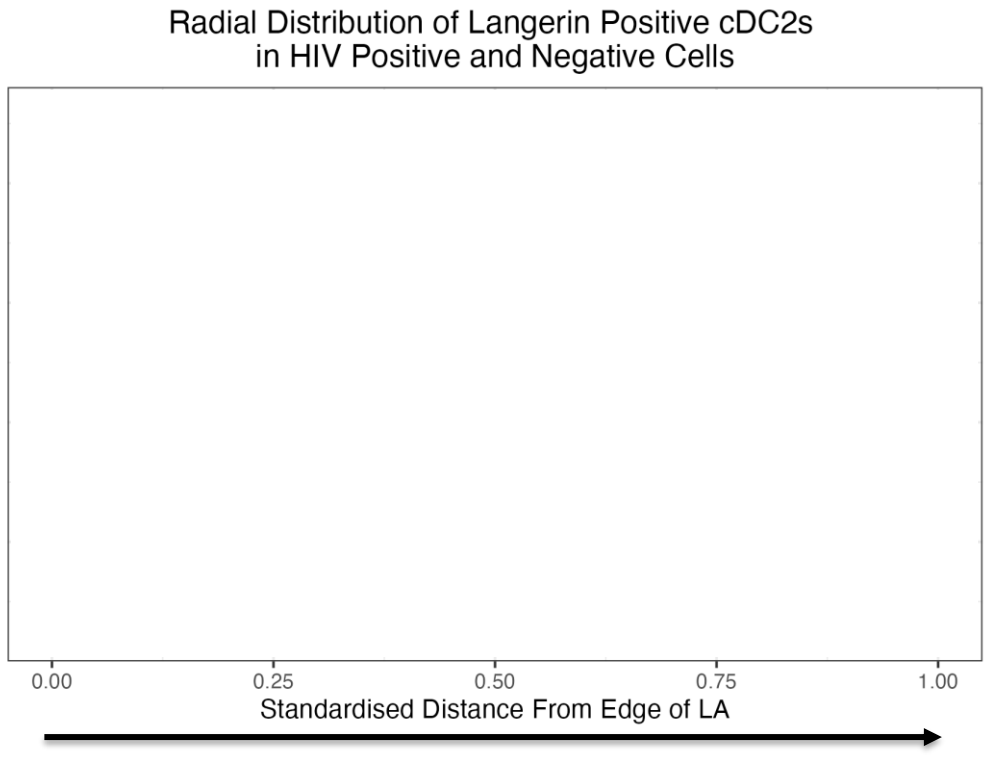
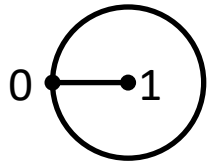
Langerin^{+} cDC2 Traffic HIV to the centre of LAs

Langerin+ cDC2 cells **carrying HIV** are more localised toward the **centre** of the LA (*p = 0.0429*)

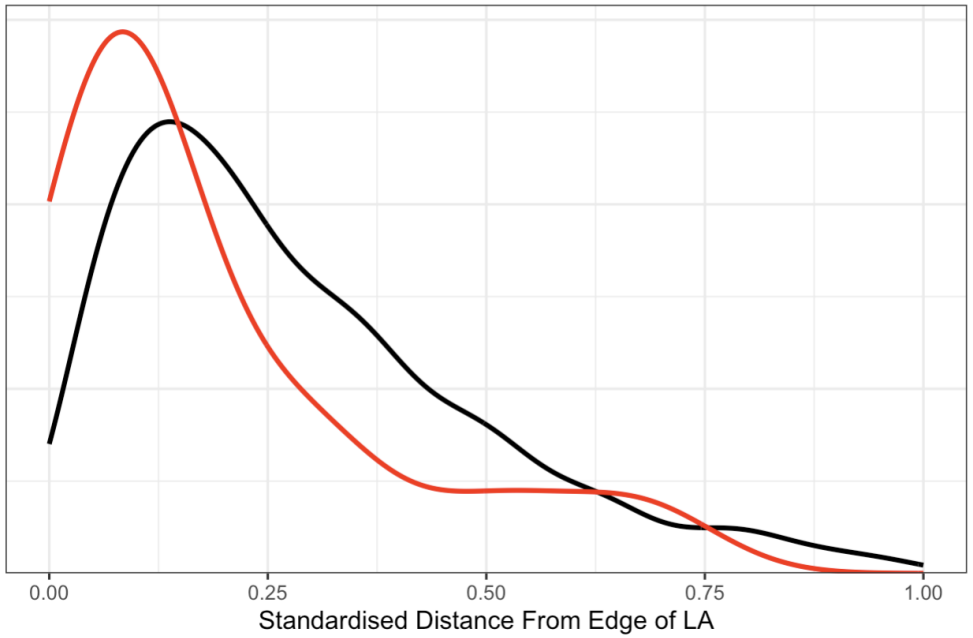


Edge of LA



Centre of LA

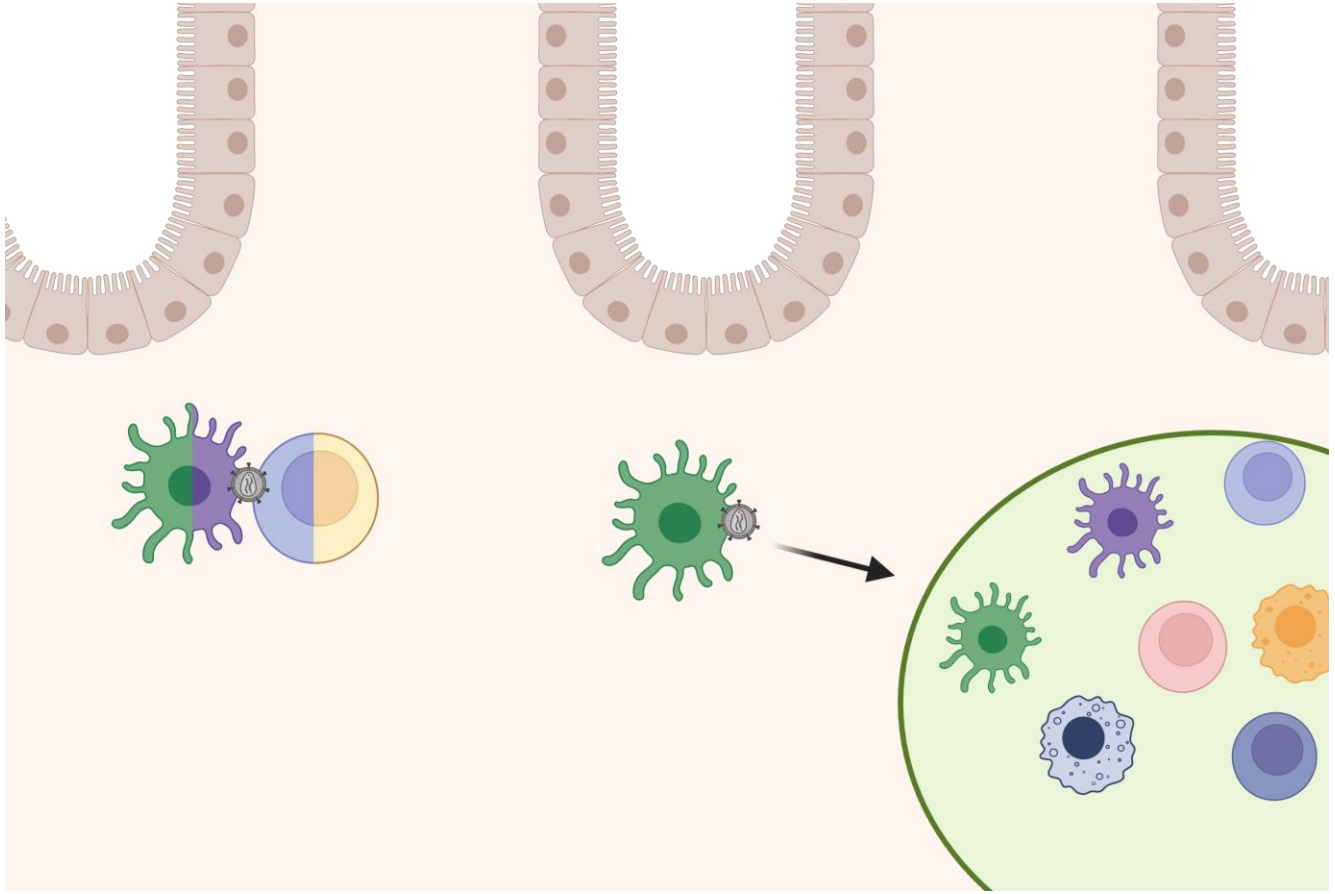
Langerin- cDC2



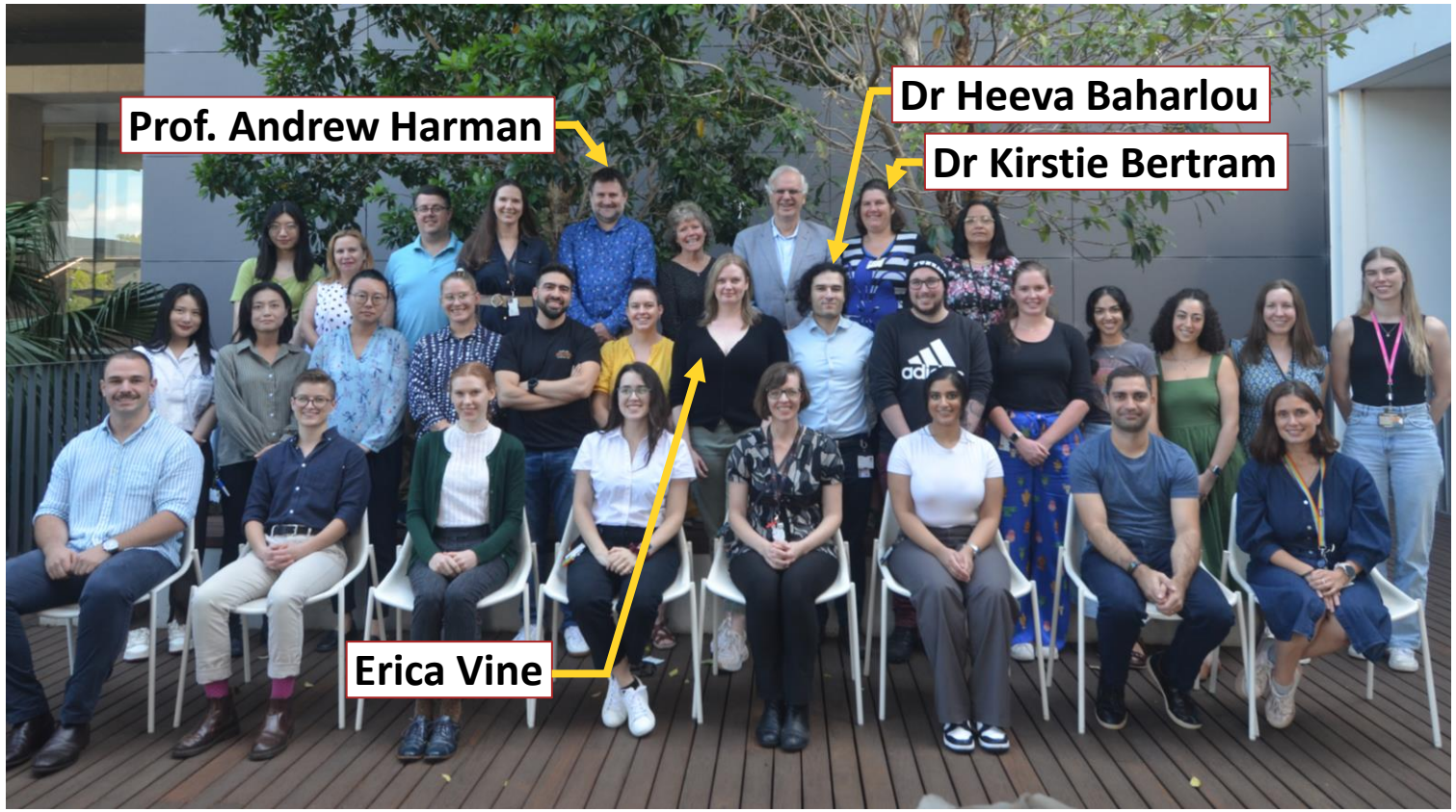
Summary

- Physiologically relevant **human** *in situ* transmission model
- An **end-to-end analysis pipeline** for imaging mass cytometry analysis, focusing on improving cell segmentation, tissue compartmentalisation and user-friendly application.
- **Lymphoid aggregates are:**
  - A **viral sanctuary** during early HIV transmission
  - A dense and **diverse microenvironment** containing several immune subsets in **organised compartments**
  - A site of rapid HIV trafficking, notably by subsets of dendritic cells such as **Langerin^{+} cDC2**

Development of mucosal vaccines



Acknowledgements



Oscar Dong

Dr Kevin Hu

Workflow:

Colorectal Surgeons

Grahame Ctercteko
Martijn Gosselink
Angelina Di Re
Geoff Collins
Toufic El Khoury
Nimalan Pathma-Nathan

WIMR & Core facilities

Dinny Graham
Barbara Guild
Grant Parnell
Ellis Patrick
Scott Byrne

Patients & Clinicians

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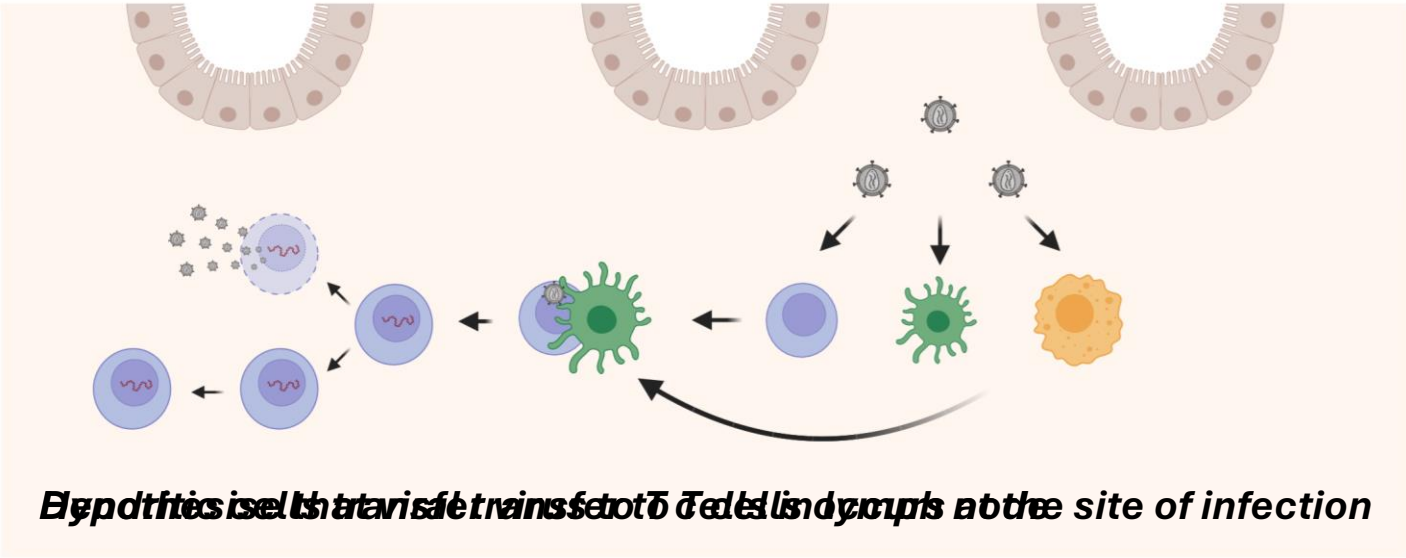
THE UNIVERSITY OF SYDNEY

Australian Government
National Health and Medical Research Council

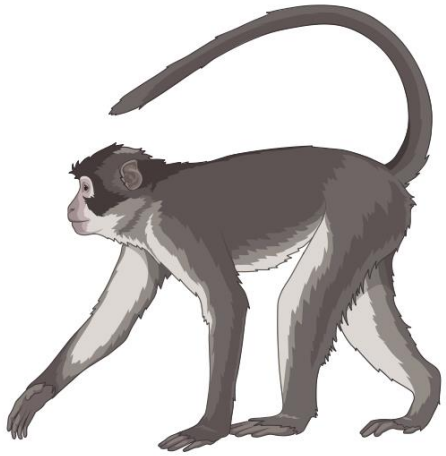
Australasian Cytometry Society

What are the early determinants in HIV infection?

Aim to explore dynamics between HIV and immune cells
***in tissue** during transmission*



Hypothesis that virus transfer to T cells occurs at the site of infection



Simian Immunodeficiency Virus Model

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Langerin⁺ cDC2 Traffic HIV to the centre of LAs

Langerin+ cDC2 cells **carrying HIV** are more localised toward the **centre** of the LA (*p = 0.0429*)

Article | Open access | Published: 12 April 2021

Human anogenital monocyte-derived dendritic cells and langerin+cDC2 are major HIV target cells

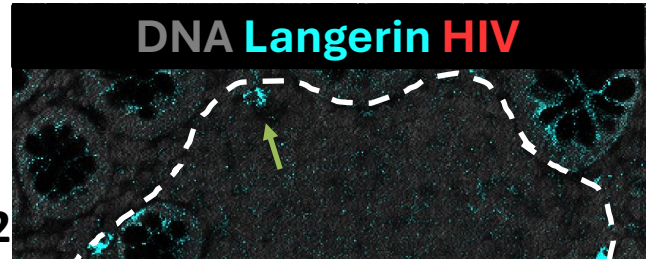
Jake W. Rhodes, Rachel A. Botting, Kirstie M. Bertram, Erica E. Vine, Hafsa Rana, Heeva Baharlou, Peter Vegh, Thomas R. O'Neil, Anneliese S. Ashhurst, James Fletcher, Grant P. Parnell, J. Dinny Graham, Najla Nasr, Jake J. K. Lim, Laith Barnouti, Peter Haertsch, Martijn P. Gosselink, Angelina Di Re, Faizur Reza, Grahame Ctercteko, Gregory J. Jenkins, Andrew J. Brooks, Ellis Patrick, Scott N. Byrne, Eric Hunter, Muzlifah A. Haniffa, Anthony L. Cunningham & Andrew N. Harman — Show fewer authors

Nature Communications **12**, Article number: 2147 (2021) | Cite this article

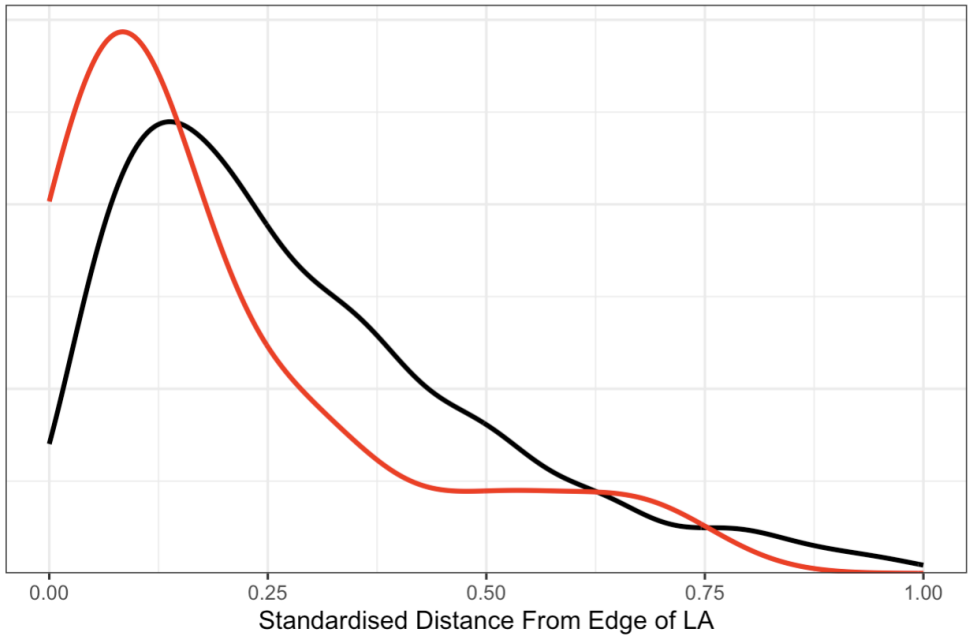
4801 Accesses | **28** Citations | **11** Altmetric | Metrics

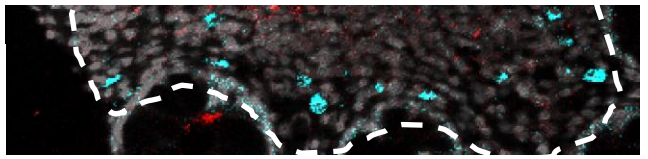
- Previous **findings supported** by new high parameter **spatial imaging**
- **Further analyses** are required to determine **exactly which** cells these **langerin⁺ cDC2** cells are **interacting** with

Untreated sample



Langerin- cDC2



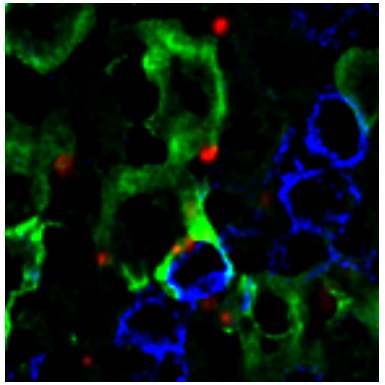


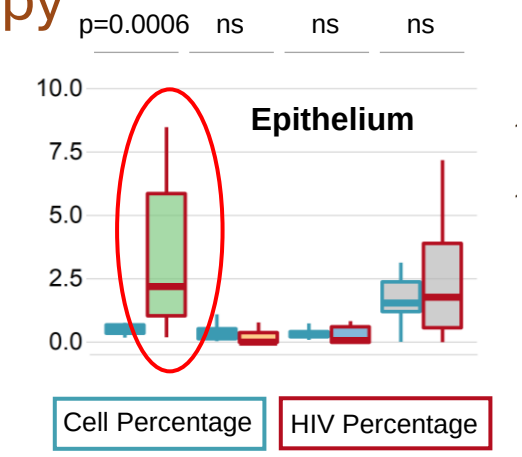
HIV primarily interacts with dendritic cells

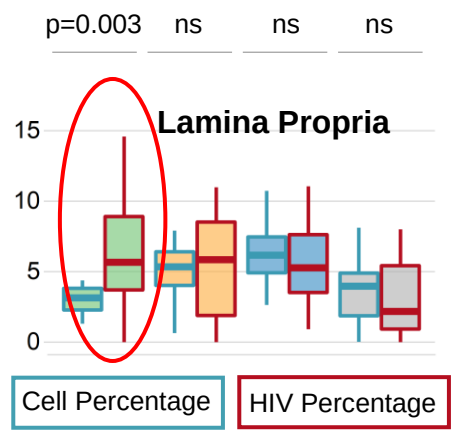
Not CD4+ T cells!

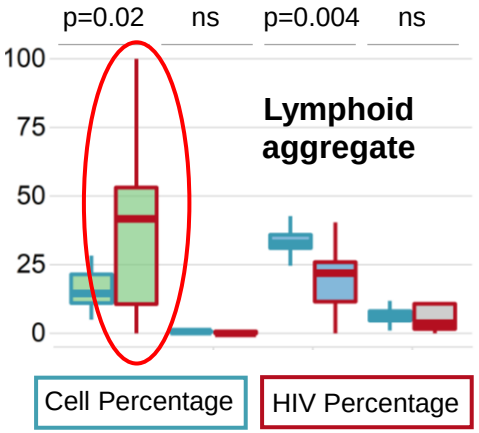
Iterative fluorescence microscopy

(high resolution images + HIV RNA)









CD4+ T cell

Dendritic Cell

Macrophage

Kevin Hu

Heeva Baharlou

Nicolas Canete

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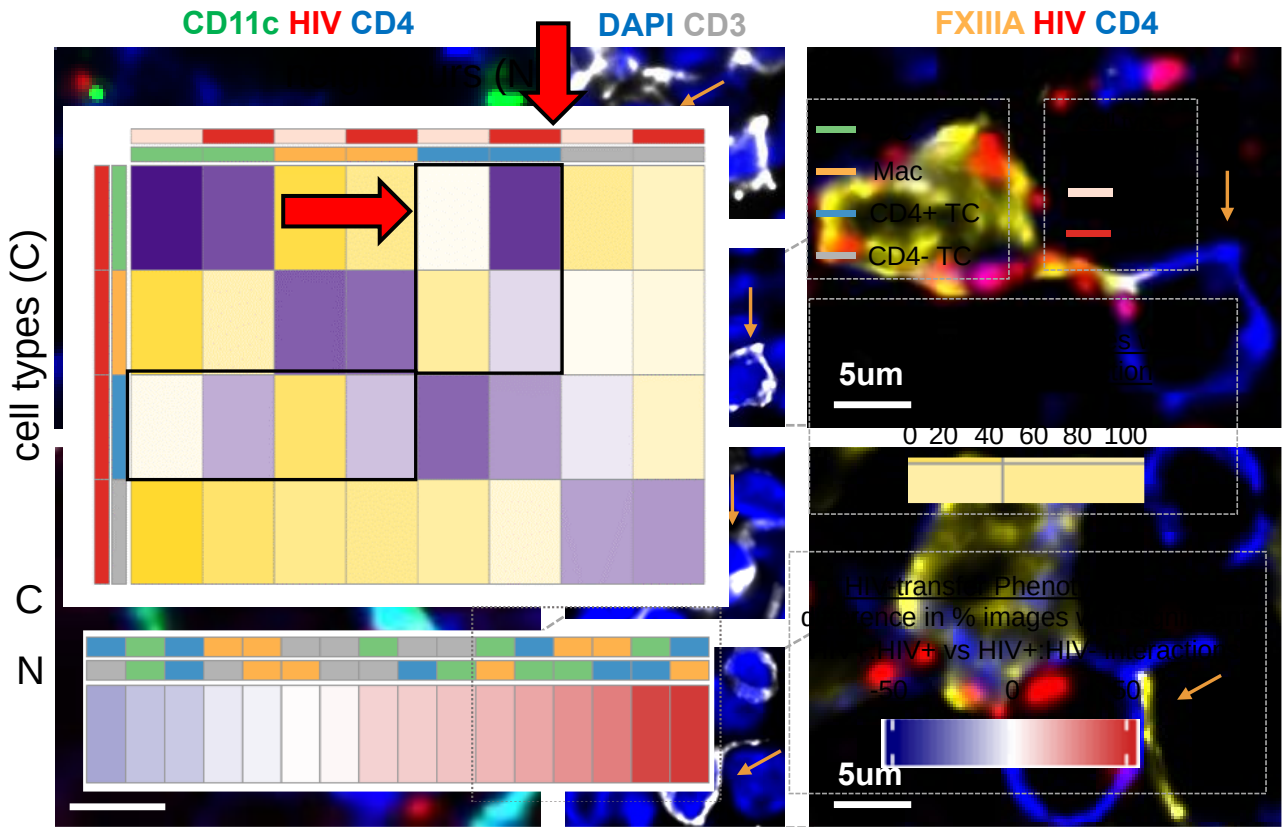
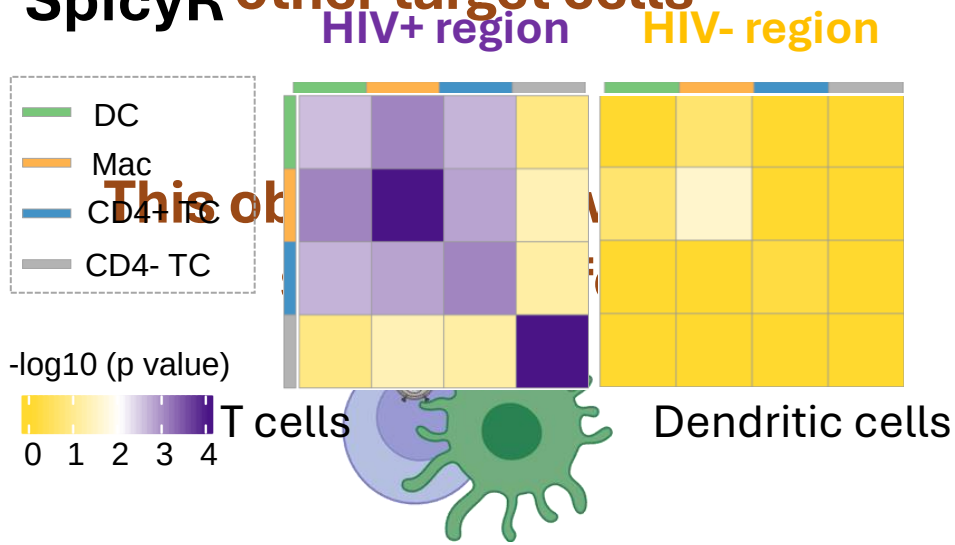
HIV induces cell-cell interactions suggestive of viral transfer

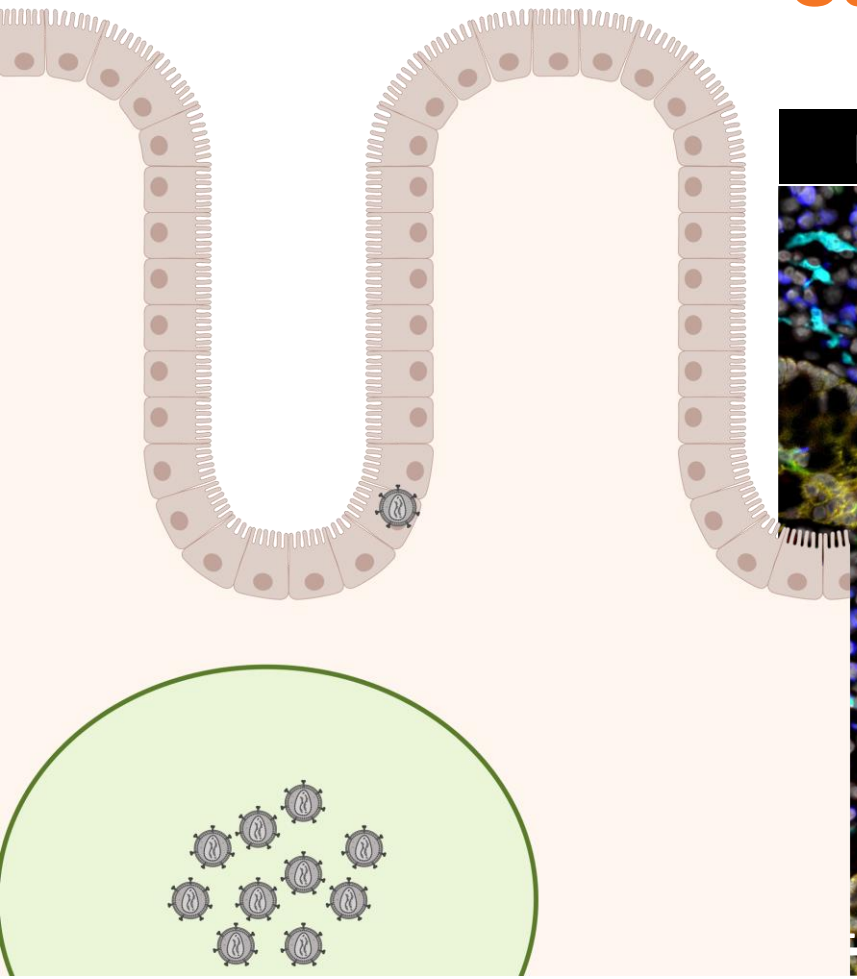


A cell with HIV was found to cluster more frequently with other target cells

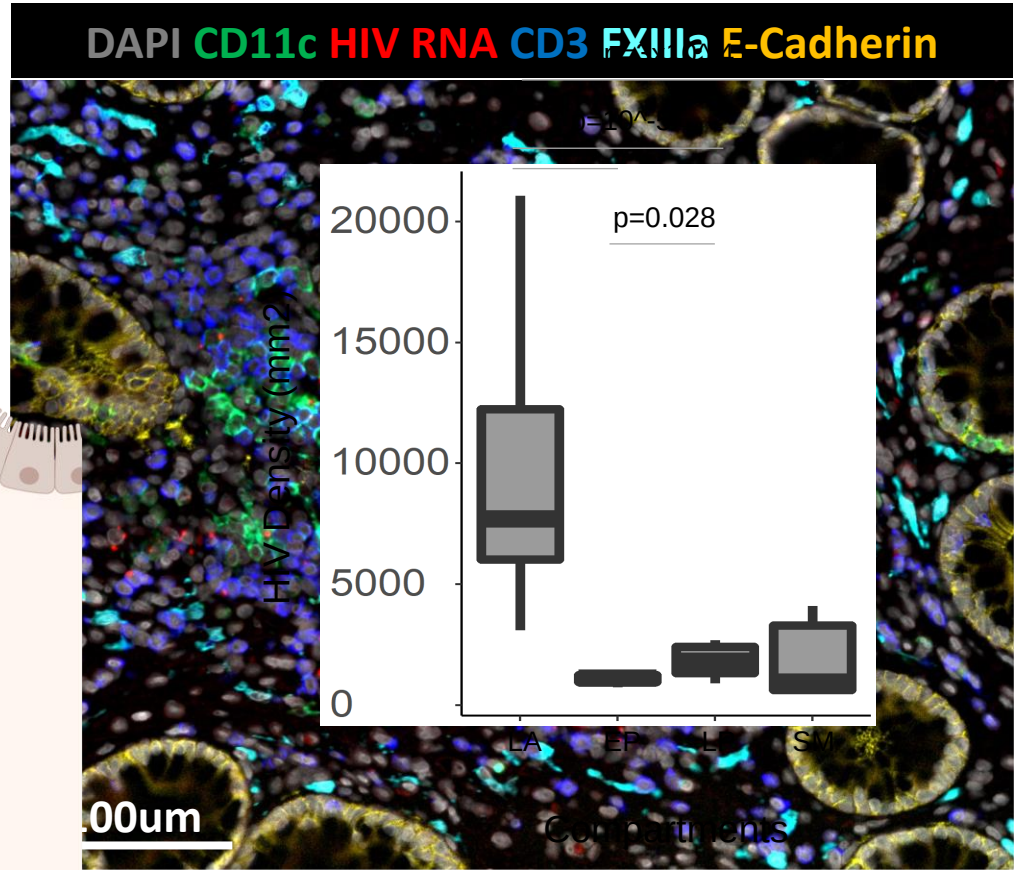
SpicyR



Lymphoid aggregate play a substantial role in early HIV transmission



30 minutes post-exposure



HIV rapidly accumulates in lymphoid aggregates

LAs are poorly understood (aka Lymphoid follicle)

Likely related to tertiary lymphoid structures

Composition and purpose in unaffected tissues unexplained

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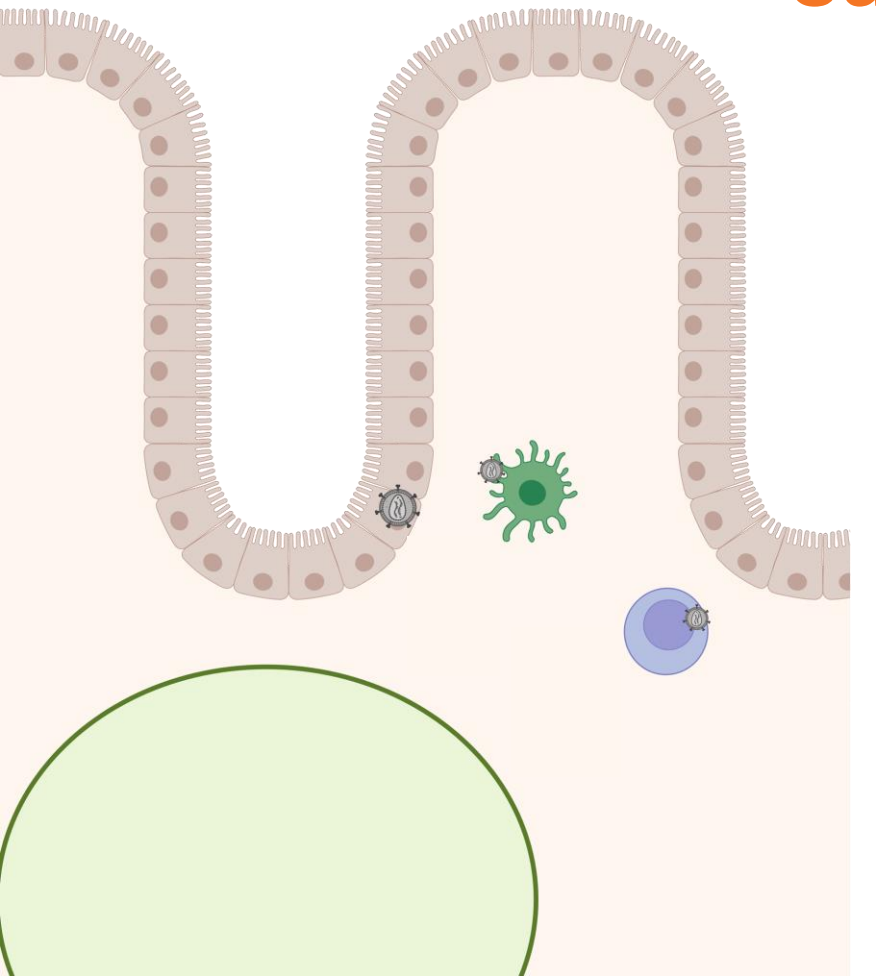
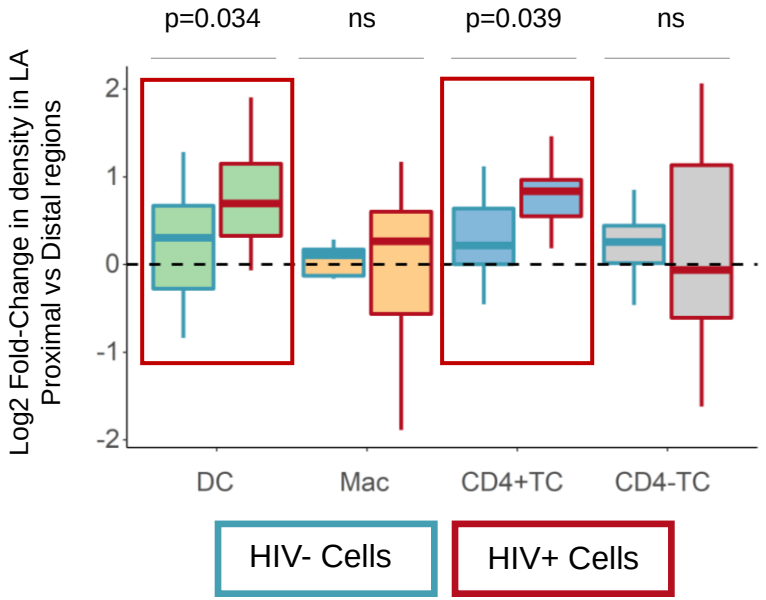
Lymphoid aggregate play a substantial role in early HIV transmission





HIV rapidly accumulates in lymphoid aggregates

CD4+ T cells and dendritic cells with HIV were accumulating around lymphoid aggregates

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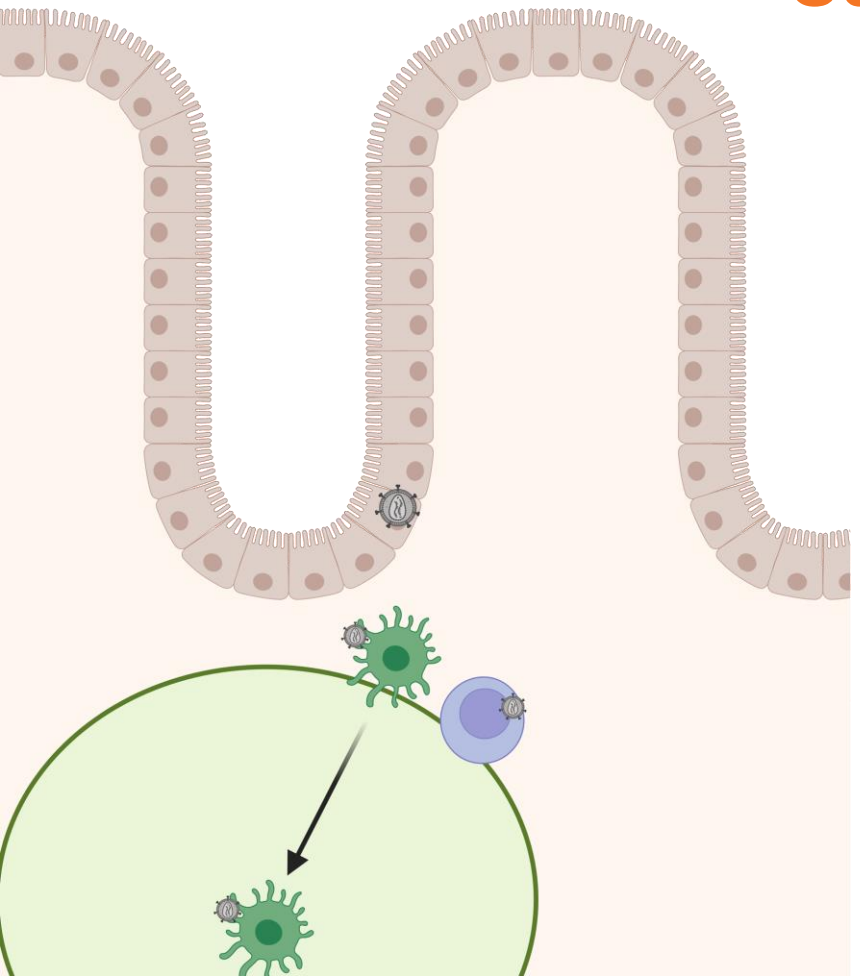
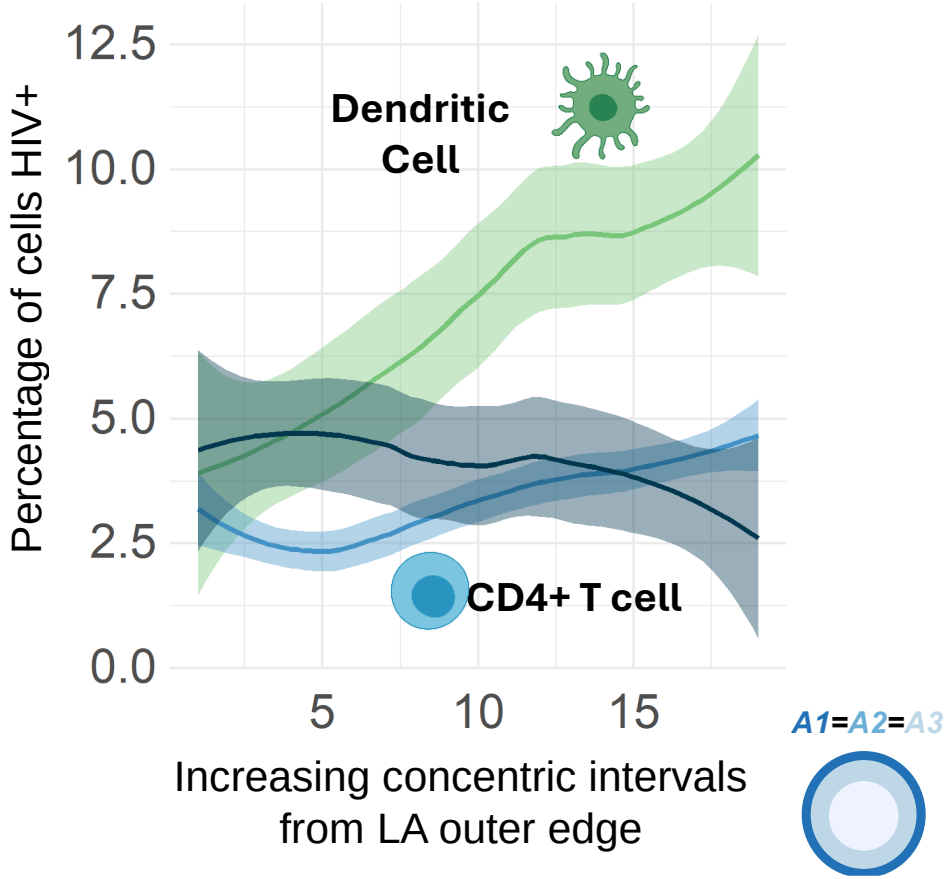
Lymphoid aggregate play a substantial role in early HIV transmission





HIV rapidly accumulates in lymphoid aggregates

CD4+ T cells and dendritic cells with HIV were accumulating around lymphoid aggregates

HIV+ dendritic cells accumulate at the centre of the lymphoid aggregate

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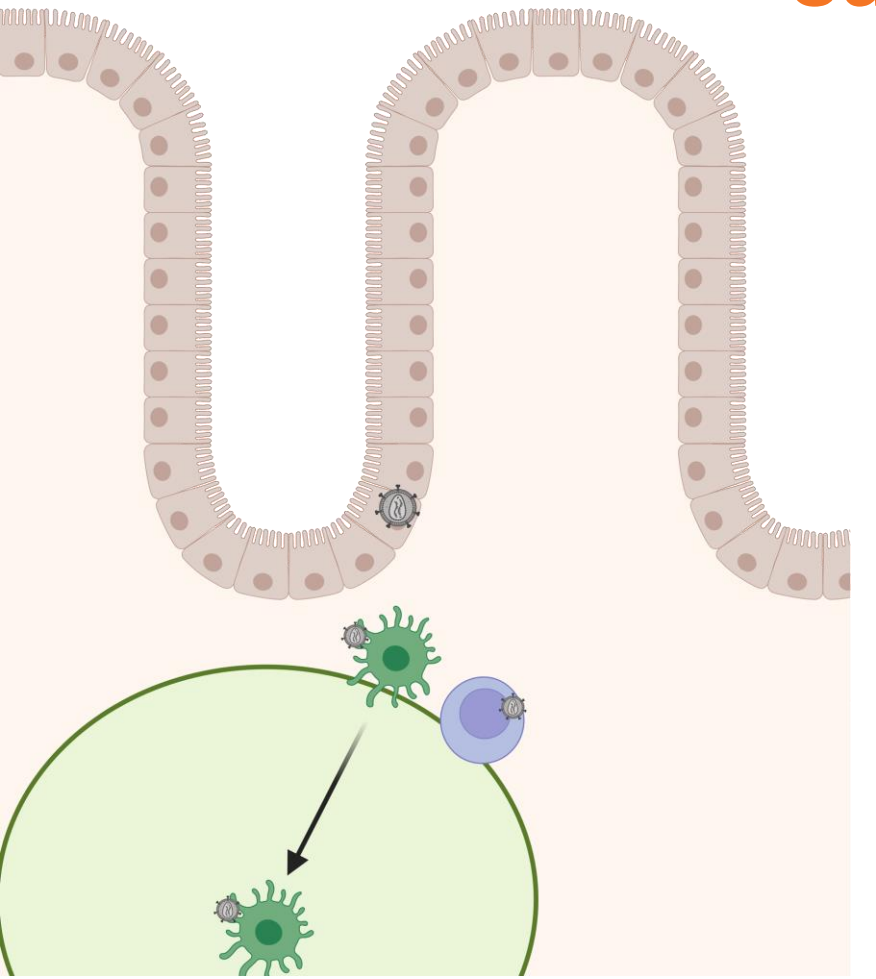
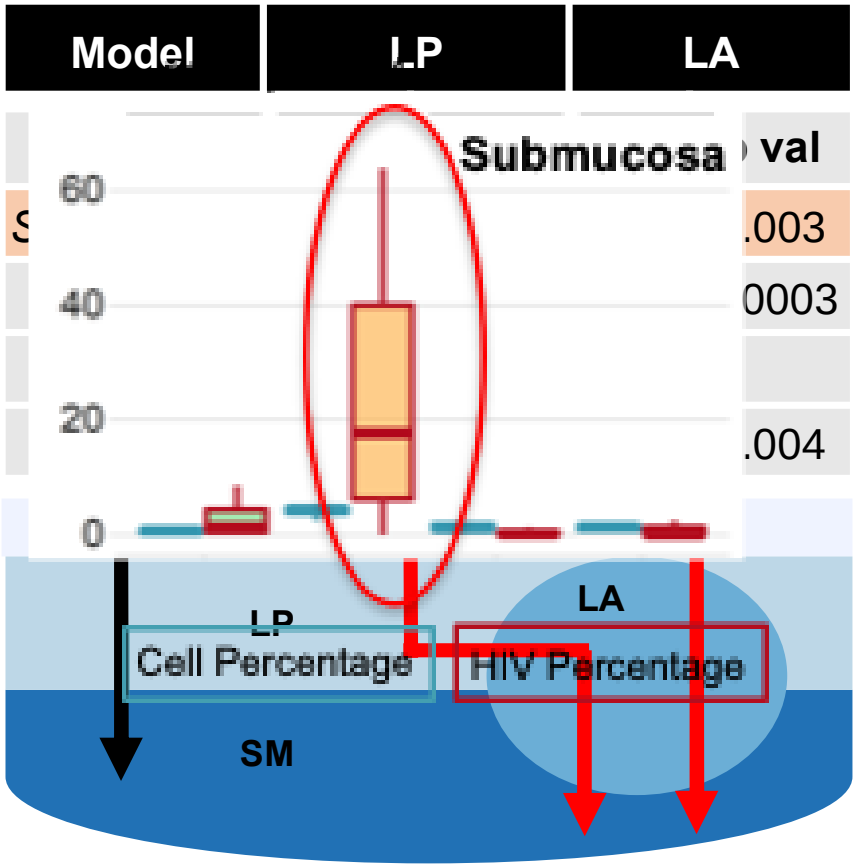
Lymphoid aggregate play a substantial role in early HIV transmission





HIV rapidly accumulates in lymphoid aggregates

CD4+ T cells and dendritic cells with HIV were accumulating around lymphoid aggregates

HIV+ dendritic cells accumulate at the centre of the lymphoid aggregate

HIV may reach the submucosa through the lymphoid aggregates

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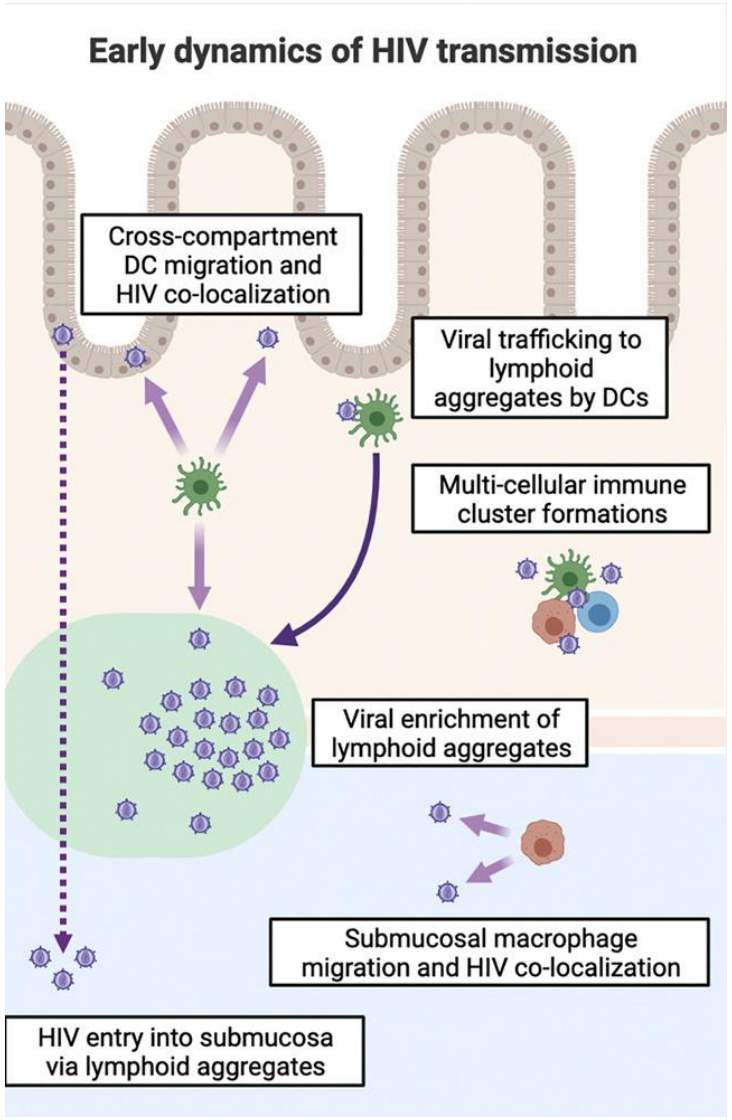

Summary

- Physiologically relevant **human** *in situ* transmission model
- HIV interacts with **all 3 target cells** in human gut tissue within 2 hours
- HIV localises to **dendritic cells** in mucosal tissues
- HIV causes it's target cells to form clusters, **inducing rapid viral transfer**
- **Lymphoid aggregates** appear to be an early viral sanctuary

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