

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

NO CONFLICTS OF INTEREST

## Spontaneous HIV expression during ART is associated with HIV-specific CD4 and CD8 T responses

Dubé Mathieu<sup>1</sup>, Tastet O<sup>1</sup>, Sannier G<sup>1,2</sup>, Brassard N<sup>1</sup>, Delgado GO<sup>1</sup>, Pagliuzza A<sup>1</sup>, Prat A<sup>1,2</sup>, Routy JP<sup>3</sup>, Fromentin R<sup>1</sup>, Chomont N<sup>1,2</sup>, Kaufmann DE<sup>1,2,4</sup>

<sup>1</sup>CRCHUM, <sup>2</sup>Université de Montréal and <sup>3</sup>McGill University, Montreal, QC, Canada, <sup>4</sup>CHUV and University of Lausanne, Switzerland

**CRCHUM**

RESEARCH CENTRE

December 15<sup>th</sup>, 2022

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

# Background

---

- There is evidence of spontaneous, **leaky** viral gene transcription and translation during ART (Dornadula et al. 1999, Gunthard et al. 2011, Halvas et al. 2020, Ishizaka et al 2016, Yukl et al. 2018, Ferdin et al. 2018, Passaes et al. 2021, Wu et al. 2021).
- HIV-specific CD4 and CD8 T cell responses persist during ART (Niessl et al. 2020, Reiss et al. 2017, Stevenson et al. 2021)
- Anti-HIV immunity is expected to play an important role in future cure strategies (Moysi et al. 2018, Trautmann et al. 2016)
  - purging reservoirs
  - exerting immunosurveillance
  - supporting development of broadly neutralizing HIV-specific antibodies

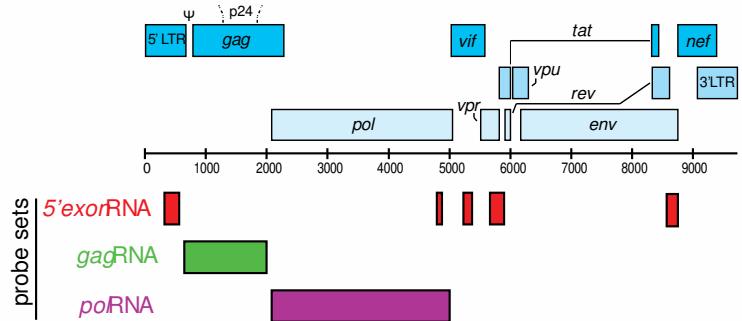
## Gaps of knowledge

---

- The quantitative and qualitative features of leaky latent reservoirs are not yet established.
- Still unclear how HIV-specific immunity can persist during ART
- Is there a connection between the **leaky latent reservoir** and the **HIV-specific immunity?**

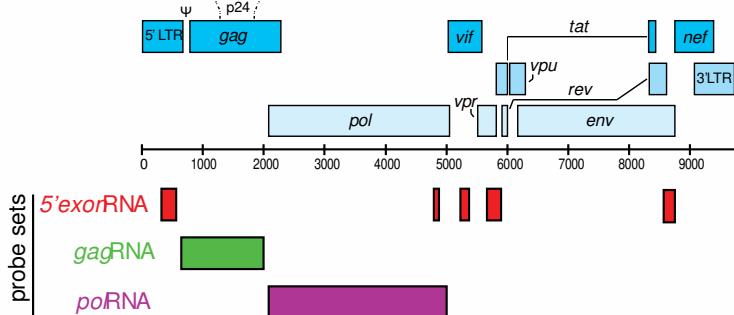
# Single-cell RNA detection by HIV RNAflow-FISH

## A. Probeset design



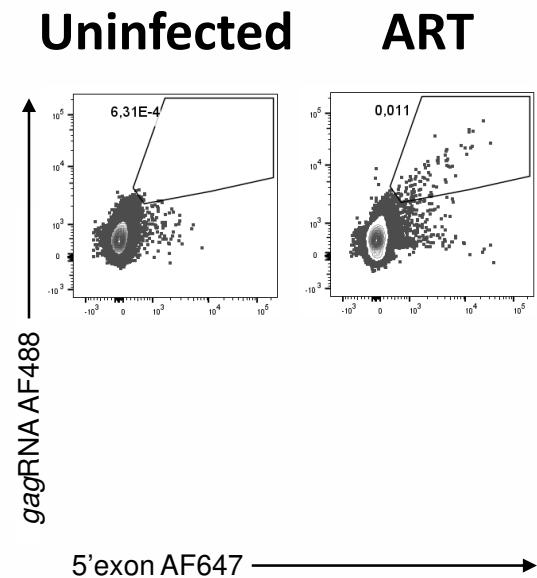
# Single-cell RNA detection by HIV RNAflow-FISH

## A. Probeset design



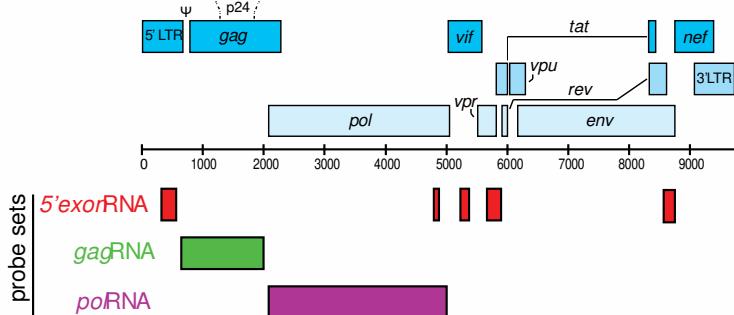
## B. vRNA+ cells gating

Type of reservoir	Stimulation
Inducible	PMA+iono.



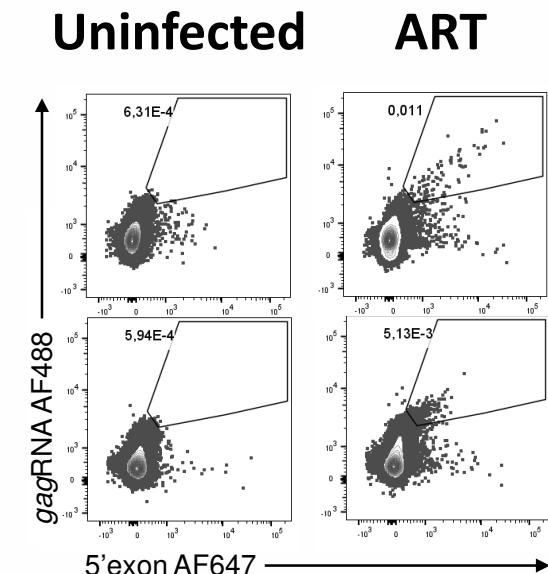
# Single-cell RNA detection by HIV RNAflow-FISH

## A. Probeset design



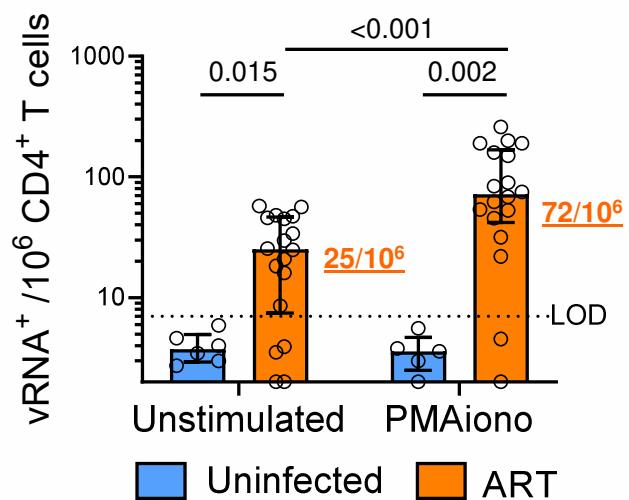
## B. vRNA+ cells gating

Type of reservoir	Stimulation
Inducible	PMA+iono.
Leaky	Unstim.



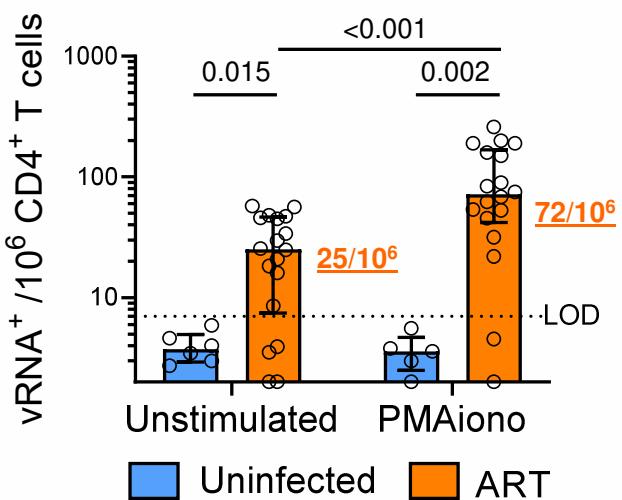
# Spontaneous vRNA expression is detectable

## A. vRNA+ cells quantification

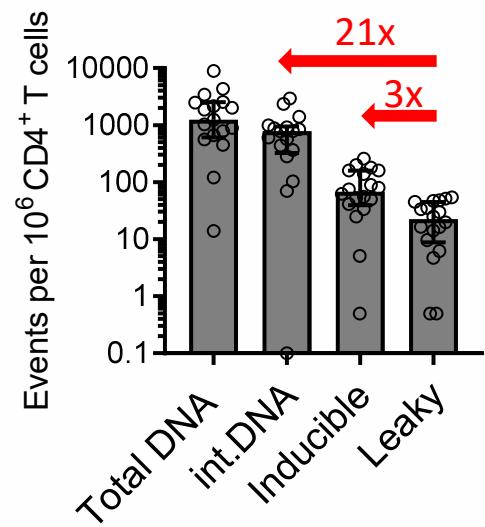


# Spontaneous vRNA expression is detectable

## A. vRNA+ cells quantification

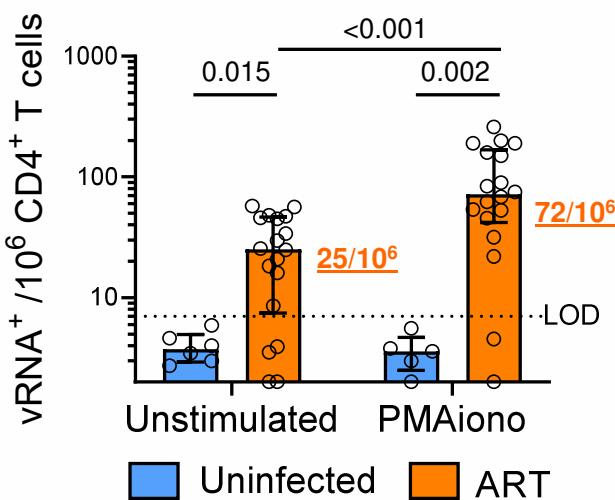


## B. Metrics comparisons

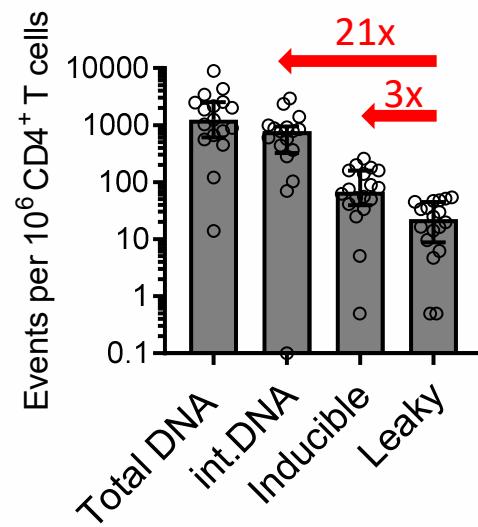


# Spontaneous vRNA expression is detectable

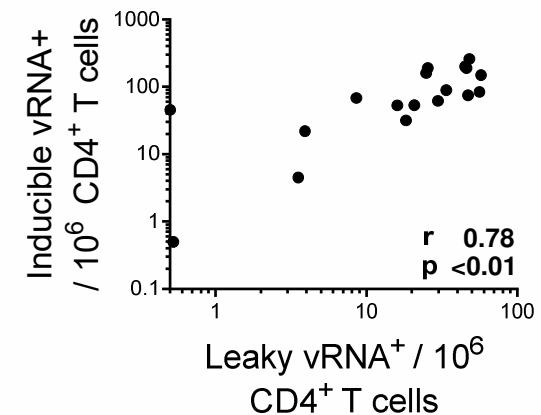
## A. vRNA+ cells quantification



## B. Metrics comparisons

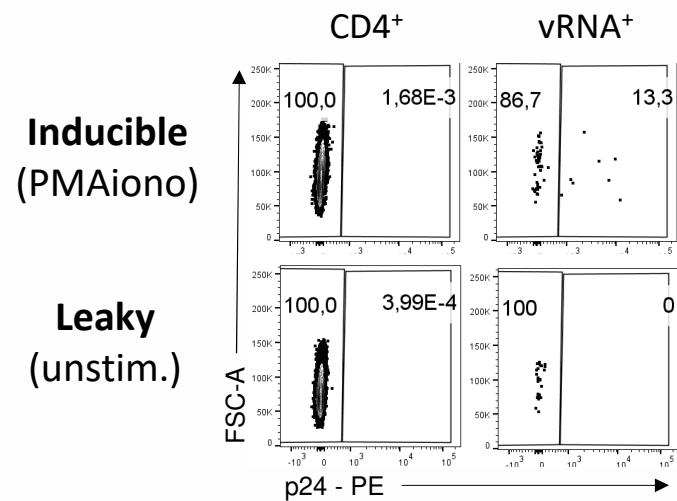


## C. Association inducible vs leaky

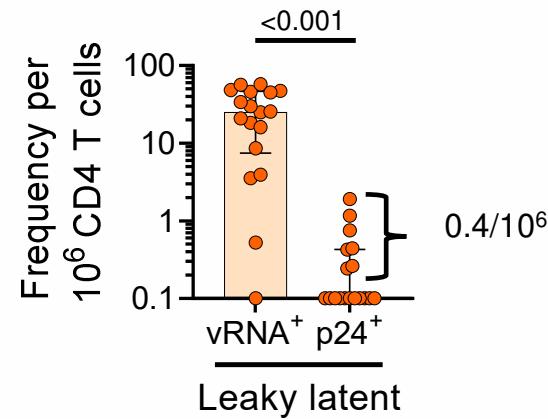


# Leaky reservoirs are dominated by abortive gagRNA<sup>+</sup> cells

## A. p24<sup>+</sup>

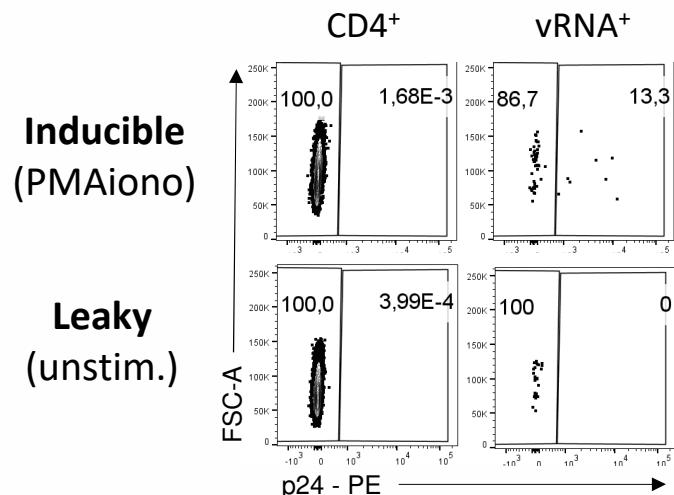


## B. Detection of leaky p24<sup>+</sup>

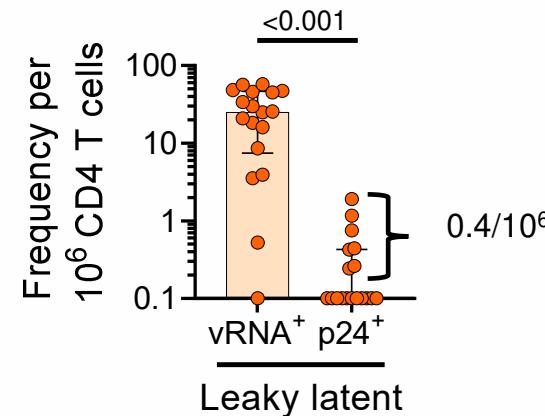


# Leaky reservoirs are dominated by abortive gagRNA<sup>+</sup> cells

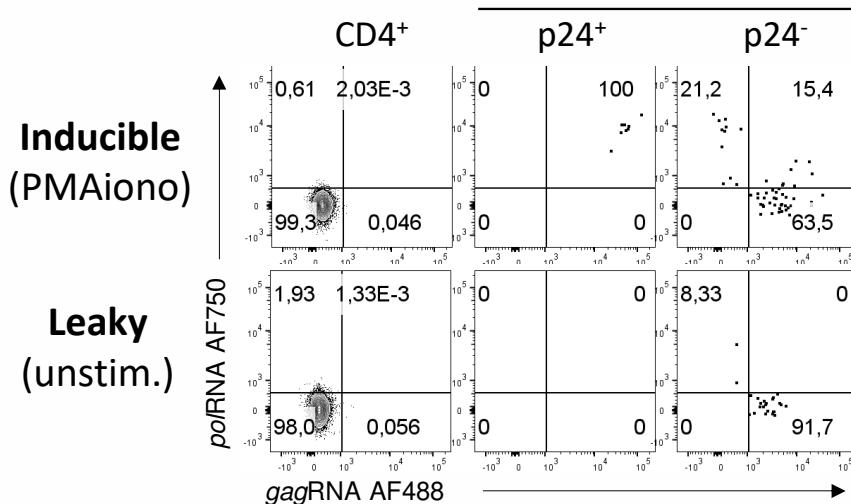
## A. p24<sup>+</sup>



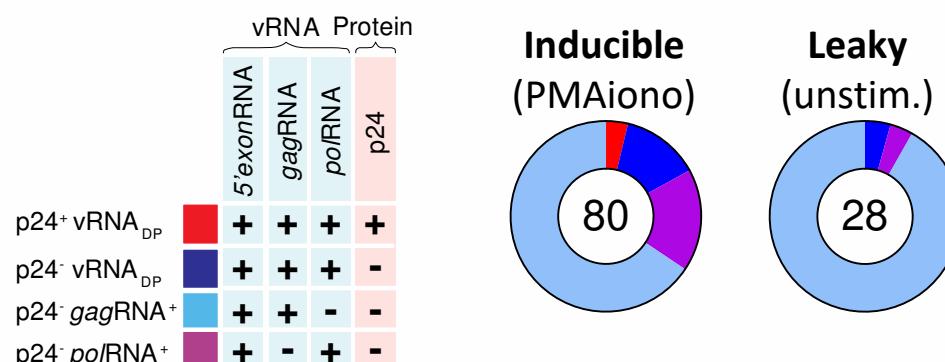
## B. Detection of leaky p24<sup>+</sup>



## C. gagRNA vs po/RNA

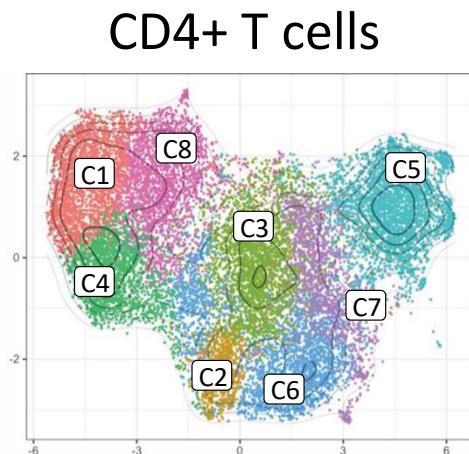


## D. Profile



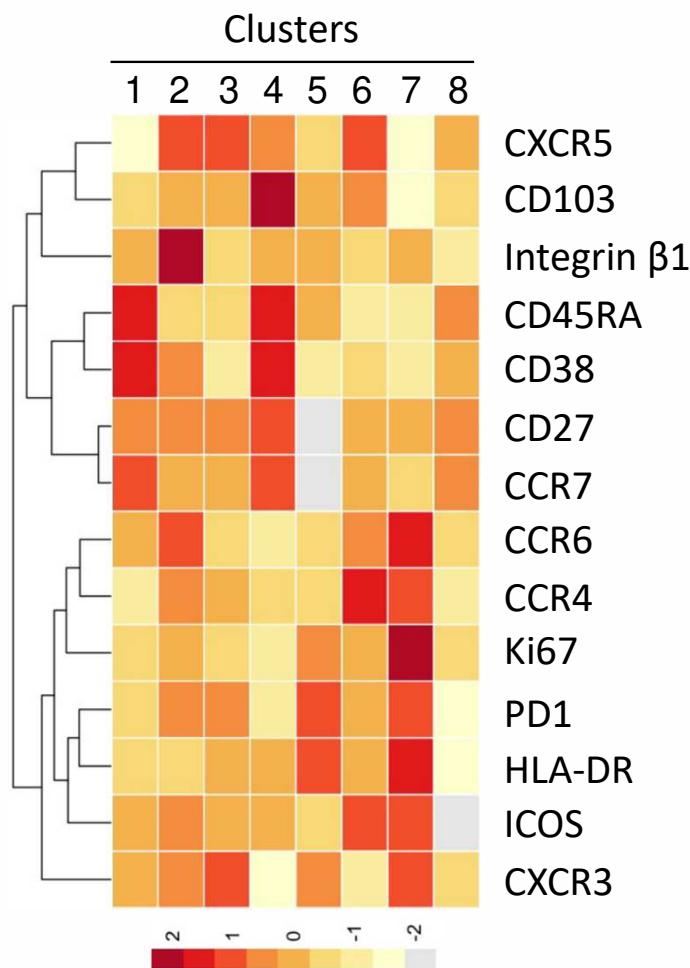
# Leaky reservoirs are phenotypically diverse

## A. UMAP representation

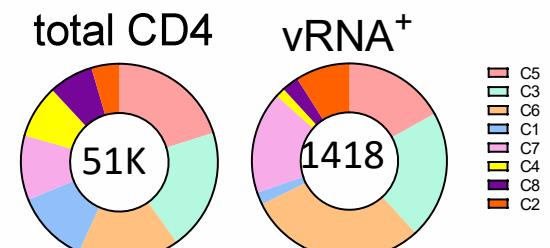


6  
UMAP 2  
UMAP 1

## B. Cluster heat map

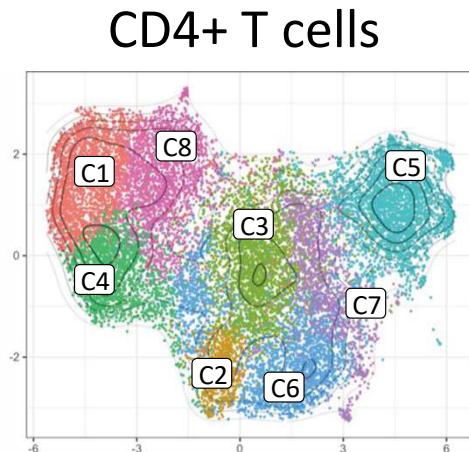


## C. Phenotyping

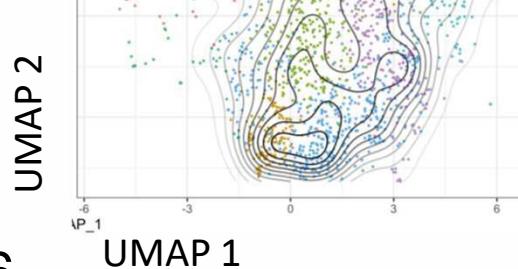


# Leaky reservoirs are phenotypically diverse

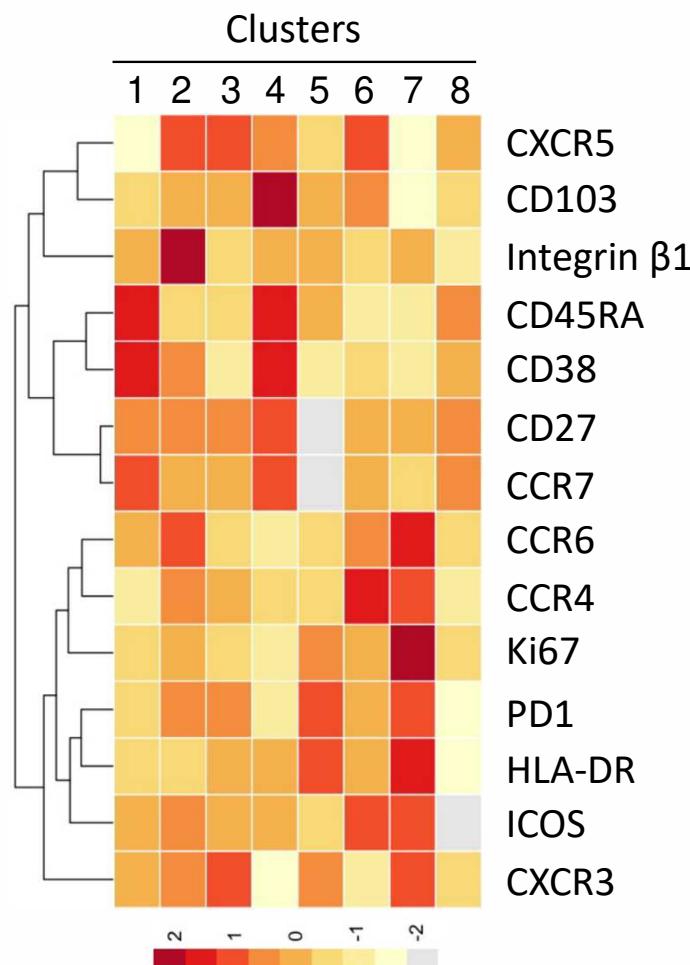
## A. UMAP representation



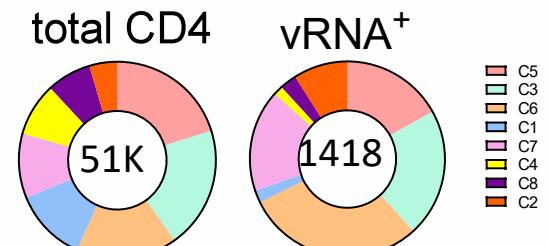
## Leaky vRNA<sup>+</sup> cells



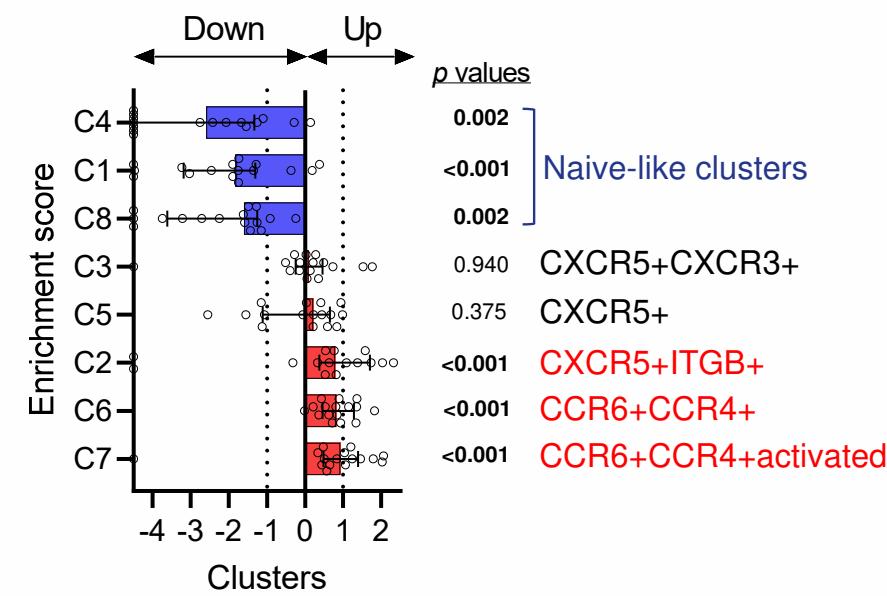
## B. Cluster heat map



## C. Phenotyping

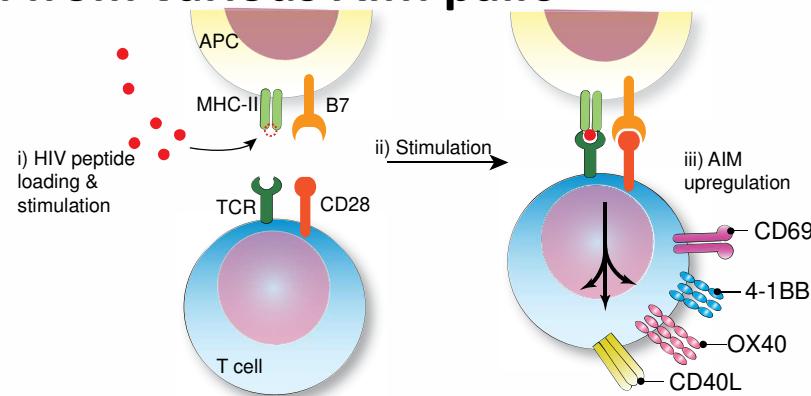


## D. Cluster enrichment

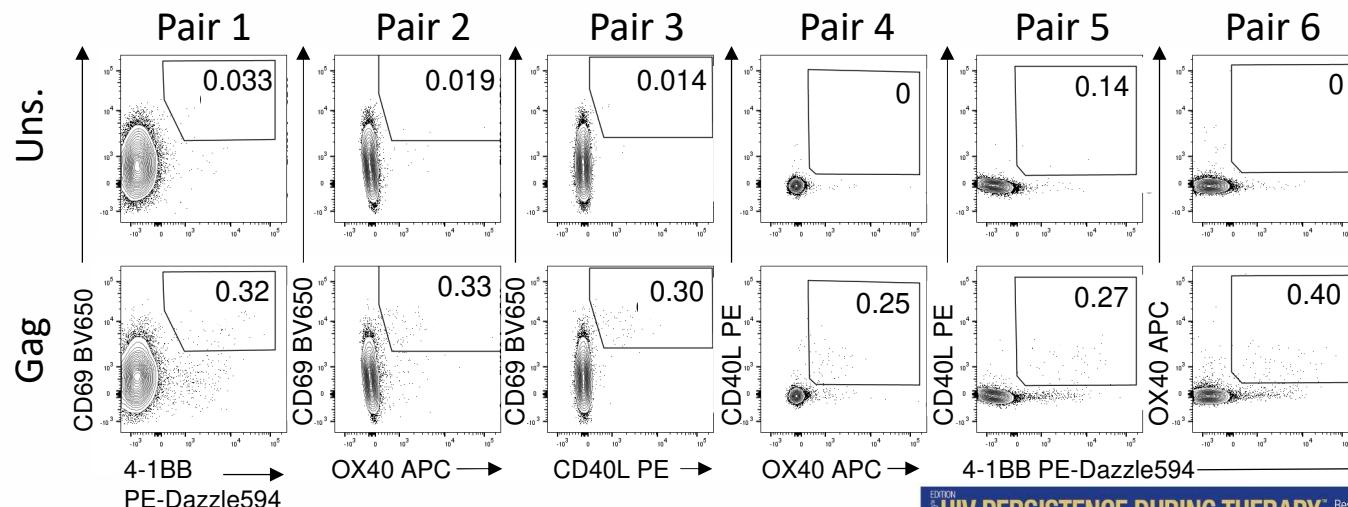


# Leaky reservoirs correlate with HIV-specific CD4 and CD8 T cells

## A. Signal from various AIM pairs

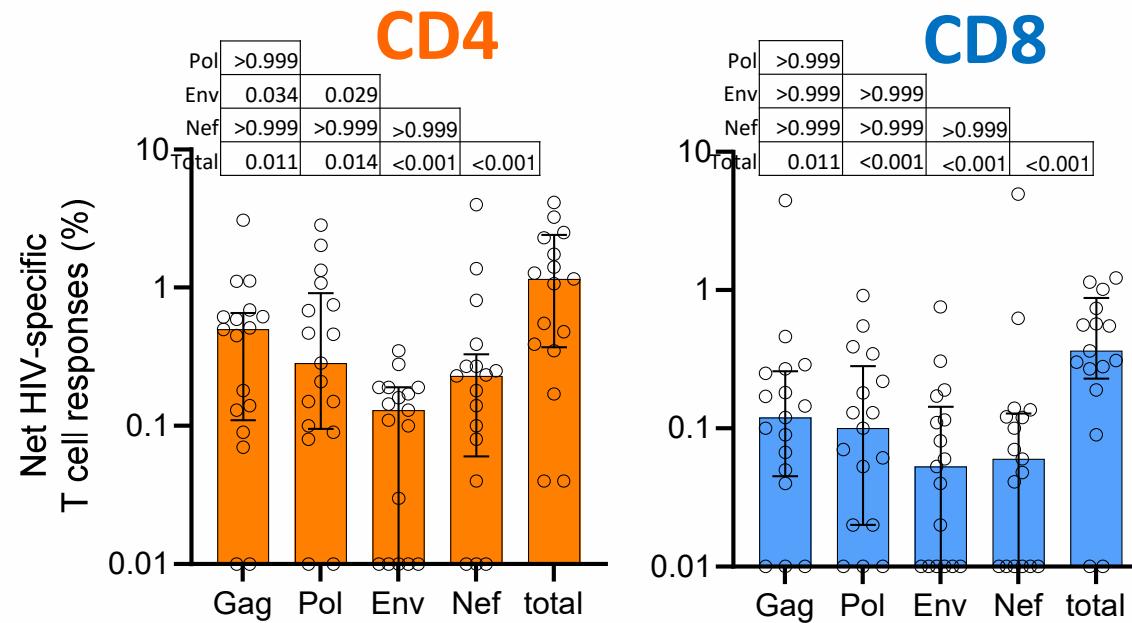


## B. Signal from various AIM pairs



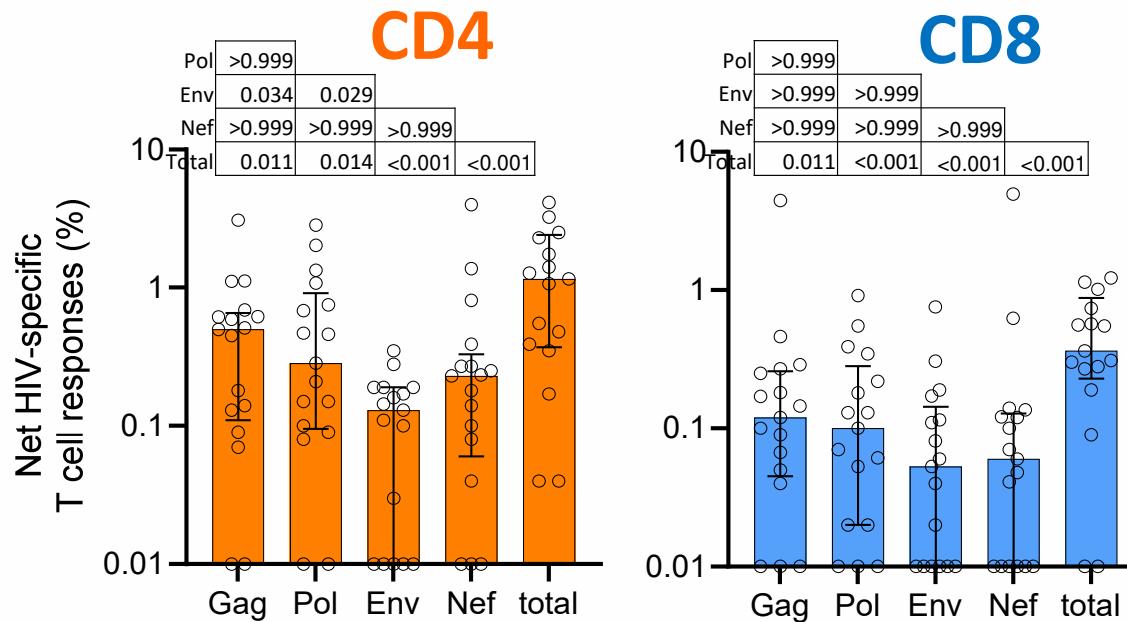
# Leaky reservoirs correlate with HIV-specific CD4 and CD8 T cells

## C. Net AIM responses

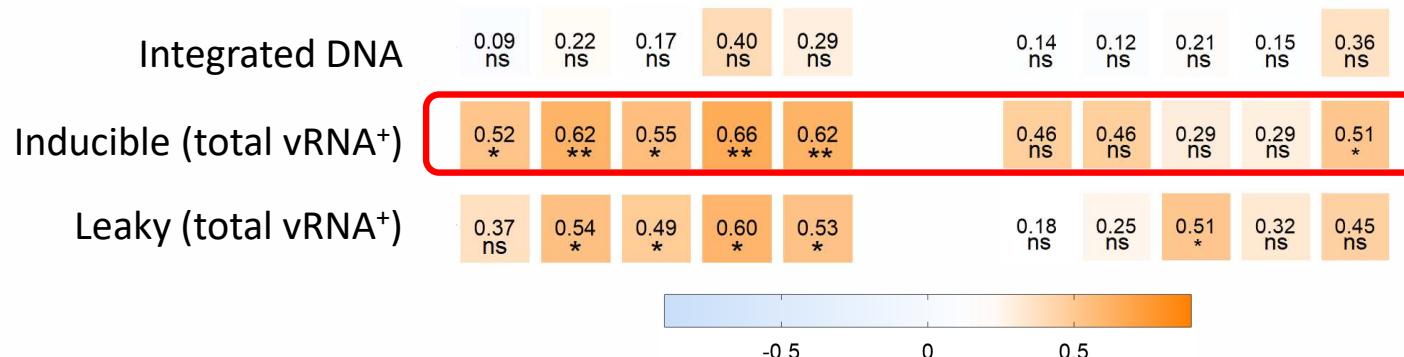


# Leaky reservoirs correlate with HIV-specific CD4 and CD8 T cells

## C. Net AIM responses

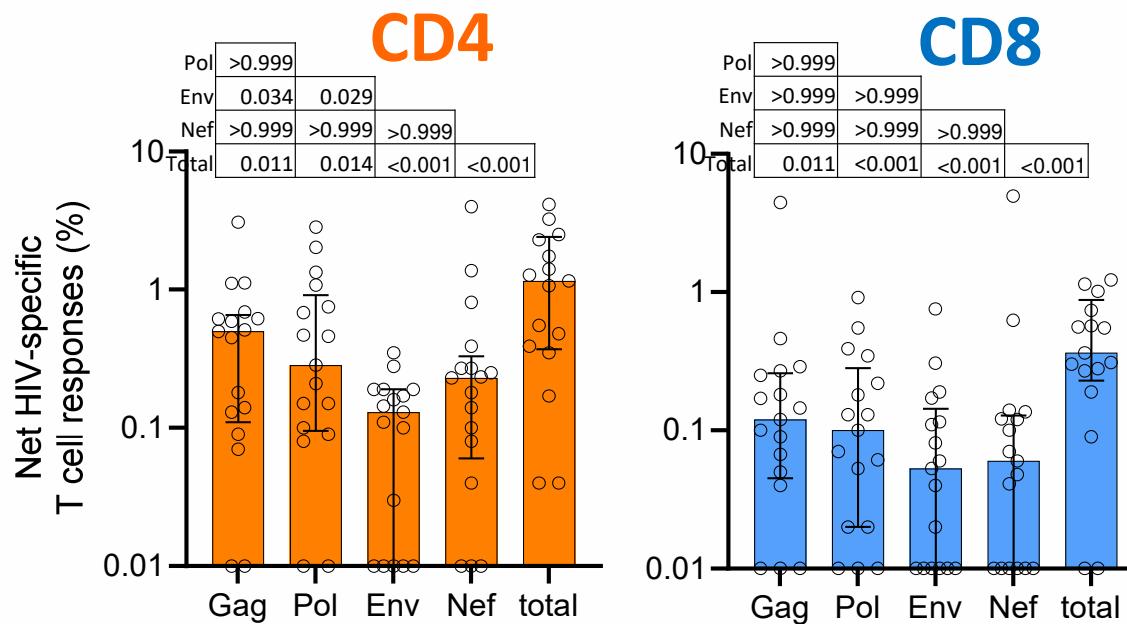


## D. Correlations



# Leaky reservoirs correlate with HIV-specific CD4 and CD8 T cells

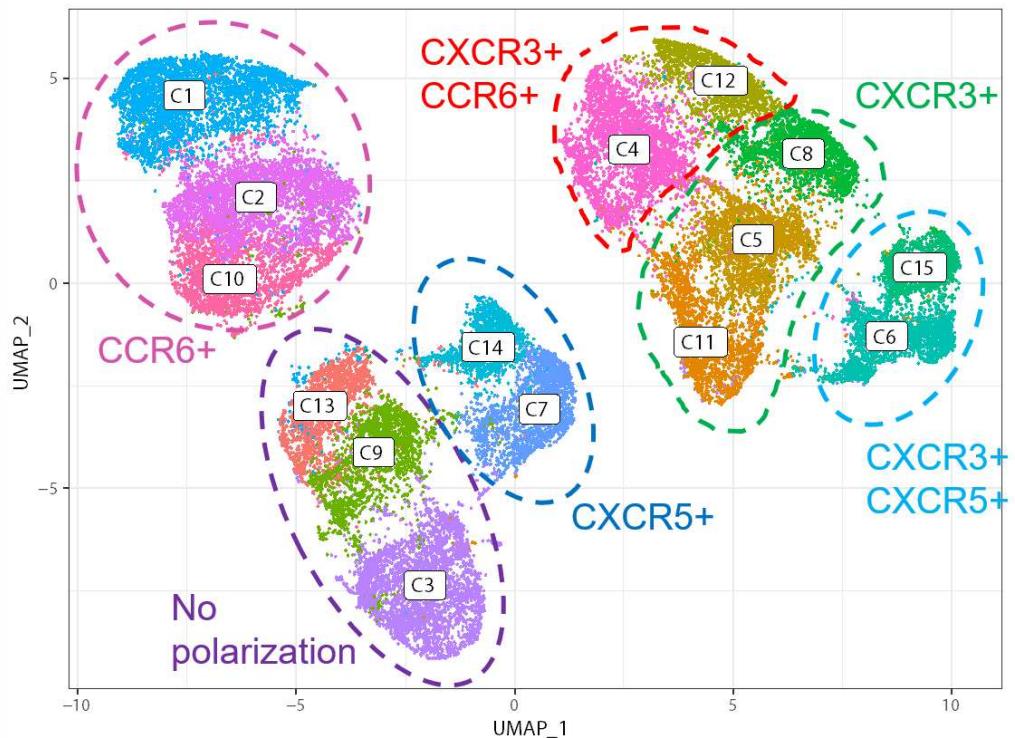
## C. Net AIM responses



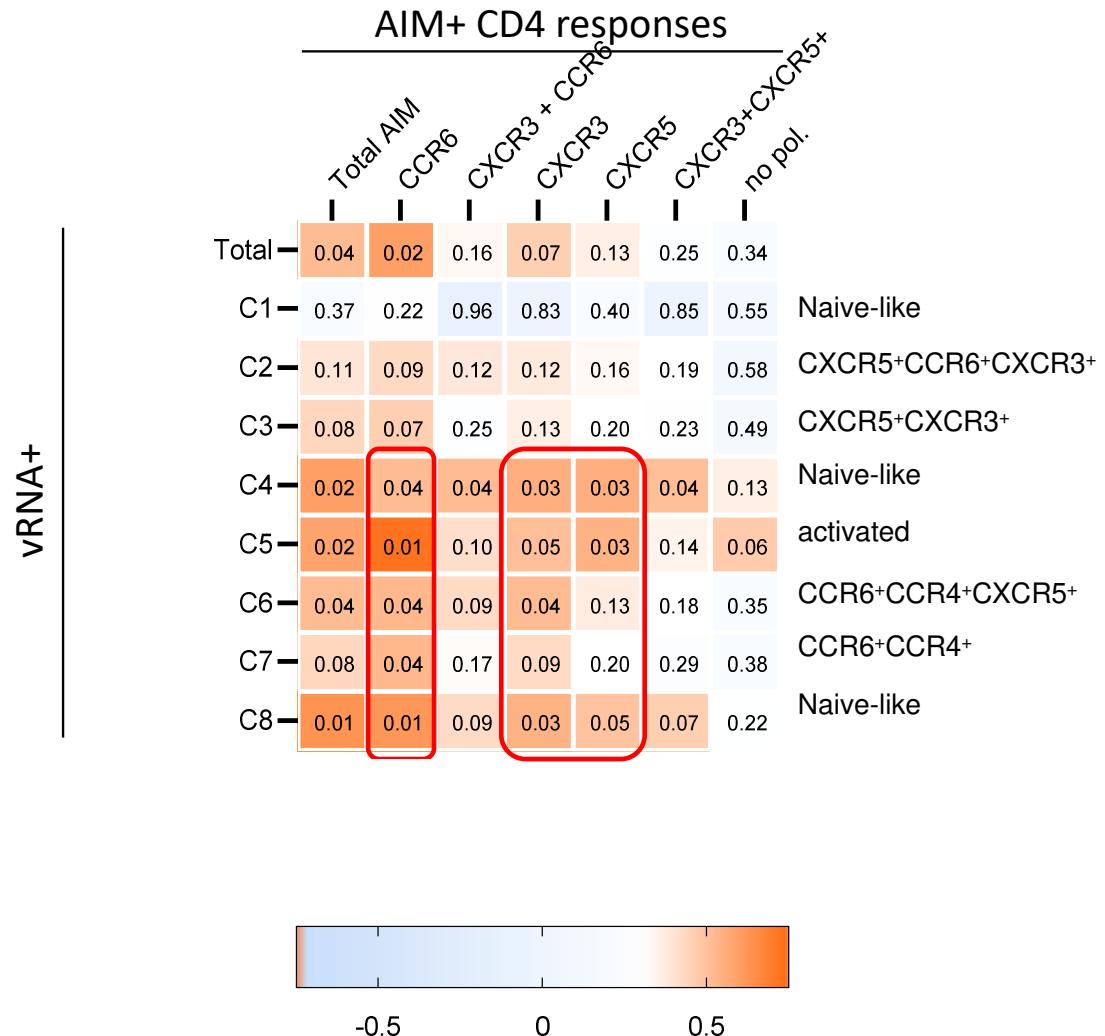
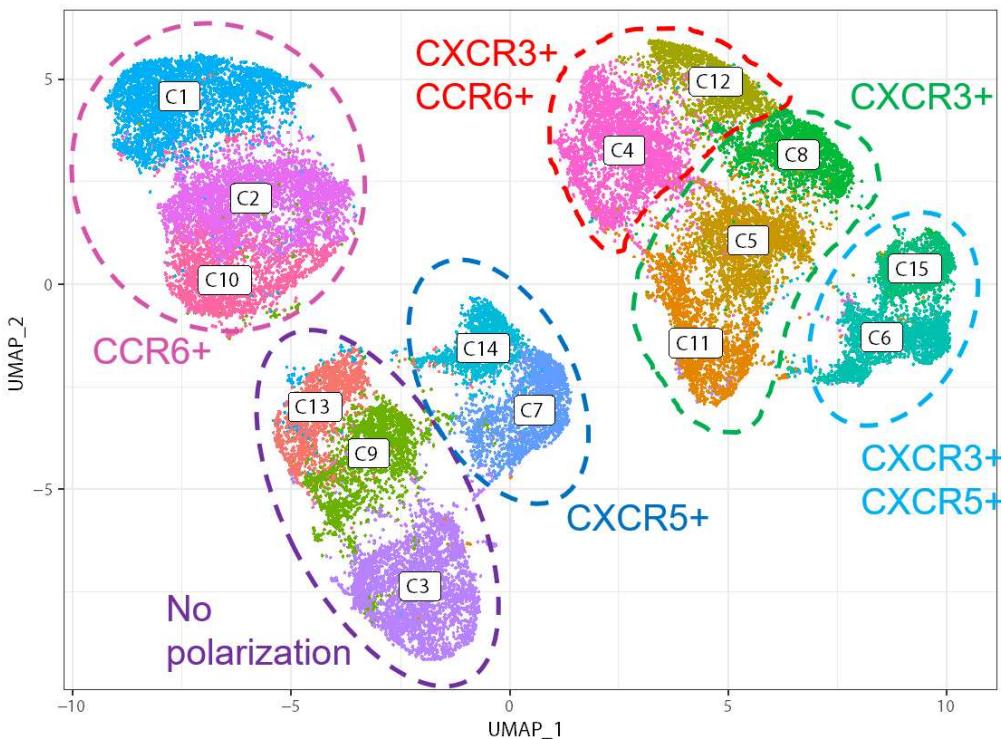
## D. Correlations



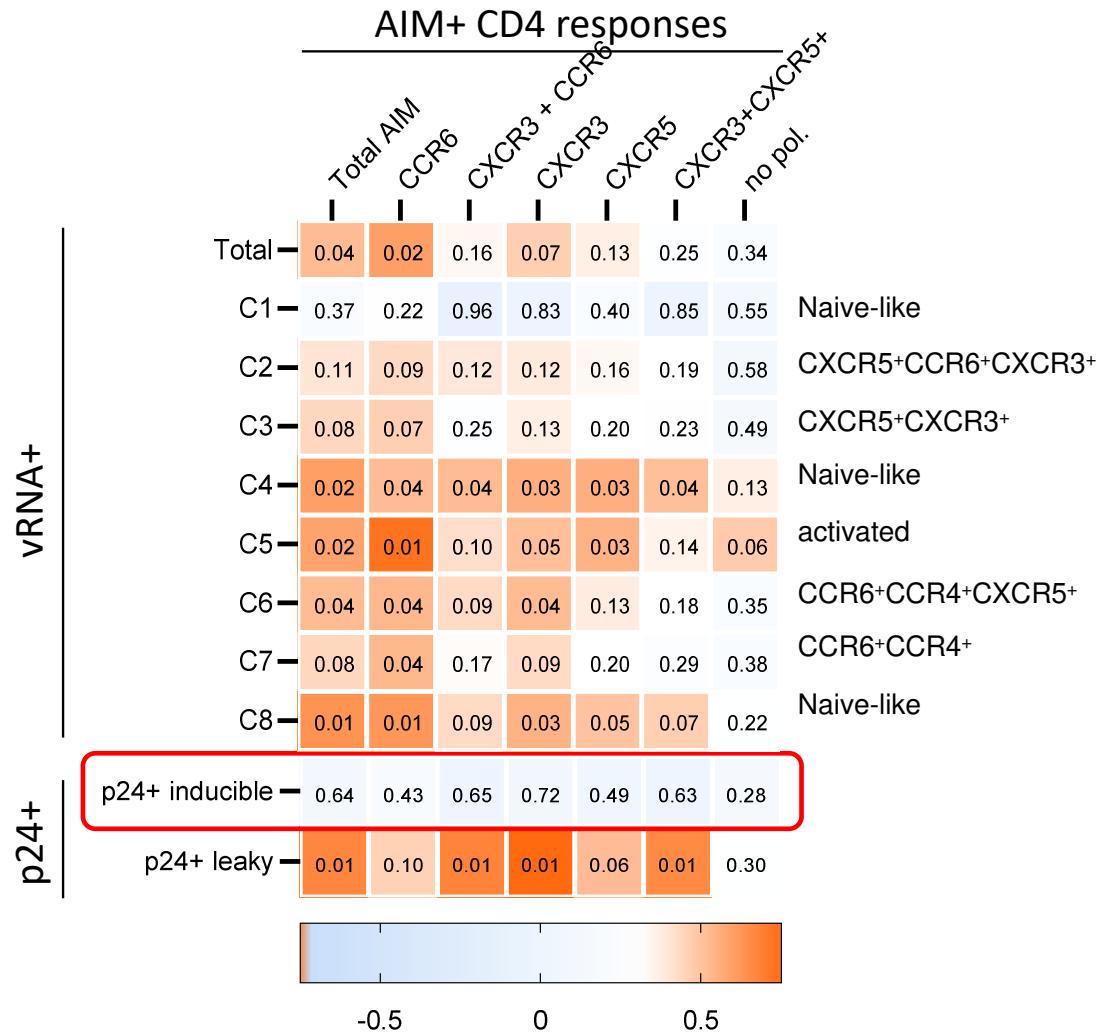
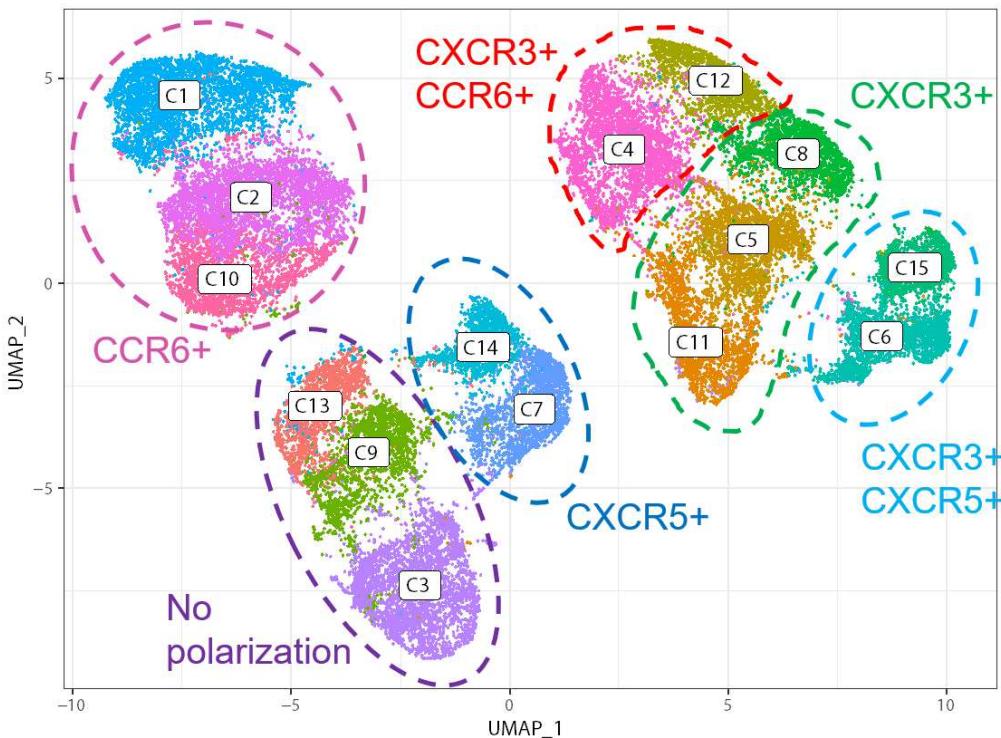
# Asymmetric network of correlations between Leaky reservoirs and CD4 T cell responses



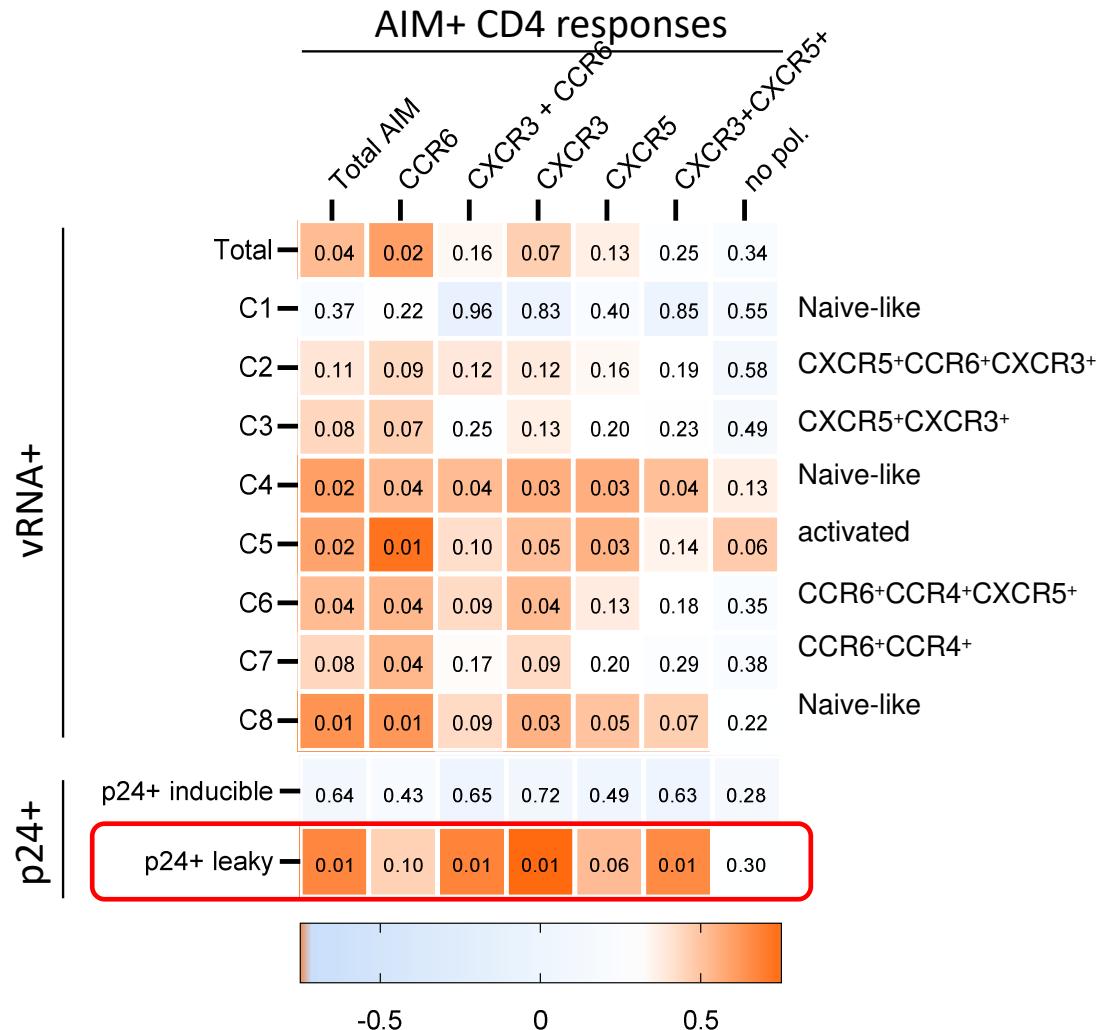
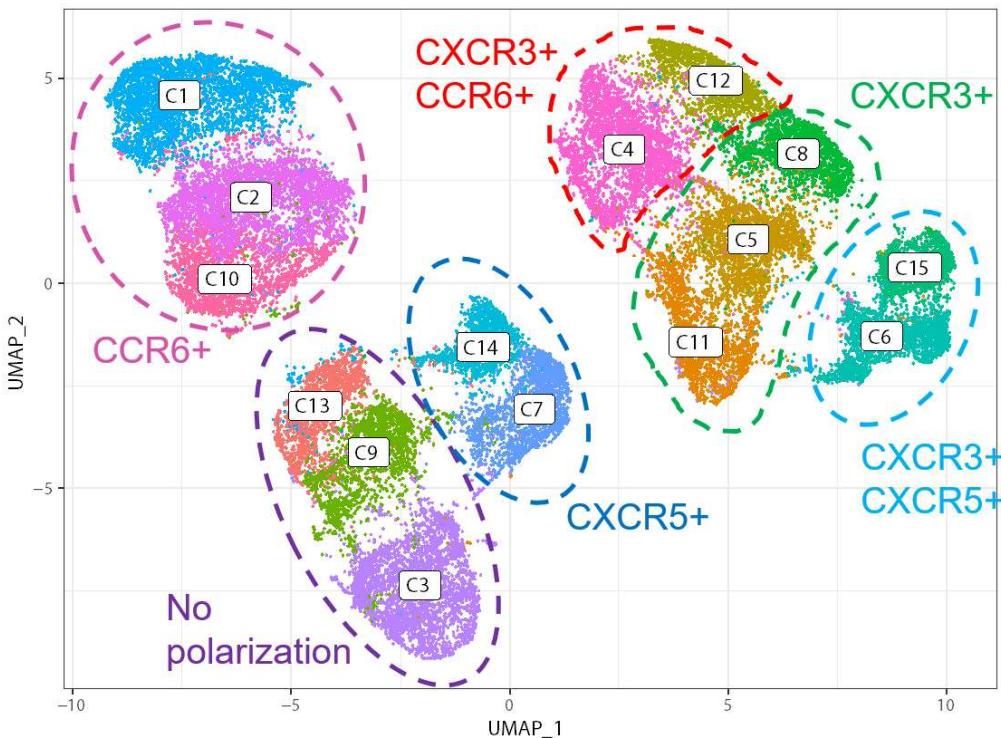
# Asymmetric network of correlations between Leaky reservoirs and CD4 T cell responses



# Asymmetric network of correlations between Leaky reservoirs and CD4 T cell responses



# Asymmetric network of correlations between Leaky reservoirs and CD4 T cell responses





## COMMUNITY SUMMARY

**Key questions:**

- What are leaky reservoirs? Do they have a biological relevance?

**Key findings:**

- Leaky reservoir cells are rare in blood (25 cell/  $10^6$  CD4), but appear detectable in most participants
- Leaky reservoirs preferentially reside in memory CD4 T cells, and enriched in CCR6+ cells
- vRNA+ and p24+ leaky reservoirs correlate with HIV-specific CD4 and CD8 T cell responses. They may maintain cellular immunity against HIV during ART.

**What next?**

- Would this mechanism be **good** (improved immunosurveillance) or **bad** (exhaustion/dysfunction)?



## Acknowledgment



CENTRE DE RECHERCHE



### Kaufmann's lab

**Daniel Kaufmann**

Géraly Sannier

Gloria Ortega-Delgado

Manon Nayrac

Amy E. Baxter

**Nathalie Brassard**

Julia Niessl

Elsa Brunet-Ratnasingham

Alexandre Nicolas

Mélanie Laporte

Jean-Pierre Routy  
Josée Girouard

## Study participants Clinical teams

### *Chomont's lab*

Nicolas Chomont

Rémi Fromentin

Amélie Pagliuzza

### *Prat's lab*

Olivier Tastet

Flow cytometry platform

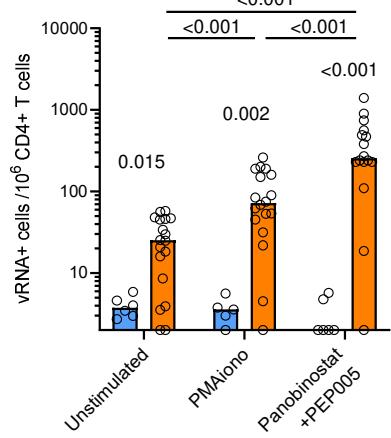
NC3 platform



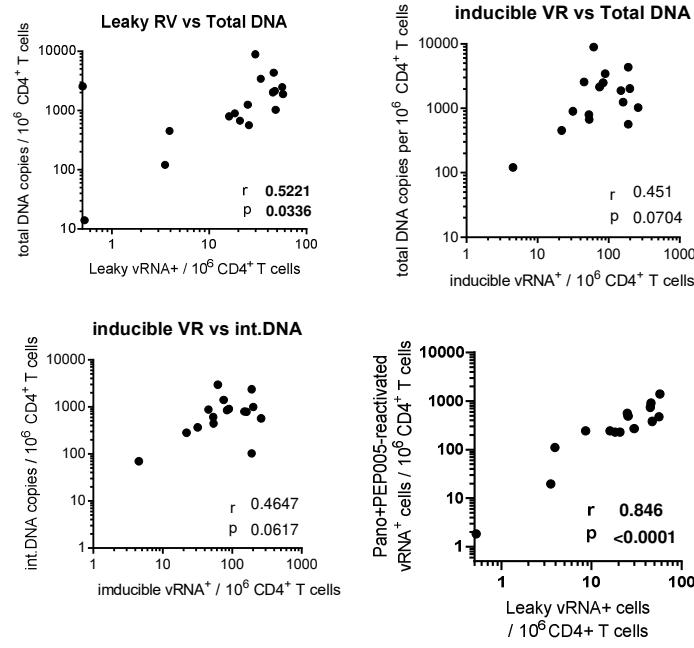


# Single-cell RNA detection by RNAflow-FISH

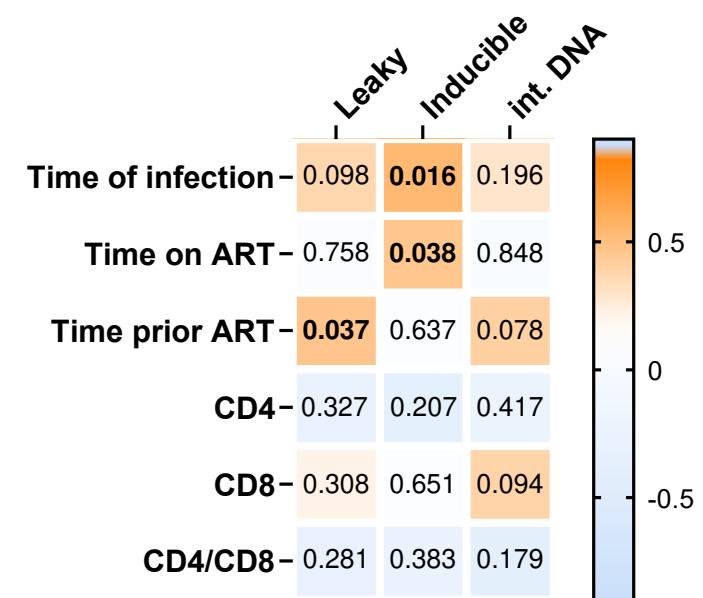
## A. vRNA+ cells quantification



## B. Association between total DNA and leaky vRNA

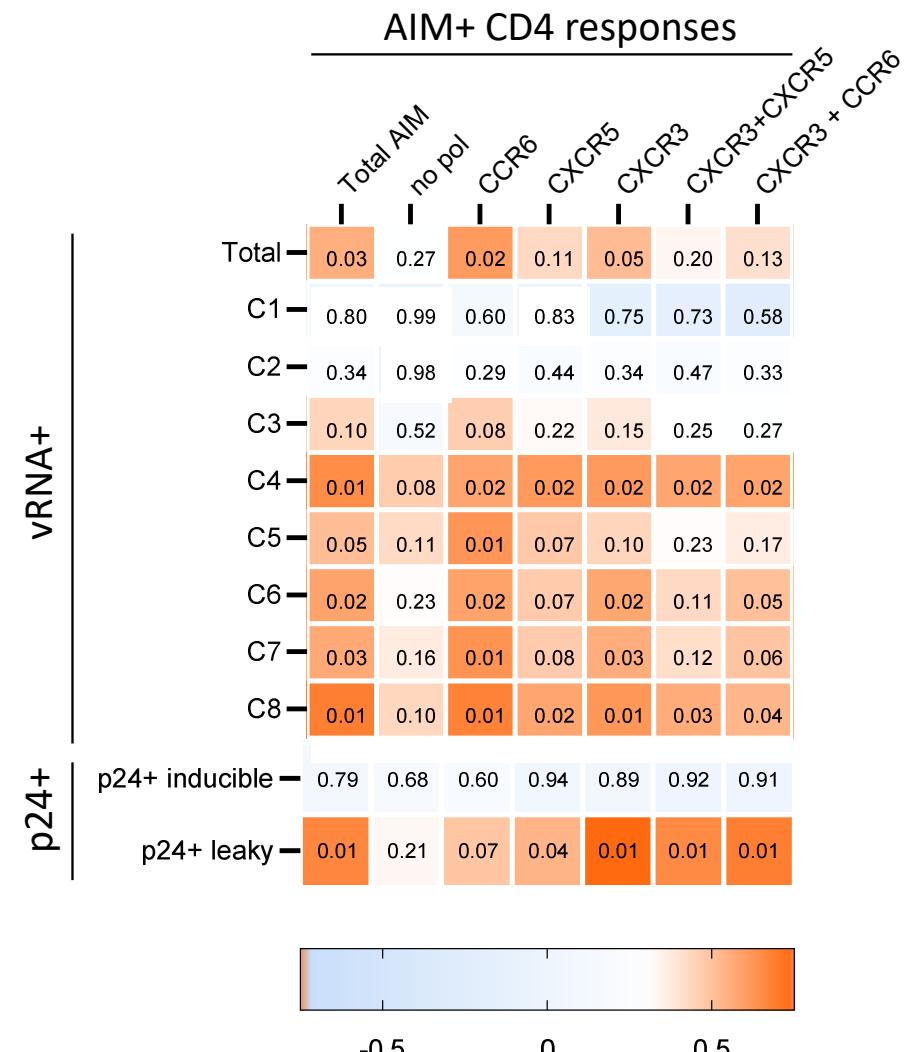
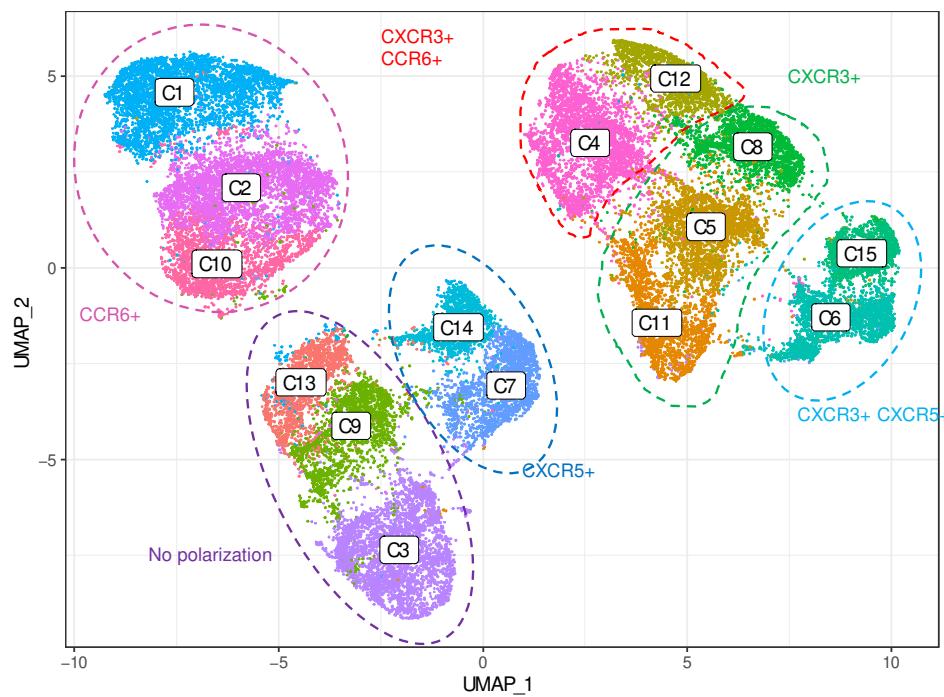


## C. Association with clinical parameters

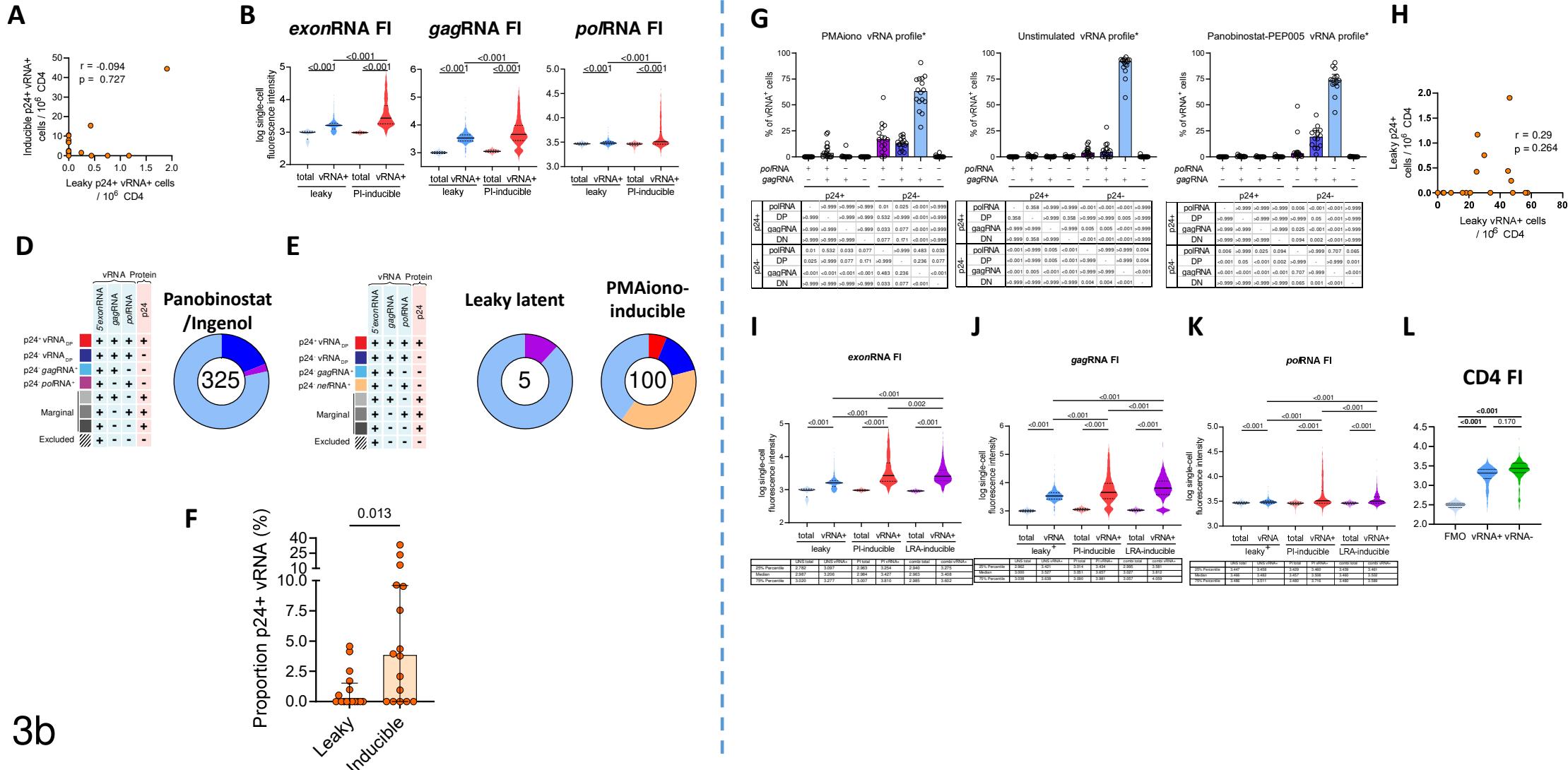


2b

# Asymmetric network of correlations between reservoirs and immune responses



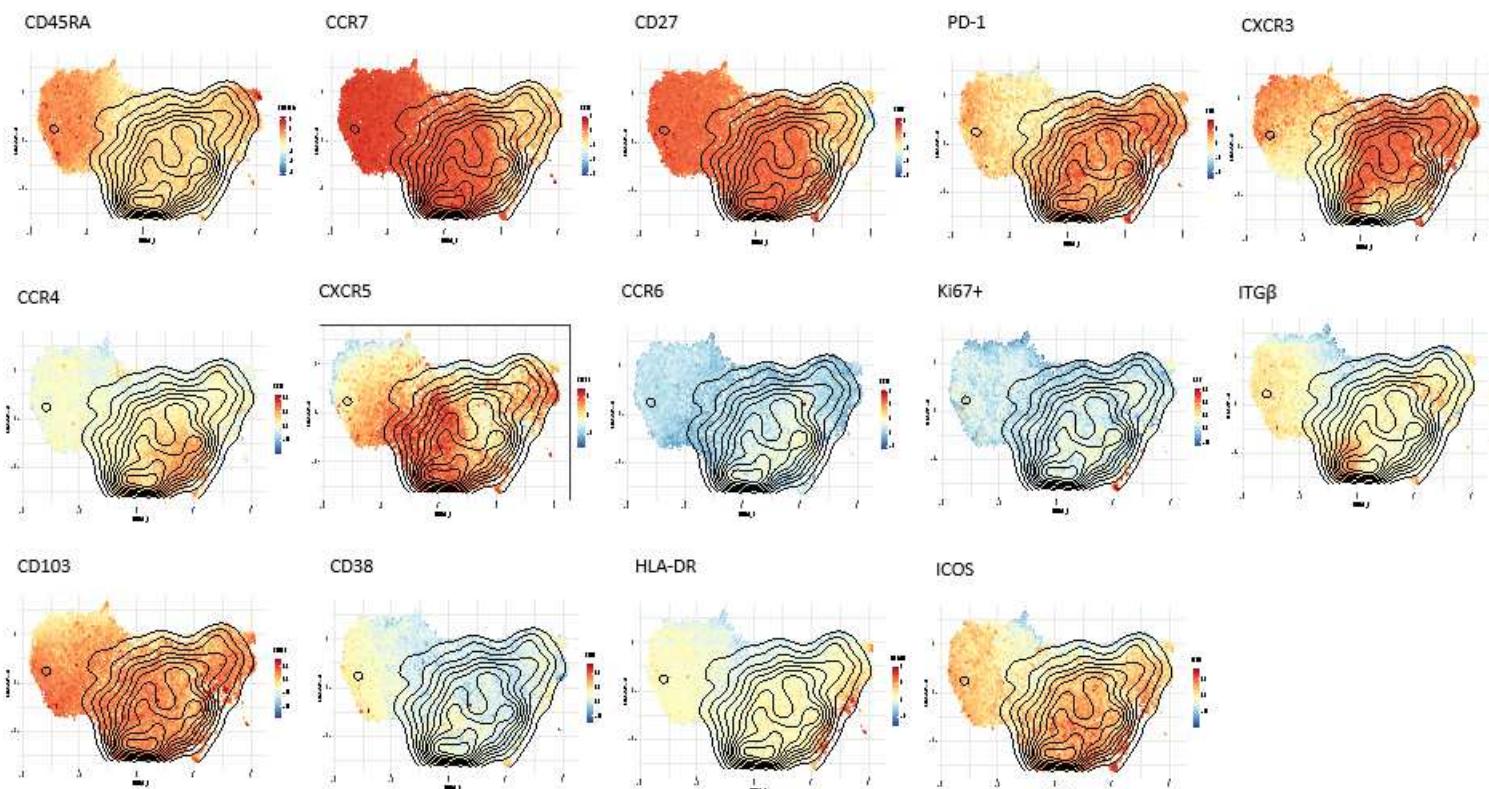
# Leaky reservoirs is dominated by abortive gagRNA+po/RNA- cells



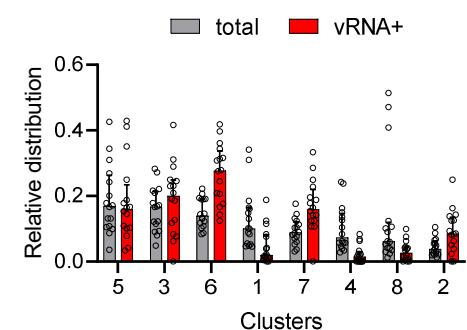
3b

# Leaky reservoirs are phenotypically diverse

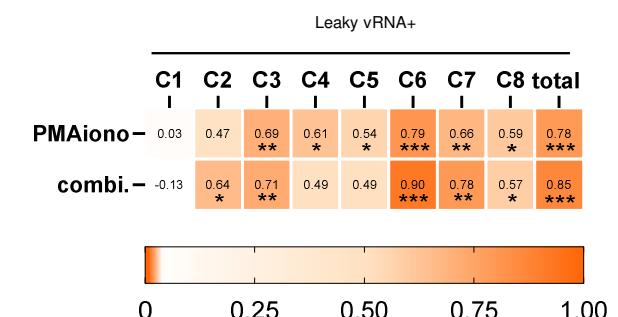
A



B

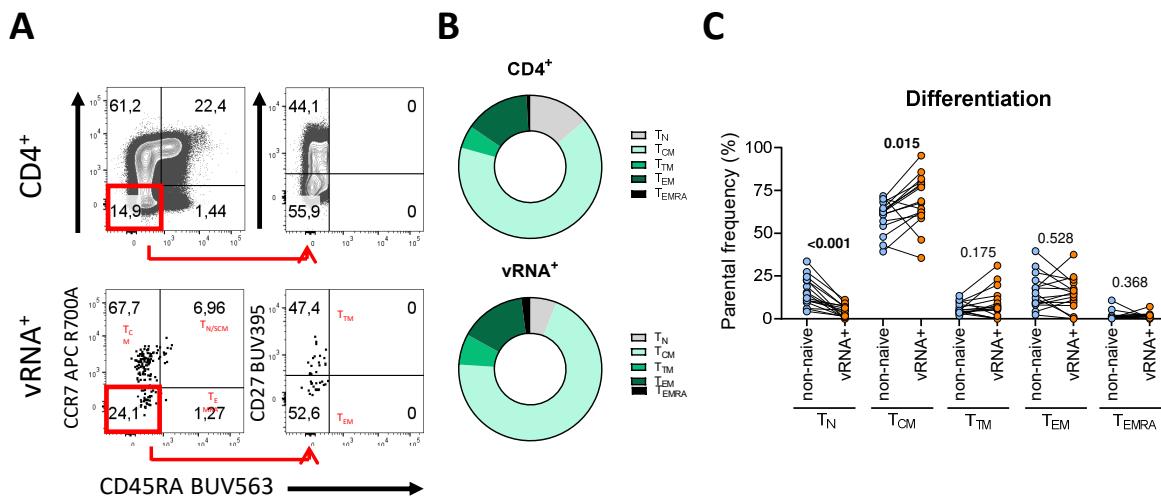


C



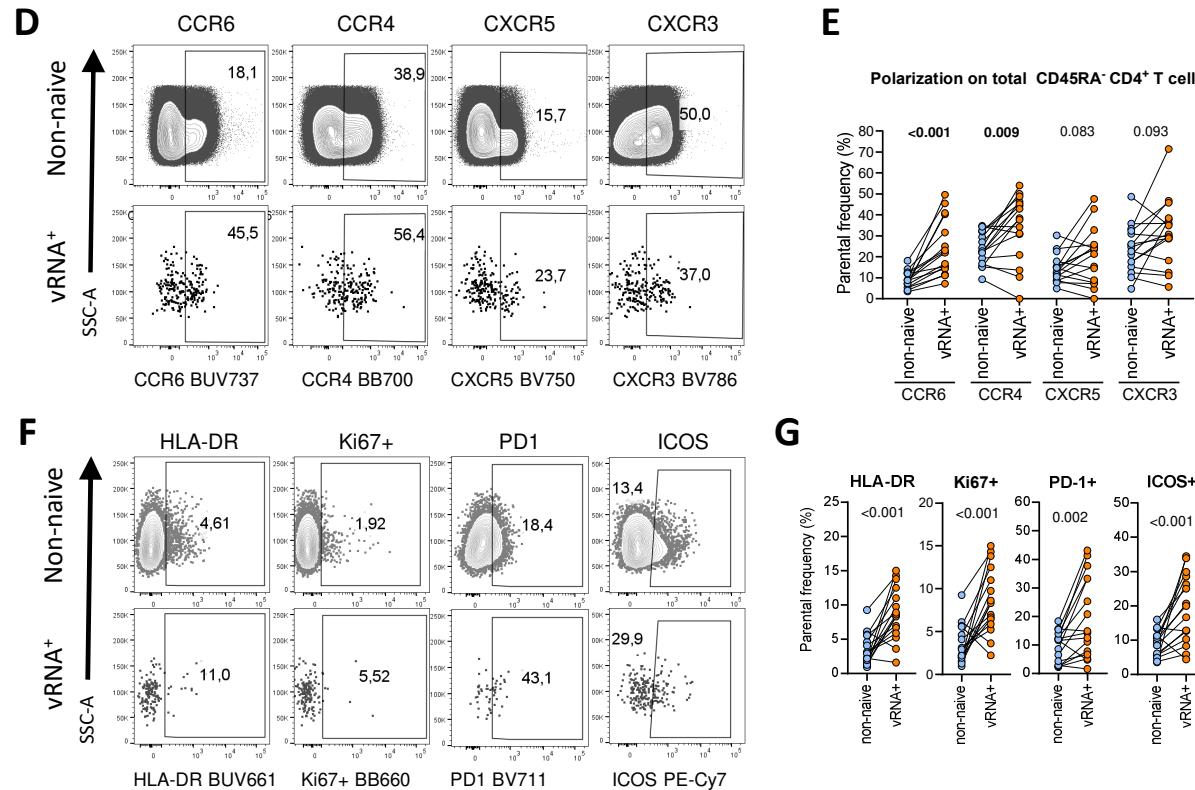
3c

# Leaky reservoirs are phenotypically diverse



4c

# Leaky reservoirs are phenotypically diverse

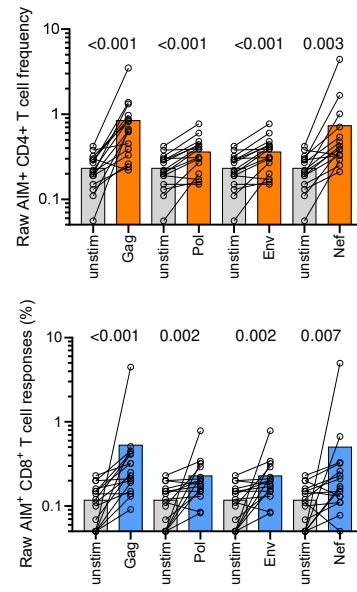


4d

# Leaky reservoirs correlate with HIV-specific CD4 and CD8 T cells

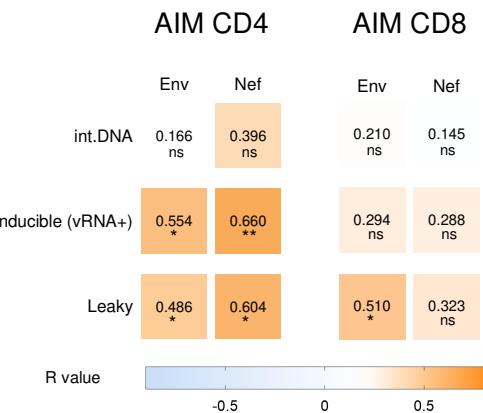
## A. Raw AIM responses

CD4



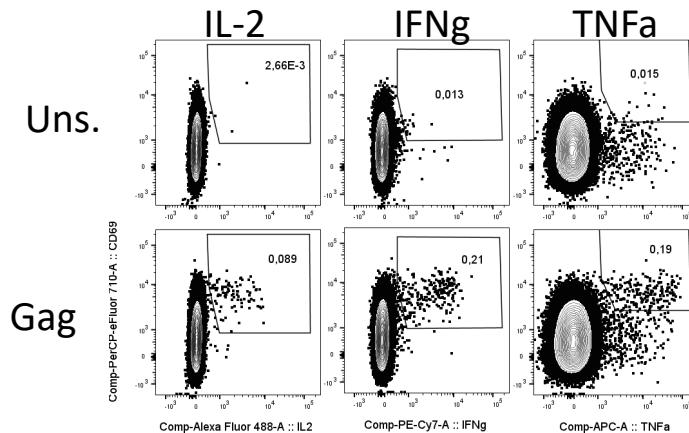
CD8

## B. Correlations



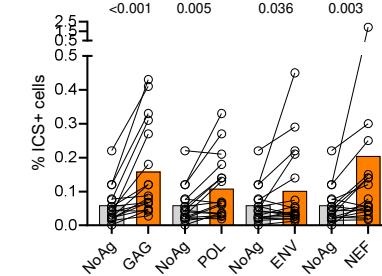
# Leaky reservoirs correlate with HIV-specific CD4 and CD8 T cells

## A. ICS gating

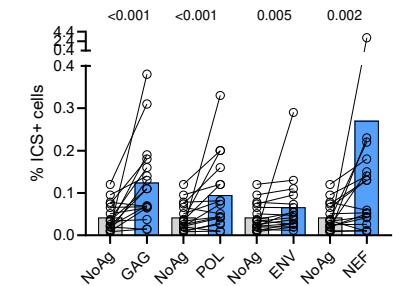


## B. Raw ICS

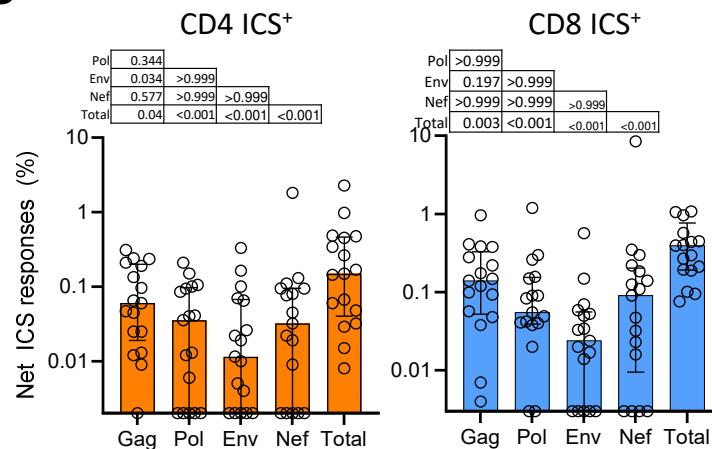
### CD4



### CD8

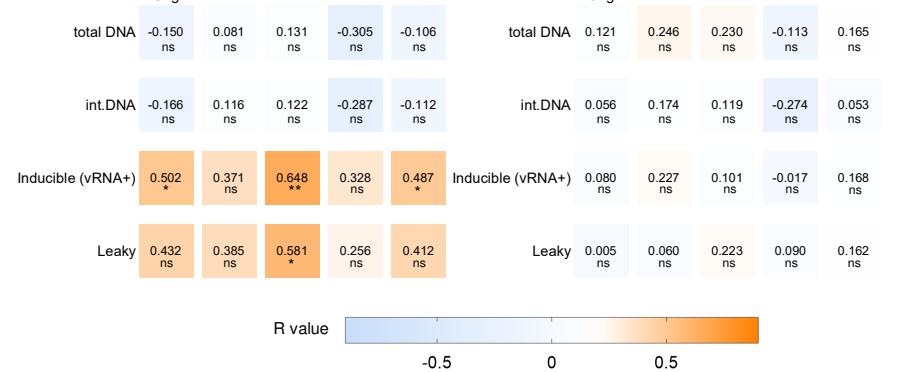


## C. Net ICS



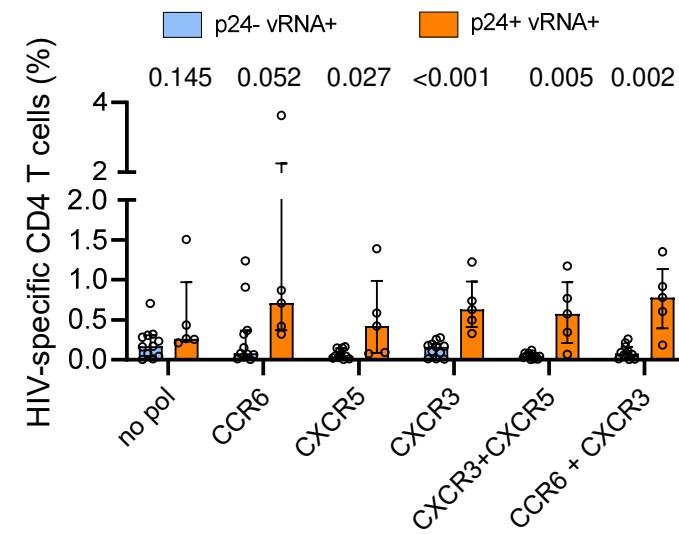
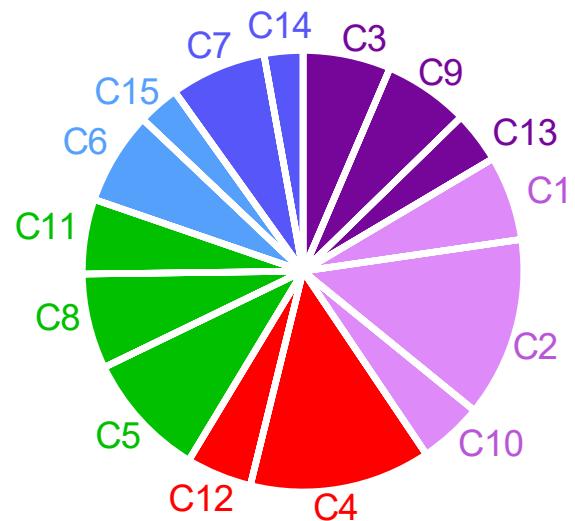
## D. Correlations

### CD4



5c

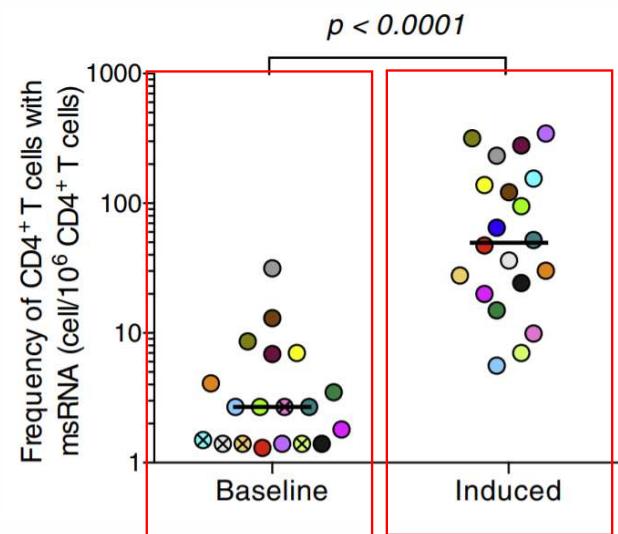
## Asymmetric network of correlations between reservoirs and immune responses



6b

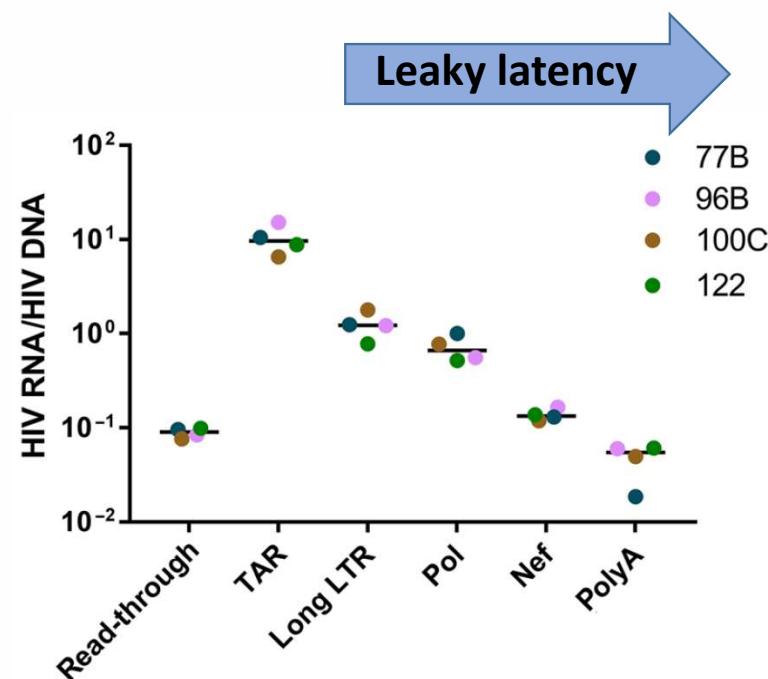
# Leaky latency

## TILDA



Procopio et al. EBioMedicine 2015

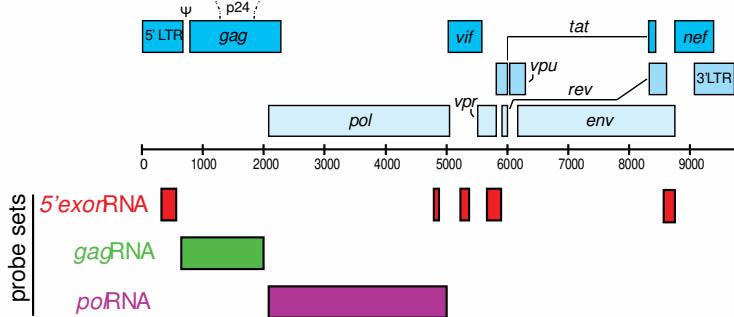
## ddRT-PCR



Yukl et al, STM 2018

# Single-cell RNA detection by RNAflow-FISH

## A. Probeset design



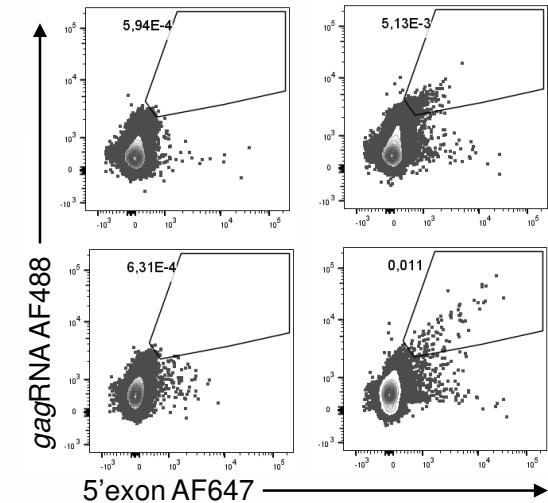
## B. vRNA+ cells gating

### Type of reservoir      Stimulation

Leaky      Unstim.

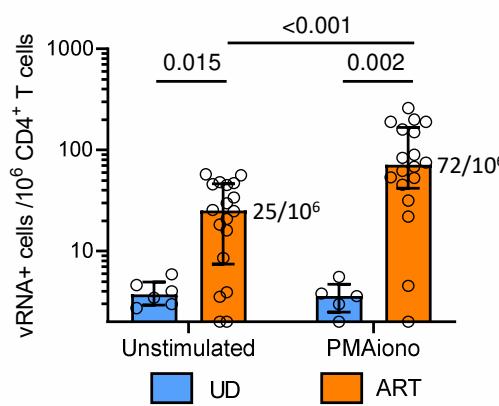
Inducible      PMA+iono.

UD      ART

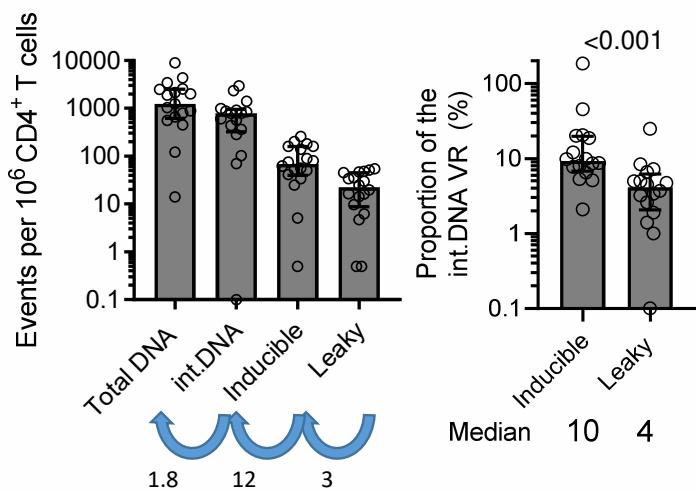


# Spontaneous vRNA expression is detectable in most participants

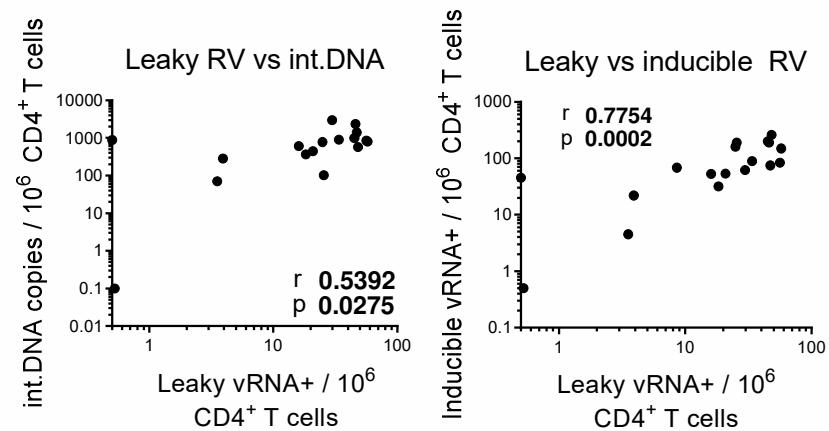
## A. vRNA+ cells quantification



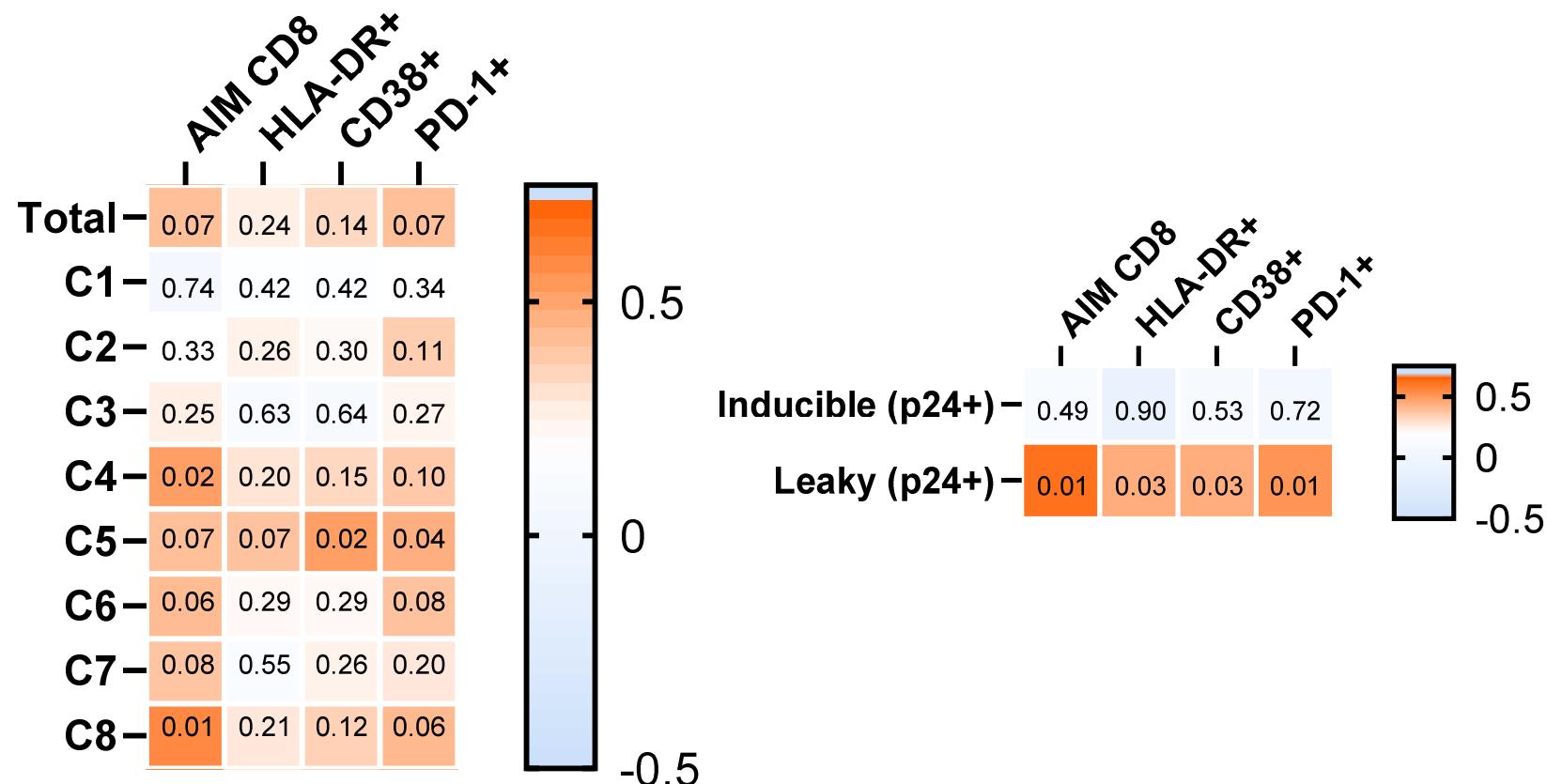
## B. Metrics comparisons



## C. Association between metrics



# Correlations with magnitude of CD8 subsets



**M** FLORIDA  
**IAIMI**  
USA

9<sup>TH</sup> EDITION

**HIV PERSISTENCE DURING THERAPY™**

Reservoirs & Eradication Strategies Workshop

**DECEMBER**  
**10-13, 2019**  
[www.hiv-persistence.com](http://www.hiv-persistence.com)