Transcriptional profiles in HIV reservoirs- new insights using HIV-seq

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Session: Discovery and Translational Science: Reservoir

Disclosure of interest: None

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

I acknowledge that we are currently in a global climate and ecological crisis and that is why our group has a strong commitment towards sustainability.

Acknowledgment

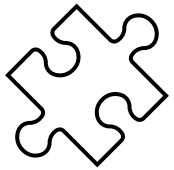
With gratitude to people living with HIV, past and present, whose contributions make this research possible

Challenges in studying the HIV reservoir

- There is currently no cure for HIV
- Despite therapy, HIV-1 persists in latently-infected CD4+ T cells
- Majority of proviruses are defective, however reservoir cells can be *transcriptionally active*

  - **low-level antigen production[1,2]**
  - **immune activation and dysfunction[3]**
  - **potential reseeding of replication-competent virus(?)[4,5]**

- Mechanisms underlying the persistence of HIV remain unclear



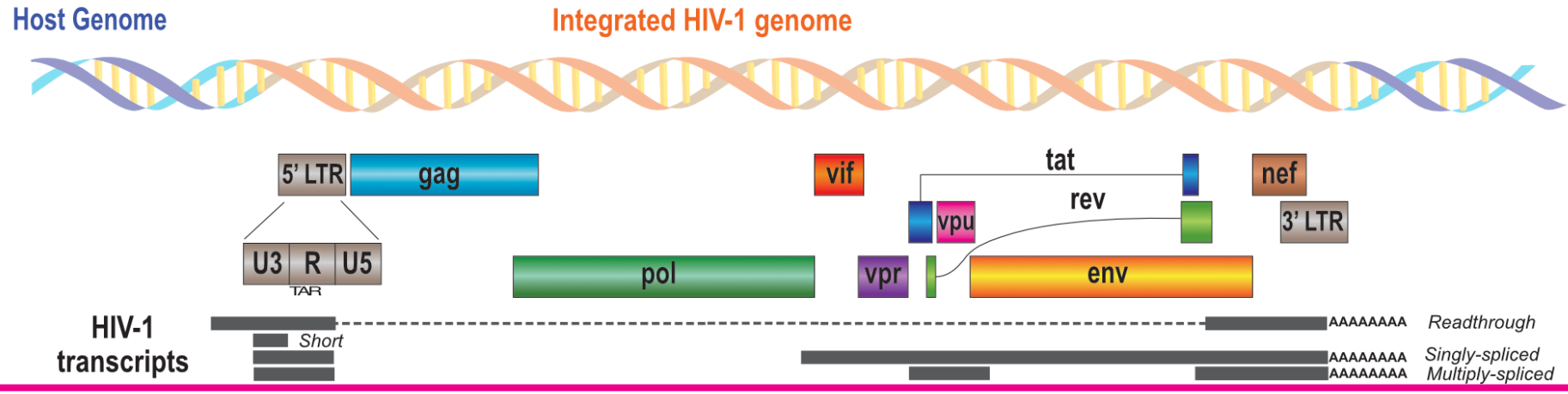
What are the blocks to HIV transcription during ART?

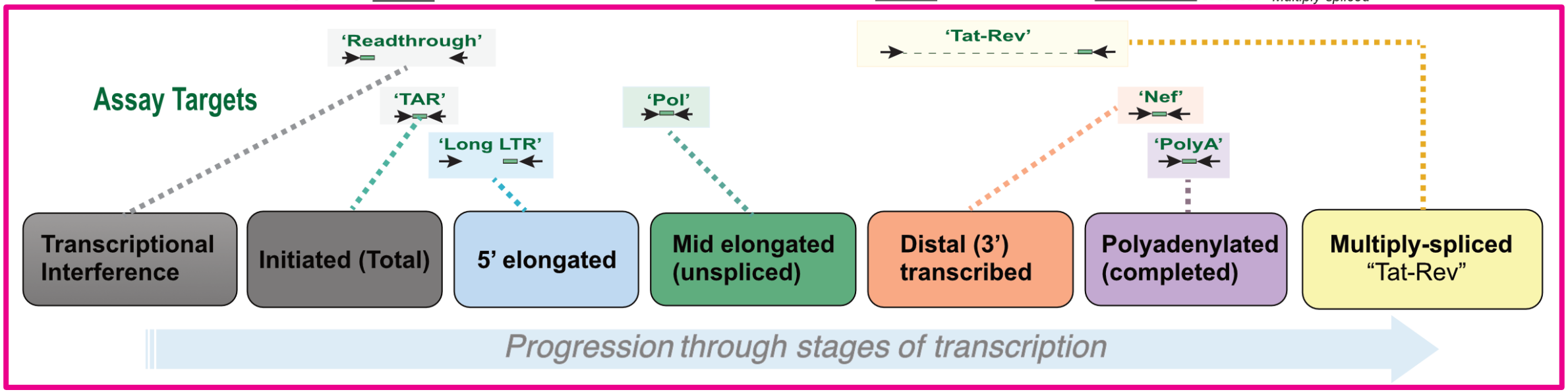
Which cellular factors are involved?

[1]Pollack Cell Host Mircobe, 2018; [2]Imamichi PNAS 2019; [3]Olson J. Infect. Dis. 2021; [4]Lorenzo-Redondo, Nature, 2018; [5]Bozzi, Sci. Adv. 2019

Understanding blocks to HIV-1 transcription

Prof. Steven Yukl, M.D. (UCSF)





Yukl et al., Science Trans Med, 2018; Telwatte et al., PLoS Path. 2018

HIV-1 transcription in the blood and gut-challenges for single cell analyses

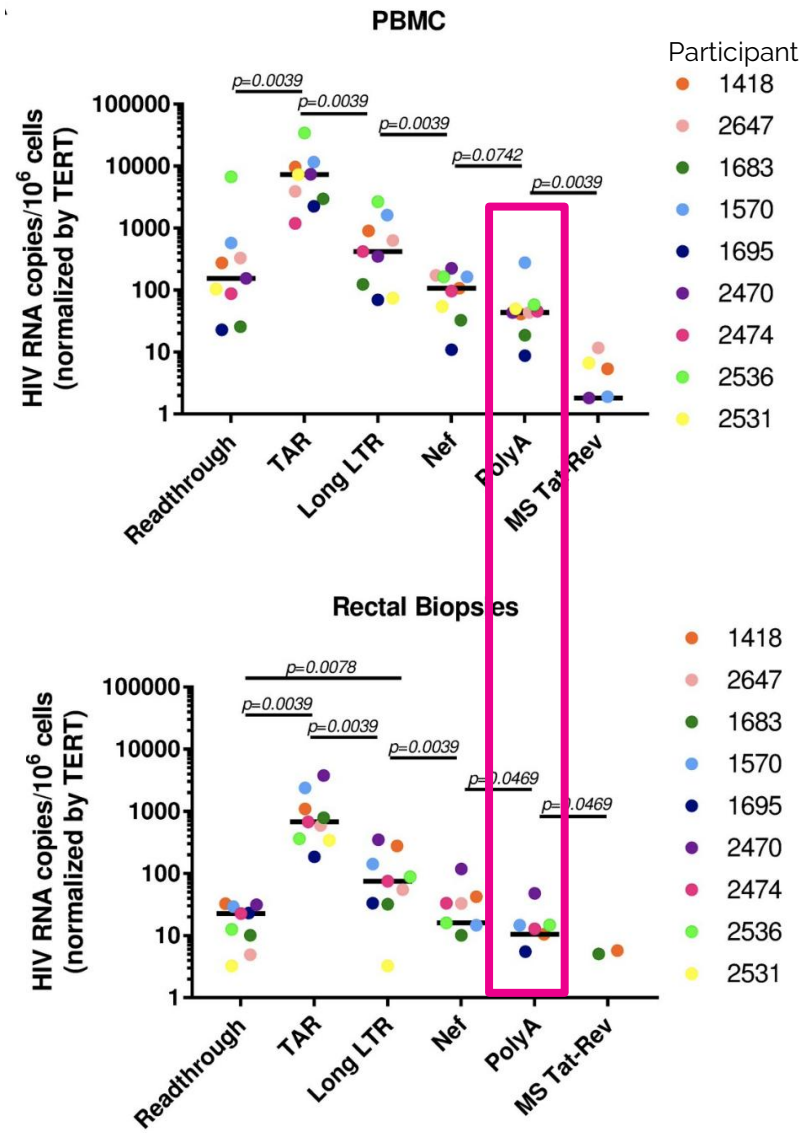
Infected cells from people living with HIV (PWH) exhibit successive blocks to HIV transcription

Blocks to transcription→ inability to complete viral life cycle

Found that only a small proportion of HIV transcripts are polyadenylated

scRNAseq relies on priming at the poly(A) tail

Need for new targeted capture approaches to sensitively identify transcriptionally active reservoir cells



Telwatte et al. 2018 PLoS Path.

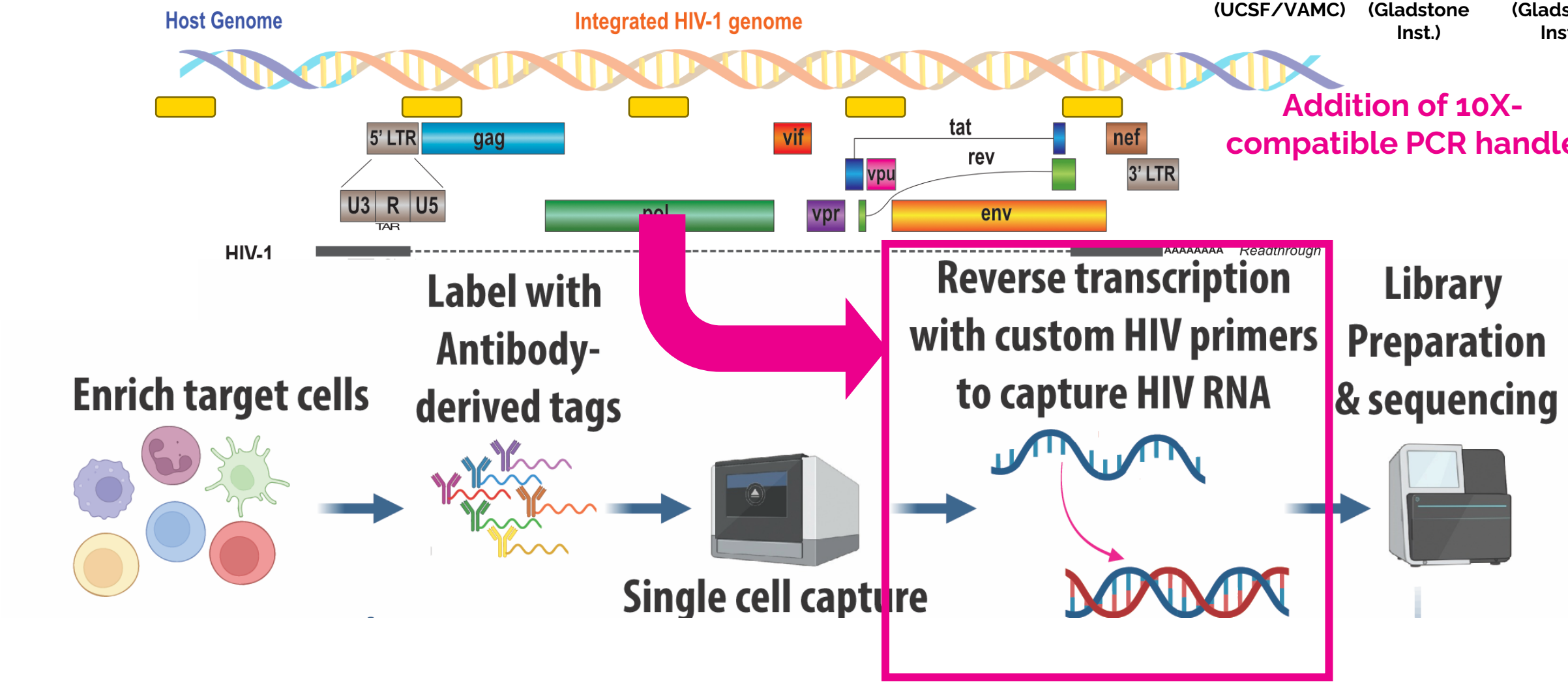
How can we study rare HIV-infected cells under ART?

Development of HIV-seq

Prof. Steven Yukl, M.D. (UCSF/VAMC)

Prof. Nadia Roan, Ph.D. (Gladstone Inst.)

Dr. Julie Frouard, Ph.D. (Gladstone Inst.)



Frouard & Telwatte, bioRxiv, 2024; Psi/RRE primers from Bruner et al, Nature 2019

Study design and cells analysed

Prof. Steven Deeks, M.D (UCSF)

A/Prof. Sulggi Lee, M.D (UCSF)

Cohorts: TreatAcute and SCOPE (San Francisco, USA)

Total Cells	HIV RNA+ cells	HIV RNA+ cells (ART suppressed)	Infected cell frequencies	HIV transcript levels (reads/cell)
85,667	1,072	**25***	0.061% - 2.42%	1 – 1063*
		**highest # of cells analysed*		HIV_{low}: 1-50 copies HIV_{high}: 51-200 copies
		Pre-ART viremic 'Week 0'	On ART suppressed 'Week 24/45'	Pre-ART viremic 'Week 0'

Differentially expressed proteins at single cell level

Cellular Indexing of Transcriptomes and Epitopes by Sequencing ('CITE-seq'): gene expression (transcriptome) and surface protein levels (epitopes) within the same cell

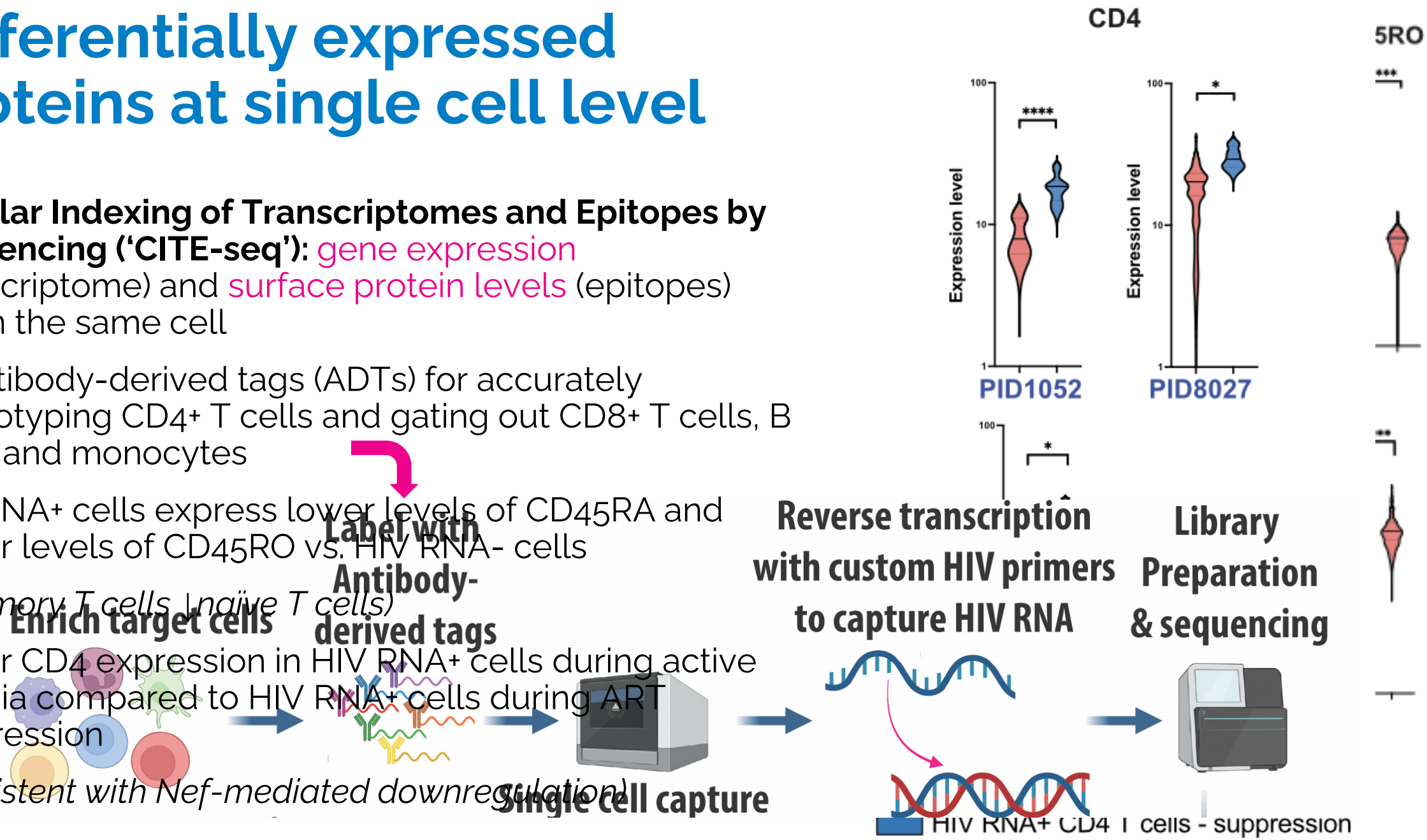
16 antibody-derived tags (ADTs) for accurately phenotyping CD4+ T cells and gating out CD8+ T cells, B cells, and monocytes

HIV RNA+ cells express lower levels of CD45RA and higher levels of CD45RO vs. HIV RNA- cells

(↑memory T cells ↓naïve T cells)

Lower CD4 expression in HIV RNA+ cells during active viremia compared to HIV RNA+ cells during ART suppression

(consistent with Nef-mediated downregulation)



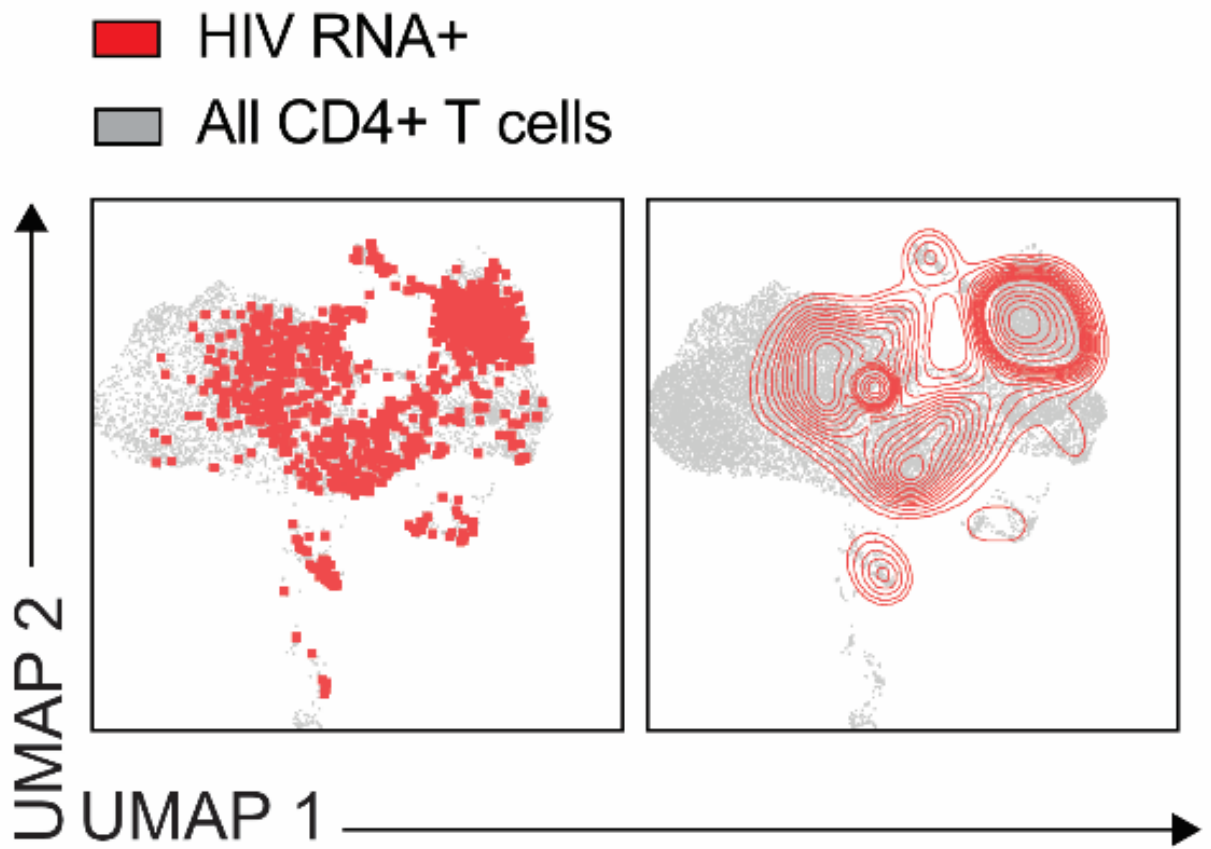
HIV RNA+ cells exhibit a heterogeneous phenotype but are enriched among Tem cells

HIV RNA+ cells:

- **overrepresented** in T effector memory
- **under-represented** among central memory (Tcm) cells and those of the CCR7+CD27- phenotype

Consistent with prior scRNAseq data[1,2]

Transitional memory (Ttm), regulatory T cells (Treg), and T follicular helper (Tfh) cells were equally represented among uninfected and HIV RNA+ CD4+ T cells



[1]Collora Immunity 2022; [2]Geretz Sci Transl Med. 2023

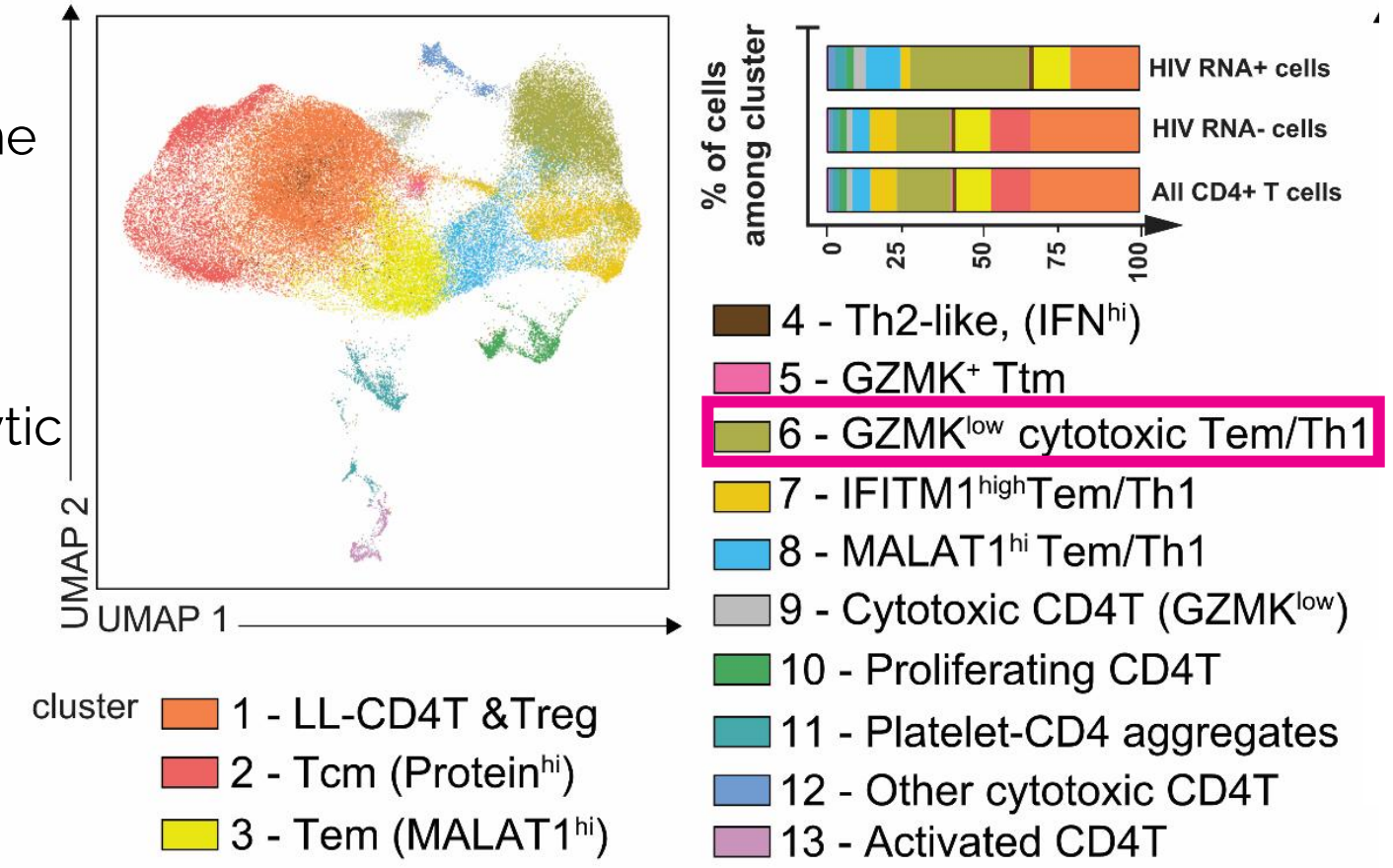
HIV RNA+ cells during viremia are enriched among cells with a cytotoxic/Th1 phenotype

Louvain clustering identified 13 clusters

Classical CD4+ T cell subsets did not define the clusters

HIV RNA+ cells from viremic PWH were enriched in Cluster 6:

- high expression of cytotoxic and cytolytic genes, including *GZMA, GZMB, GZMH, GZMM, PRF1, GNLY, NKG7*
- Th1-defining factors *IFNG* and *TBX21*
- Cytotoxic Th1 phenotype[1,2]



[1]Collora Immunity 2022; [2]Geretz Sci Transl Med. 2023

Upregulation of both NFAT, PKC, and chemokine signalling pathways in HIV RNA+ cells

Upregulation of the NFAT pathway (*MAF*, *CLTA4*) in HIV RNA+ cells

- consistent with NFAT as a driver of HIV transcription[1,2]

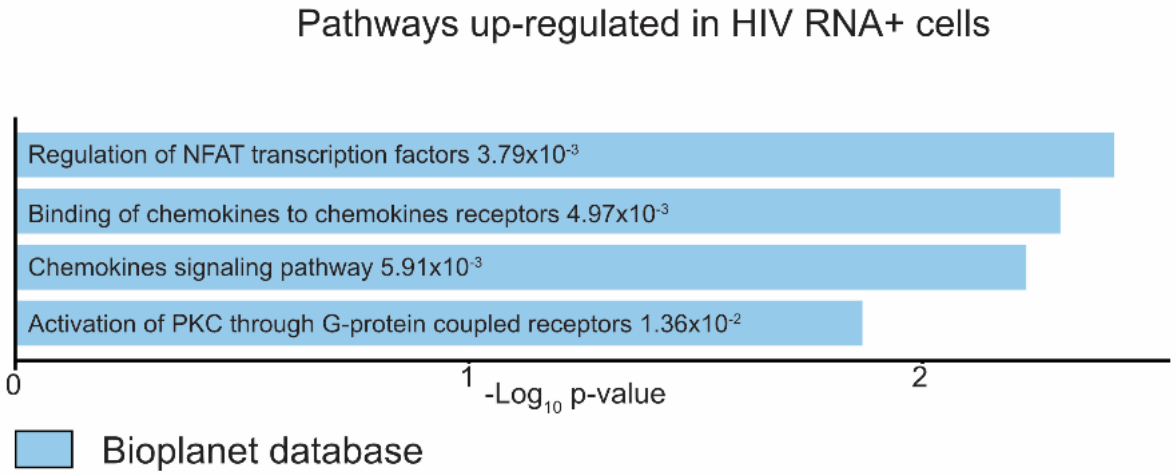
Upregulation of the PKC pathway

- involved in HIV gene expression and latency reversal[3,4]

Upregulation of chemokine signaling pathways (*CXCR6*, the CXCR6 ligand *CXCL13*, and *PLCB1*)

Cellular state conducive to viral replication

E.



[1]Robichaud, J. Biol. Chem. 2002; [2]Bosque, Blood 2009; [3]Nguyen, Front. Immunol, 2015, [4]Telwatte, AIDS 2020

Lower restriction factor expression in HIV RNA+ vs. HIV RNA- cells from same individuals

Table 4. Differentially expressed genes between HIV RNA+ cells vs. HIV RNA- cells during viremia (n=4)

Gene	Log FC	FDR
APOBEC3A	-4.074	4.36E-07
SERPINA1	-3.813	1.40E-13
IFITM3	-1.026	1.14E-08

Lower restriction factor expression may be conducive to HIV persistence and likely favors HIV transcription at the single-cell level

*Unable to assess sex-based differences

Frouard & Telwatte, et al., bioRxiv 2024

HIV RNA+ cells under ART suppression show anti-inflammatory signature

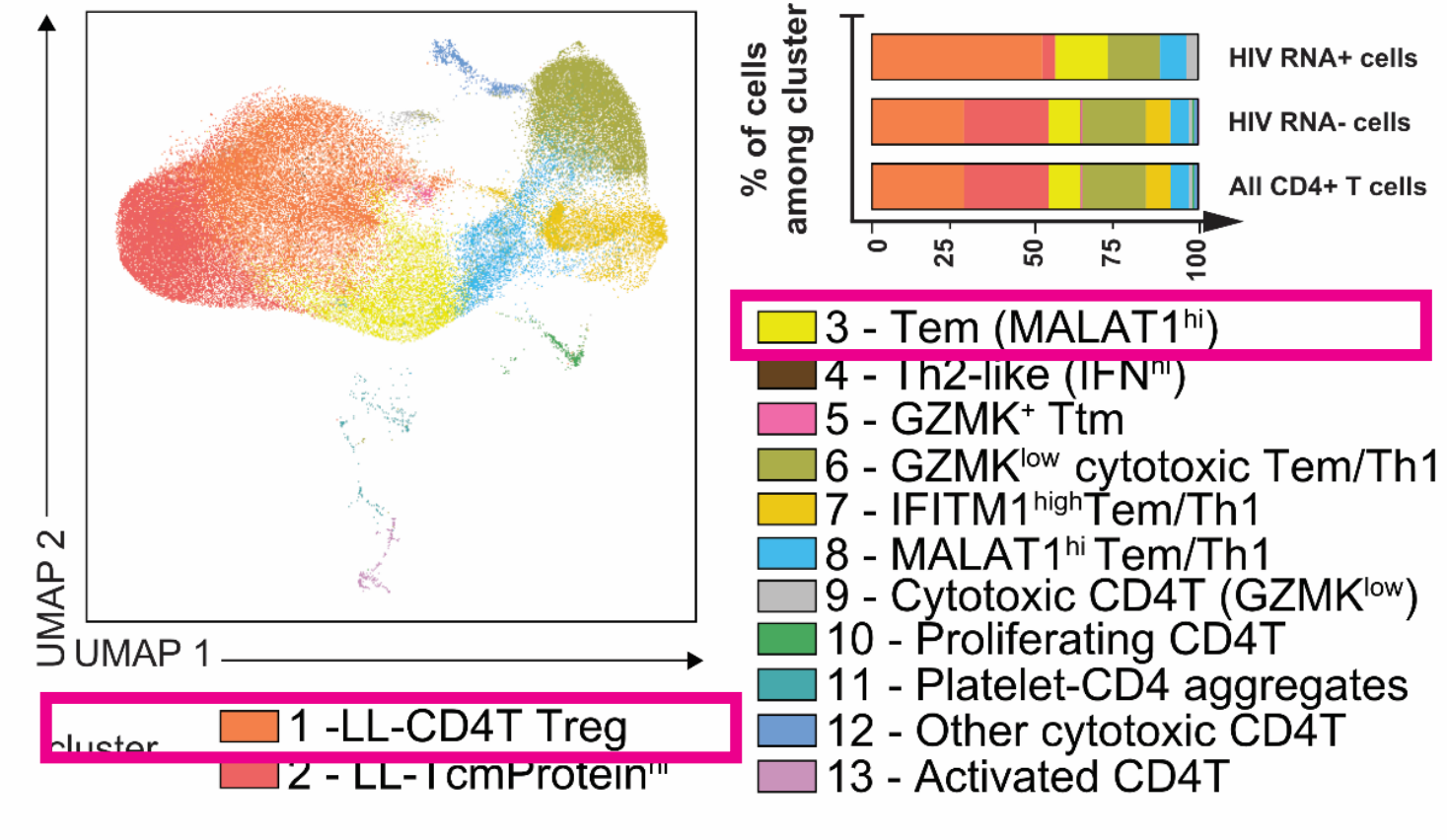
HIV-transcribing cells from ART-suppressed timepoints:

Cluster 1 (long lived CD4+ T cells) & **Cluster 3** (Tem MALAT1^{high})

Distinct anti-inflammatory signature:

- elevated TGF-β
- diminished IFN signaling

HIV RNA+ cells during ART suppression do not show a cytotoxic phenotype (as found during viremia)



Summary

HIV RNA+ cells from viremic PWH are heterogeneous but exhibit shared features

- More likely to be effector memory T cells (Tem)
- Display previously undescribed state more conducive to viral replication, with low expression of restriction factors and increased activation of cellular pathways promoting HIV gene expression
- Exhibit a cytotoxic signature characterized by higher expression of granzymes, perforin, and granulysin and a Th1 signature

HIV RNA+ cells from suppressed PWH:

- Preferentially Tem cells but do not exhibit a cytotoxic signature
- TGF-β-associated signature is a phenomenon that only emerges in the context of ART suppression

Key Populations and Impact on Community

Key Population: People living with HIV

Impact: HIV has important differential effects on cells based on whether people are on or off treatment

Understanding these differences is key to designing more targeted and effective cure strategies

Supports the long-term goal of safe, effective, and accessible cure for everyone living with HIV

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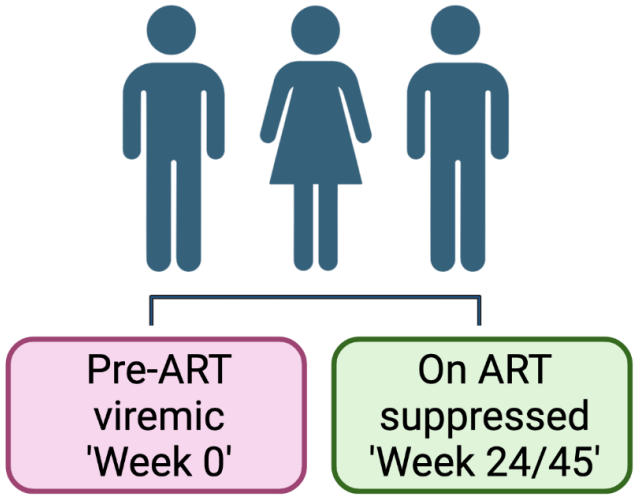
Antiviral factor expression in CD4+ T cells is higher during viremia than ART-suppression



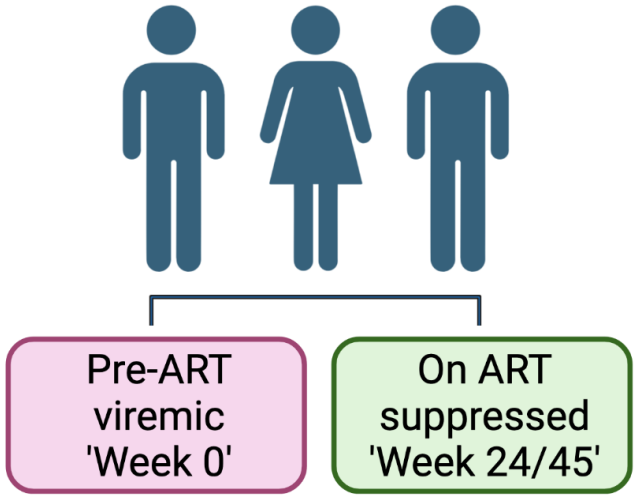
In agreement with findings in non-paired studies of non-controller, viremic, and ART-suppressed individuals (Abdel-Mohsen et al., Retrovirol. 2013)

Table 2. Paired samples of CD4+ T cells during viremia vs. ART suppression (n=3)

Gene	Log FC	FDR
IFI6	0.618	0
ISG15	0.577	0
IFITM1	0.575	0
MX1	0.525	0
TRIM22	0.309	0
IFITM2	0.306	0
IFITM3	0.260	2.42E-99
BST2	0.256	0

Frouard & Telwatte, et al., bioRxiv 2024

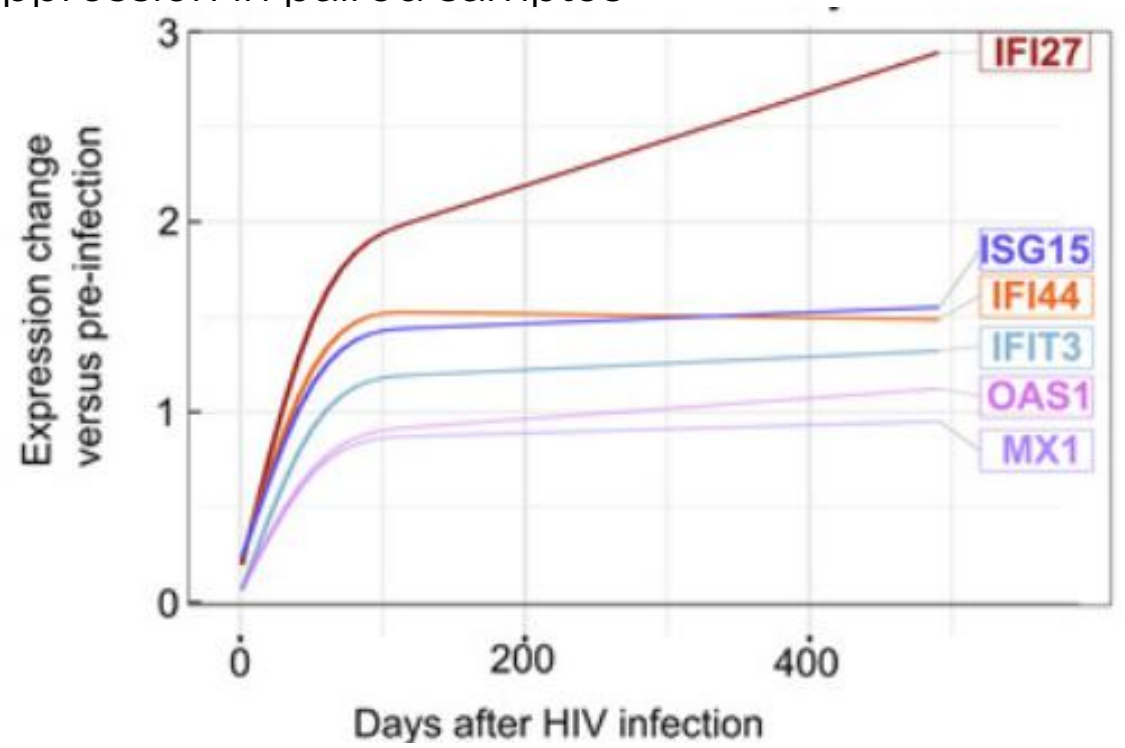
Upregulation of restriction factors during viremia vs. ART-suppression in HIV RNA+ CD4+ T cells



Interferon-stimulated genes, including MX1, IFI27 and ISG15 are all upregulated in HIV RNA+ CD4+ T cells from viremia vs. ART suppression

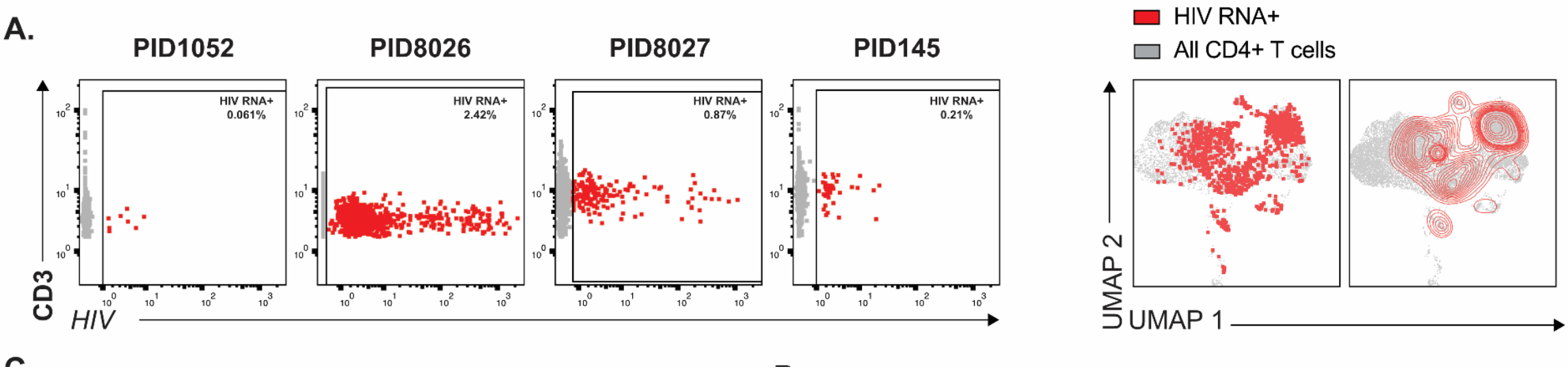
These ISGs were upregulated during acute infection but also remained chronically elevated during ART suppression (Mackelprang et al., iScience 2023)

Table 3. HIV RNA+ CD4+ T cells in viremia vs. suppression in paired samples



Mackelprang et al., iScience 2023

Figure 2


A.
PID1052
PID8026
PID8027
PID145
HIV RNA+
0.061%
HIV RNA+
2.42%
HIV RNA+
0.87%
HIV RNA+
0.21%
CD3
HIV
HIV RNA+
All CD4+ T cells
UMAP 2
UMAP 1