Understanding the landscape of lymph node reservoirs during ART through single cell analysis

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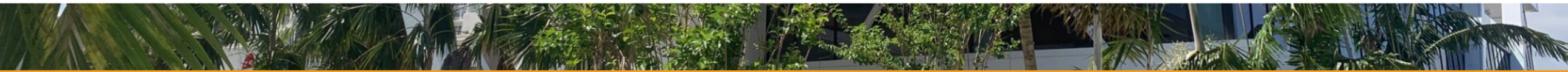
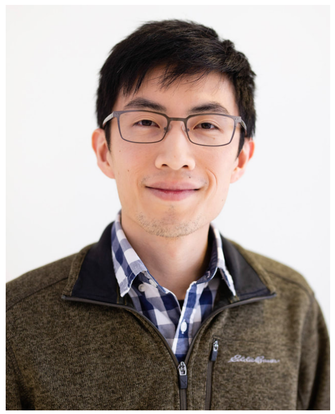
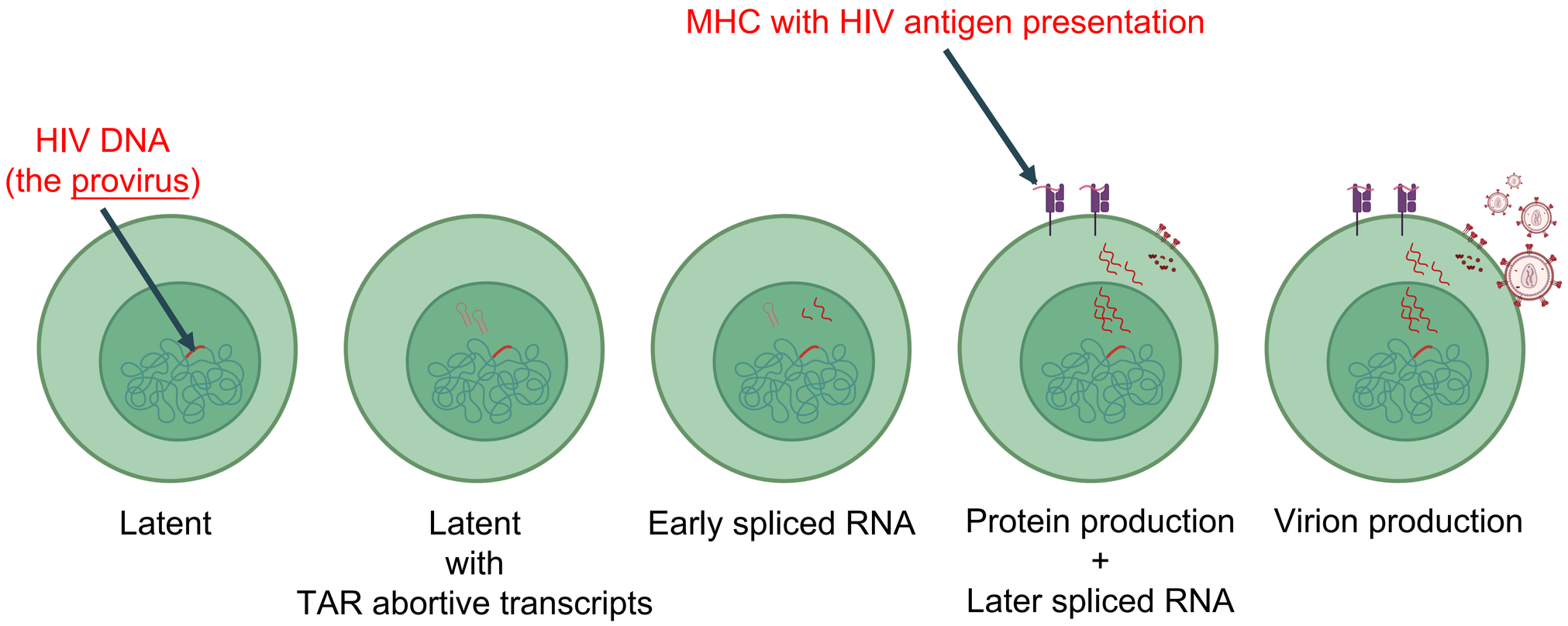


Disclosures and cautionary statements

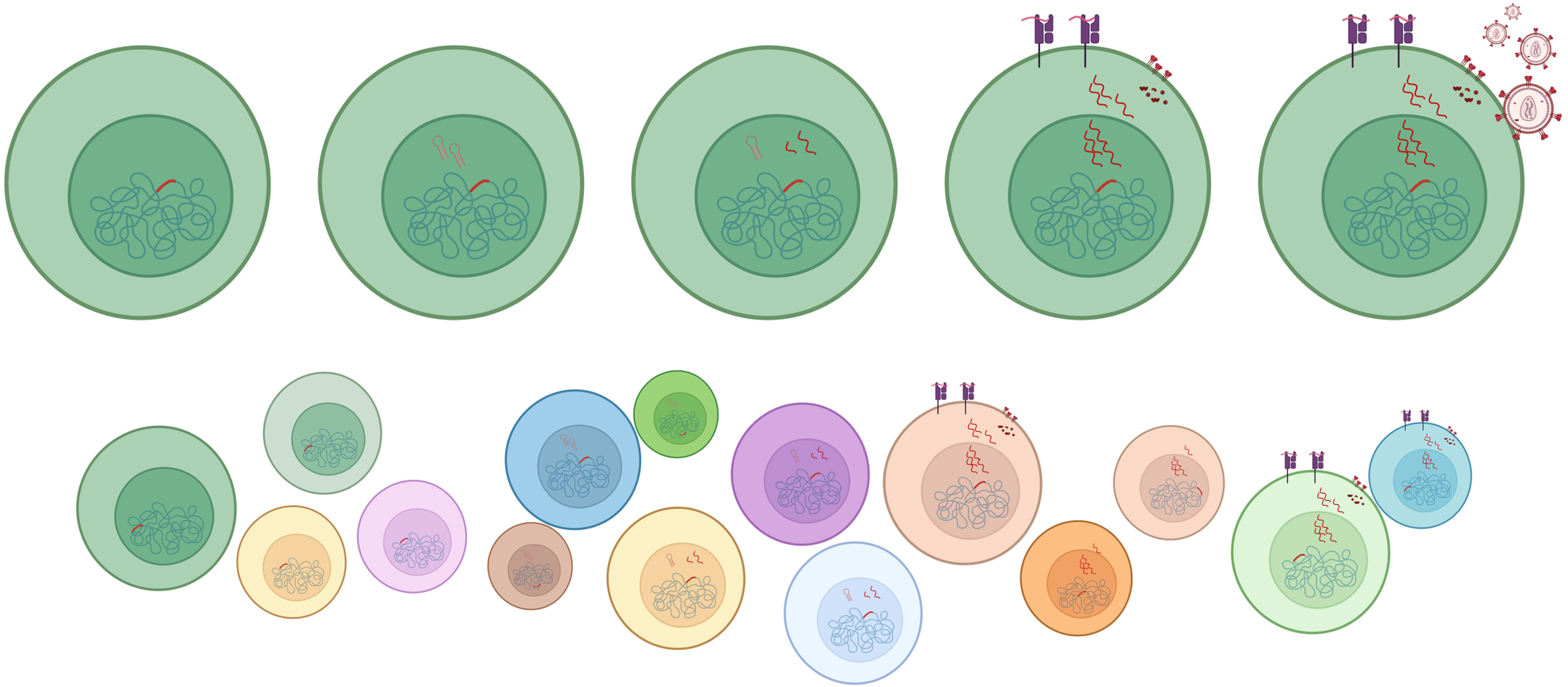
- MRB consults for Interius Biotherapeutics, Capstan, Merck, Gilead
- Entirety of data and concepts in this presentation are unpublished, preliminary, and may be updated as we accumulate more data Skepticism, ideas, and novel interpretations welcome.
- The work in this talk was performed by Jayme Nordin, PhD student, and Vincent Wu, PhD.



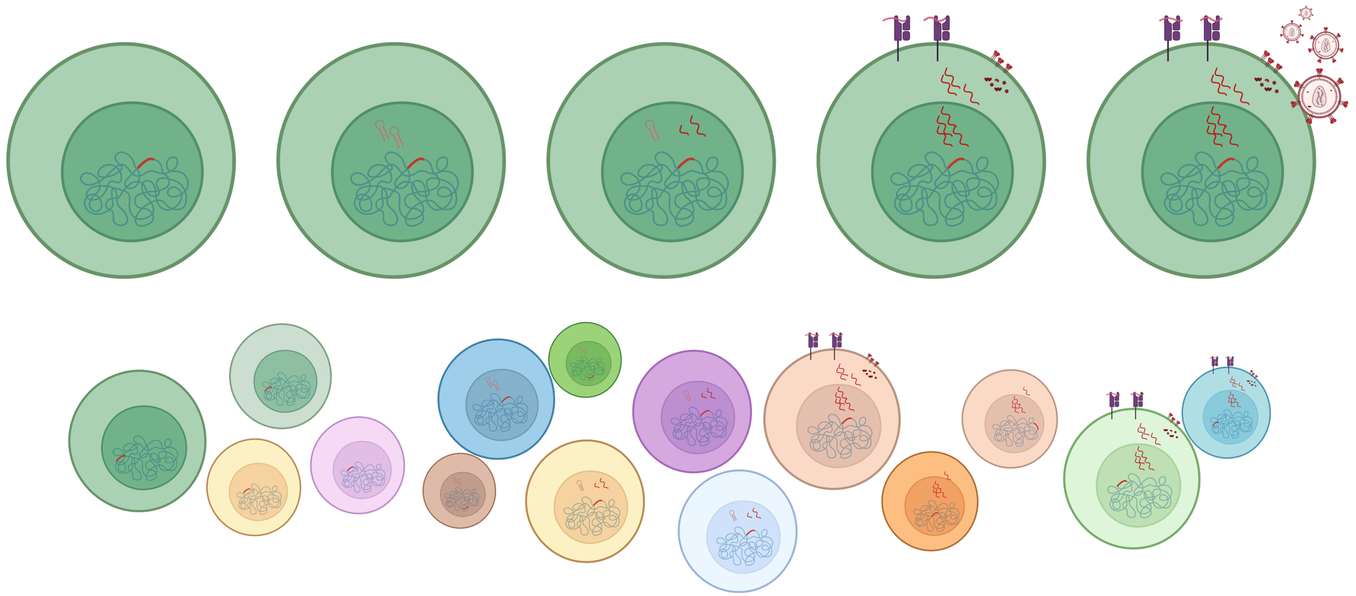
The virologic state of the HIV reservoir is heterogenous



To compound the issue, the cellular state of the HIV reservoir is heterogenous



The heterogeneity of the HIV reservoir necessitates single cell analysis but has several challenges



High CD4+ T-cell heterogeneity (including in context of HIV)

Multiple including Chomont et al., Nat Med 2009; Szabo et al., Nature Comm 2019; Gálvez et al., mBio 2021; Wu et al., JCI Insight 2020, Wu et al., Nature Immuno 2022

Latent infection + rarity of infected cells

Chun et al., Nature 1997; Finzi et al., Science 1997; Eriksson et al., PLoS Pathog 2013

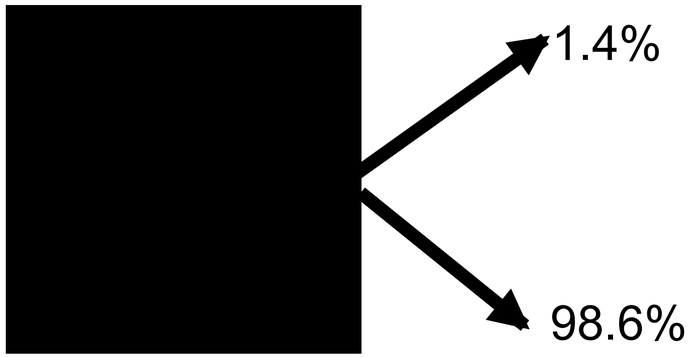
No known surface markers for infected cells (without relying on vRNA or viral protein)

Cohn et al., Nature Med 2018; Liu et al., Sci Transl Med 2020, Wu et al., Nature Immuno 2022

Made with biorender.com.

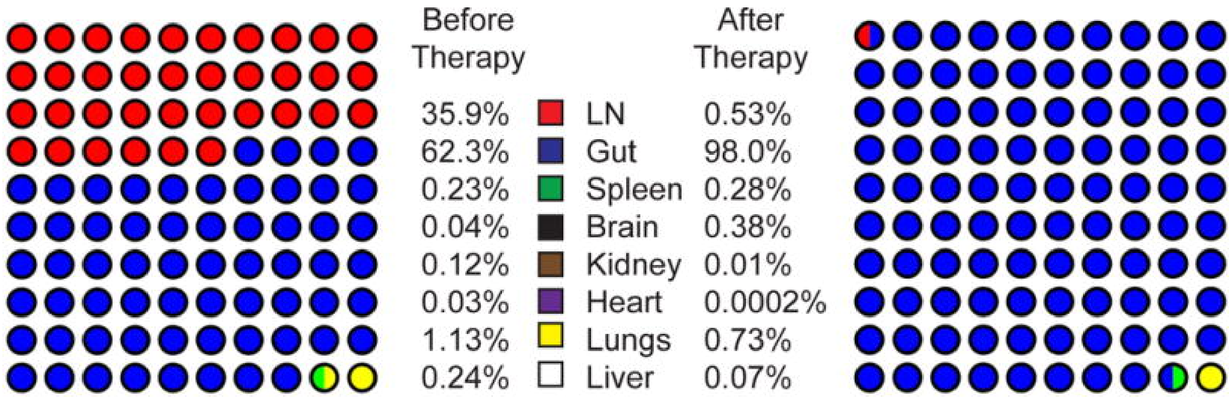
Nearly all the HIV reservoir during ART is in tissues

What is the reservoir?



Blood - well studied, though varies somewhat depending on the publication

Tissues: We know a lot is in lymph nodes and gut lymphoid structures and tissues.



Estes et al., *Nat Med* 2017

Estimated 2.5 x 10^9 viral DNA+ cells in lymphoid tissue during ART (70 Kg human; T. Shacker, U. Minnesota)

Study Goal: to define HIV reservoir composition in LN of ART-treated PLWH

- Determine the phenotypic, transcriptomic, and epigenetic profile of HIV-infected memory CD4+ T cells in lymph nodes of ART-PLWH (DOGMAseq)
- Determine what are the predominant infected cell subsets in ART LN… is it Tfh cells?
    - Tfh cells have been shown in a variety of studies to be the dominant infected population in lymph nodes in viremic infection, but this mostly assumed in ART
- Determine whether the provirus in HIV infected LN CD4+ T cells during ART is quiescent or transcriptionally active, and potential differential characteristics thereof

Samples (n = 26) used for lymph node (LN) studies

ART

Participant ID	Date of sample collection (mm/dd/yy)	Days on ART	pVL (copies/mL)
A002	6/20/16	476	<40
A024*	9/2/16	784	Undetectable
A170	6/13/18	770	Undetectable
A213	10/29/18	7326	Undetectable
A262	7/15/19	1197	Undetectable
A304	11/23/22	1035	Undetectable
A025*	2/9/15	489	Undetectable
A303	9/21/15	355	Undetectable
A127	11/10/17	749	<40
A256	3/30/15	1027	<40

Chronic

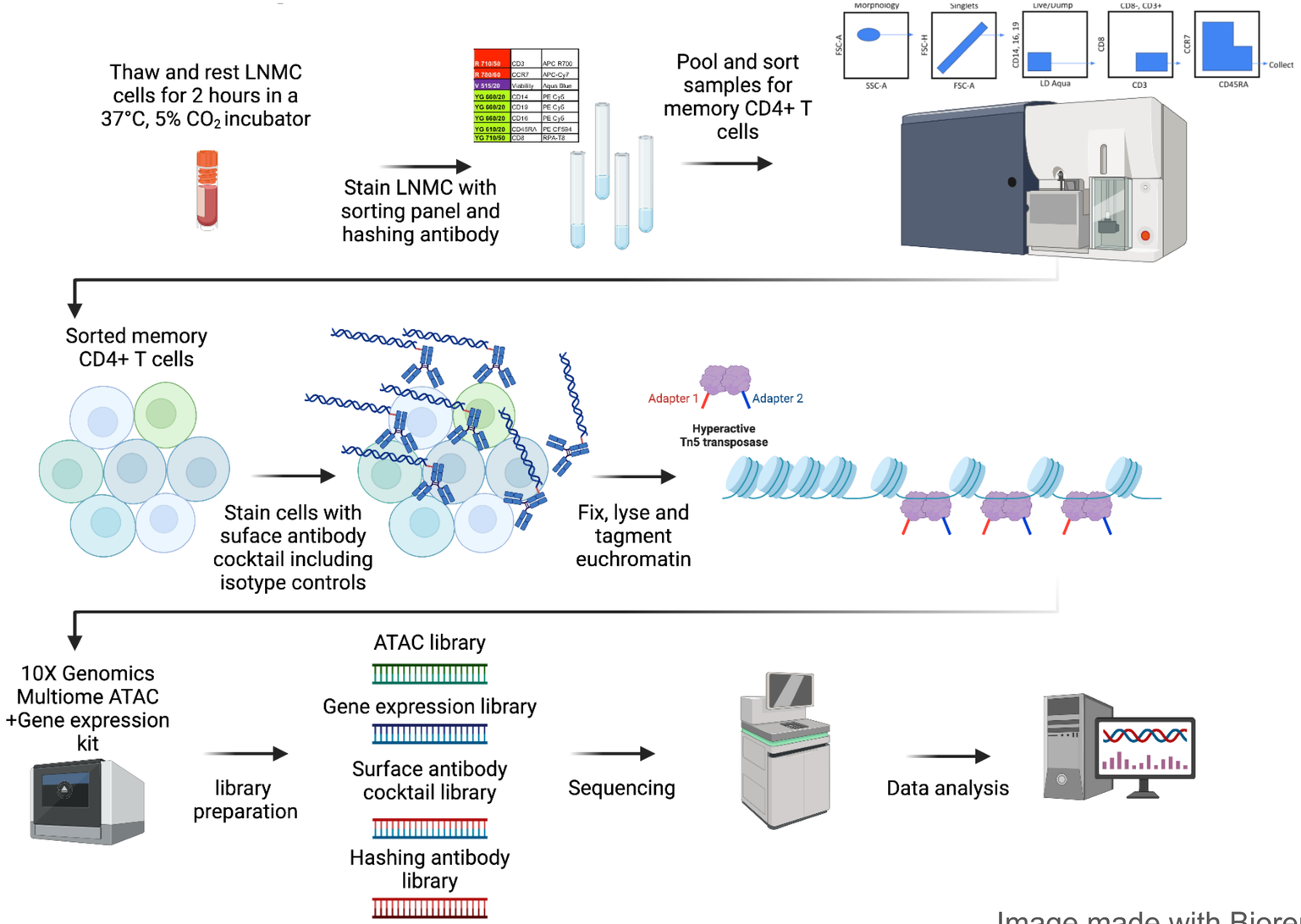
Participant ID	Date of sample collection (mm/dd/yy)	Days on ART	pVL (copies/mL)
C028	9/12/16	-	25,951
C087	6/12/17	-	1,771,593
C098	7/21/17	-	1,019,989
C102	7/27/17	-	1,692,571
C107	8/30/17	-	6,326,452
C111	9/4/17	-	552,134
C229	1/28/19	-	3,882,624
C242	3/27/19	-	715,509
C282	1/29/20	-	3,087,470
C101*	7/26/17	-	1,935,095
C235*	5/14/19	-	259,708
C250*	2/14/19	-	1,067,647

Without HIV

Participant ID	Date of sample collection (mm/dd/yy)	Days on ART	pVL (copies/mL)
U287*	2/24/20	-	Undetectable
U294*	7/7/21	-	Undetectable
U190	7/18/18	-	Undetectable
U191	7/19/18	-	Undetectable

*Inguinal LN. All others are cervical LN.
Collaboration with CIENI (Mexico)

DOGMAseq: single cell assessment of protein+RNA+DNA composition



live sorted memory CD4+ T cells for analysis

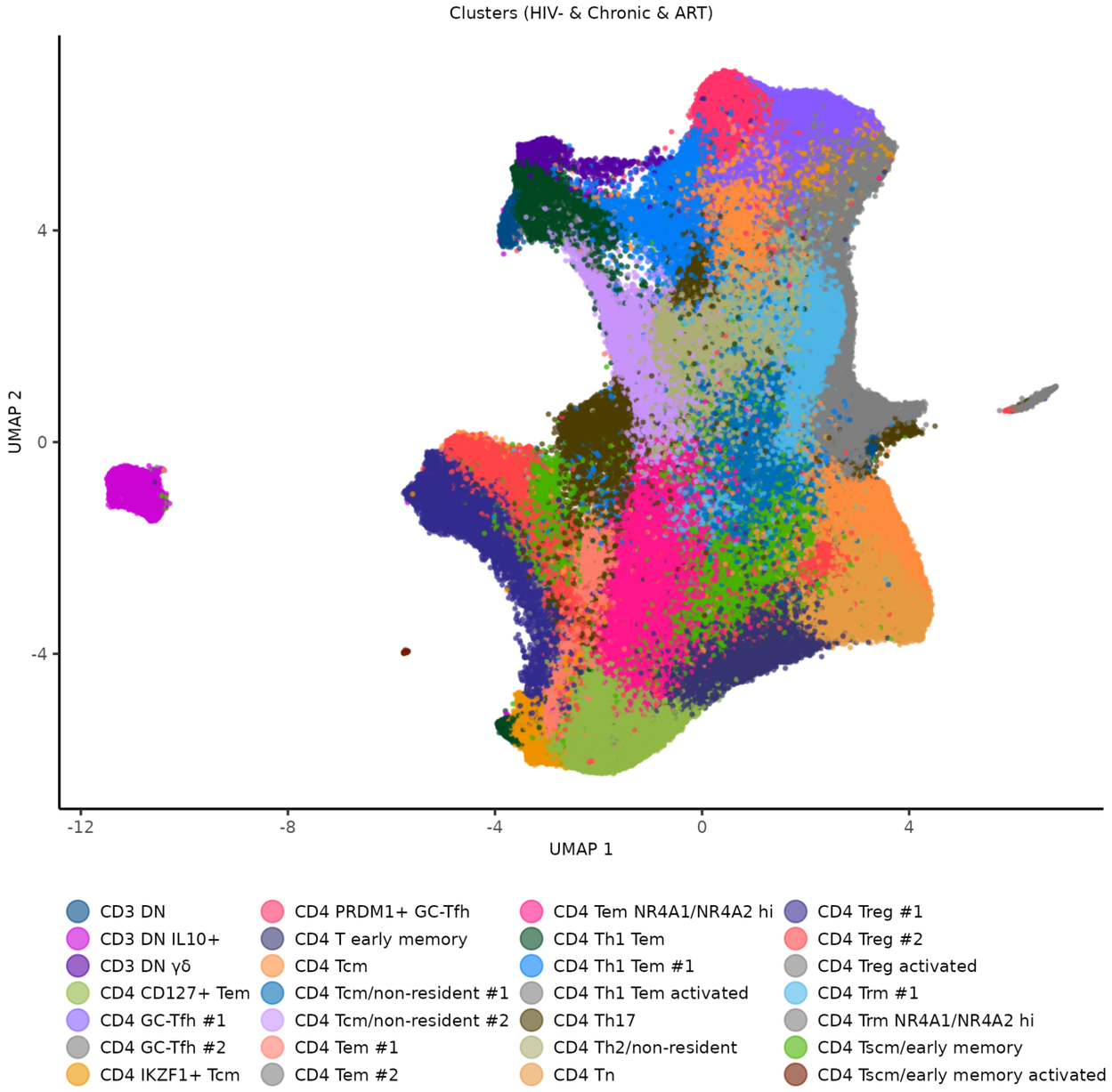
Targeted ~26667 cells for each chronic LN sample

Targeted ~56000 cells for each ART LN sample

Total cells passing QC:
ART: 294,465
Chronic: 163,061
w/o HIV: 102,234

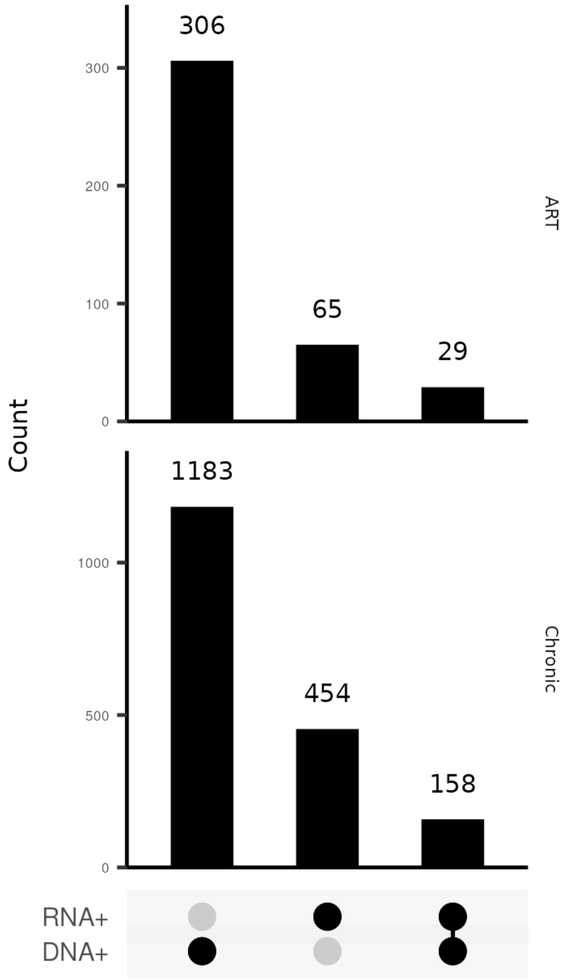
Image made with Biorender

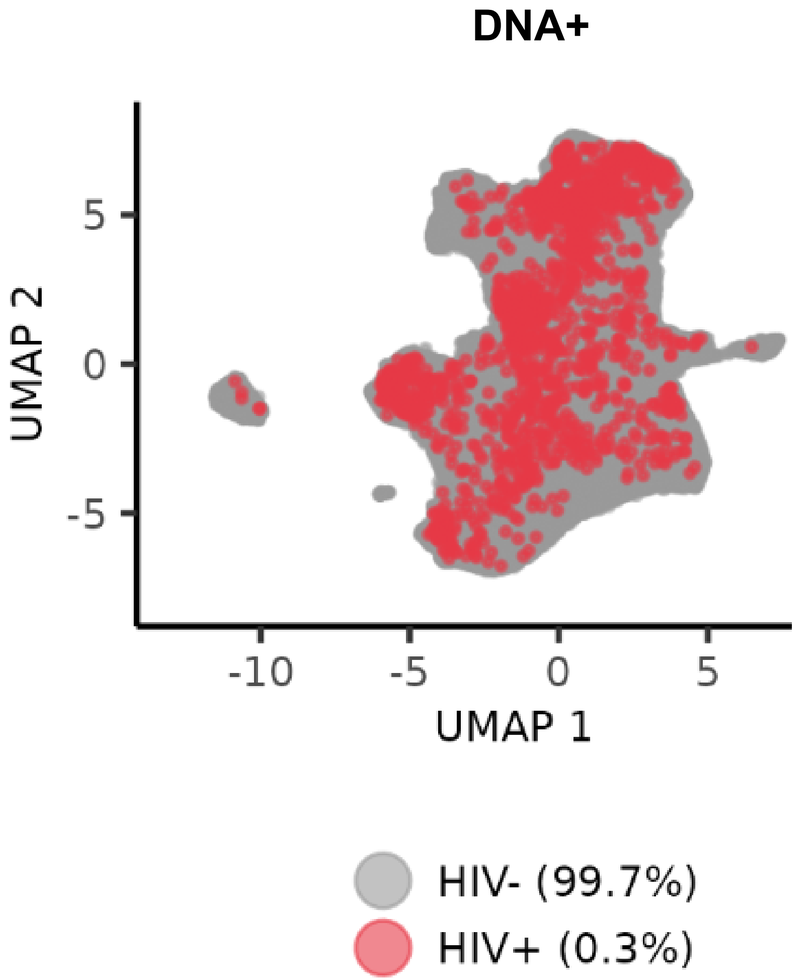
Definition of LN memory CD4 + CD3 DN T cell subsets

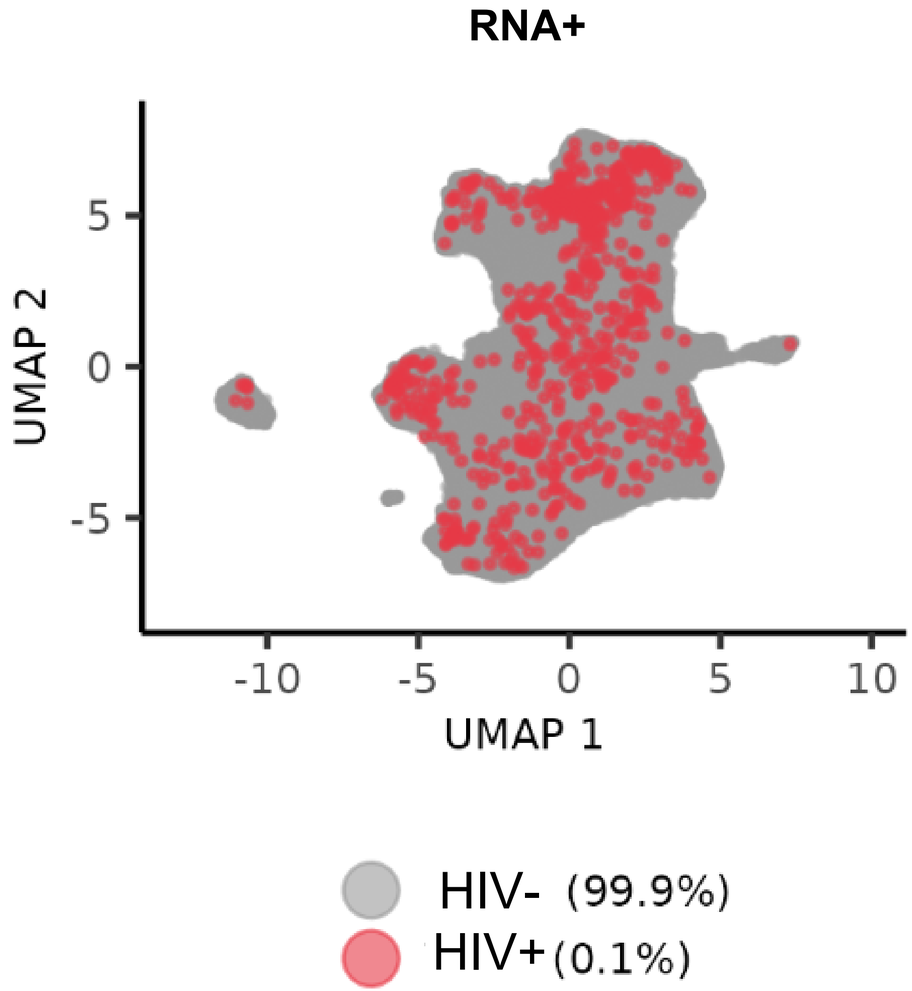


- T follicular helper (Tfh)
  - Three subsets
- T regulatory cells (Treg)
  - Three subsets
- Resident memory T cells (Trm)
  - Two subsets
- Effector memory T cells (Tem)
  - Five subsets
- Central/early memory T cells (Tcm, Tscm)
  - Eight subsets
- Naive T cells
- CD3 DN subsets (three)

Detection of viral DNA+ and RNA+ cells

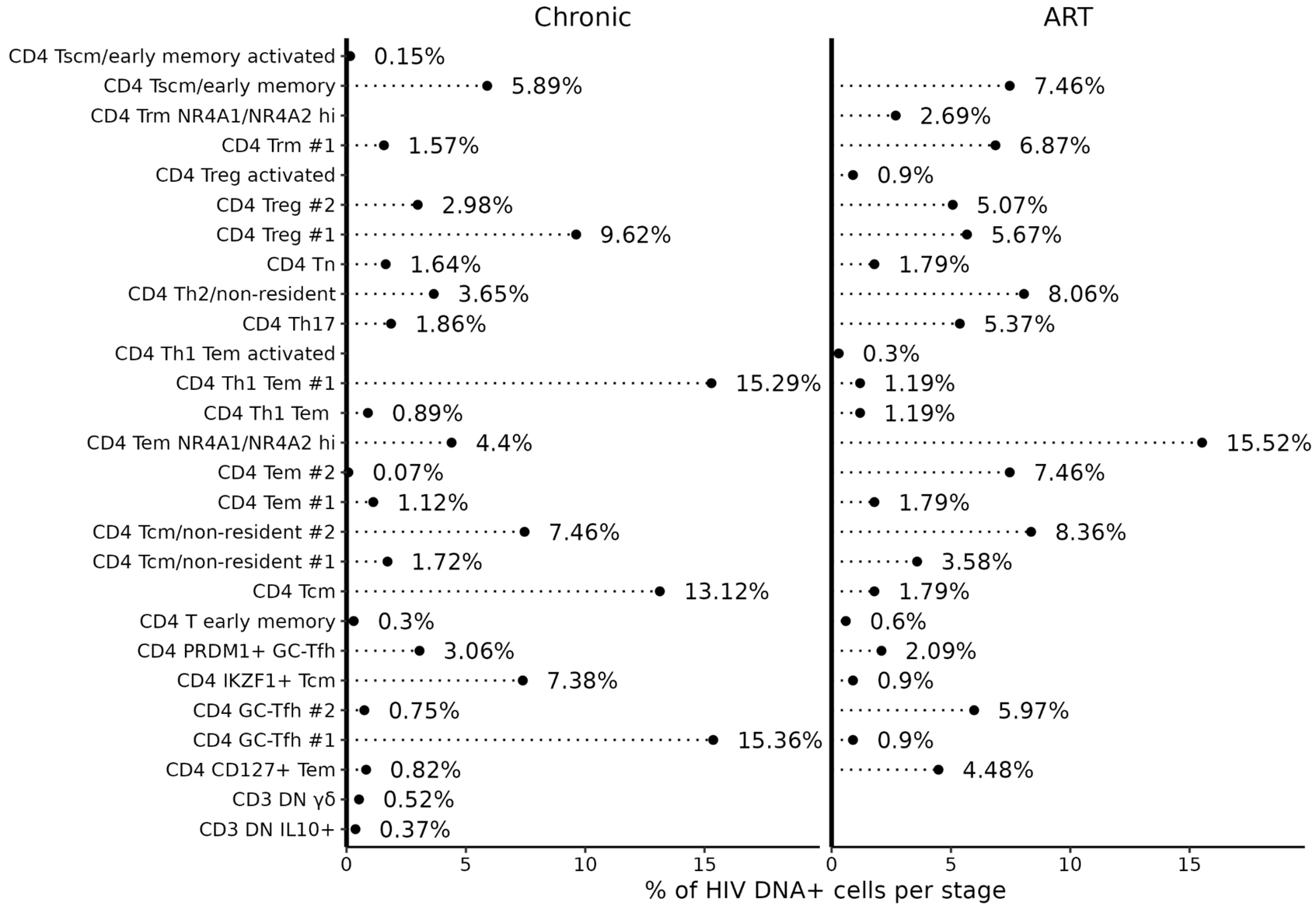




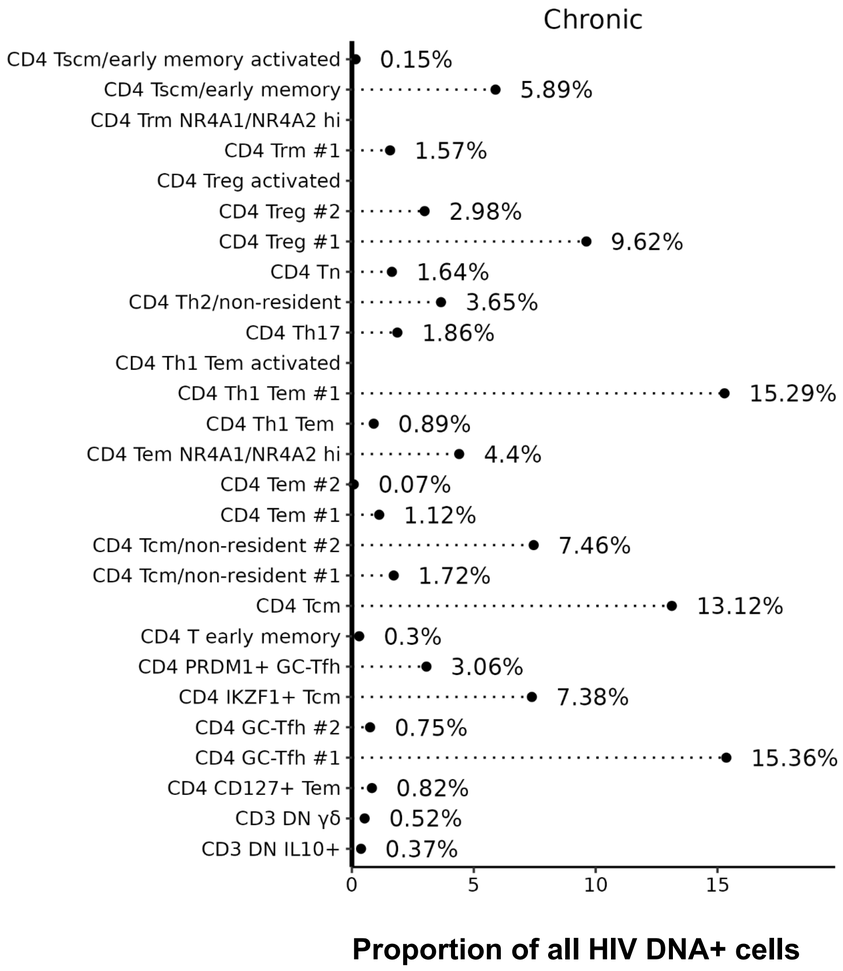


UMAP plots show aggregation of chronic+viremic+w/o HIV samples

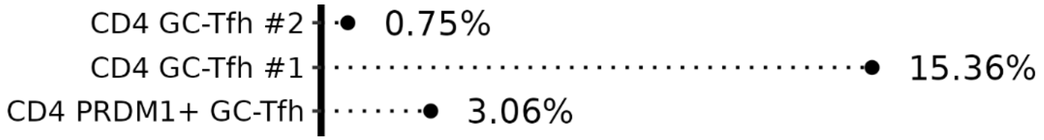
1. The lymph node proviral DNA reservoir landscape is very heterogeneous and differs between ART and viremic PLWH

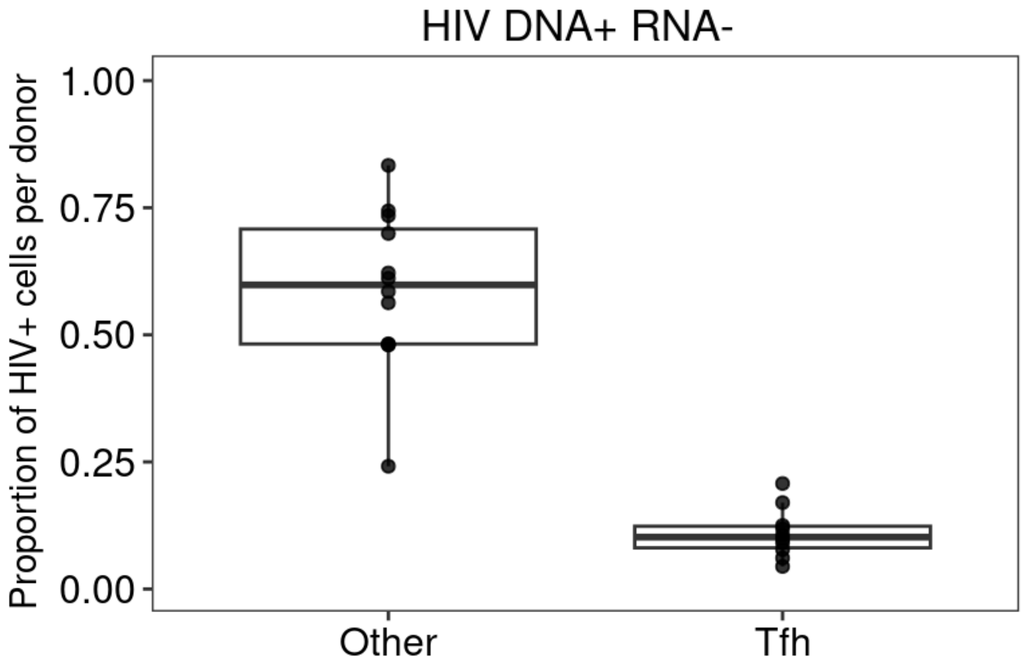


2. Tfh cells comprise a large, but not dominant, proportion of the HIV DNA+ cells in viremic PLWH

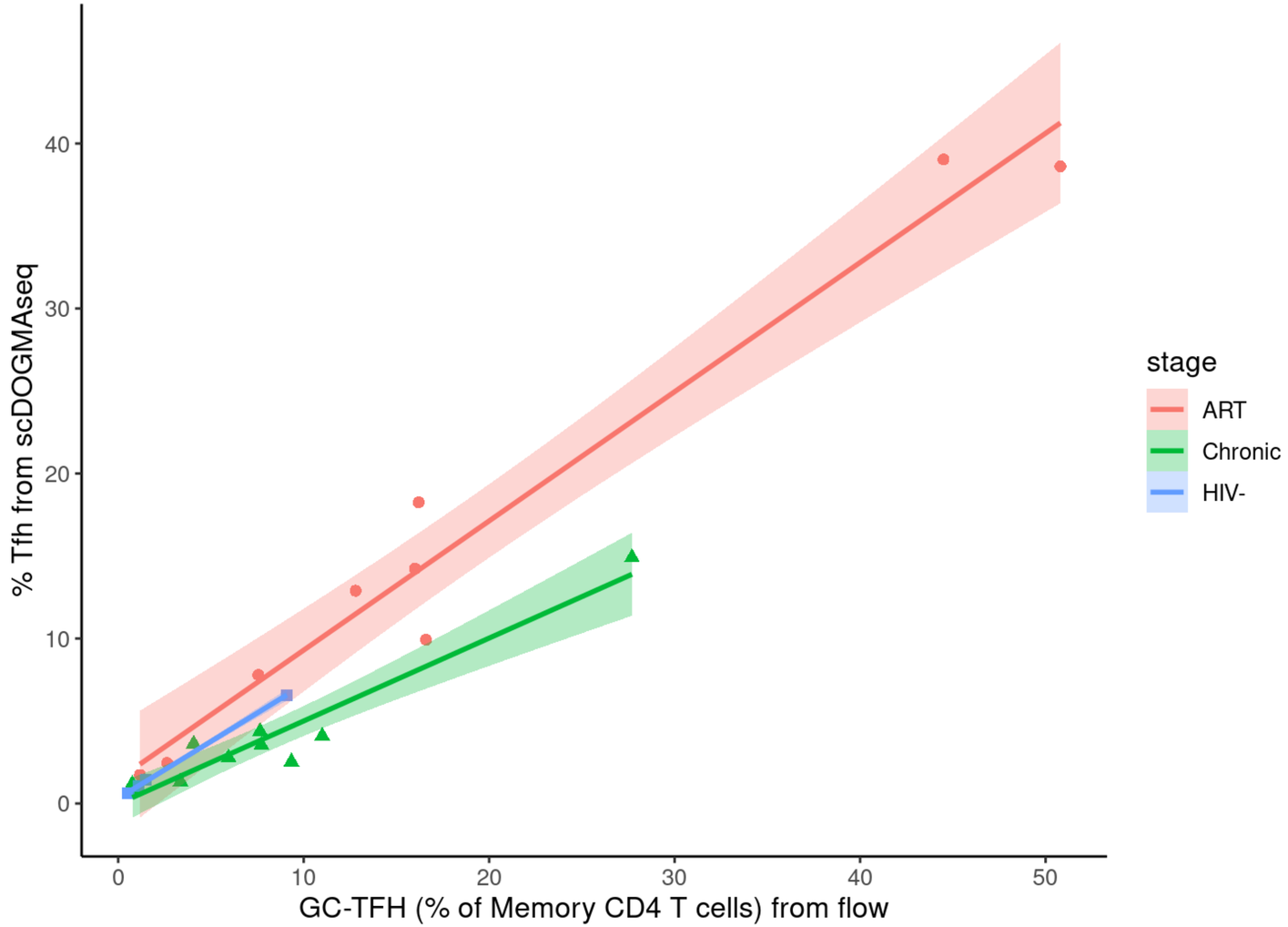


- **Tfh cells comprise a large proportion (~20%) of the proviral DNA+ cells in viremic LN**

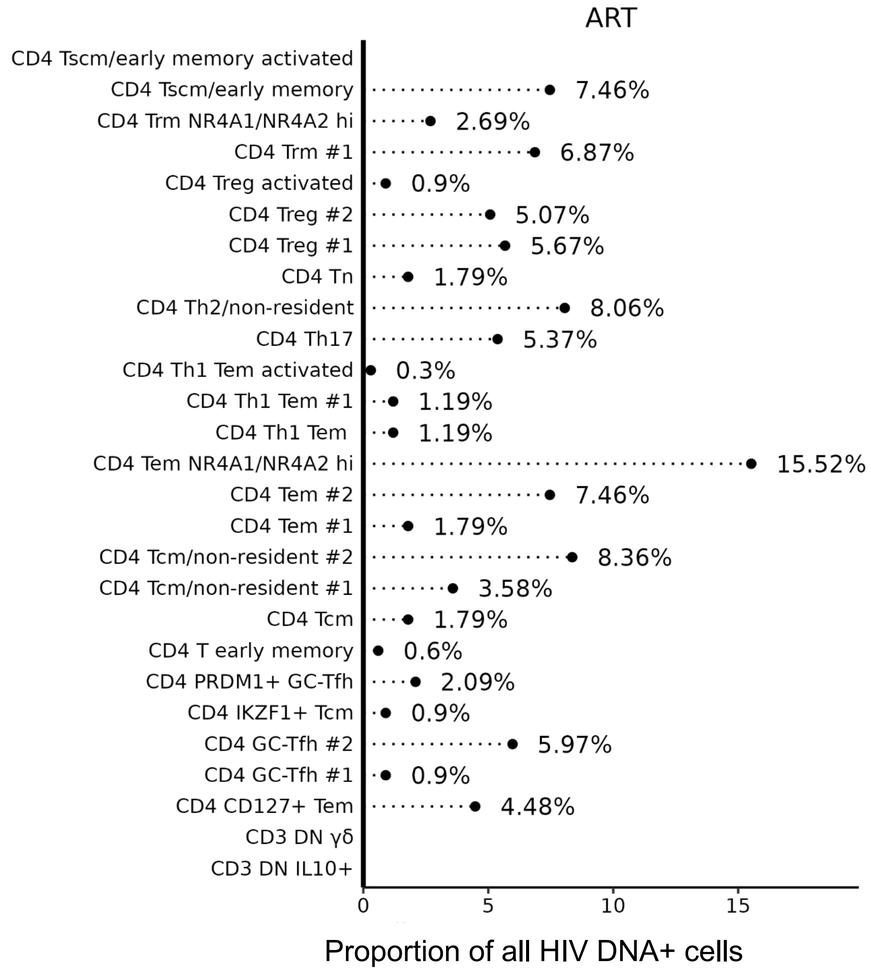




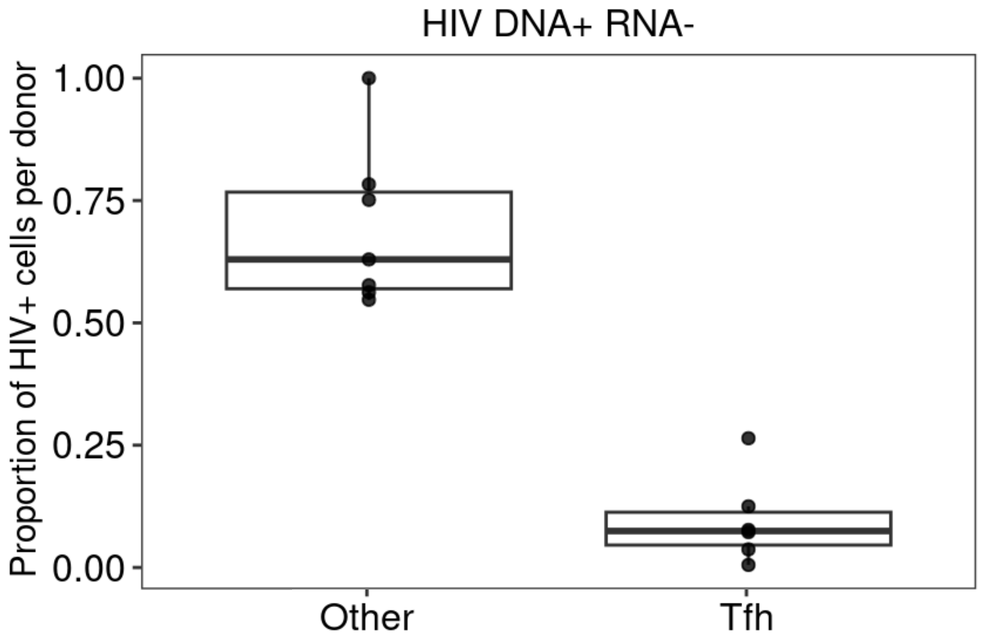
GC-Tfh definition by single cell quantification by single cell analysis is directly correlated to flow cytometry



3. The lymph node proviral DNA reservoir landscape during ART is not dominated by Tfh cells

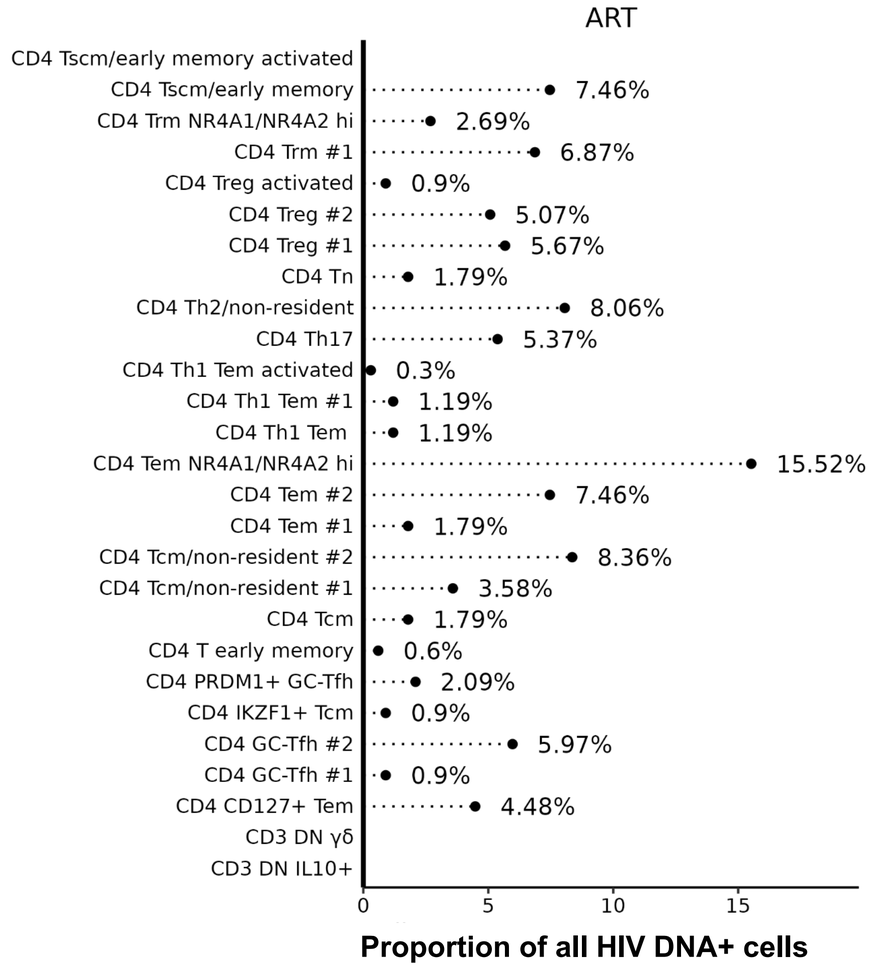


- **Tfh cells only comprise ~9% of the proviral reservoir (vs. 20% in viremia)**

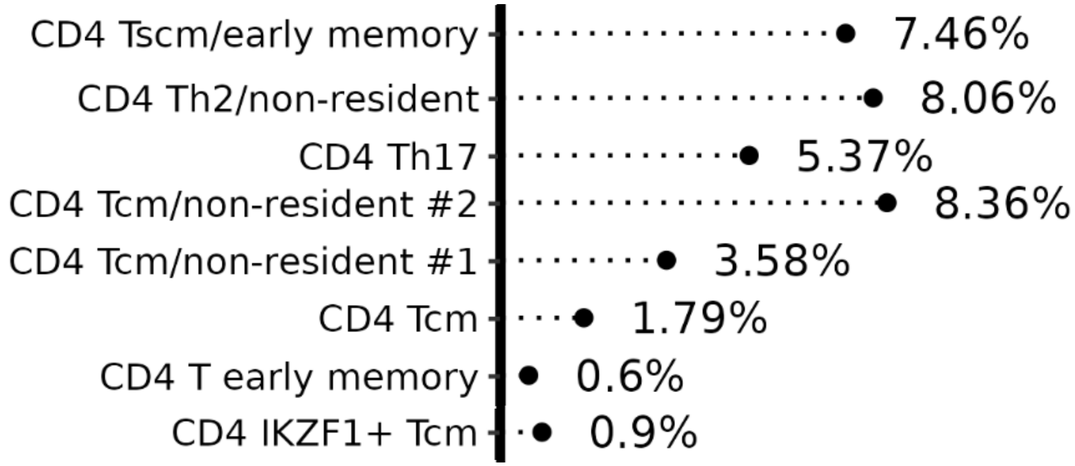


Data shown by M. Pardons, oral presentation 1.2, confirm this

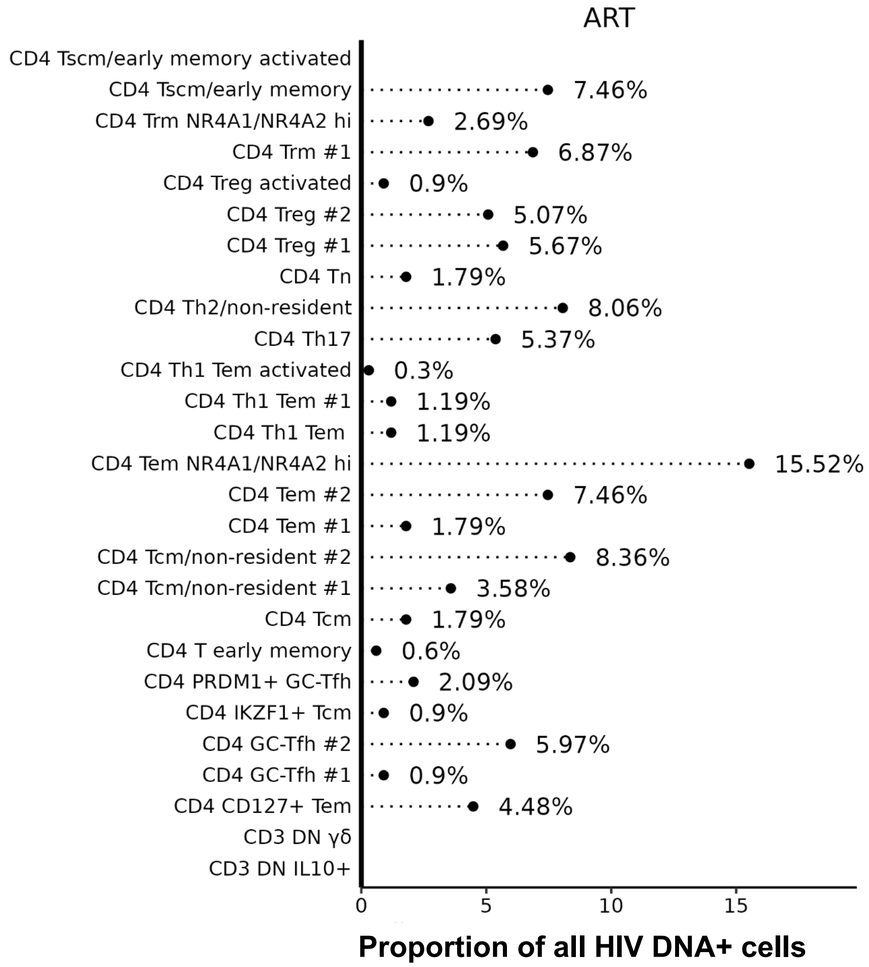
4. Central/early non-resident memory populations comprise a large proportion of the lymph node proviral DNA reservoir



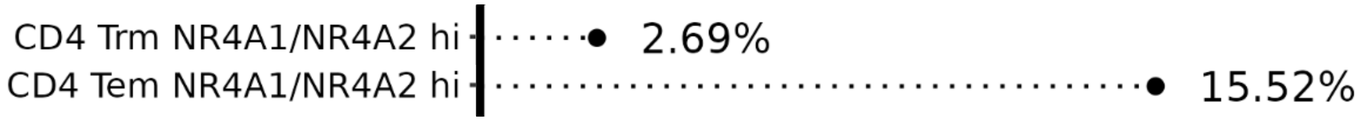
- **Central/early memory populations comprise a large proportion (36%) of the proviral DNA reservoir:**



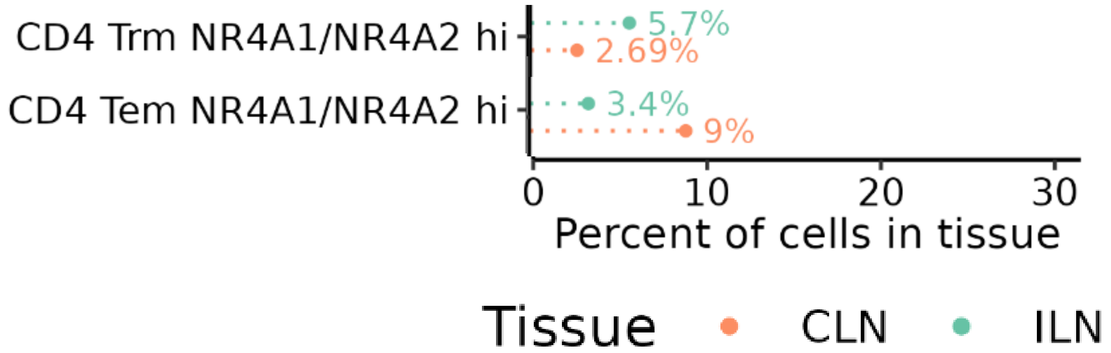
5. NR4A1/NR4A2 hi effector memory CD4+ T cells are a large component of the proviral DNA reservoir



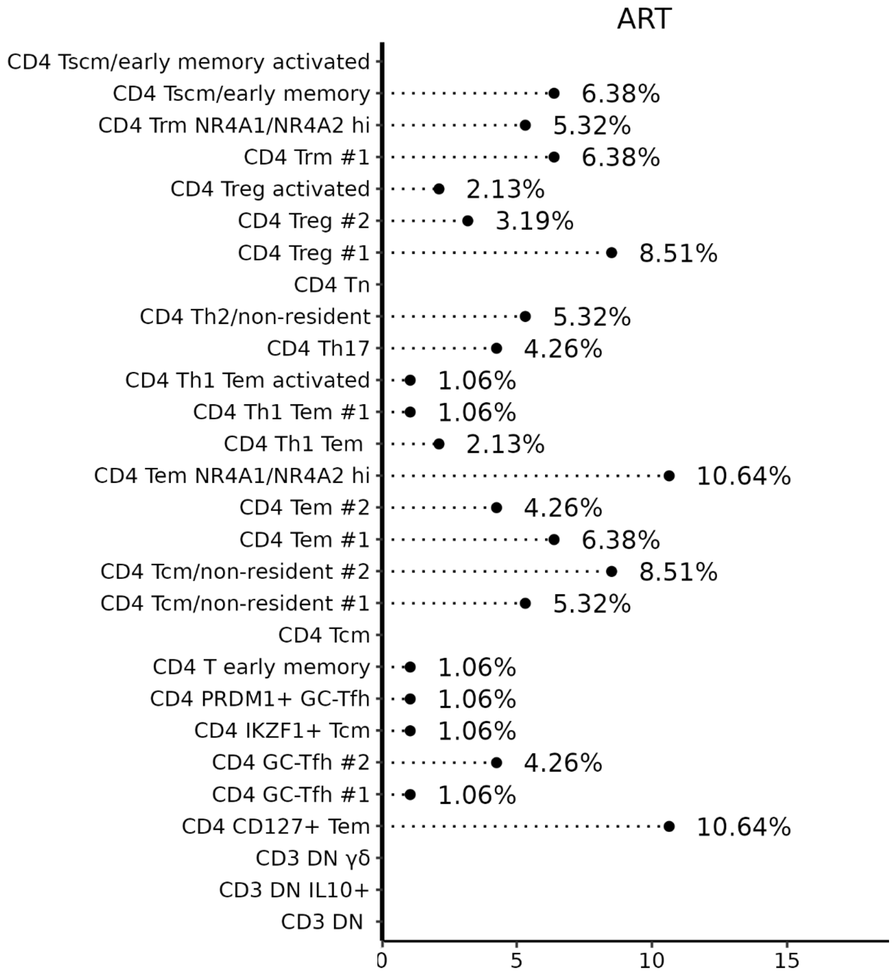
- **NR4A1/NR4A2 hi Tem cells comprise ~18% of the proviral DNA reservoir:**



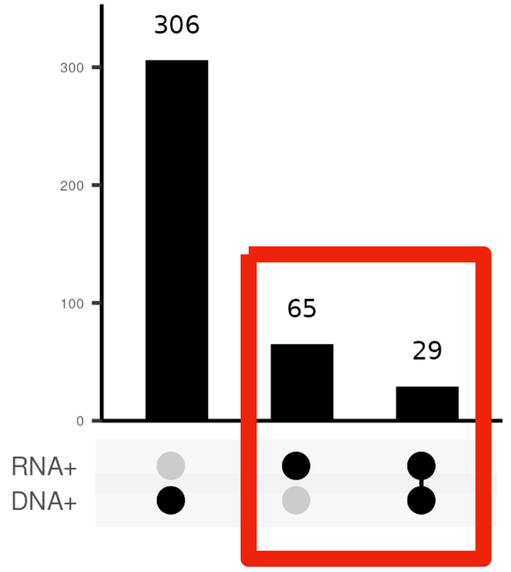
- **NR4A1/NR4A2 hi Tem subsets are 8-10% of total lymph node CD4+ cells in the tissues**



6. The viral reservoir during ART can be transcriptionally active, but is low frequency



1 RNA+ cell = ~1%

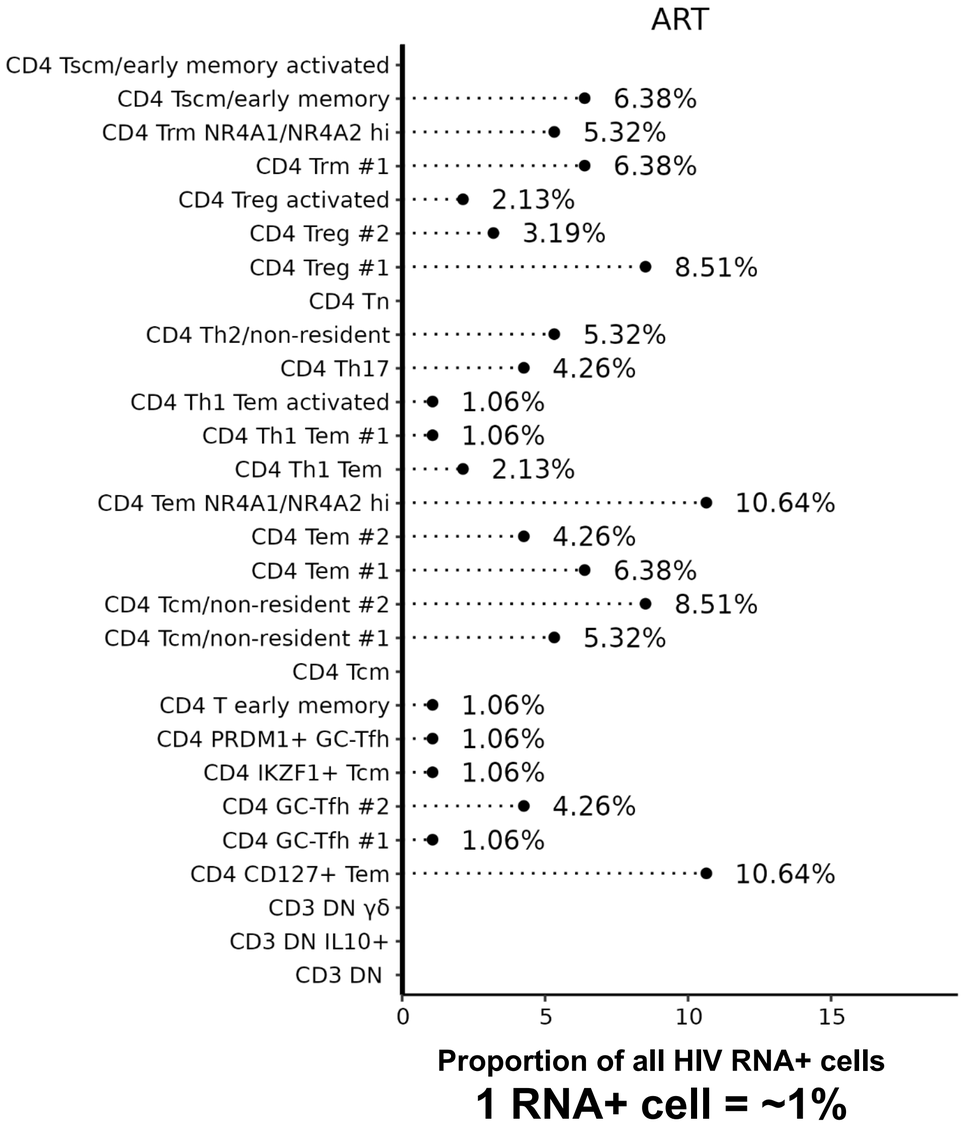


Total LN cells measured= 294,465

94 vRNA+ cells

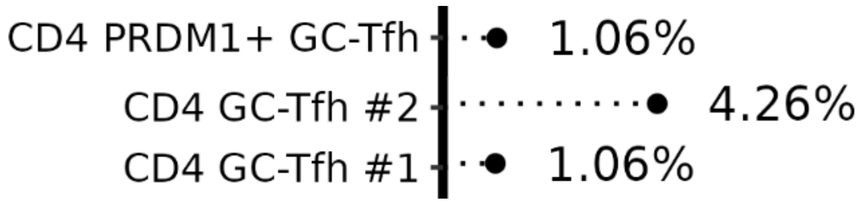
0.03% of total cells vRNA+

7. Tfh cells are a minor component of the transcriptionally active reservoir during ART

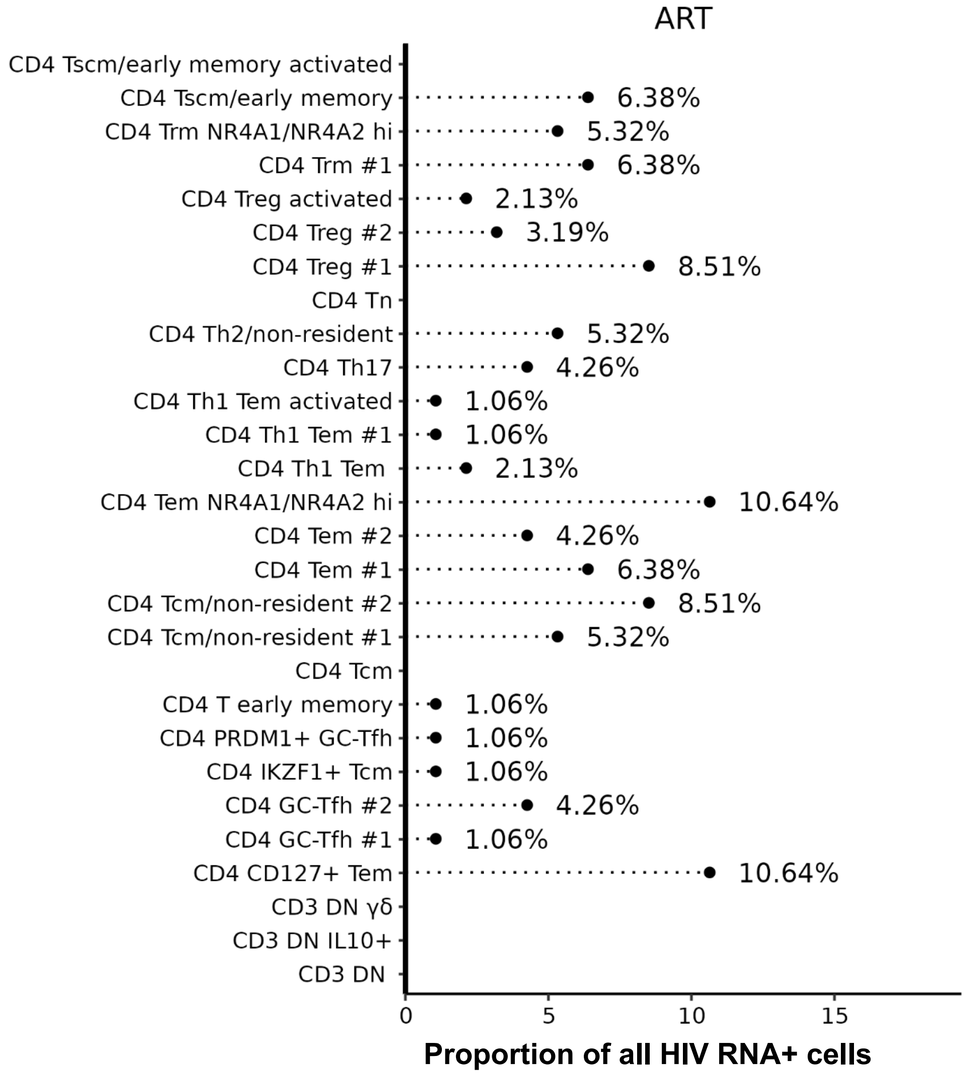


1 RNA+ cell = ~1%

- **Tfh comprise a small component (6.4%) of the viral RNA+ reservoir in ART**

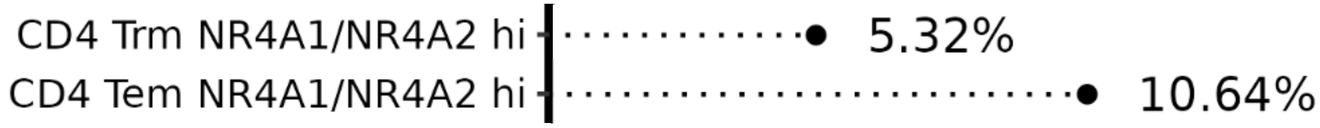


8. NR4A1/NR4A2 hi effector memory CD4+ T cells comprise a large proportion of the transcriptionally active reservoir during ART



1 RNA+ cell = ~1%

- **Largest proportion of the viral RNA+ reservoir is CD4 NR4A1/NR4A2+ cells**



What are NR4A1/NR4A2 hi Tem CD4+ T cells?

- Phenotypic characteristics: central memory (CD28+ CD127hi CD45RO+ CD27+) non-resident (CD69 low). Appears resting (low for HLA-DR, ki67, CD71)
- RNA profile: Top DEG NR4A1 (Nur77), Jun, Fos, NR4A2 also top DEGs. Lowest bcl2 RNA

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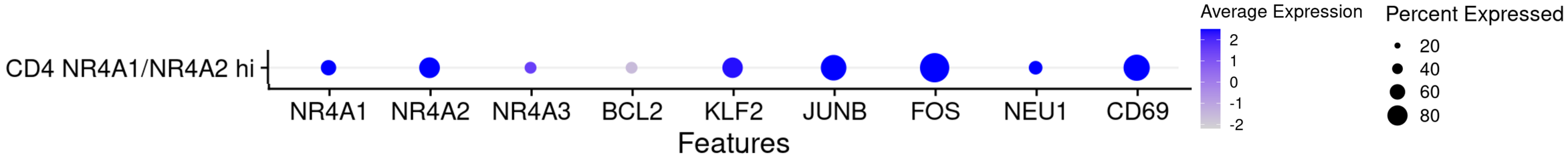
- RNA profile: Top DEG NR4A1 (Nur77). Jun, Fos, NR4A2 also top DEGs. Lowest bcl2 RNA compared to all other clusters
  - NR4A1 expression requires T cell receptor triggering, lasts 3-18hrs. Not bystander activation.
  - Context dependent functions: transcription factor and/or facilitator of apoptosis (binds to Bcl2)
  - Migratory profile, with high KLF2 and high CD69 RNA (but low CD69 protein)



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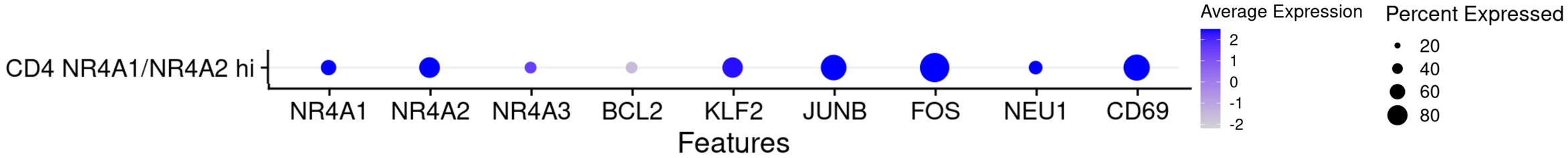

- Shows evidence of very recent activation… BUT APPEARS PHENOTYPICALLY TO BE RESTING MEMORY; may be a major reason for viral rebound propensity upon ART interruption. Very likely also contributes to dissemination of infected cells across the body in both viremia and ART.

Conclusions

- The HIV reservoir in lymph nodes of viremic vs ART PLWH differs in composition
  - This discrepancy may be due to depletion of cells with active viral replication in the LN at the time of ART initiation; dead cells can’t form a reservoir!
- While Tfh cells are a major infected cell type in viremic PLWH, other populations dominate in ART PLWH.
- In ART LN, transcriptionally active infected cells are observed, indicating a continual effort for the virus to replicate
- Very recently antigen-specific activated CD4+ T cells harbor a disproportionately high level of virus during ART
- It is possible that other lymph nodes, of which there are >600 in humans, may have a different reservoir composition, especially mucosal draining lymphoid tissues

Acknowledgements

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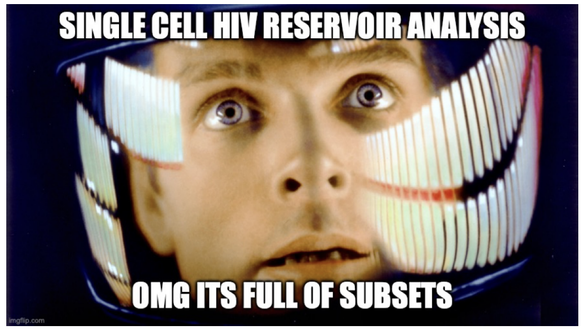
  - NIAID R01 AI184285 (Betts, Søgaard, Jones)
  - NIAID R21 AI172629 (Betts)
  - NIAID UM1AI169633 (Williams/Ferrari)



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