

HIV persistence in bacteria specific CD4+ T cells

11th HIV Persistence during therapy workshop

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Conflicts of interest

None to disclose

A summary for all

- **What was our question?**

Can cells that recognize bacteria found in the gut contribute to HIV pathogenesis and persistence?

- **What did we find?**

These cells can be infected by HIV, are preferentially lost during untreated infection and not fully restored by treatment initiation

Moreover, infected bacteria-specific cells can carry genetically intact HIV genomes in PWH on ART

- **Why is it important?**

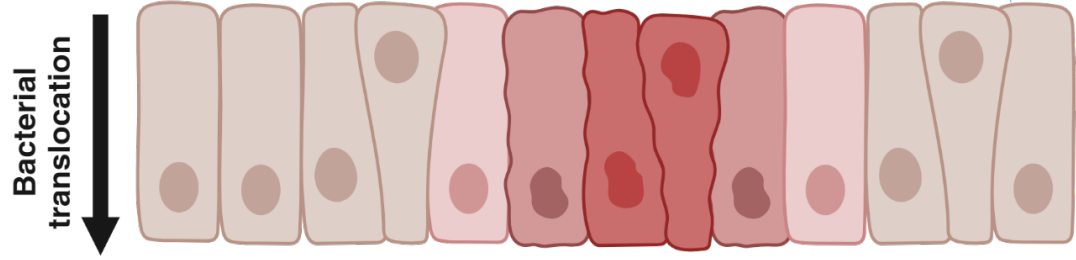
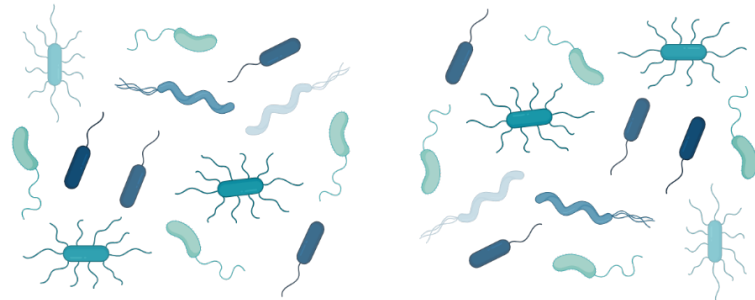
A better characterization of the reservoir cells can help us design new therapies. For example, by modulating the bacterial flora of PWH on ART

Rationale and hypothesis

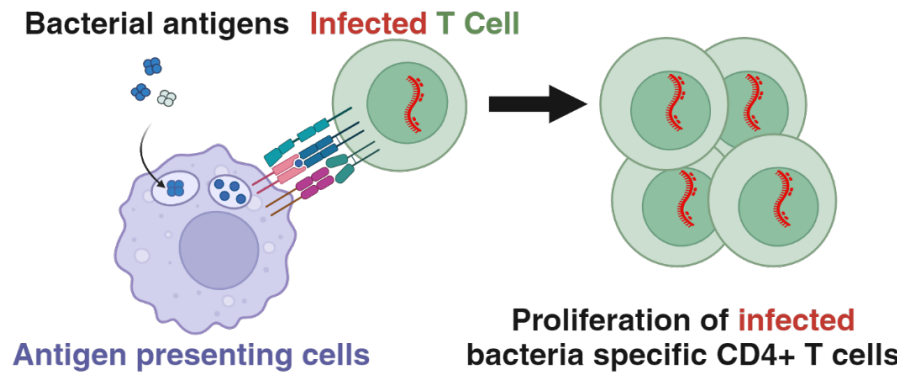
- Most HIV reservoir cells are clonal however, the majority of the antigen specificities of these cells are still unknown (Maldarelli, 2014; Mendoza, 2020; Simonetti,2021; Gantner,2020)

Rationale and hypothesis

Gut lumen



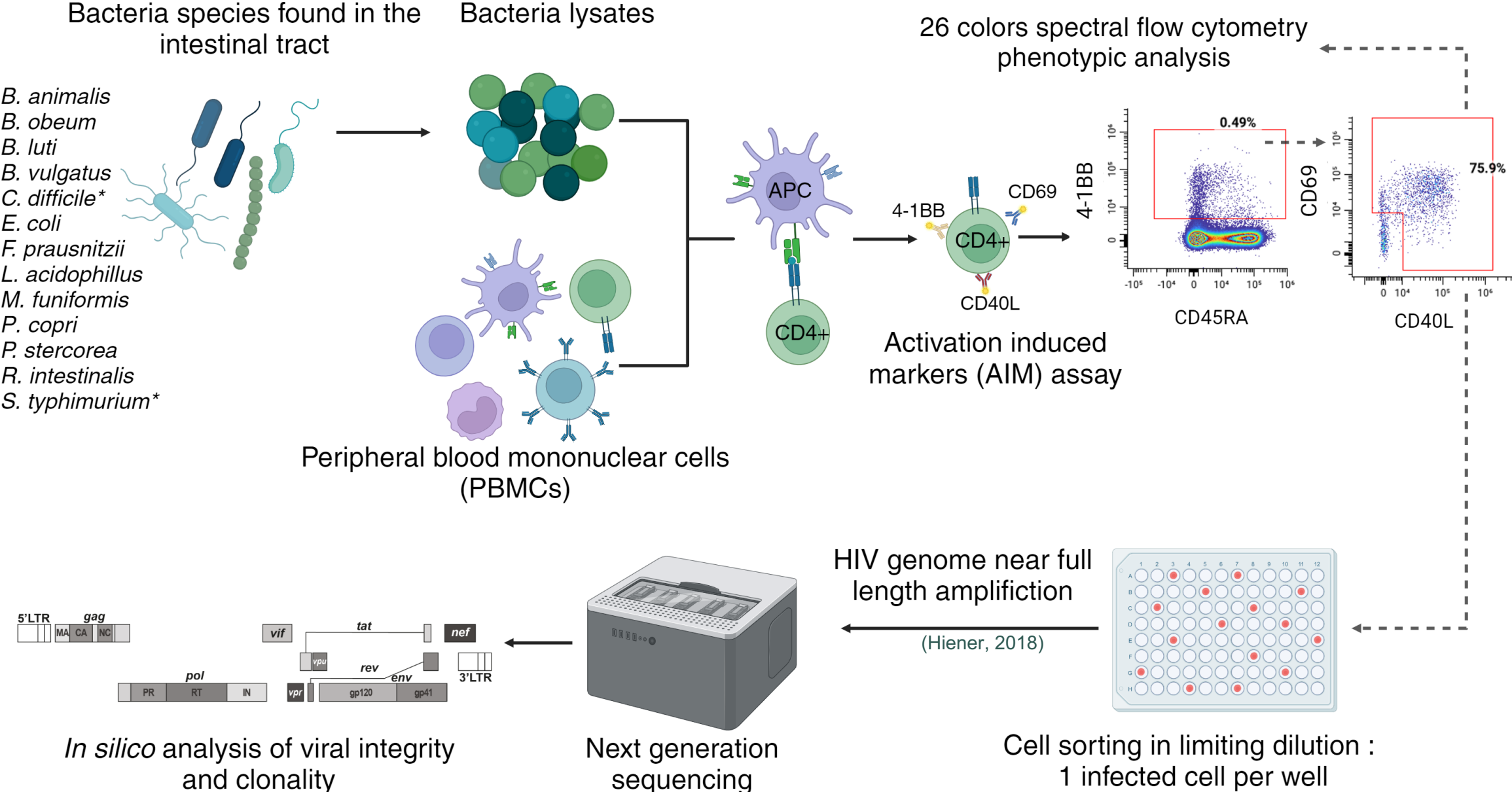
Blood



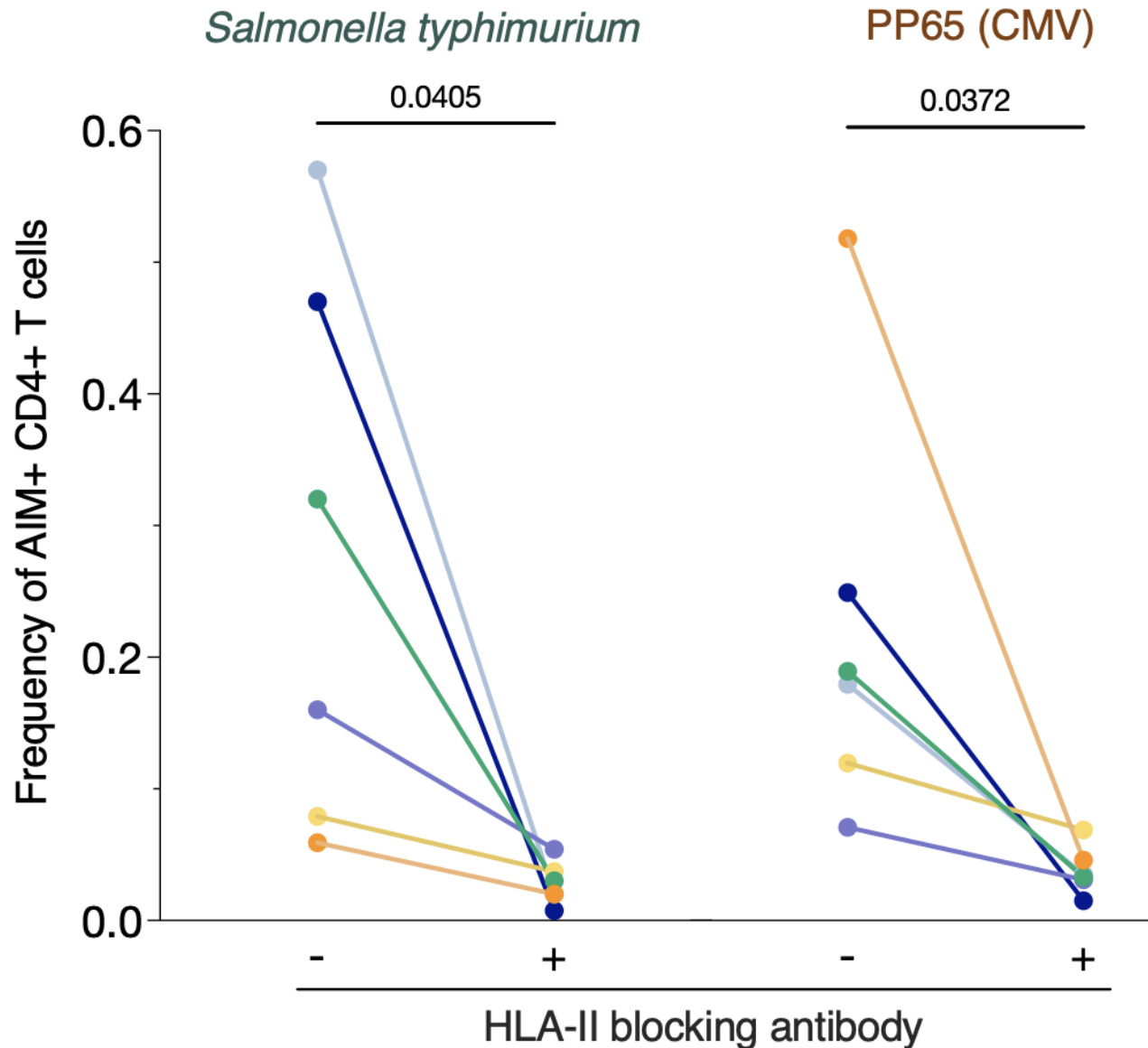
- The gut is home to an important number of CD4+ T cells. Notably cells with high susceptibility for HIV infection (Chun, 2008; Brenchley, 2006; Planas, 2017)
- The gut is a major source of antigens
- During HIV infection the gut epithelia is altered leading to the translocation of bacterial products into the blood (Brenchley, 2006)

We hypothesized that gut-bacteria specific CD4+ T cells are preferentially infected and are HIV reservoirs in PWH on ART

Methods

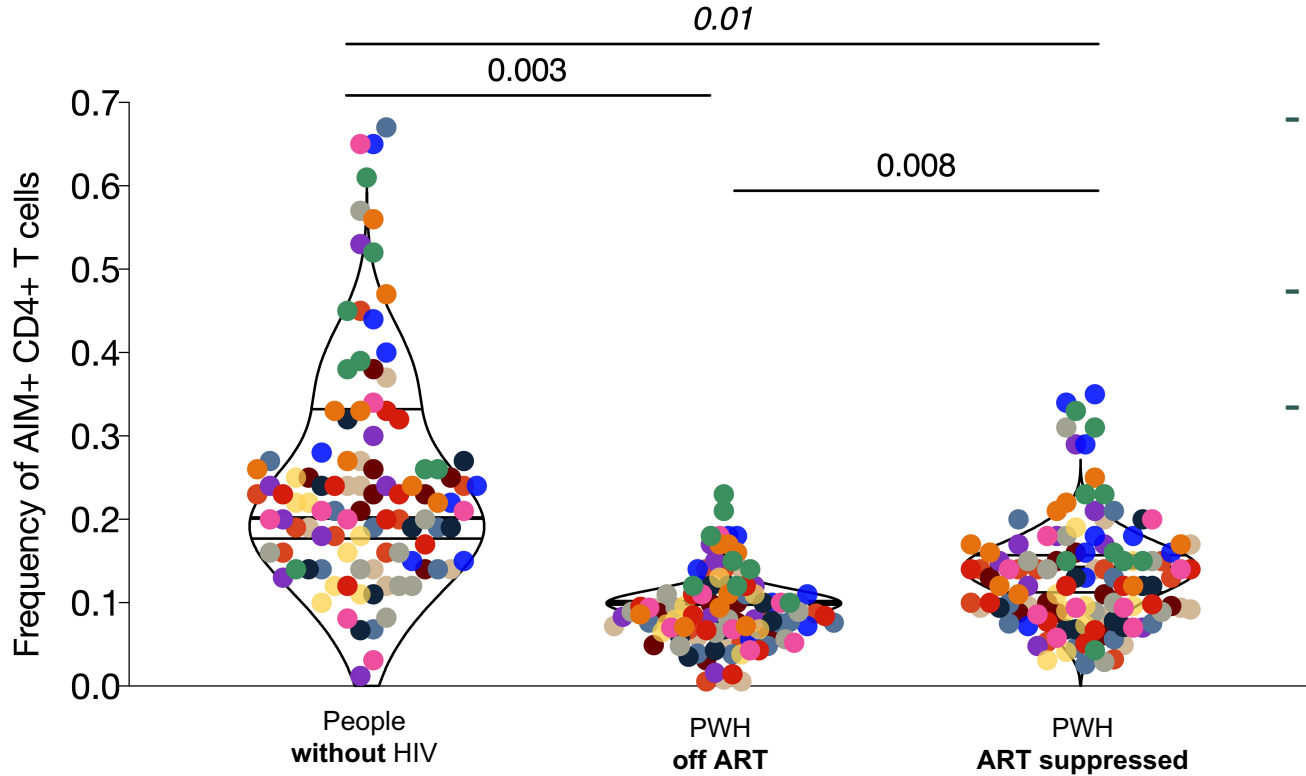


Validation of the AIM assay



- Antibody blockade of HLA-II a loss of the AIM signal
- This suggest that the activation by the AIM assay is TCR-mediated

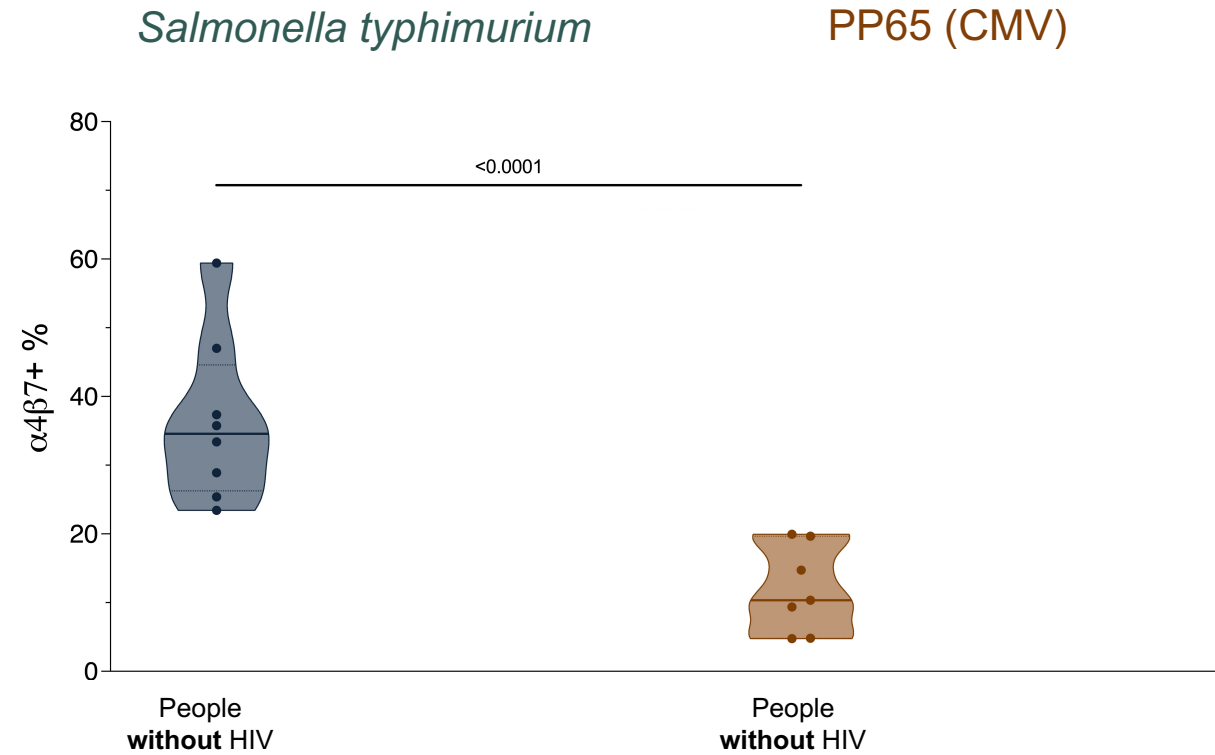
Frequencies of gut-bacteria specific CD4+ T cells



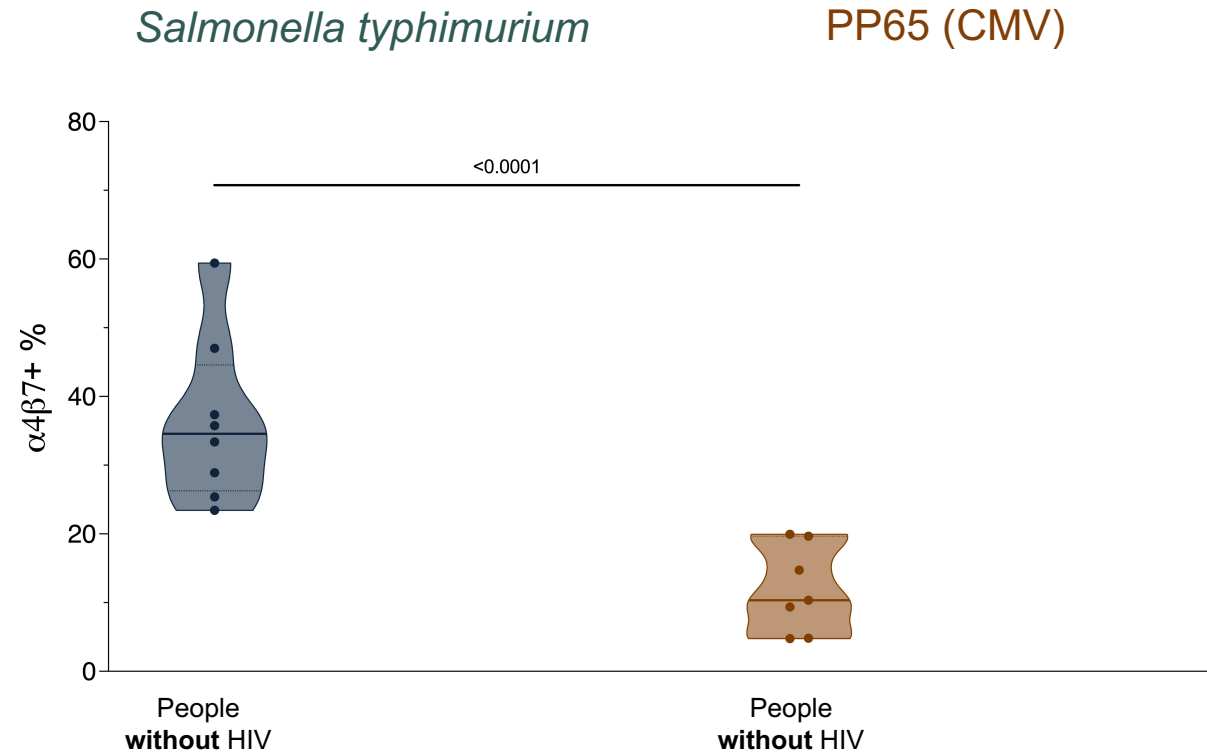
- HIV infection is associated with lower frequencies of gut-bacteria specific CD4+ T cells
- ART only partially restores this phenomenon
- This depletion may be due to the preferential infection by HIV and subsequent death of these cells

● *B. animalis* ● *B. luti* ● *C. difficile* ● *L. acidophilus* ● *P. copri*
● *B. obeum* ● *E. coli* ● *M. uniformis* ● *S. typhimurium*
● *B. vulgatus* ● *F. prausnitzii* ● *P. stercorea* ● *R. intestinalis*

High levels of gut homing molecules on *Salmonella typhimurium* specific CD4+ T cells



High levels of gut homing molecules on *Salmonella typhimurium* specific CD4+ T cells

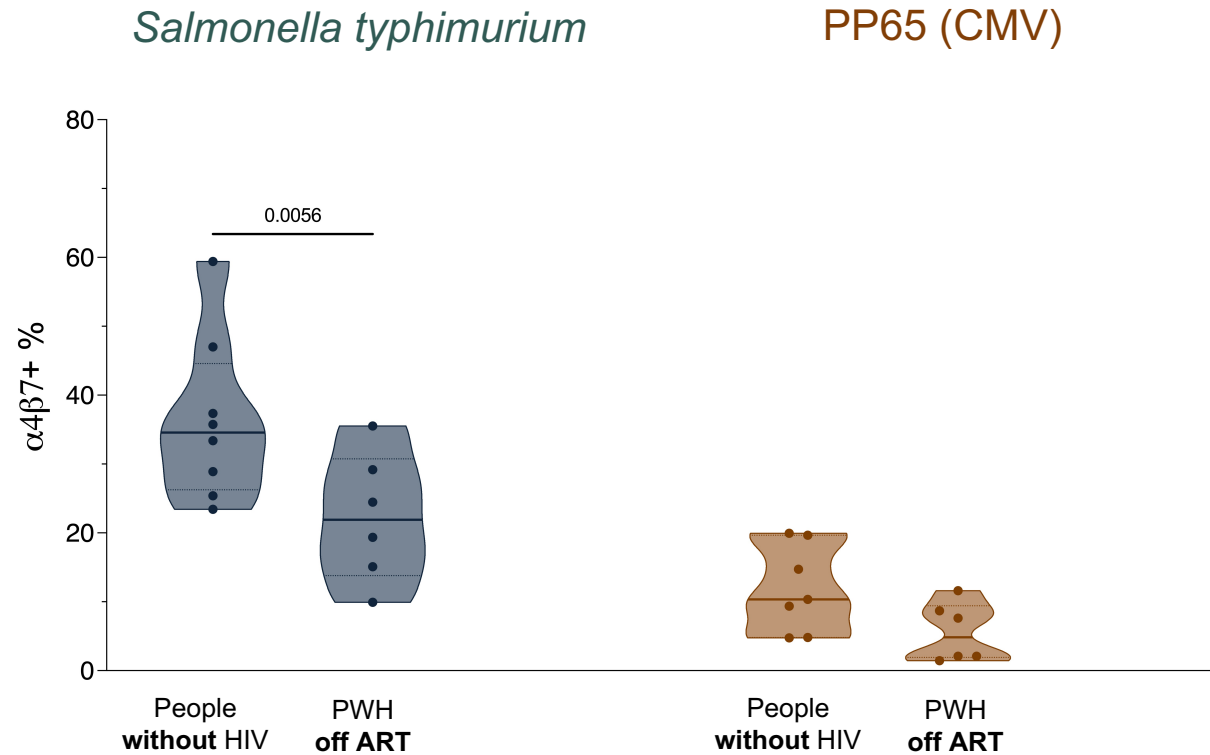


- $\alpha 4\beta 7+$ cells have been shown to be enriched in total HIV DNA

(Xie, 2021; Asowata, 2021; Jiminez-Leon, 2024)

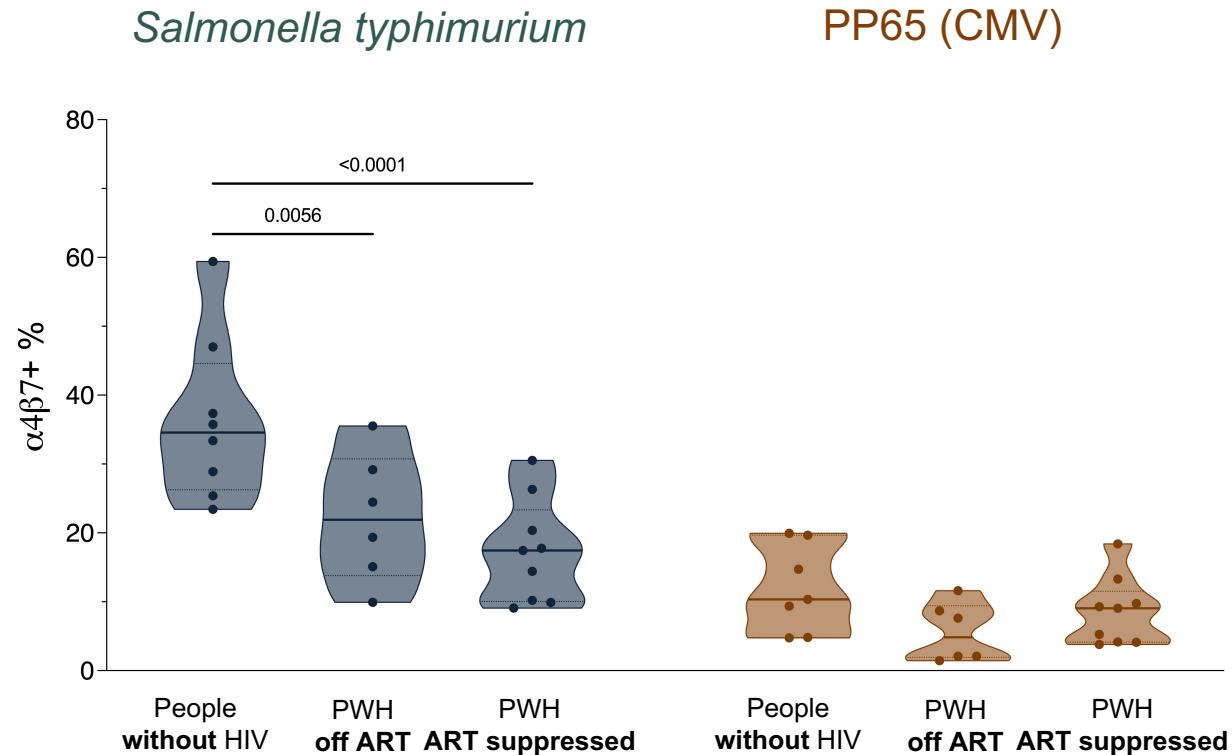
- *Salmonella Typhimurium* specific CD4+ T cells expressed higher level of this gut homing integrin than CMV specific CD4+ T cells

High levels of gut homing molecules on *Salmonella typhimurium* specific CD4+ T cells



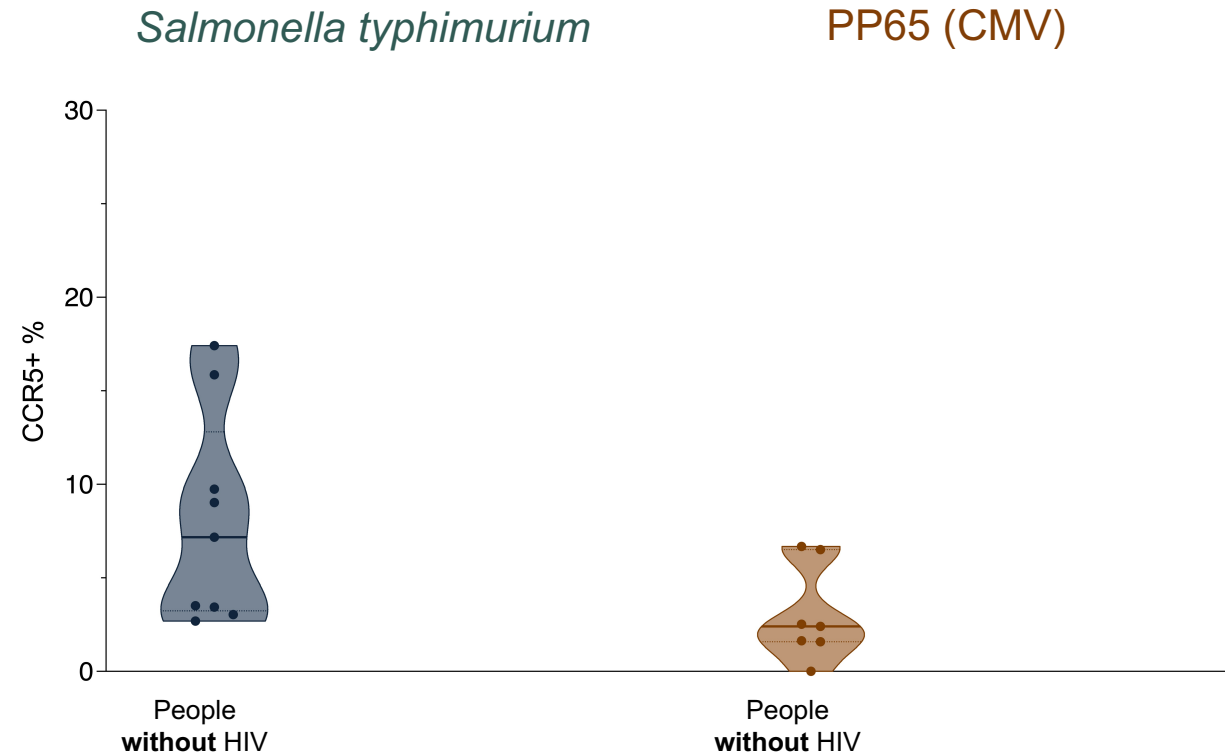
- We see a decrease of the frequency of $\alpha 4\beta 7+$ cells in PWH off ART

High levels of gut homing molecules on *Salmonella typhimurium* specific CD4+ T cells



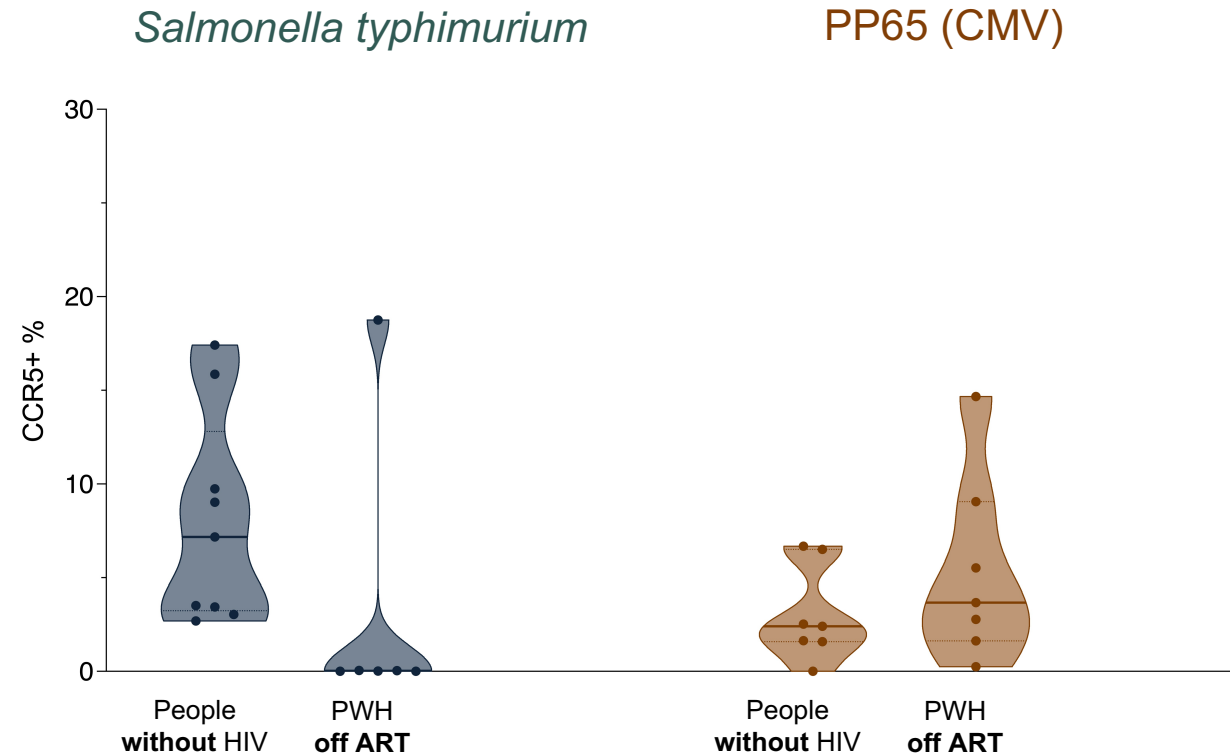
- This is maintained in ART suppressed individuals for bacteria specific CD4+ T cells but not CMV specific cells

Depletion of susceptible CD4+ T cells



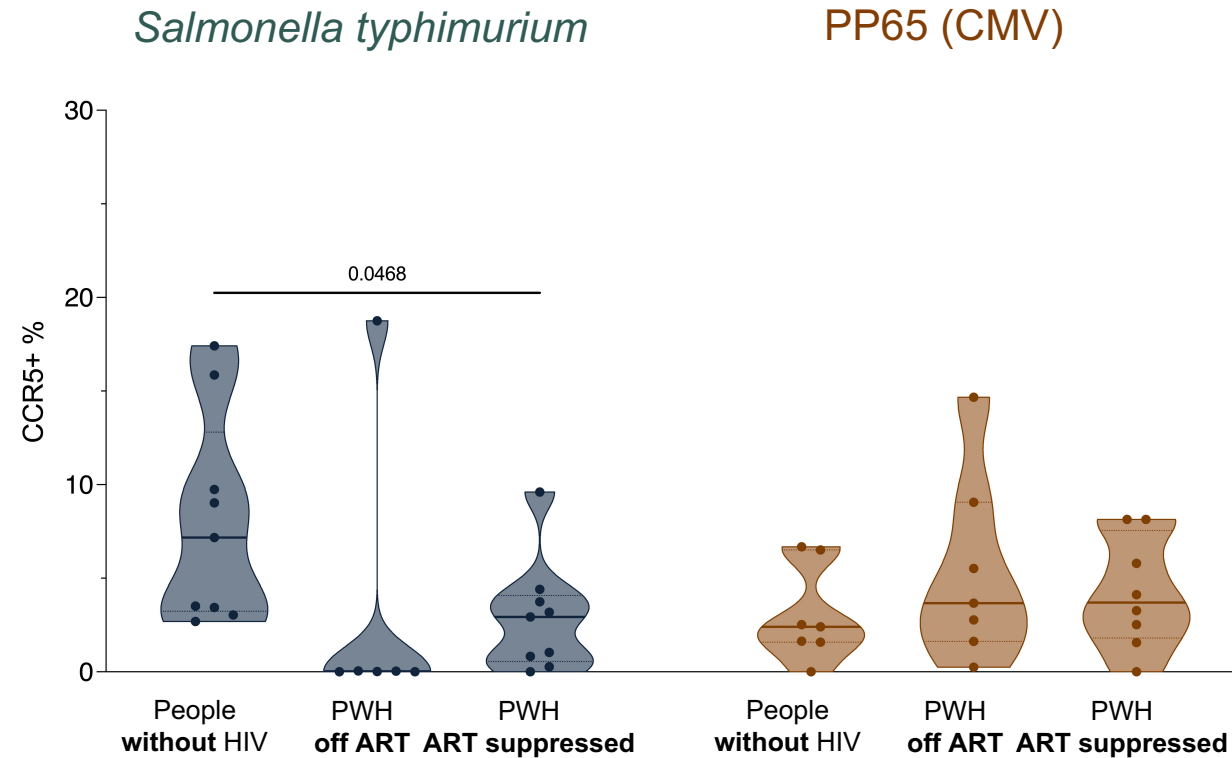
- There was a trend for higher CCR5 expression in *Salmonella typhimurium* specific CD4+ T cells rather than in CMV specific CD4+ T cells

Depletion of susceptible CD4+ T cells



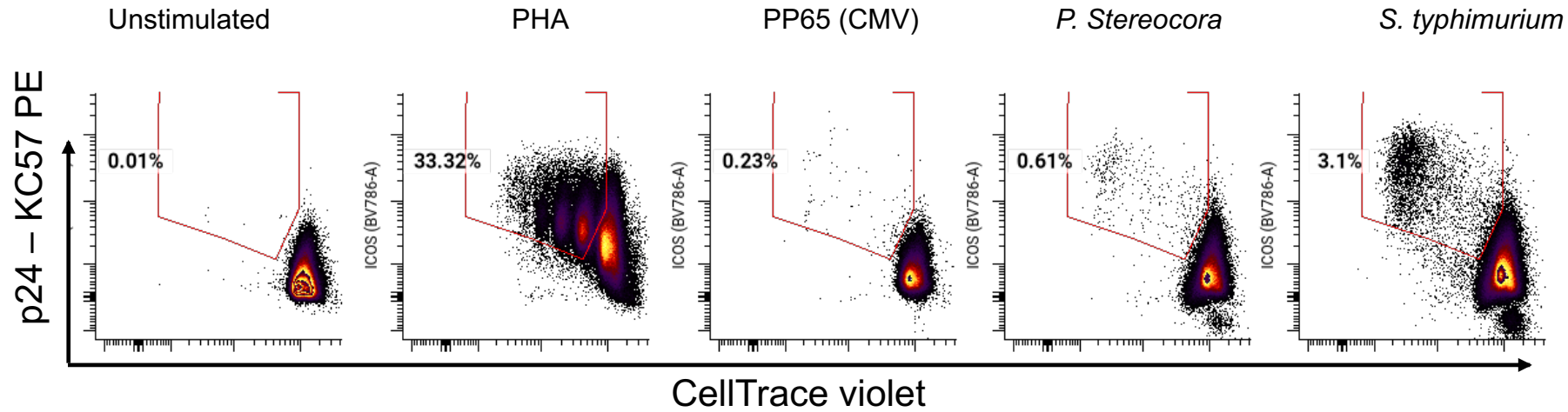
- CCR5 expression on *Salmonella typhimurium* is decreased in PWH
- This suggest a depletion of CCR5+ *Salmonella typhimurium* specific CD4+ T cells

Depletion of susceptible CD4+ T cells



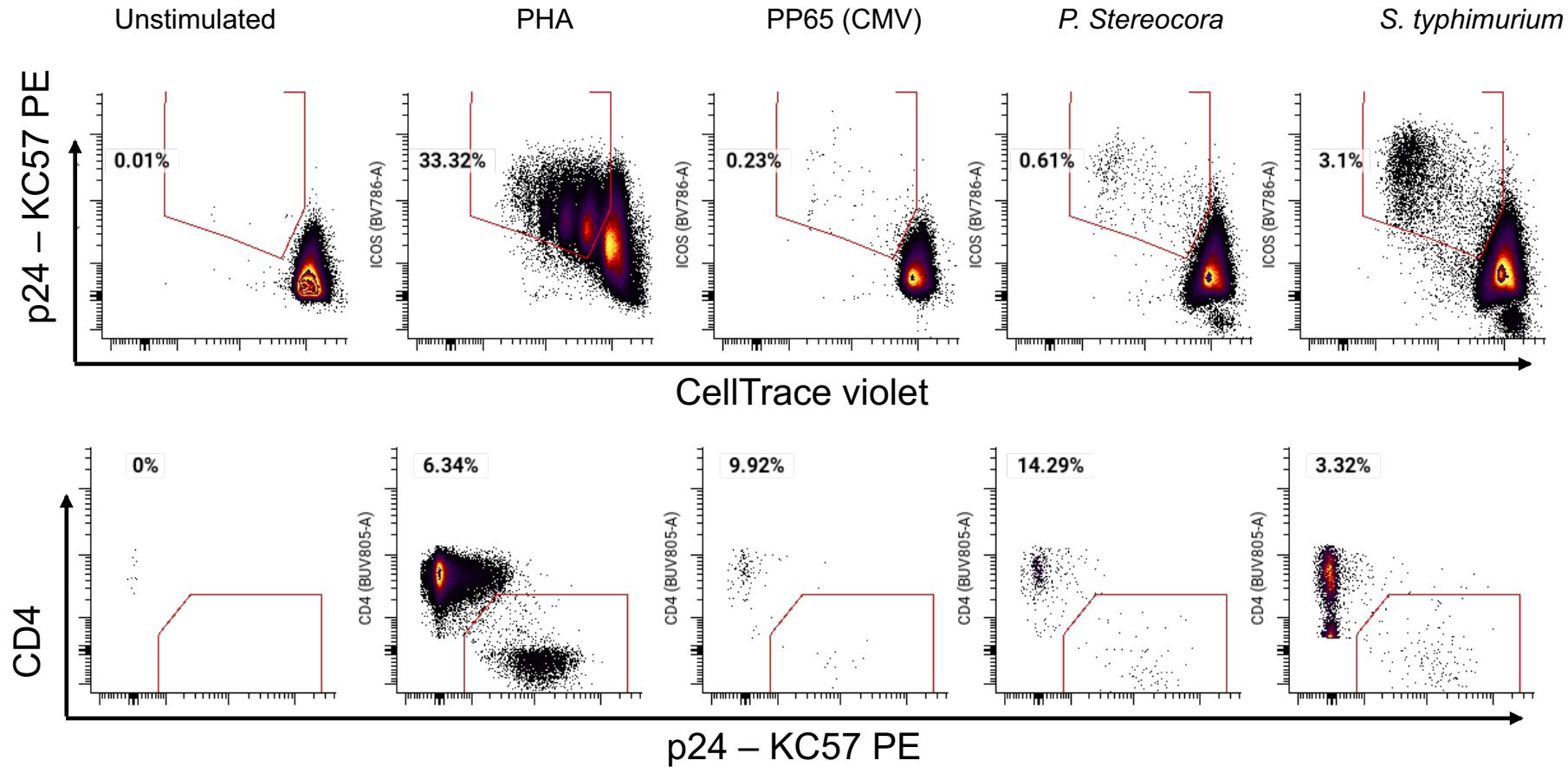
- This phenomenon is maintained in ART suppressed individuals for *Salmonella Typhimurium* specific CD4+ T cells but not for CMV specific CD4+ T cells

In vitro infection of gut-bacteria specific CD4+ T cells



CCR5 tropic TF virus
(HIV-1 33931N)

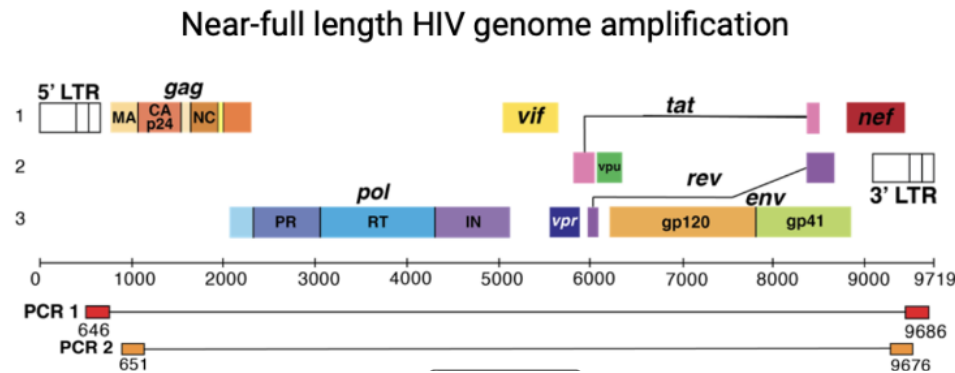
In vitro infection of gut-bacteria specific CD4+ T cells



CCR5 tropic TF virus
(HIV-1 33931N)

Contribution to HIV reservoir

We performed near-full length amplification of HIV genomes on sorted bacteria specific CD4+ T cells using an approach developed by the Palmer group, and adapted in our lab (Hiener et al. 2018, Dufour et al. 2023)

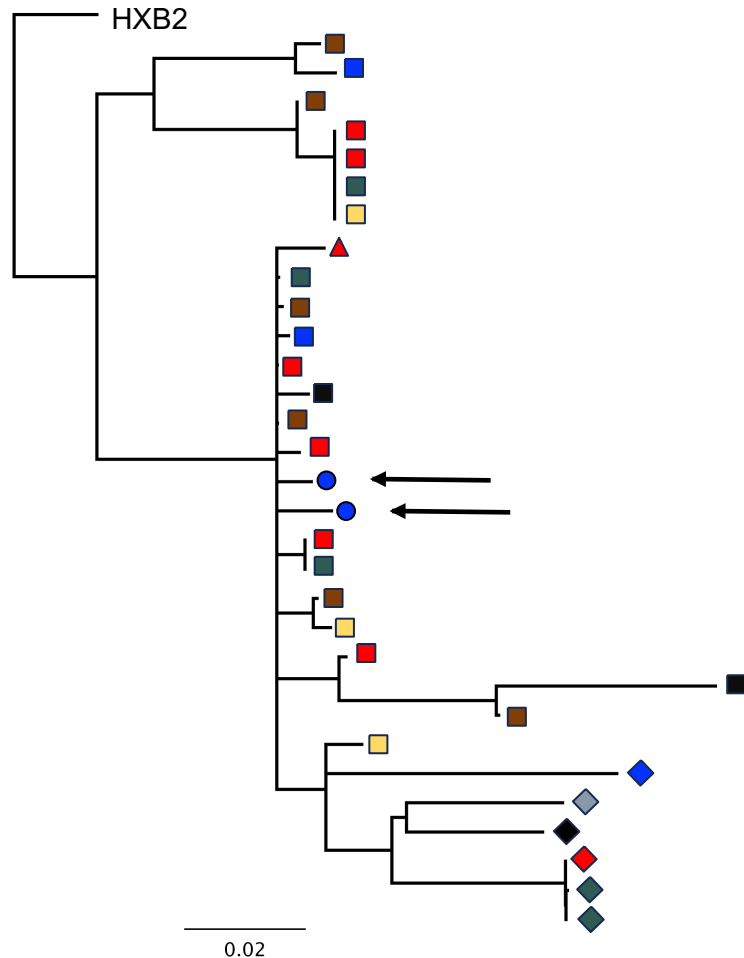


This approach followed by next-generation sequencing (PacBio and Nanopore) allows us to determine:

- Intactness of proviruses
- Clonality of HIV reservoir cells

Proviral landscape

One participant



Proviral landscape

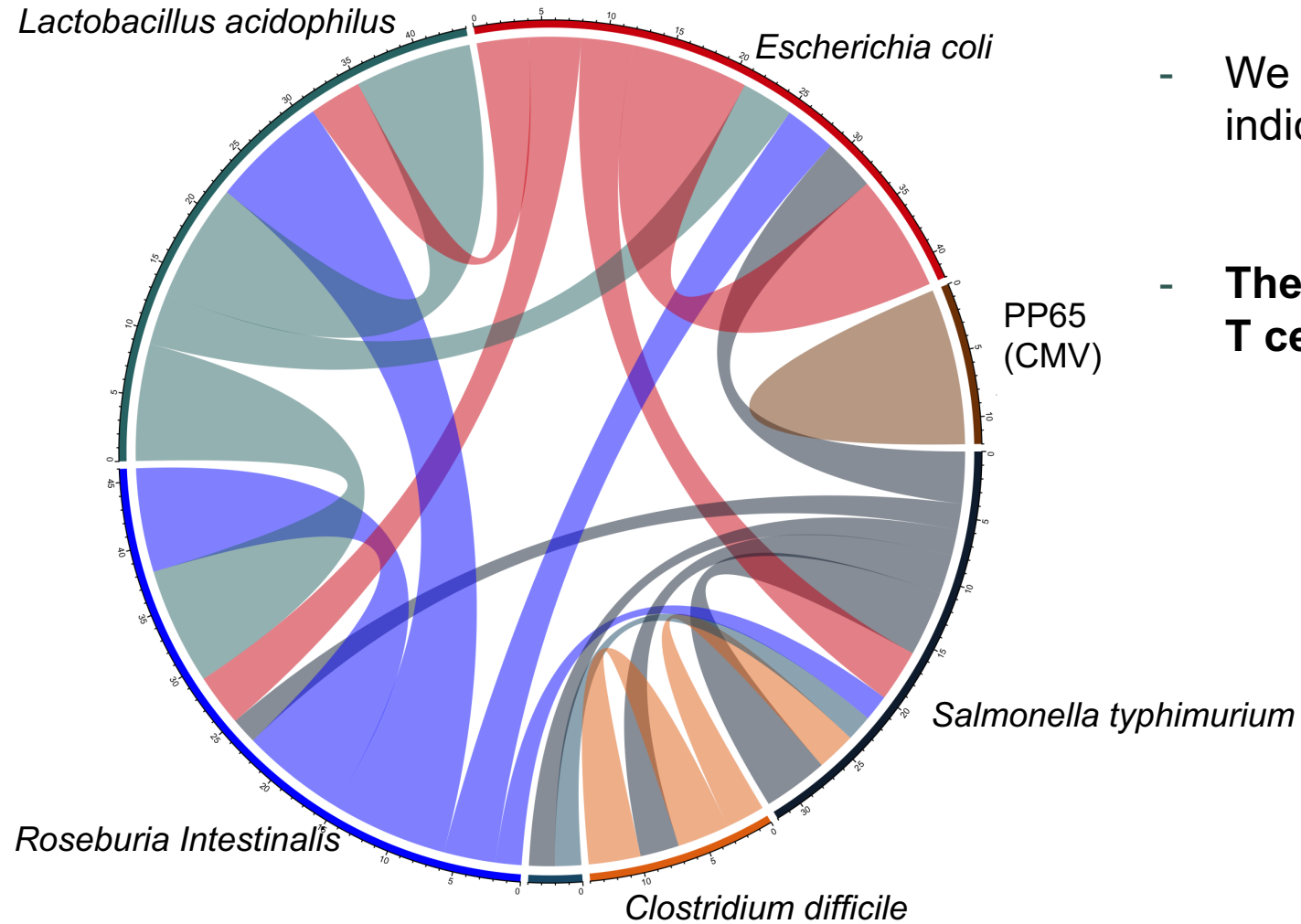
- Major Deletion
- △ MSD Missing
- Intact
- ◇ Hypermutations

Antigen Specificity

- Total CD4
- CMV – PP65
- *Lactobacillus animalis*
- *Roseburia intestinalis*
- *Escherichia coli*
- *Salmonella typhimurium*

- We detected proviruses from all bacterial specificities tested, indicating that gut bacteria specific CD4+ T cells harbor HIV DNA in ART-suppressed individuals
- We saw shared HIV clones between bacterial species indicating **cross-reactivity**

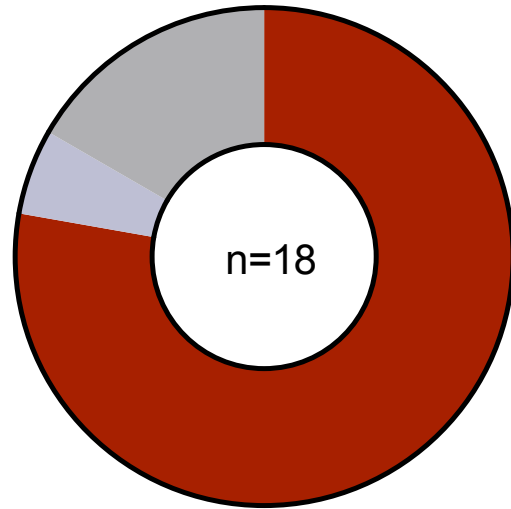
Cross reactivity



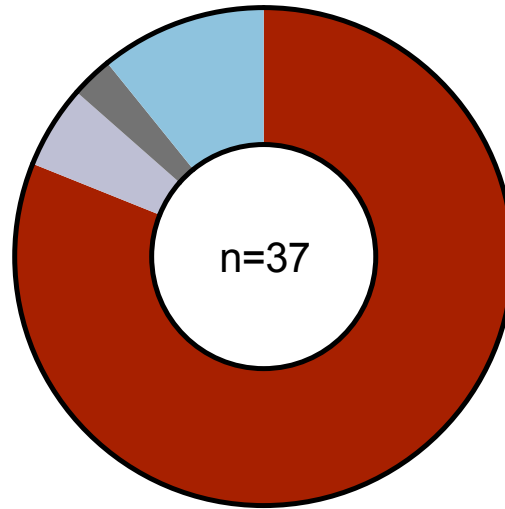
- We saw shared HIV clones between bacterial species indicating **cross-reactivity**
- **These clones were not found in PP65 specific CD4+ T cells**

Interactivity map showing clonal proviruses only

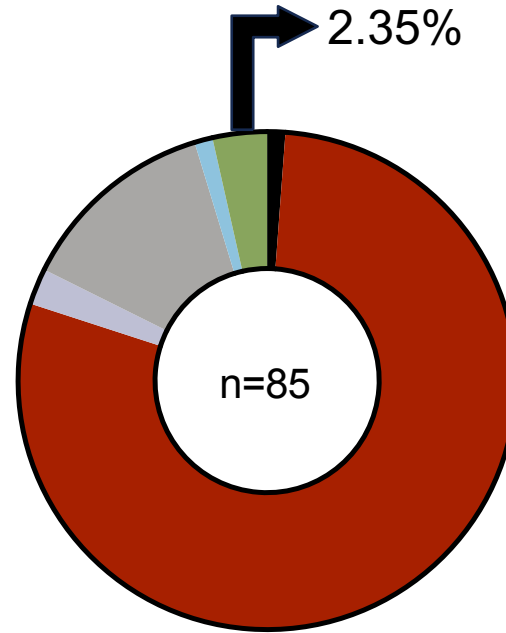
Provirus integrity



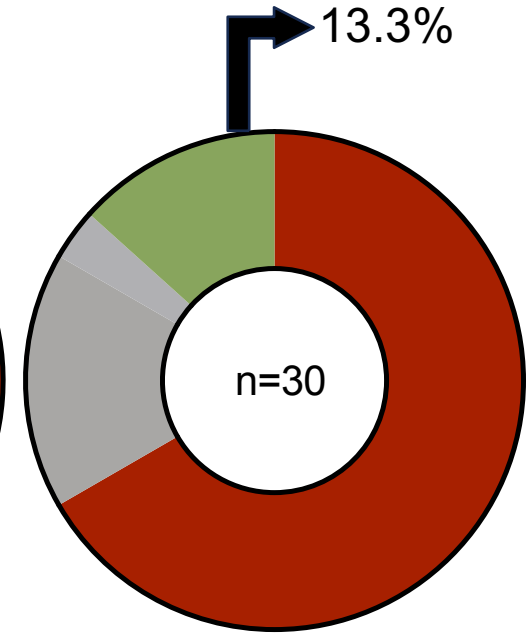
Roseburia intestinalis



PP65 (CMV)



CD4



Salmonella typhimurium



- Among 200 sequences retrieved from 5 participants, we detected 6 distinct genetically inferred intact proviruses in 3 participants
- **4** of them were retrieved in **S. typhimurium specific cells from 2 participants**
- Why S. Typhimurium specific CD4+ T cells would be a preferential reservoir for HIV?

Conclusions

- Bacteria specific CD4+ T cells are preferentially depleted during untreated HIV infection and not fully restored by ART
- These cells express markers associated with HIV permissiveness and can be infected in vitro
- We retrieved proviruses from all bacterial specificities tested, demonstrating that **gut bacteria specific CD4+ T cells carry HIV DNA**
- These bacteria-specific infected cells can undergo clonal proliferation
- **Intact proviruses were found in *S. typhimurium* specific CD4+ T cells.**
- Immune reconstitution is incomplete since the functions of gut-bacteria specific CD4+ T cells remain altered in ART-suppressed individuals

Next steps

- We plan to sort gut bacteria-specific CD4+ T cells from participants in acute HIV infection (<2 months) to determine if the depletion of these cells occurs at the earliest stage
- We will perform TCR sequencing on gut bacteria specific CD4+ T cells to measure cross-reactivity between bacterial species
- In addition, we are planning to perform AIM assays followed by provirus sequencing in gut isolated cells from ART suppressed individuals

Acknowledgments

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To all participant !

