# CD8+ T Cells Shape the HIV Integration Site Landscape According to the Degree of T Cell Pressure

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## Abstract

**In rare individuals who exhibit CD8-mediated control of HIV replication and people on long-term antiretroviral therapy (ART), the integration sites of intact HIV proviruses become skewed towards transcriptionally inactive genomic loci but the underlying mechanisms remain to be elucidated. Here we determine the role of elite controller CD8+ T cell pressure in shaping the HIV integration site landscape.**

## Introduction

- During HIV infection, **proviral DNA integrates into human chromosomes** – preferentially into transcriptionally active genes.
- In HIV elite controllers, proviral integration sites in **transcriptionally silent genomic loci are overrepresented**.
- Understanding the **processes that drive the selection** for cells with such integration characteristics will support the interpretation of clinical studies.

## Key Findings

**Elite controller CD8 T cells shape the proviral landscape, promoting control-associated features**.
This includes favoring proviruses that are:

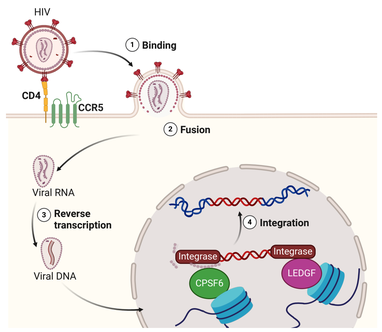
- Highly **clonal**
- Located **outside of genes**
- Positioned **further from genes**
- Enriched in **repetitive DNA elements**

The extent of these features correlates with the degree of CD8+ T cell control in a given PDX mouse. This selection process can be modeled using a Bayesian mixture model.

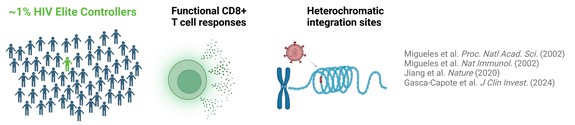
## Acknowledgments

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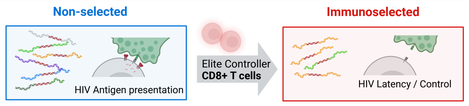
## Background



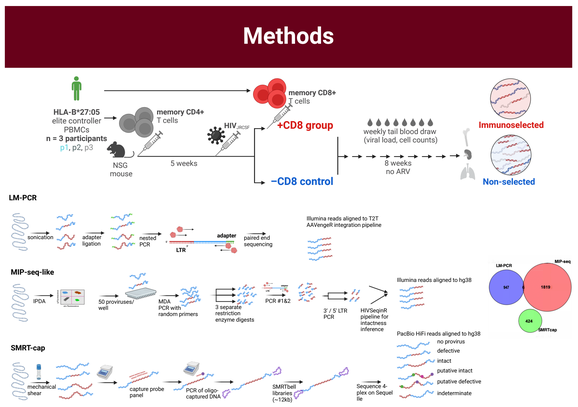

**Mechanism of HIV genomic insertion:** Host proteins including LEDGF and CPSF6 recruit HIV proviral DNA to actively transcribed genes.



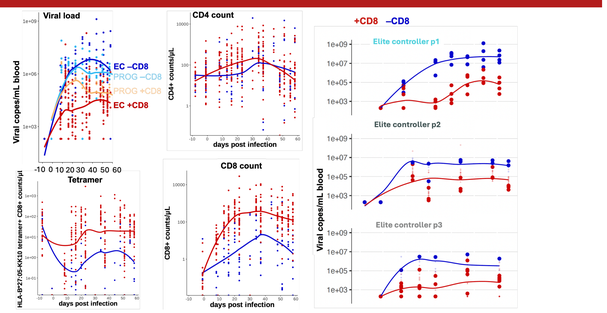

**Hypothesis: CD8+ T cell responses impact the HIV integration site landscape, selecting for less transcriptionally active provirus.**



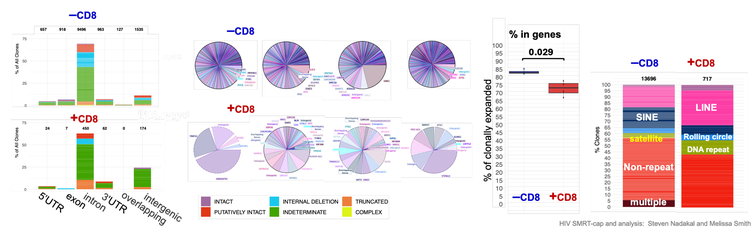

## Methods




## Sustained CD8+ T cell pressure eliminates the vast majority of infected cells




## Enrichment in HIV integrations in intergenic regions




HIV SMRT-cap and analysis: Steven Nadakal and Melissa Smith

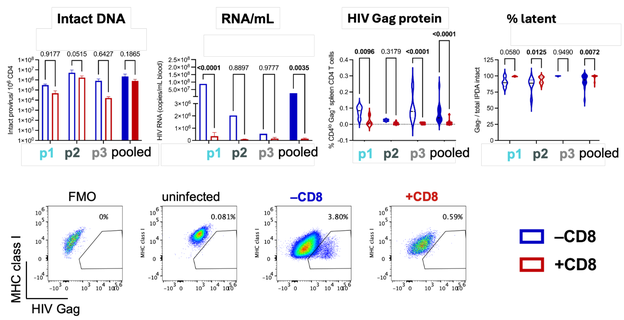
## Conclusion

**Elite controller CD8+ T cells are sufficient to select for more clonal integration sites and prune the total pool of integrations in favor of proviruses:**

1. Outside of genes,
2. Further away from genes,
3. In repetitive DNA elements.

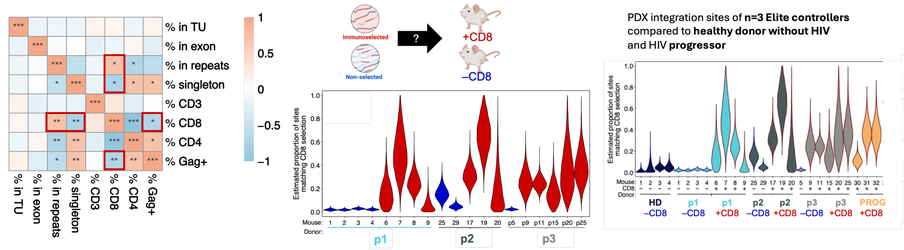
This selection is associated with a reduction in the overall number of infected cells and an increase in HIV latency. Selection at the integration site level correlates with measures of CD8+ T cell pressure in PDX mice. This can be modeled using Bayesian inference. Our data opens the possibility that the HIV integration site landscape may be harnessed as a readout of CD8+ T cell pressure, which could be a useful tool for clinical trials aimed at an HIV cure.

## Elite controller CD8+ T cells selectively eliminate cells that are actively transcribing and producing HIV proteins




## CD8+ T cells shape the HIV integration site landscape according to the degree of T cell pressure

- Correlation of **%CD8+ T cells** in spleen and integrations **in repeats**
- Negative correlation of **%CD8+ T cells** in spleen and **unique integration sites**
- **Bayesian mixture model** distinguishes +CD8 mice from −CD8 mice
- Estimates proportion of integrations selected by CD8+ T cell pressure

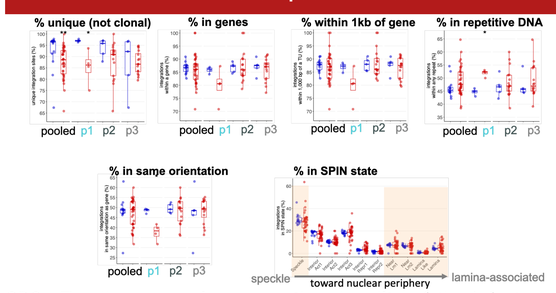



**Integration sites of endogenous HIV** in total memory CD4+ T cell of n=3 human elite controller donors

| | sites | sonicLengths (mean \| sd) | in TUs (%) | in exons (%) | in repeats (%) | in same orientation (%) | singletons (%) |
|---|---|---|---|---|---|---|---|
| p1 | | | | | | | |
| GTSP6119 | 5 | 6 (1.2)[0.4) | 4 (80) | 0 (0) | 2 (40) | 4 (80) | 4 (80) |
| p2 | | | | | | | |
| GTSP6120 | 13 | 43 (3.3)[4) | 11 (84.6) | 0 (0) | 9 (69.2) | 6 (46.2) | 8 (61.5) |
| p3 | | | | | | | |
| GTSP6121 | 12 | 49 (4.1)[5.1) | 10 (83.3) | 0 (0) | 8 (66.7) | 5 (41.7) | 6 (50) |

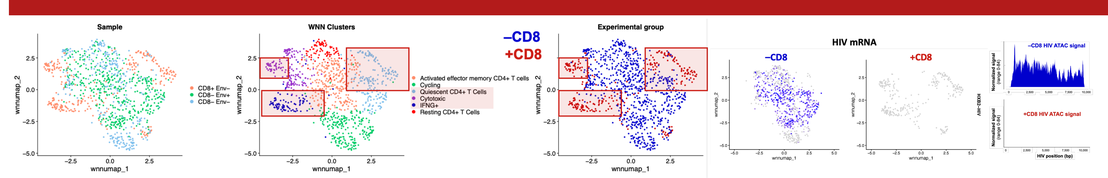
**Future directions**: Test the effect of individual CD8+ T cell clones of known antigen specificity, sensitivity, and avidity on the integration site landscape.

## CD8+ T cells select for HIV integration sites in loci associated with transcriptional silence



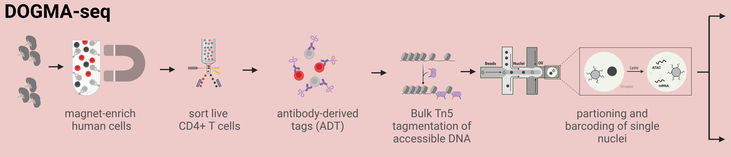

CD8+ T cell pressure **increases the clonality** of the infected cells and selects for provirus **outside of transcriptional units** (TU), **further away from the nearest TU**, and preferentially located **in repeats**.

## Phenotypic changes of infected cells under CD8+ T cell pressure




- Absence of HIV RNA+ and HIV ATAC+ cells in the +CD8 samples sampled.

**DOGMA-seq**




**Future directions**: DOGMA-seq to determine changes in gene expression in the presence of CD8+ T cells, determine clonality of uninfected cells, characterize phenotype of HIV+ killing-resistant cells.

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