In-depth single-cell analysis of translation-competent HIV-1 reservoirs identifies cellular sources of residual viremia and rebound viruses

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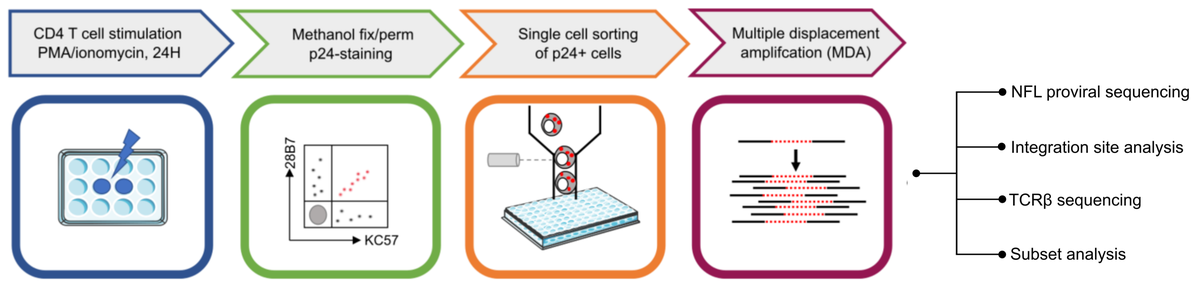
1 Background

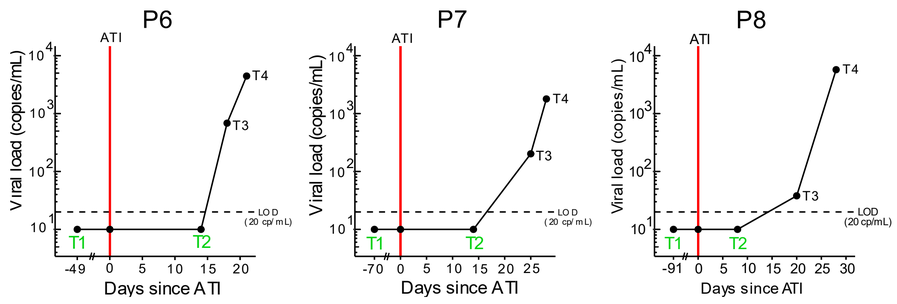
- HIV-1 infection remains incurable due to the establishment of a **persistent viral reservoir**, capable of causing viral rebound upon antiretroviral treatment interruption.
- Different mechanisms of **clonal expansion** of infected cells contribute to the long-term maintenance of the reservoir: antigen-driven, homeostatic and integration site-driven proliferation.
- Previous studies have shown that large, infected cell clones can harbor replication-competent proviruses, though their **contribution to residual viremia and rebound viremia** is largely unknown.
- We developed a single-cell assay for in-depth characterization of the translation-competent reservoir, called **STIP-seq: S**imultaneous **T**CR, **I**ntegration site and **P**rovirus sequencing. Using this assay in the context of an analytical treatment interruption (ATI) study, we found matches between proviruses recovered with STIP-seq and plasma sequences obtained before and during the ATI.

2 Methods

1. STIP-seq was performed on 8 cART-suppressed individuals.



2. STIP-seq was performed on 3 participants that underwent an ATI, both before (T1) and during (T2) the ATI.



3. Single genome sequencing (V1-V3 *env*) was performed on plasma virus at timepoints T1-T4, and these sequences were phylogenetically compared to the STIP-seq sequences.

3 Results

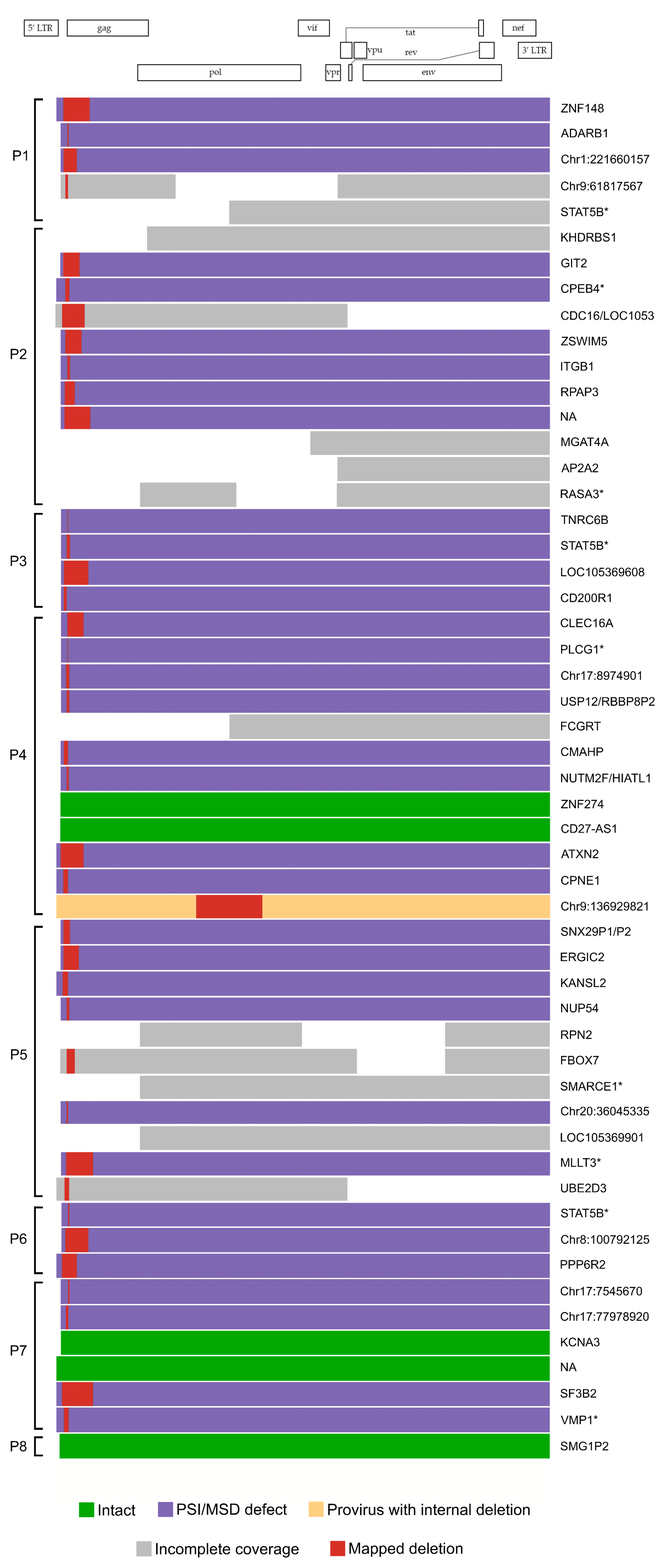


Figure 1: Virogram of near full-length proviral sequences obtained with STIP-seq on 8 cART-suppressed individuals. Proviral genomes were reconstructed using a 5-, 2-, or 4-amplicon approach. Corresponding integration sites are indicated at the right-hand side of each proviral genome. Cancer-related genes are indicated with an asterisk.

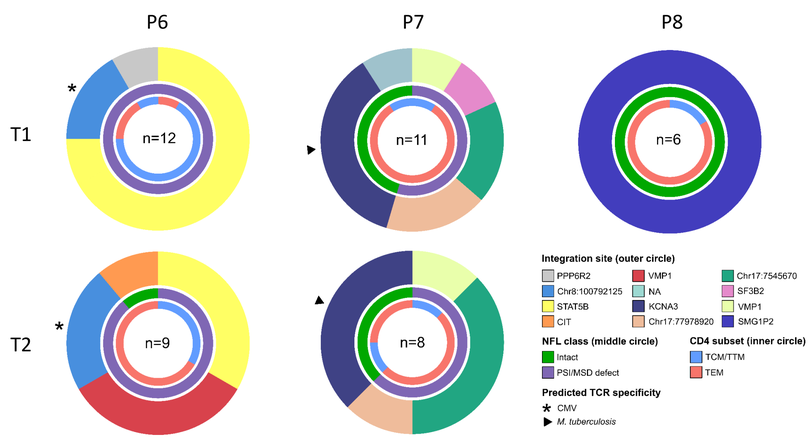


Figure 2: Donut charts displaying integration sites, NFL class, and memory subsets of p24-producing cells recovered before (T1) and during (T2) the ATI. The number of analyzed p24+ cells is indicated for each participant. Predicted pathogen-specificities are indicated with symbols.

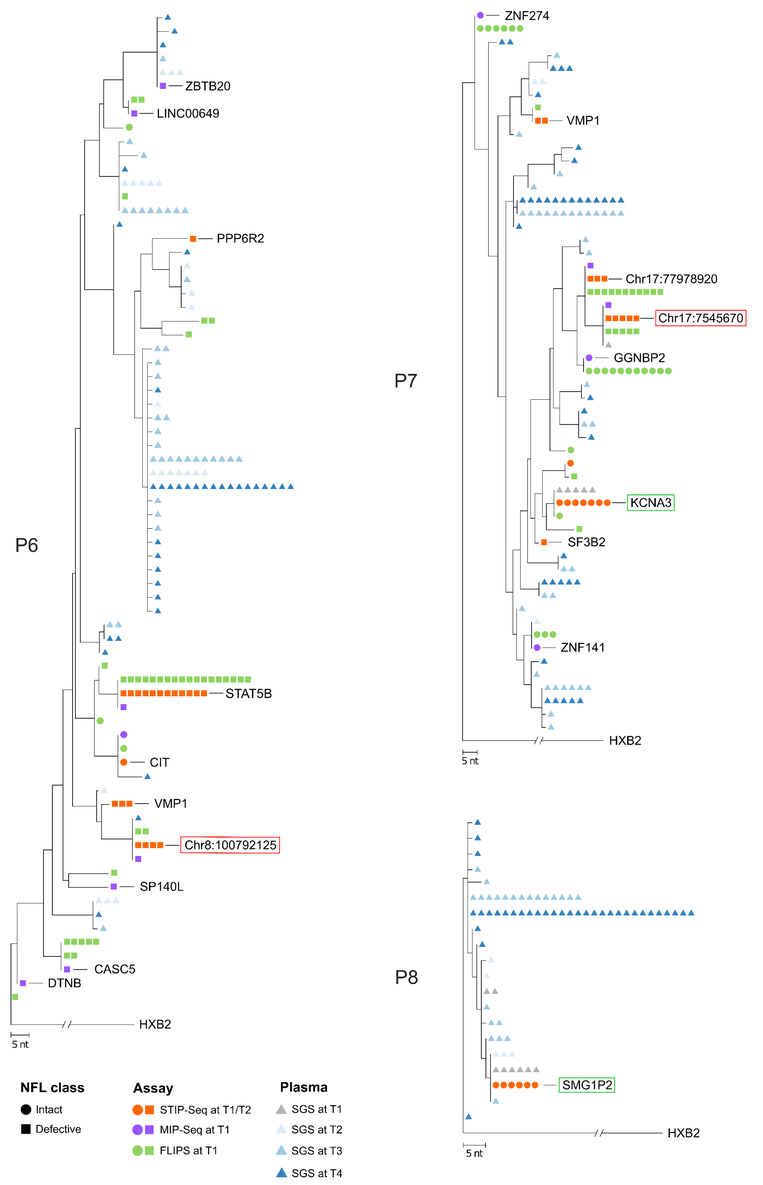


Figure 3: Maximum-likelihood phylogenetic trees (V1-V3 *env)* for 3 participants that underwent an analytical treatment interruption. The trees include V1–V3 *env* plasma sequences from before (T1) and during (T2, T3, and T4) the treatment interruption (P6, P7, and P8), as well as STIP-Seq, MIP-Seq and FLIPS sequences (T1) that were trimmed to the V1–V3 env region (P6, P7). Clones displaying a match between defective and intact STIP-Seq sequences and plasma sequences are indicated by red and green frames, respectively.

- A total of 40 distinct proviral genomes with full coverage were obtained from 8 cART-treated individuals (**Figure 1**), falling into three categories: genome-intact (5/40), packaging signal (PSI) and/or major splice donor (MSD) defects (34/40) or large internal deletion (1/40).
- Nine proviruses were integrated into a cancer-related gene (**Figure 1**, indicated with asterisk). Some of these were found in cells with predicted pathogen-specificity (Influenza, *M. tuberculosis*).
- In the context of an ATI, 29 p24+ cells at T1 and 17 p24+ cells at T2 were recovered (**Figure 2**). Little differences between both timepoints were observed, but a new clone was detected at T2 in participant P6, integrated in the *VMP1* gene.
- In participants P6 and P7, a link between a defective provirus obtained with STIP-Seq and a plasma virus could be found (**Figure 3**).
- Participant P7 and P8 both display a match between an intact STIP-Seq provirus and plasma virus obtained at T1. The provirus from P7 is integrated in *KCNA3,* which is a gene involved in cell proliferation. Furthermore, this clone has a predicted specificity towards *M. tuberculosis.* The provirus from P8 (IS in *SMG1P2*) also matches to rebounding plasma virus obtained at T2 and T3 (**Figure 3**).

4 Conclusions

- We developed a single-cell assay that captures 4 layers of information of the translation-competent reservoir: TCR, integration site, proviral sequence and phenotype of the cell.
- p24+ cells are enriched in NFL proviral genomes that have deletions at the 5’ end of the genome. Our data suggest that the MSD is a particular hotspot for deletion.
- Cells with predicted TCR specificity towards pathogens can harbor proviruses integrated into cancer-related genes, suggesting that several mechanisms can synergize to favour the peristence of the translation-competent reservoir.
- Our data indicate that STIP-Seq can capture clones that contribute to low-level viremia and viral rebound.

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