Clonally expanded HIV-1 proviruses with 5'-Leader defects can give rise to nonsuppressible residual viremia.

Jennifer A. White^{*}, Fengting Wu^{*}, Saif Yasin, Milica Moskovljevic, Joseph Varriale, Filippo Dragoni, Angelica Camilo Contreras, Jiayi Duan, Mei Y. Zheng, Ndeh F. Tadzong, Heer B. Patel, Jeanelle Mae C. Quiambao, Kyle Rhodehouse, Hao Zhang, Jun Lai, Subul A. Beg, Michael Delannoy, Christin Kilcrease, Christopher J. Hoffmann, Sébastien Poulin, Frédéric Chano, Cécile Tremblay, Jerald Cherian, Patricia Barditch-Crovo, Natasha Chida, Richard Moore, Michael F. Summers, Robert F. Siliciano, Janet D. Siliciano, Francesco R. Simonetti.

Supplementary Materials

Table of contents

Supplementary Results

-Extended clinical history of study participants.
-Integration site analysis of proviruses causing viremia.
-CD4⁺ T cell repertoire analyses from participant P1.
-Analysis of autologous neutralization

Supplementary Methods

-5'-Leader RNA studies.

-Western blots.

-Transmission Electron Microscopy.

-Duplex quantification of total LTR copies and specific provirus.

-T cell subset analysis.

Supplementary References

Supplementary Figures

Figure S1. Clinical history and HIV-1 population analysis of Participant 3.

Figure S2. Clinical history and HIV-1 population analysis of Participant 4.

Figure S3. Additional HIV-1 sequence analyses related to Figure 1.

Figure S4. Viral outgrowth assay from P1 shows lack of exponential outgrowth.

Figure S5. 5'-Leader deletions cause a modest decrease in the number of nucleocapsid binding sites.

Figure S6. Validation of digital PCR assays that selectively amplify proviruses of interest, related to Figure 4.

Figure S7. Deletions do not alter tRNA binding propensity to the 5'-Leader.

Figure S8. Production of viral particles upon transfection with 5'-Leader-defective NL4-3.

Figure S9. Impact of controlled shearing on genomic DNA used in integration site-specific digital PCR assays.

Figure S10. TCR analyses of CD4⁺ T cell central and effector memory subsets.

Figure S11. Isolation of antigen-responding CD4⁺ T cells from PBMCs in P1.

Supplementary Tables

-Table S1. Study participant characteristics.

-Table S2. Integration site analysis of proviruses contributing to NSV.

-Table S3. ITC isotherm fitting data of NC to NL4-3 WT, d21, d22 to full saturation.

-Table S4. Analysis of CTL mutations.

-Table S5. Oligos used in this study.

Supplementary Results

Extended clinical history of study participants. P1 is an HLA-B*57:03⁺ slow-progressor who initiated ART with a CD4 nadir of 454 cells/µL more than 20 years after HIV-1 diagnosis. Over time, he developed comorbidities including type-2 diabetes, hypertension, mixed hyperlipidemia, and hypogonadism. At 6.8 years on ART, he had an HIV-1 reservoir size significantly below average (<0.06 infectious units per million (IUPM) by the quantitative viral outgrowth assay (QVOA) (1), and 3.5 copies of intact proviruses/10⁶ total CD4+ T cells by the intact proviral DNA assay (IPDA) (2). P2 had a diagnosis of HIV-1/AIDS in 1991, a nadir of 197 cells/µL, and has been on ART for more than 26 years. He had a Roux-en-Y gastric bypass in 2010 and a history of hyperlipidemia, hypogonadism, and benign prostatic hyperplasia. His reservoir size falls on the opposite side of the spectrum, with 15 IUPM by QVOA and 311 intact proviruses/10⁶ total CD4⁺ T cells. P3 initiated ART shortly after diagnosis in 2007 with a nadir of 221 cells/µL and maintained an undetectable viral load for almost 10 years. Her reservoir size falls within the average of most individuals who started ART during chronic infection (161 copies of intact proviruses/10⁶ total CD4⁺ T cells) (3). P3 is on chronic treatment with azathioprine due to type-I autoimmune hepatitis. P4 had a diagnosis of HIV-1/AIDS and Kaposi's Sarcoma around 1990 and has been on ART for more than 26 years. Although medical records before 2016 are limited, he maintained prolonged suppression until 2020, when his viral load reached a plateau on the order of 10³ copies/mL. This was initially interpreted as virological failure, and his clinical care providers thoroughly assessed drug resistance, drug concentrations, and adherence, which were all unremarkable. ART optimization, intensifications, and one month of directly observed therapy failed to decrease NSV. His comorbidities include type-2 diabetes, psoriasis, and bilateral nephrolithiasis. P4's reservoir size is also within the typical range of PLWH on ART (58 intact proviruses/10⁶ total CD4⁺ T cells) (3). At the time of this report, all four participants are in overall good health and show stable CD4⁺ T cell counts above 600 cells/µL, while HIV-1 RNA in plasma remains above 20 copies/mL despite ART.

Integration site analysis of proviruses causing viremia. The site of HIV-1 integration into particular host genes, for example host genes involved in cell proliferation, may affect the persistence of infected cells as it can lead to aberrant expression of the host gene in a way that promotes the survival and proliferation of a clone of infected cells (4). In rare cases, the site of HIV-1 integration can contribute to silencing of the integrated provirus (5). In addition, the analysis of HIV-1 sub-genomic sequences can lead to an overestimation of proviral clonality (6, 7). Therefore, we s confirmed the clonal nature of proviruses causing viremia by integration site analysis. Through paired provirus and integration site data based on limiting dilution and whole genome amplification (7, 8), we characterized proviruses matching variants found in plasma (Figure 2C and Supplementary Table S2) from total or effector memory cells, as the latter have been linked to higher infection frequency and clonal expansion (9-11). The predominant plasma clone found in P1 is integrated the first intron of the Adenosine Kinase (ADK) gene, downstream of the translation start site of both long and short ADK splice variants (nuclear and cytoplasmic forms, respectively). In P2, we recovered the integration sites for 3 proviruses causing viremia at one or more time points. These proviruses were located in the AAK1, DNAJB14, and RRM1 genes (see Supplementary Table, S1). In uninfected individuals, these four genes show inducible expression at medium to high levels in CD4⁺ T cells. The provirus causing NSV in P3 is integrated within the ZFYVE9 gene, which encodes for a zinc finger-containing protein involved in TGF β signaling, is poorly expressed in CD4⁺ T cells. In P4, the provirus responsible for NSV is integrated into the CCND3 gene, a highly expressed gene involved in G1/S phase transition and cell proliferation. Interestingly, all 6 proviruses were integrated in opposite orientation relative to the host gene transcription, a proviral feature that is selected for in

individuals on long-term ART (12, 13). Viral integration in these genes have been previously identified both in infection *in vitro* as well as in PLWH before and on ART. However, none of these genes have been linked to HIV-1 persistence due to insertional mutagenesis (12, 14), suggesting that immune stimuli are the main drivers of their proliferation and viral production.

CD4⁺ T cell repertoire analyses from Participant 1. To investigate whether

compartmentalization within effector memory cells is common among all CD4⁺ T cells, and not unique to the two infected clones causing viremia in P1 and P2, we analyzed TCR^β repertoires from total, CM, and EM cells from P1 (~6000 productive TCRβ sequences per sample). While CM cells showed striking richness, EM cells had the highest degree of clonality, as previously described (15). This latter subset was dominated by very large clones: the top 50 clonotypes ranked by abundance contributed to more than 60% of all sequences, and the most expanded clone among all CD4⁺ T cells (CASSDLGQGHTEAFF, 11%) represented 24% of all CDR3β sequences in EM cells (~1 cell out of 4), compared to only 0.2% in the CM subset Supplementary Fig S9B). We then compared TCR β sequences found in both CM and EM subsets versus those found solely in EM cells (4027 versus 9010 unique sequences, respectively, supplementary Figure S9D). Sequences found in both subsets were on average significantly more abundant in EM cells, supporting the hypothesis of a differentiationproliferation flux directed from CM to EM (16) (Figure S9E). TCRβ sequences found only in the EM subset had a cumulative abundance of 21% among all EM cells, but were significantly less expanded than total EM clonotypes (p<0.0001, Figure S9 F and G), likely a reflection of the lower proliferative capacity of EM clonotypes not relying on differentiation from CM cells.

Analysis of autologous neutralization. Antibody-mediated immune pressure can potentially affect HIV-1 reservoir dynamics, including the selection of which proviruses lead to viral rebound upon treatment interruption (17). Our site-directed mutagenesis experiments suggest that the small 5'-L deletions in ADK.d22 and DNAJB14.d21 proviruses resulted in reduced Envelope levels in virus-producing cells and virions (Figure 5E-I). However, low-level Env expression on productively-infected cells could still result in engagement by neutralizing and/or effector antibodies and affect cell survival (18, 19). Therefore, we investigated whether viruses pseudotyped with ADK.d22 and DNAJB14.d21 full-length Envelopes could be neutralized with autologous IgG in a TZM-bl cell-based assay, as previously described (17). As shown in Figure 8C, the Envelope from both proviruses is infectious but substantially resistant to autologous neutralization. At the latest time points, inhibition of ADK.d22-Env and DNAJB14.d21-Env viral entry did not reach 50% at 100 ug/mL purified IgGs, the highest concentration tested in the assay. Plasma samples from P1 obtained before, or years after, the onset of NSV (3.8 versus 4.9, and 7.8 years on ART, respectively) showed no change in ADK.d22-Env neutralization (IC50 >100 ug/mL). Similarly, the lack of DNAJB14.d21-Env neutralization was comparable between two plasma samples collected 3 months apart (IC50 >100 ug/mL).

Supplementary Methods

Preparation of DNA Templates. The NL4-3 5'-Leader wildtype DNA template was generated from a pUC19 plasmid containing the Top17 sequence (5'-TAATACGACTCACTATA-3') and the RNA encoding sequence (5'-

GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCAC TGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTCAAAGTAGTGTGTGCCCGTCTGTTGTG TGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGT GGCGCCCGAACAGGGACTTGAAAGCGAAAGTAAAGCCAGAGGAGATCTCTCGACGCAGG ACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGC TACGCCAAAAATTTTGACTAGCGGAG-3', and reverse primers: 5'-

CGCCGCCCTCGCCTCTT-3' and 5'-CTCTTGCCGTGCGCGCTT-3', respectively. tRNA^{Lys3} template was generated from a pUC57 plasmid (Genewiz) containing Top17 sequence and the RNA encoding sequence (5'-

GCCCGGCTAGCTCAGTCGGTAGAGCATCAGACTTTTAATCTGAGGGTCCAGGGTTCAAGT CCCTGTTCGGGCGCCA-3'). DNA templates were generated by standard PCR amplification (EconoTaq PLUS 2x Master Mix, Lucigen) of plasmids described above. A forward amplification primer 80 nucleotides upstream of the T7 promoter were used for all constructs (5'-GGGATGTGCTGCAAGGCGATTAAGTTGGG-3'). The reverse amplification primer for WT,

d21, d22 5'-Leader constructs was 5'-mUmACCGACGCTCTCGCACCCATC-3', while constructs truncated within the AUG hairpin for ITC experiments used 5'-

mCmGCACCCATCTCTCTCCTTCTAGCCT-3' (20, 21). Truncations were used for ITC experiments to highlight the endothermic contribution associated with initial NC binding sites. Reverse amplification primer for tRNA^{Lys3} was 5'-mUmGGCGCCCGAACAGGGAC-3'. Methylated reverse primers were used to reduce self-templated run on during in vitro transcription (20). All DNA templates were subsequently validated by Sanger sequencing (Eurofins Genomics).

RNA in Vitro Transcription. RNAs were prepared via T7 RNA polymerase (purified in-house) in 15-mL reactions. A 15-mL reaction contained ~1 mg of PCR-amplified DNA template, 20 mM MgCl₂, 3 mM NTPs, 2 mM spermidine, 2 mM DTT, 20% (vol/vol) DMSO, 80 mM Tris·HCI (pH 9.0), and T7 RNA polymerase. Amounts of each component were optimized via small-scale (30 μ L) transcription reactions. The reaction was quenched after 8-hours of incubation at 37 °C by addition of an EDTA mixture (250 mM EDTA, pH 8.0) and was boiled for 5 min, then snap cooled on ice for 5 min prior to addition of glycerol (final concentration, 6% [vol/vol]). RNAs were purified by electrophoresis on 7.5 M urea-containing polyacrylamide denaturing gels (SequaGel; National Diagnostics) at 30 W for 12 hours, visualized by UV shadowing, and eluted using the Elutrap electroelution system (Whatman) at 130 V overnight. The eluted RNAs were concentrated and washed twice with 2 M high-purity NaCl followed by extensive desalting (~40 mL Water) using Amicon Ultra Centrifugal Filter Device (Millipore).

NC Purification. HIV-1_{NL4-3} NC (55 amino acids: MQKGNFRNQRKTVKCFNCGKEGHIAKNCRA PRKKGCWKCGKEGHQMKDCT ERQAN) was placed into a pET-3a plasmid and transformed into BL21 (DE3) pLysE. The protein was overexpressed and purified as previously described (22). Cells were lysed via freeze-thawing and microfluidization (6 times). Lysate was treated with 10% polyethylenimine (added 4% of lysate volume) to remove excess nucleic acids. After centrifugation, supernatant was applied to ion exchange chromatography using tandem Q- and SP- columns (Q-column was removed before elution). Final protein was isolated using size exclusion chromatography on a Superdex 30 column in 20 mM Tris·HCl, pH 7.5, 140 mM KCl, 10 mM NaCl, 5 mM MgCl₂, and 5 mM TCEP.

Isothermal Titration Calorimetry. ITC experiments were carried out using a MicroCal PEAQ-ITC Automated (Malvern Panalytical). To reduce non-specific NC binding and NC-induced unwinding caused by the chaperone activity of NC, titrations were conducted in the presence of 5 mM MgCl₂ (23, 24). A volume of 40 μ L of NC (200-250 μ M) in ITC buffer (20 mM Tris·HCl, pH 7.5, 140 mM KCl, 10 mM NaCl, 5 mM MgCl₂, and 5 mM TCEP) was loaded into the injection syringe. The calorimetry cell was loaded with 200 μ L of RNA (1 μ M for titration to saturation and 3 μ M for assessment of initial binding) in the same buffer as NC. After thermal equilibration at 25°C and the initial 60-s delay, a single injection of 0.4 μ L followed by 18 serial injections of 2

µL were made into the calorimetry cell with a 120-s delay between injections. Protein to Buffer and Buffer to Buffer controls were subtracted from data. Data was baseline corrected. The raw data integration, and curve fitting was done using Malvern's MicroCal PEAQ ITC analysis software to get stoichiometry, affinities, and thermodynamic parameters.

In Vitro Dimerization Assay. NL4-3 WT, d21, and d22 5'-Leaders (12.5 μ M in water) were heat denatured for 5 minutes and cooled on ice for 5 minutes to prevent nonnative higher order structures. Samples were then prepared in physiological ion buffer with 20 mM Tris·HCl (pH 7.5), 140 mM KCl, 10 mM NaCl, and 1 mM MgCl₂ at 10 μ M RNA concentration. Samples were subsequently diluted to desired concentrations (0.1, 0.5, 1, 5 μ M). 350-ng RNA samples were loaded onto 1% agarose gels prestained with ethidium bromide and run for 75 minutes at 115 V in 1× TB buffer (44.5 mM Tris–boric acid, pH 7.5) at room temperature.

tRNA Annealing Gel Shift Assay. Gel Shift Assay between NL4-3 WT, d21, and d22 5'-Leaders and tRNA^{Lys3} were conducted at either 1:0 or 1:1 ratio in annealing buffer (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 1 mM MgCl₂, and 140 mM KCl). RNA was kept at a 20 μ M concentration to ensure dimerization. To promote tRNA binding, samples were heat annealed in a thermocycler (Bio-Rad) at 94 °C for 5 min, 85 °C for 15 min, 75 °C for 15 min, and 65 °C for 60 min as previously described (25). 100 ng of 5'-Leader RNA were loaded onto 2% agarose gels pre-stained with ethidium bromide and run for 75 minutes at 115 V in 1× TB buffer (44.5 mM Tris–boric acid, pH 7.5) at room temperature.

Western blots. Transfection supernatants were normalized based on p24 and resuspended in RIPA buffer. Protein lysate recovery was quantified by BCA (ThermoFisher). Western blots were carried out as previously described (26). The same membrane was assayed for p24 and gp41. We used the following primary antibodies: murine monoclonal anti-p24 (Abcam, clone 39/5.4A, 1:2000 dilution) and human monoclonal anti-gp41 (AIDS reagent program, clone 246-D, 1:1000 dilution). After incubation over night at 4C, the membranes were washed three times and incubated for one hour with respective secondary antibodies: HRP-conjugated goat anti-murine IgG (Biolegend, 1:1000 dilution) and HRP-conjugated goat anti-human IgG (Invitrogen, 1:1000 dilution). Membranes were washed and imaged by ECL (ThermoFisher) on an iBright imager (ThermoFisher).

Transmission Electron Microscopy. Cells were transfected as described above in 35 mm tissue culture dishes (Falcon 3001). After 24 hours cells were rinsed with 37°C PBS for 1 min, and then fixed with 2% paraformaldehyde 2% glutaraldehyde (both EM grade) 100 mM Sorenson's phosphate and 5 mM MgCl2 pH 7.4 for 1hr at room temperature on a slow rocker. After a 30 min buffer rinse containing 3% sucrose, cells were post-fixed in 1% osmium tetroxide reduced with 0.8% potassium ferrocyanide 100 mM phosphate 5 mM MgCl2 at 4°C for 1hr in the dark. Samples were then rinsed with 100 mM maleate buffer 3% sucrose and en-bloc stained with 2% aqueous uranyl acetate (0.22 um filtered) in the same buffer, for 1 hr in the dark. Plates were dehydrated in a graded series of ethanol then infiltrated in Eponate 12 (Pella) overnight without catalyst. The next day cells were further embedded with fresh epon + 1.5% DMP-30 (catalyst) then cured at 37°C for three days, and further polymerized at 60°C overnight. Epon was removed from the plastic dish and 3 mm circles punched out and glued to epon blanks for sectioning, 60 nm thin compression free sections were obtained with a Diatome diamond knife (45 degree). Sections were picked up on 1x2 mm formvar coated copper slot grids (Polysciences), and further stained with uranyl acetate followed by lead citrate. Grids were examined on a Hitachi H-7600 TEM operating at 80Kv. Images were digitally captured with an AMT XR-80 (8 mega pixel) CCD camera.

Duplex quantification of total LTR copies and specific provirus. As previously described (9, 27, 28), we optimized a duplex digital PCR assay that selectively amplifies the integration site (*ADK*.d22 or *DNAJB14*.d21), together with all proviruses retaining the R-U5 LTR region

(irrespectively of its 5' or 3' location). To ensure that most partitions contain only a single LTR copy, we subjected genomic DNA to controlled physical shearing to yield fragments of ~6000 nucleotides or shorter (Figure S9). To do so, we used q-tubes (Covaris) following manufacturer's instructions (11000 rpm for 30 seconds, twice). Extent of shearing was tested by RPP30 as previously described (2). Due to the proximity of the integration site to the 5' R-U5 junction, >90% of proviruses of interest are detected as double-positive events (Figure 6B). To confirm the specificity of the assay, we duplexed the same integration site-specific assay with primers and probes targeting the 5'-L deletions unique to ADK.d22 and DNAJB14.d21. This second assay design yielded comparable frequencies of double-positive partitions (Figure 6B). This assay can be used to calculate the relative abundance of a provirus of interest among all infected cells. However, estimating the fraction of proviruses retaining both LTRs is challenging, and previous studies suggest that solo LTRs can represent a large fraction of the proviral landscape (9). Total LTR copies were quantified with a set of primers and probes annealing to the R-U5 region as previously described (29). To selectively quantify proviruses of interest, we designed primers surrounding the site of HIV-1 integration (27, 28). The probe was designed to anneal both to the provirus and its flanking human genome (Supplementary Table S5). Digital PCR was carried out with either the Biorad QX200 with cycling conditions as previously described (27) or the Qiagen Qiacuity with the following cycling parameters: 95°C for 2', and 40 cycles of 95°C for 15" and 58°C for 30"; default parameters were used for the detection of positive partitions. Frequencies of LTR and integration site copies were calculated based on cell equivalent input by RPP30. The abundance of a provirus of interest relative to the total pool of infected cells was calculated as the percentage of LTR copies belonging to that provirus (integration site copies x2) over the total LTR copies.

T cell subset analysis. Cryopreserved PBMCs were thawed and rested for 4 hours in RPMI with 10% FBS. We incubated cells with FcgR block (BD Pharmingen) at room temperature for 10 minutes. Cells were stained, with a 30 minutes incubation on ice, with an APC-labelled antibody to CD3 (Biolegend; clone UCHT1), phycoerythrin (PE)-Cy7-labelled antibody to CD4 (Biolegend; clone RPA-T4), BV421-labelled antibody to CD45RA (BD Biosciences; clone HI100), PE-labelled antibody to CCR7 (Biolegend; clone G043H7) and PE-Cy5-labelled antibodies to CD14 (Thermo Fisher; clone 61D3), CD16 (Biolegend; clone 3G8) and CD20 (Biolegend; clone 2H7). Dead cells were excluded using propidium iodide. Cells stained with single fluorophore-labelled antibodies were used to set sorting gates. CD45RA expression was used to distinguish Naïve-like and terminally differentiated cells from memory cells. Central memory cells were distinguished from effector memory cells by the expression of CCR7. Naïvelike (Na), Central Memory (CM), Effector Memory (EM) and Effector Memory CD45RA positive (EMRA) subsets were sorted using either the Beckman Coulter MoFlo Legacy or XDP cell sorters. Sorted cell fractions were pelleted and stored at -80C until used for gDNA extraction with QIAamp DNA mini kit (Qiagen). TCR repertoires were obtained via Adaptive Biotechnologies (Seattle, WA) as previously described (27).

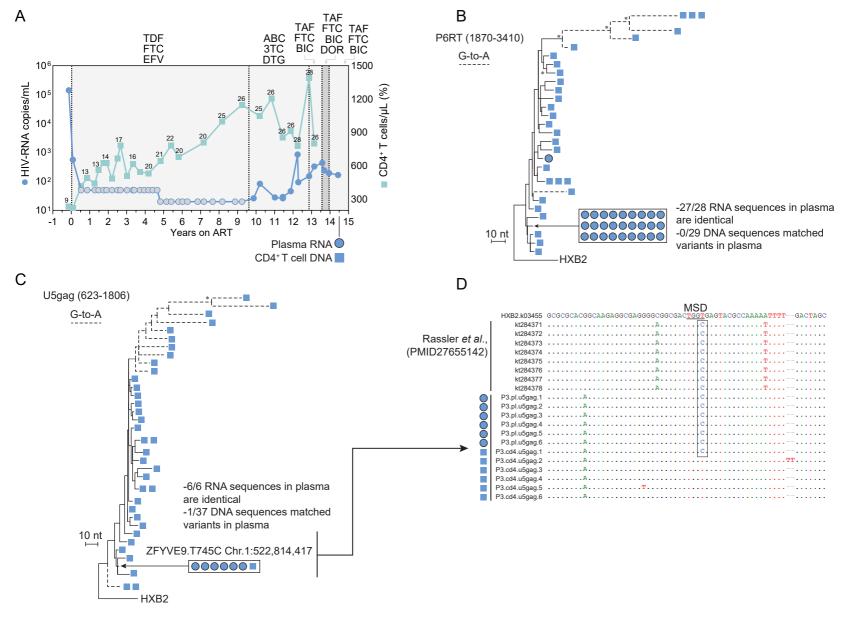
Supplementary References

- 1. Laird GM, Eisele EE, Rabi SA, Lai J, Chioma S, Blankson JN, et al. Rapid Quantification of the Latent Reservoir for HIV-1 Using a Viral Outgrowth Assay. *PLOS Pathogens.* 2013;9(5):e1003398.
- 2. Bruner KM, Wang Z, Simonetti FR, Bender AM, Kwon KJ, Sengupta S, et al. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature*. 2019;566(7742):120-5.
- 3. Simonetti FR, White JA, Tumiotto C, Ritter KD, Cai M, Gandhi RT, et al. Intact proviral DNA assay analysis of large cohorts of people with HIV provides a benchmark for the

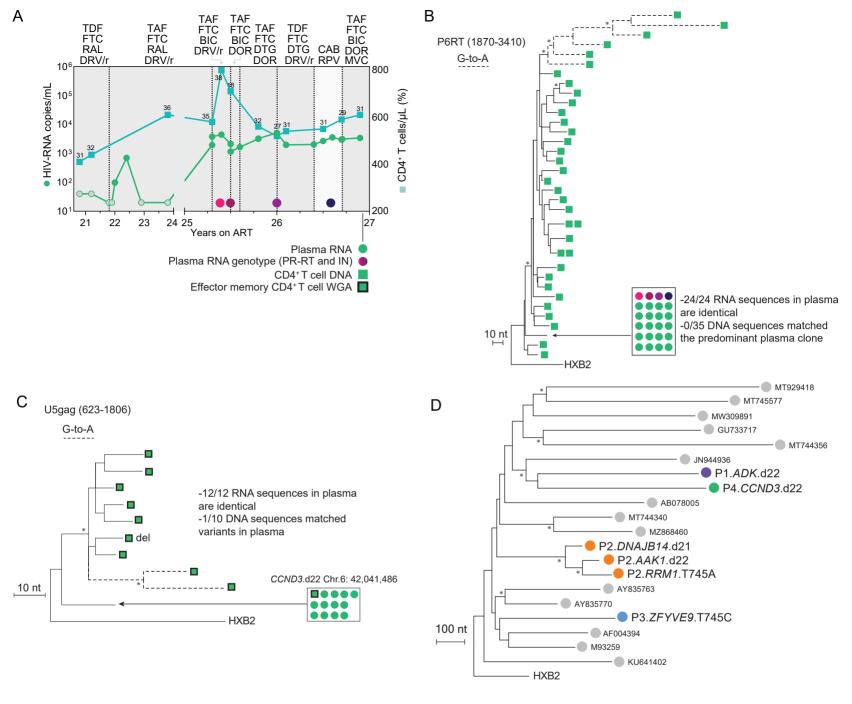
frequency and composition of persistent proviral DNA. *Proc Natl Acad Sci U S A.* 2020;117(31):18692-700.

- 4. Coffin JM, and Hughes SH. Clonal Expansion of Infected CD4+ T Cells in People Living with HIV. *Viruses.* 2021;13(10).
- 5. Jiang C, Lian X, Gao C, Sun X, Einkauf KB, Chevalier JM, et al. Distinct viral reservoirs in individuals with spontaneous control of HIV-1. *Nature*. 2020;585(7824):261-7.
- 6. Laskey SB, Pohlmeyer CW, Bruner KM, and Siliciano RF. Evaluating Clonal Expansion of HIV-Infected Cells: Optimization of PCR Strategies to Predict Clonality. *PLoS Pathog.* 2016;12(8):e1005689.
- 7. Patro SC, Brandt LD, Bale MJ, Halvas EK, Joseph KW, Shao W, et al. Combined HIV-1 sequence and integration site analysis informs viral dynamics and allows reconstruction of replicating viral ancestors. *Proc Natl Acad Sci U S A.* 2019;116(51):25891-9.
- 8. Einkauf KB, Lee GQ, Gao C, Sharaf R, Sun X, Hua S, et al. Intact HIV-1 proviruses accumulate at distinct chromosomal positions during prolonged antiretroviral therapy. *J Clin Invest.* 2019;129(3):988-98.
- 9. Anderson EM, Simonetti FR, Gorelick RJ, Hill S, Gouzoulis MA, Bell J, et al. Dynamic Shifts in the HIV Proviral Landscape During Long Term Combination Antiretroviral Therapy: Implications for Persistence and Control of HIV Infections. *Viruses.* 2020;12(2).
- 10. Bacchus-Souffan C, Fitch M, Symons J, Abdel-Mohsen M, Reeves DB, Hoh R, et al. Relationship between CD4 T cell turnover, cellular differentiation and HIV persistence during ART. *PLoS Pathog.* 2021;17(1):e1009214.
- 11. Morcilla V, Bacchus-Souffan C, Fisher K, Horsburgh BA, Hiener B, Wang XQ, et al. HIV-1 Genomes Are Enriched in Memory CD4(+) T-Cells with Short Half-Lives. *mBio.* 2021;12(5):e0244721.
- 12. Coffin JM, Bale MJ, Wells D, Guo S, Luke B, Zerbato JM, et al. Integration in oncogenes plays only a minor role in determining the in vivo distribution of HIV integration sites before or during suppressive antiretroviral therapy. *PLoS Pathog.* 2021;17(4):e1009141.
- 13. Einkauf KB, Osborn MR, Gao C, Sun W, Sun X, Lian X, et al. Parallel analysis of transcription, integration, and sequence of single HIV-1 proviruses. *Cell.* 2022;185(2):266-82 e15.
- 14. Bushman FD. Retroviral Insertional Mutagenesis in Humans: Evidence for Four Genetic Mechanisms Promoting Expansion of Cell Clones. *Mol Ther.* 2020;28(2):352-6.
- 15. Miron M, Meng W, Rosenfeld AM, Dvorkin S, Poon MML, Lam N, et al. Maintenance of the human memory T cell repertoire by subset and tissue site. *Genome Med.* 2021;13(1):100.
- 16. Grossman Z, Singh NJ, Simonetti FR, Lederman MM, Douek DC, Deeks SG, et al. 'Rinse and Replace': Boosting T Cell Turnover To Reduce HIV-1 Reservoirs. *Trends Immunol.* 2020;41(6):466-80.
- 17. Bertagnolli LN, Varriale J, Sweet S, Brockhurst J, Simonetti FR, White J, et al. Autologous IgG antibodies block outgrowth of a substantial but variable fraction of viruses in the latent reservoir for HIV-1. *Proc Natl Acad Sci U S A.* 2020;117(50):32066-77.
- Williams KL, Stumpf M, Naiman NE, Ding S, Garrett M, Gobillot T, et al. Identification of HIV gp41-specific antibodies that mediate killing of infected cells. *PLoS Pathog.* 2019;15(2):e1007572.
- 19. Rajashekar JK, Richard J, Beloor J, Prevost J, Anand SP, Beaudoin-Bussieres G, et al. Modulating HIV-1 envelope glycoprotein conformation to decrease the HIV-1 reservoir. *Cell Host Microbe.* 2021;29(6):904-16 e6.
- 20. Keane SC, Van V, Frank HM, Sciandra CA, McCowin S, Santos J, et al. NMR detection of intermolecular interaction sites in the dimeric 5'-leader of the HIV-1 genome. *Proc Natl Acad Sci U S A.* 2016;113(46):13033-8.
- 21. Tran T, Liu Y, Marchant J, Monti S, Seu M, Zaki J, et al. Conserved determinants of lentiviral genome dimerization. *Retrovirology*. 2015;12:83.

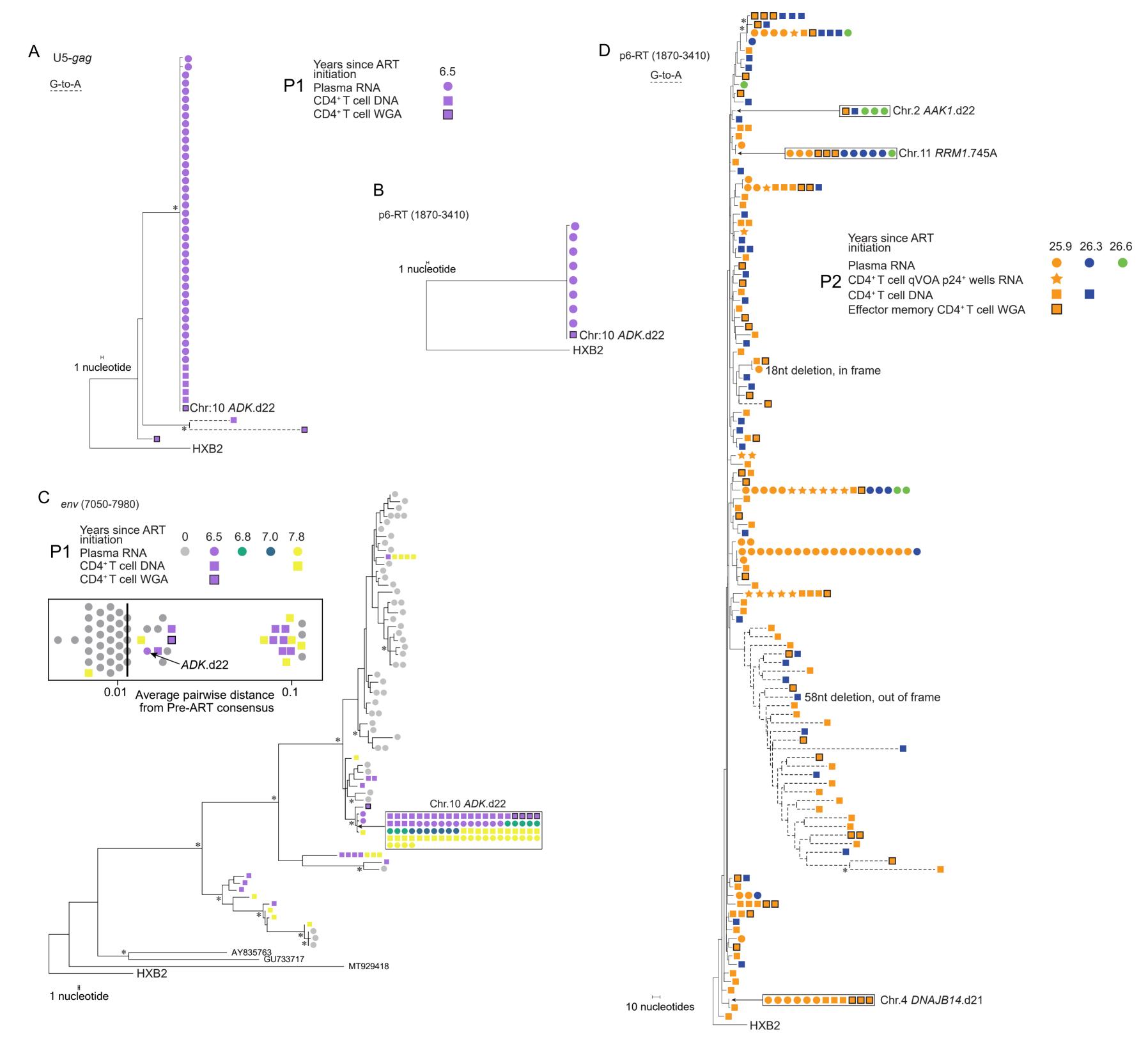
- 22. Lee BM, De Guzman RN, Turner BG, Tjandra N, and Summers MF. Dynamical behavior of the HIV-1 nucleocapsid protein. *J Mol Biol.* 1998;279:633-49.
- 23. Ding P, Kharytonchyk S, Waller A, Mbaekwe U, Basappa S, Kuo N, et al. Identification of the initial nucleocapsid recognition element in the HIV-1 RNA packaging signal. *Proc Natl Acad Sci U S A*. 2020;117(30):17737-46.
- 24. Keane SC, Heng X, Lu K, Kharytonchyk S, Ramakrishnan V, Carter G, et al. RNA structure. Structure of the HIV-1 RNA packaging signal. *Science*. 2015;348(6237):917-21.
- 25. Gremminger T, Song Z, Ji J, Foster A, Weng K, and Heng X. Extended interactions between HIV-1 viral RNA and tRNALys3 are important to maintain viral RNA integrity. *International Journal of Molecular Sciences.* 2020;22(1):58.
- 26. Kirchherr JL, Hamilton J, Lu X, Gnanakaran S, Muldoon M, Daniels M, et al. Identification of amino acid substitutions associated with neutralization phenotype in the human immunodeficiency virus type-1 subtype C gp120. *Virology.* 2011;409(2):163-74.
- 27. Simonetti FR, Zhang H, Soroosh GP, Duan J, Rhodehouse K, Hill AL, et al. Antigendriven clonal selection shapes the persistence of HIV-1-infected CD4+ T cells in vivo. *J Clin Invest.* 2021;131(3).
- 28. Brandt LD, Guo S, Joseph KW, Jacobs JL, Naqvi A, Coffin JM, et al. Tracking HIV-1-Infected Cell Clones Using Integration Site-Specific qPCR. *Viruses.* 2021;13(7).
- 29. Anderson EM, and Maldarelli F. The role of integration and clonal expansion in HIV infection: live long and prosper. *Retrovirology*. 2018;15(1):71.



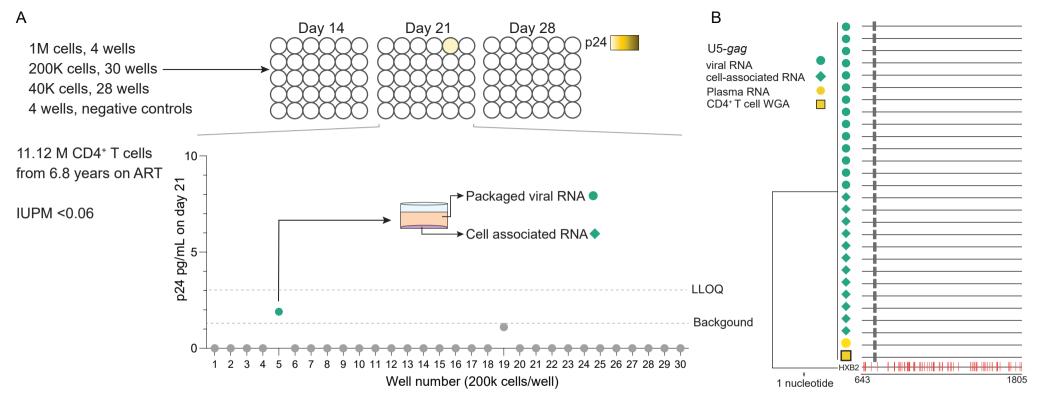
Supplementary Figure S1. Clinical history of Study Participant P3 with nonsuppressible viremia (NSV), and analysis of HIV-1 populations in plasma and CD4⁺ T cells. (A) Plasma HIV-1 RNA and CD4⁺ T cell counts over time for P3; grey circles indicate values below the limit of quantification of the clinical assay; numbers above squares indicate CD4⁺ T cell percentage; light grey areas indicate standard ART, dark grey areas indicate ART intensification; ART regimens are indicated above the graph; time points and samples analyzed in this study are shown below the graph. (B) Maximum likelihood tree analysis of *P6*-RT single genome sequences from P3; dashed branch lines indicate sequences with APOBEC3G/F-induced hypermutation; tree nodes with bootstrap values above 80 are marked by star symbols; identical sequences matching proviruses with integration and full genome data are highligthed in boxes. (C) Maximum likelihood tree analysis of U5-*gag* single genome sequences from P3; chromosomal location is indicated above boxed area. (D) 5'-L sequences of reisdual plasma virus published by Rassler *et al.* aligned to the predominant plasma clone found in P3; sequences are aligned to HXB2. 3TC lamivudine, ABC abacavir, FTC emtricitabine, TDF tenofovir disoproxil fumarate, TAF tenofovir alanfenamide, DTG dolutegravir, BIC bictegravir, EVF efavirenz, DOR doravirine.



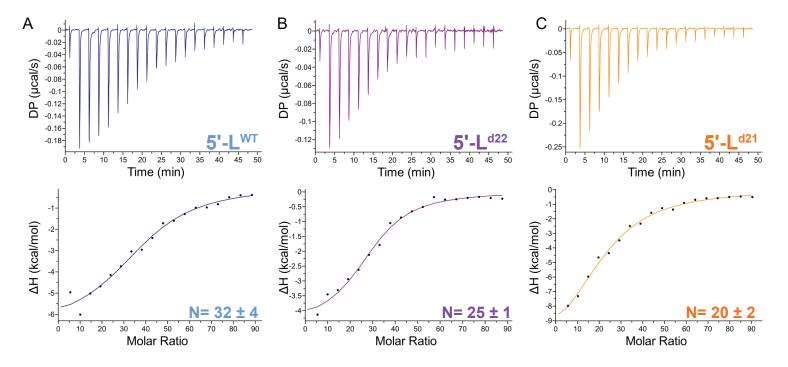
Supplementary Figure S2. Clinical history of study participant P4 with nonsuppressible viremia (NSV), and analysis of HIV-1 populations in plasma and CD4⁺ T cells. (A) Plasma HIV-1 RNA and CD4⁺ T cell counts over time for P4; grey circles indicate values below the limit of quantification of the clinical assay; numbers above squares indicate CD4⁺ T cell percentage; light grey areas indicate standard ART, dark grey areas indicate ART intensification; ART regimens are indicated above the graph; time points and samples analyzed in this study are shown below the graph. (B) Maximum likelihood tree analysis of P6-RT single genome sequences from P4; dashed branch lines indicate sequences with APOBEC3G/F-induced hypermutation; tree nodes with bootstrap values above 75 are marked by star symbols; identical sequences matching proviruses with integration and full genome data are highligthed in boxes. (C) Maximum likelihood tree analysis of U5-gag single genome sequences from P4; chromosomal location is indicated above boxed area. (D) maximum likelihood tree analysis of near full length genomes of the proviruses causing viremia from all participants together with 15 subtype B references. FTC emtricitabine, TDF tenofovir disoproxil fumarate, TAF tenofovir alanfenamide, RAL raltegravir, DTG dolutegravir, BIC bictegravir, CAB cabotegravir, DOR doravirine, RPV rilpivirine, DRV/r darunavir/ritonavir, MVC, maraviroc.



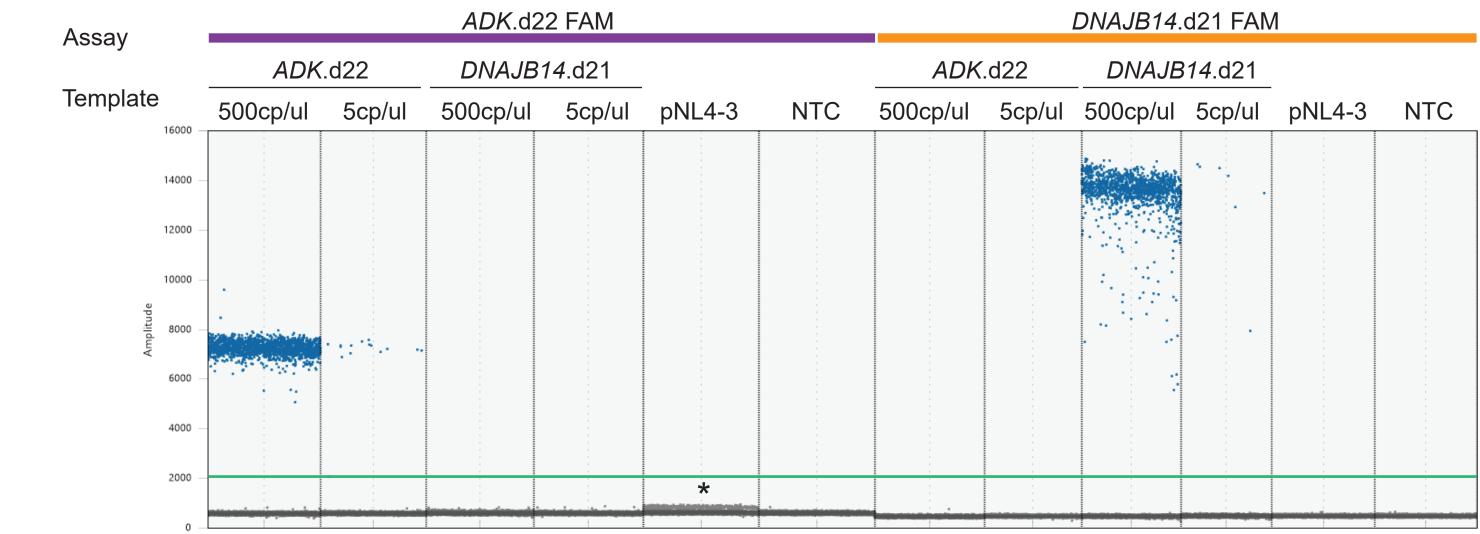
Supplementary Figure S3. Additional HIV-1 sequence analyses from P1 and P3 related to Figure 1. (A) Maximum likelihood tree analysis of U5-*gag* single genome sequences from P1; dashed branch lines indicate sequences with APOBEC3G/F-induced hypermutation; tree nodes with bootstrap values above 75 are marked by star symbols; HBX2 reference sequence is used as outgroup; timepoints and sample types are color-coded as indicated in the legend. (B) Maximum likelihood tree analysis of P6-RT single genome sequences from P3. (C) Maximum likelihood tree analysis of *env* single genome sequences from P1 (related to Figure 1C) including RNA sequences found in plasma at the time of ART initiation (grey circles); hypermutated sequences are excluded from this analysis; boxed scatter plot shows average pairwise distances from the consensus of all pre-ART plasma sequences; identical sequences are sampled only once; the black vertical bar indicates median distance; (D) Complete maximum likelihood tree analysis of P6-RT single genome sequences from P2, related to Figure 1D; proviruses of interest with 5'-Leader deletion are highilighted by black boxes.



Supplementary Figure S4. Quantitative viral outgrowth assay from P1 shows lack of exponential replication of predonimant plasma clone. (A) Experimental set up of viral outgrowth assay from total CD4⁺ T cells from P1; the graph show p24 levels in each of the wells with 200k cells at day 21; RNA was extracted from cells and pelleted viral particles from the supernatant of well 5, which had low p24 signal; LLOQ, lower limit of quantification, is based on the lowest values of the standard curve. (B) Neighbor-joining tree of U5-*gag* single genome sequences recovered from QVOA well number 5 aligned to the predominant vairant found in plasma; HXB2 reference sequences is used as outgroup; highlighter plot on the right shows mutations relative to predominant plasma clone in P1; 22-nucleotide deletion is indicated in grey.

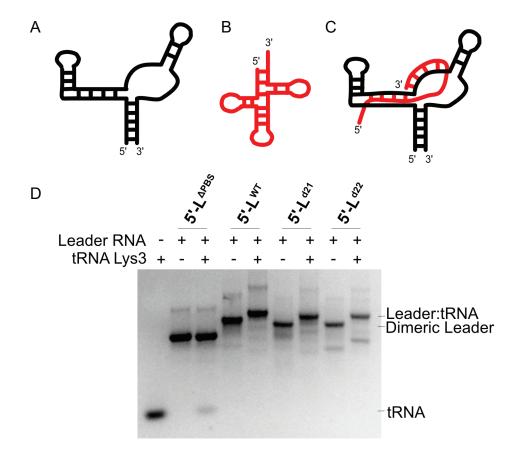


Supplementary Figure S5. 5'-Leader deletions cause a modest decrease in the number of nucleocapsid binding sites. (A, B, C) ITC isotherms of WT (A), d22 (B), and d21 (C) truncated leader constructs with high ratios of NC to reach saturation. Fitted N values are indicated with standard deviation of values across four replicates. The fitted Kd values for WT< d21, and d22 constructs were 1.8, 3.9 and 1.7 μ M, respectively; however, these values reflect a range of affinities for the >20 individual NC bindings sites. See Table S3 for detailed statistics.



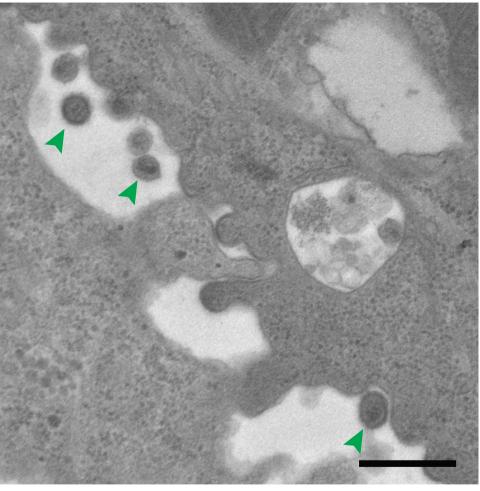
Α

Supplementry Figure S6. Validation of digital PCR assays that selectively amplify proviruses of interest, related to Figure 4. (A) Results of droplet digital PCR using primers and probe sets to amplify sequences across 5'-L deletions of proviruses of interest. To assess specificity, each assay was tested with synthetic double strand DNA identical to ADK.d22 or DNAJB14.d21, plasmid containing wild type NL4-3, or water (NTC). The green bar indicates the threshold for positive partitions. The star symbol indicates dim partitions from pNL4-3 non-spefic signal, correctly classified as negative for ADK.d22.

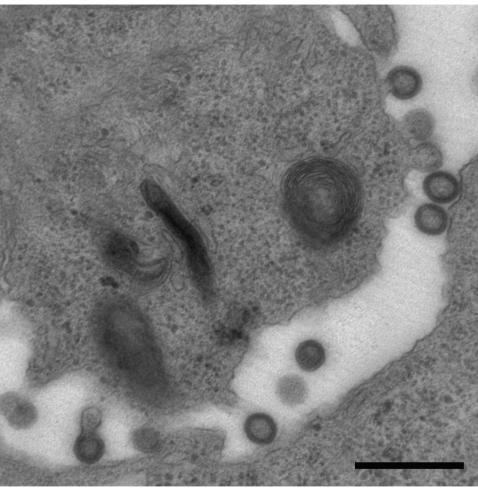


Supplementary Figure S7. Deletions do no alter tRNA binding propensity to the 5'-Leader. (A) Proposed PBS secondary structure. (B) tRNA Lys3 secondary structure. (C) Model of tRNA binding to PBS in an extended conformation proposed by Gremminger et al. (25) (D) EMSA of 5'-Leader constructs in presence and absence of tRNA. Lane 1 is tRNA with increase material loaded compared to subsequent lanes to appropriately visualize tRNA on the gel. Lane 2, 4, 6, 8 are 5'-Leader constructs with deletion of PBS, wildtype, d21 mutation, and d22 mutation respectively without tRNA, while Lanes 3, 5, 7, 9 are in presence of tRNA^{Lys3}.

Wild type NL4-3

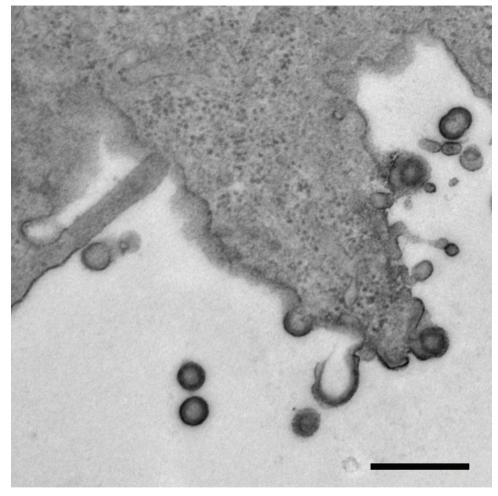


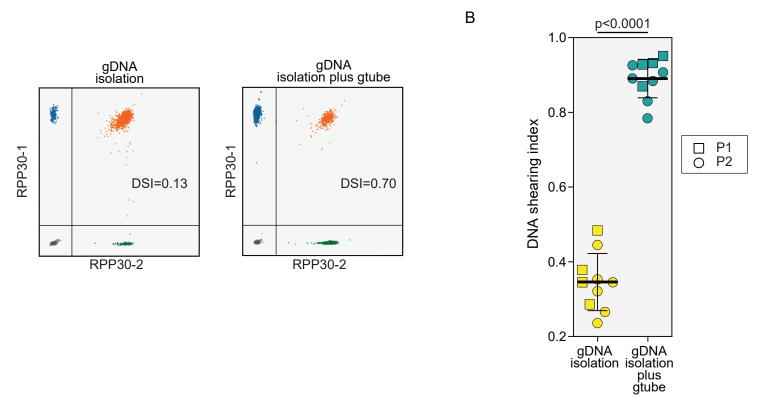
NL4-3 d22



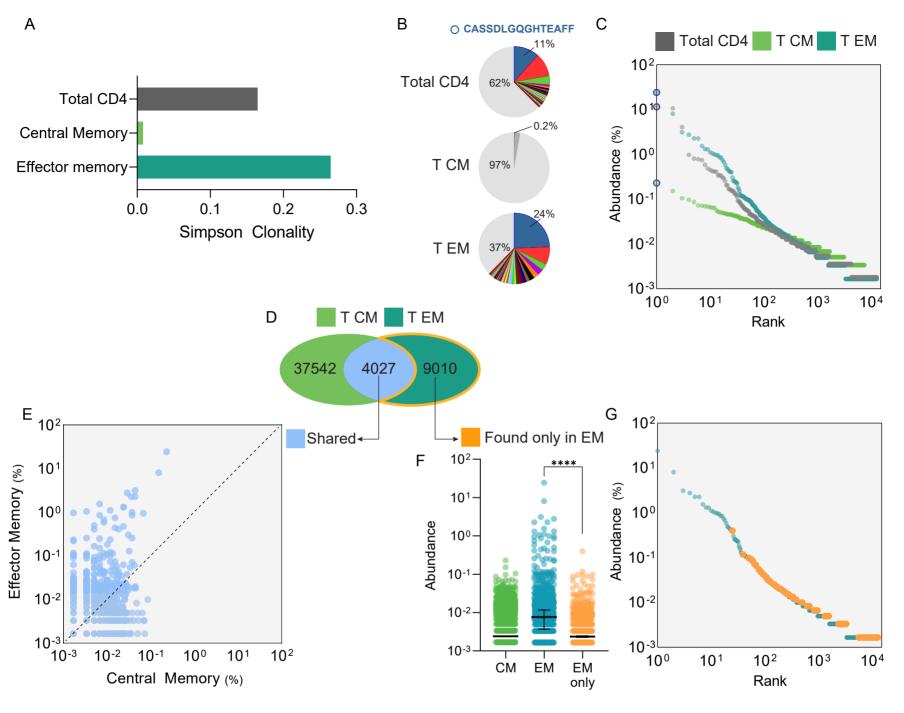
Supplementary Figure S8. Production of viral particles upon transfection with 5'-Leader defective NL4-3. (A) 293T cells were transfected with 5'-Leader wild type or mutant NL4-3 plasmids; at 24 hours cells were rinsed with warm PBS and fixed for thin-section electron microscopy. Black bars indicate 500nm. Green arrow heads indicate mature virions with conical morphology of the capsid.

NL4-3 d21

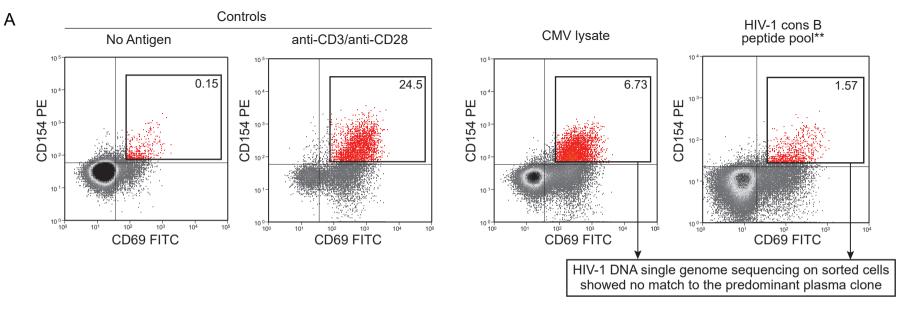




Supplementary Figure S9. Impact of controlled shearing on genomic DNA used in integration site-specific dPCR assays. (A) Droplet digital PCR 2D plots showing RPP30 amplification before and after controlled DNA shearing; DSI, DNA shearing index. (B) Controlled shearing leads to singificant increase in DSI values; symbols indicate DSI values of matched DNA samples used in Figures 6 and 7, before and after physical shearing with g-tubes (see methods); statistical significancw was tested by parametric paired t-test.



Supplementary Figure S10. TCR β sequence analysis of total, Central, and Effector memory CD4⁺ T cell subsets from P1. (A) Higher Simpson Clonality index of TCR β sequences in EM than in CM cells. (B) Relative abundance (%) of the 50 most expanded CDR3 aminoacid sequences across the three groups of cells; sequences below the top 50 rank are grouped together in grey; the percentage of the most expanded CDR3 sequence CASS-DLGQGHTEAFF is shown above each pie chart. (C) Rank-aundance plot of TCR β sequences; steeper curves indicate higher clonal dominance; the most abundant sequences is highlighted in blue. (D) Analysis of repertoire overlap between CM and EM cells; numbers indicate unique TCR β sequences; light blue represents shared sequences while orange indicates those unique to EM cells. (E) Analisys of differential abundance of TCR β sequences than total EM cells; errora bars indicate 95% confidence interval. (G) Rank-aundance plot of TCR β sequences from cell found only in EM cells (orange) versus total EM cells.



Supplementary Figure S11. Isolation of antigen-responding CD4⁺ T cells from PBMCs in P1.

(A) Stimulation of CD8-depleted PBMCs for 16 hours with CMV and HIV-1 Gag antigens leads to upregulation of activation markers CD154 and CD69; left panels show negative and positive controls carried out with no antigen and anti-CD3/anti-CD28 antibody-coated beads, respectively; numbers in gates indicate percentage of doulbe positive events; **cells stimulated with HIV-1 Gag were analyzed and sorted on a Backman Coulter MoFlo instrument, while all other conditions were run on a XDP instrument.

Characteristics	P1	P2	P3	P4	Median ^d
Sex	Male	Male	Female	Male	
Age (y)	63	60	58	60	60
Race	African American	Caucasian/White	African American	Caucasian/White	
Years since diagnosis	30	31	15	32	31
Years on ART	7.8	26.4	14.5	27	20
CD4 ⁺ T cell count nadir (cells/mm ³)	454	197	221	na	
CD4 ⁺ T cell count, last (cells/mm ³)	828	793	803	610	798
HIV-1 RNA, setpoint (copies/mL)	8771	na ^c	141667	na	
HIV-1 RNA, last (copies/mL) ^a	58	20	167	3400	113
Years with detectable viremia	5	11	5	5	5
ART regimen, last ^b	TAF,FTC,BIC	TAF,FTC,BIC,FTR	TAF,FTC,BIC	TAF, FTC, BIC, DOR, MVC	
Infectious units per million (QVOA)	<0.06	15 (10-21)	na	na	
Intact proviruses/10 ⁶ CD4 ⁺ T cells (IPDA)	3.5	311	161	58	110
HLA-B	53:01, 57:03	44:02	44:03	44:03, 57:02	

Supplementary Table S1. Participant characteristics. a, measured with limit of detection of 20 copies/mL; b, TAF tenofovir alafenamide fumarate, FTC emtricitabine, BIC bictegravir, FTR fostemsavir, DOR doravirine, MVC maraviroc; c, not available. d, median values were calculated when available for all 4 participants.

	Sample			Provirus	6						Host gen	e	
Participant ID	Provirus ID	Chromosome	Position ^a	5 nt duplication	Host junctions recovered	Strand	Orientation relative to gene	Gene symbol	Gene ID (NCBI)	Gene transcription orientation	Provirus location ^b	Expression levels in effector memory CD4 ⁺ cells ^c	Pathway ^c
P1	p1.ADK.d22	10	74192042	GTTAC	5', 3'	-	opposite	ADK	132	+	intron 1/9	medium	Purine metabolism
	p2.AAK1.d22	2	69478200	CTATG	5', 3'	+	opposite	AAK1	22848	-	intron 17/21	medium	Clathrin-mediated endocytosis, Vesicle- mediated transport
P2	p2.DNAJB14.d21	4	99910773	TTCAT	5', 3'	+	opposite	DNAJB14	79982	-	intron 3/7	medium	Hsp70 protein binding
	p2.RRM1.T745A	11	4117665	GCCAC	5', 3'	-	opposite	RRM1	6240	+	intron 7/18 (1/12)	medium	Pyrimidine and purine nucleotides <i>de novo</i> biosynthesis
P3	p3.ZFYVE9.T745C	1	522814417	GGCTC	5', 3'	-	opposite	ZFYVE9	9372	+	intron 9/18	vey low	TGF-beta receptor signaling
P4	p4.CCND3.d22	6	42041486	GTAAC	5', 3'	+	opposite	CCND3	896	-	intron 1/3	high	Cyclin-dependent protein serine/threonine kinase activity, G1/S transition

Number refers to the third nucleotide of the 5nt duplication, using GRCH38/hg38 assembly Numbers in parentheses refer to alternative isoforms Based on Immune Cell Atlas (http://immunecellatlas.net/) population RNA-seq, normalized expression values (0-5 trace, very low 5-20, low 20-80, 80-800 medium , 800-8000 high) Based on Gene Onthology Project (http://geneontology.org/) a b c

d

Supplementary Table S2. Integration site analysis of defective proviruses causing NSV

RNA	[Nucleocapsid]	[Dimer]	N	N Error	Kd	Kd Error	$\Delta \mathbf{H}$	ΔG	-TAS	Offset	Red. Chi-Sqr.				
Construct	(µM)	(µM)	(sites)	(sites)	(µM)	(µM)	(kcal/mol)	(kcal/mol)	(kcal/mol)	(kcal/mol)	(kcal/mol) ²				
		0.49	33.7	1.4	1.63	0.55	-5.02	-7.90	-2.87	0.11	4.10E-02				
NL4-3 WT	225		28.9	1.0	1.21	0.39	-4.87	-8.07	-3.20	-0.13	3.20E-02				
NL4-5 W1	225	0.45	36.2	1.3	1.62	0.49	-4.76	-7.90	-3.14	-0.11	3.00E-02				
			28.0	2.0	2.75	1.32	-6.54	-7.59	-1.05	0.07	8.10E-02				
			24.9	1.0	1.14	0.35	-3.60	-8.11	-4.50	0.08	1.80E-02				
NL4-3 d22	225	0.50	0.50	0.50	0.50	0.50	25.3	1.3	1.19	0.49	-3.28	-8.08	-4.81	0.06	2.60E-02
NL4-5 UZZ	225	0.50	22.8	2.1	2.75	1.47	-4.71	-7.59	-2.88	0.16	4.60E-02				
			25.3	1.1	1.67	0.53	-3.50	-7.88	-4.38	0.05	1.50E-02				
			22.9	1.0	2.95	0.68	-9.55	-7.55	2.00	0.28	3.20E-02				
NL4-3 d21	225	0.48	18.6	1.3	3.76	1.02	-11.50	-7.40	4.06	0.26	3.90E-02				
NL4-3 UZ I	225	0.40	17.9	1.7	5.68	1.49	-13.30	-7.16	6.10	0.38	2.40E-02				
			19.5	1.2	3.23	0.84	-10.70	-7.49	3.21	0.12	4.00E-02				

Supplementary Table S3. ITC Isotherm Fitting Data of NC to NL4-3 WT, d21, d22 to full saturation. Data was fit using the MicroCal PEAQ-ITC analysis software provided by Malvern. A converged fit was achieved using the Levenberg-Marquardt algorithm. Errors of fit are indicated.

Participant ID	A	B	c				
P1 P2 P3	34:02, 74:01 02:01, 32:01 02:01, 30:02	57:03, 53:01 44:02 44:03	04:01, 07:01 05:01, 07:04 04:01, 14:03	-			
P4	03:01, 29:02	57:02, 44:03	16:01, 18:01				
P1	region	relevant MHC 8*53:01 8*53:01	expected TPODLNTML ETINEEAAEW	observed TPODLNTML ETINEEAAEW	impact	WT?	B*57 B*57 WT?
	gag	B*53:01 B*57:03 B*57:03	AISPRTLNAW KAFSPEVIPMF(S)	ETINEEAAEW PLSPRTLNAW KAFSPEVIPMF(T)	A1P, IZL documented escape S+1T literature escape, reduced fitness	0	1
		B*57:03 B*57:03	TSTLQEQIGW QASQEVKNW	TSNLQEQIAW QASQDVKNW	T3N documented escape, reduced fitness Weak Binder	0	1
	pol	B*57:03 B*57:03	STTVKAACWW PIVLPEKDSW	SNVVKAACWW PILLPEKDSW	T2N documented escape Weak Binder	0	1
ADK.d22	vif vpr	A*74:01 B*57:03 B*57:03	SQMPGIKVR ISRKAKGWF AVRHFPRIW	SQIYPGIKVR ISRKAKNWL AVRHFPRIW	Weak Binder	0	1
-	rev	B*57:03 C*04:01	LRAVRIKI SFNCGGEFF	LKAVRLIKF TFNCGGEFF	19F diminished response S1T documented escape	0	1
	env	C*04:01 C*07:01 B*57:03	OFRNKTIVF VYYGVPVWKEA KAAFDLSFF	OFHGPIAF VYYGVPVWKEA KGAVDLSHF	Non Binder T2G diminished response	0	
	nef	B*57:03 C*07:1	KAAFDLSFF HTQGYFPDW KRQDILDLWVY	HTQGYLPDW KRODILDLWVH	126 diminished response Strong binder Binding unchanged	1	1 1
total		C*07	RYPLTFGWCY 19	RYPLTFGWCF	Y10F binding unchanged	1 42.1	11 18.2
P2	region	relevant MHC A*02:01 A*02:01	expected SLYNTVATL TLNAWVKVV	observed SLYNTVATL TLNAWVKVV	impact	WT?	
	828	A*02:01 A*02:01	MTSNPPIPV VLAEAMSQV	MTSNPPIPV VLAEAMSQV		1	
		B*44:02 A*02:01 A*02:01	AEQASQDVKNW KMIGGIGGFI VLVGPTPVNI	AEQCTQEVKNW KMIGGIGGFI VLVGPTPVNI	Strong binder	1	
		A*02:01 A*02:01	ALVEICTEM	ALVEICTEM		1	
	pol	A*02:01 A*02:01	ILKEPVHGV ALQDSGLEV	ILKEPVHGV ALQDSGLEV		1 1	
		A*32:01 B*44:02 B*44:02	PIQKETWETW EEMNLPGRW QEEHERYHSNW	PIQRETWDTW EDMTLPGRW QDDHEKYHSNW	Non binder E2D inferred/documented escape Non binder	0	
AAK1.d22	vpr	C*05:01 A*02:01	HTDNGSNF RILQQLLFI	HTDNGSNF		1	
	rev	B*44:02 C*05:01	EELLKTVRL SAEPVPLQL	EELIKTVRL PAEPVPLQL	L4I diminished response, escape Non binder	0	
		A*02:01 A*02:01 A*02:01	KLTPLCVTL TLSQIVTKL SLLNATDIAV	KLTPLCVTL TLDQIFKKL SLLNATAIAV	Non binder	1	
	env	A*02:01 A*32:01	RVIEVAQRV RIKQIINMW	RIIEVLORA RIKQIINLW	Weak binder Strong binder	0	
		B*44:02 C*07:04	AENLWVTVY VYYGVPVWKEA	TEKLWVTVY VYYGVPVWKEA	A1T, N3K documented escape	0	
	nef	A*02:01 A*02:01 C*07:04	LEWRFDSTL AFHHVAREL KRODILDLWVY	LVWRFDSRL AFHHMAREL KRODILDLWVY	Non binder Non binder	0	
total		C*07:04	RYPLTFGWCY 29	RYPLTFGWCF	Y10F binding unchanged	1 1 65.5	
P2	region	relevant MHC A*02:01	expected SLYNTVATL	observed SLYNTVATL	impact	WT?	
	gag	A*02:01 A*02:01 A*02:01	TLNAWVKVV MTSNPPIPV VLAEAMSOV	TLNAWVKVV MTSNPPIPV VLAEAMSOV		1	
		B*44:02 A*02:01	AEQASQDVKNW KMIGGIGGFI	AEOCTOEVKNW KMIGGIGGFI	Strong binder	1	
		A*02:01 A*02:01 A*02:01	VLVGPTPVNI ALVEICTEM VIYQYMDDLYV	VLVGPTPVNI ALVEICTEM VIYQYMDDLYV		1	
	pol	A*02:01 A*02:01	ILKEPVHGV ALQDSGLEV	ILKEPVHGV ALQDSGLEV		1 1	
		A*32:01 B*44:02	PIQKETWETW	PIQRETWETW EDMTLPGRW	Weak binder E2D inferred/documented escape	0	
DNAJB14.d21		B*44:02 C*05:01	QEEHERYHSNW HTDNGSNF	QDDHEKYHSNW HTDNGGNF	Non binder Strong binder	0	
	rev	A*02 B*44:02 C*05:01	EELLKTVRL SAEPVPLQL	EELLOTVRL PAEPVPLQL	KSO documented escape Non binder	0	
		A*02:01 A*02:01	KLTPLCVTL TLSQIVTKL	KLTPLCVTL TLDQIFKKL	Non binder	1	
	env	A*02:01 A*02:01 A*32:01	SLINATDIAV RVIEVAQRV RIKQIINMW	SLLNATAIAV RIIEVLQRA RIKQIINLW	Weak binder Strong binder	1	
		B*44:02 C*07:04	AENLWVTVY VYYGVPVWKEA	TEKLWVTVY VYYGVPVWKEA	A1T, N3K documented escape	0	
	nef	A*02:01 A*02:01	LEWRFDSTL AFHHVAREL	LVWRFDSRL AFHHMAREL	Non binder Non binder	0	
total		C*07:04 C*07:04	KRQDILDLWVY RYPLTFGWCY 29	KRQDILDLWVY RYPLTFGWCF	Y10F binding unchanged	1 65.5	
P2	region	relevant MHC	expected	observed	impact	WT?	
		A*02:01	SLYNTVATL	SLYNTVATL		1	
	gag	A*02:01 A*02:01 A*02:01	SLYNTVATL TLNAWVKVV MTSNPPIPV	TLNAWVKVV MTSNPPIPV		1 1 1 1	
	gag	A*02:01 A*02:01 A*02:01 A*02:01 B*44:02 A*02:01	SLYNTVATL TLNAWVKVV MTSNPPIPV VLAEAMSQV AEQASQDVKNW KMIGGIGGFI	TLNAWVKVV MTSNPPIPV VLAEAMSQV AEQCTQEVKNW KMIGGIGGFI	Strong binder	1 1 1 1 1 1	
	gag	A*02:01 A*02:01 A*02:01 B*44:02 A*02:01 A*02:01 A*02:01	SLYNTVATL TLNAWVKVV MTSNPPIPV VLAEAMSQV AEQASQDVKNW KMIGGIGGFI VLVGPTPVNI ALVEICTEM	TLNAWVKVV MTSNPPIPV VLAEAMSQV AEQCTQEVKNW KMIGGIGGFI VLVGPTPVNI ALVEICTEM	Strong binder	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
	gag	A*02:01 A*02:01 A*02:01 B*44:02 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01	SLWITVATL TLNAWVKVV MTSNPPIPV VLAEAMSQV AEQASQDVKNW KMIIGGIGGI VLVIGFTVNI ALVEICTEM VYQYMDDLVV ILKEPVHSV ALQDSGLEV	TLNAWVKVV MTSNPPIPV VIAEAMSQV AEQCTQEVKNW KMIGGIGGFI VLVGPTPVNI ALVEICTEM VIYQYMDDLVV ILKEPVHGV ALQDSGLEV	Strong binder	1 1 1 1 1 1 1 1 1 1	
		A*02:01 A*02:01 A*02:01 B*44:02 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 B*44:02 B*44:02	SLINTVATL TLAAUWKW MTSNPPIPW VLAEAMSQV AEQASQDVKNW KUNGPTPMII ALVELTEM WYRQYMDDLYV ILKEPVHSV ALQDSGLEV PRQKETWETW EEMMLPGRW	TURAWVKIV MTSNPPIPV VUAEAMSQV AEQCTQEVINW KMIGGIGGFI VLVGPTPVNI ALVEICTEM VITQYMDDLVV ILKEPVHGV ALQDSGLEV PIQRETWDTW EDMTLPGRW	Strong binder Nach binder 120 internet/documented eucace	1 1 1 1 1 1 1 1 1 1 1 0 0	
RRM1.745A	pol	A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 B*44:02 B*44:02 B*44:02 C*05:01	SUNTUATL TUNAWUKW MTSNPRPV VLAEANSQV AEQASQUVSIW AUGSGGGH VLVGPTPVNI ALVEICTEM VLVGPTPVNI ALVEICTEM ULKEPVHGV ALQDSGLEV PRQLETVETW EEMNLRGRW QEEHERVISIW DOGSNF	TUNAWNIVY MTSNPPIPY VIAEMISQV ARQCTOEWNIW ARQCGGGFI VIVGPTPNI ALVEICTEM VICQPTPNI ALQDSGLEY PIQRETWOTW EDMTLNGRW QDDIECKYISHW	Stopy bloder	1 1 1 1 1 1 1 1 1 1 1 0	
RRM1.745A		A *02:01 A *02:01 A *02:01 A *02:01 B *44:02 A *02:01 A *02:01 A *02:01 A *02:01 A *02:01 A *02:01 B *04:02 B *04:02 B *04:02 B *04:02 B *04:02 B *02:01	SINTUATI TUNAWING MTSIAPPIN AGASGWIN AGASGWIN AGASGWIN ALVERTPNI ALVERTPNI ALVERTPNI ALVERTPNI ALVERTPNI ALQOSELEV PROMUNGV ALQOSELEV ROMENGI BELIKTYRE SARROQL EELIKTYRE SARPHOL	TUNAWAYAY MISNIPIPY VIAEANSGV AEQCTGEVANW KIMGGIGGT VINGPTPANI AUXIDICTEM VINGPTPANI AUXIDICTEM UREPHISV ALQOSCLEV PURETWOTW EDMITURGNV QDHERYNSW HTDMGGNF AIRIGQQ EELIKTYRL PAEPYRQL	Stops binder Non binder E20 inferret/dccunet occupe Non binder	1 1 1 1 1 1 1 1 1 1 1 0 0	
RRM1.745A	pol vpr	A *02.01 A *02.01 B *44.02 C *05.01 A *02 B *44.02 C *05.01 A *02.01	SUNTUATL TUNAWAKW MTSNPPIPV VALEANSGV ALGASGOVENW MINGGIGGI VUVEPTPNI ALGGSGGI VUVEPTPNI ALGGSGI ULEEPVHGV ALGGSLETW VICTIMBOLV EEELEPVHSW EEELEVTSW EEELEVTSU SAEFVYGL	TUNAWAYAY MISNPRIPV VUASANSQV ASQCTGEVXWW KMIGGIGGFI VUXGPTPNII ALVEICTEM VUXQPTPNII ALVEICTEM VUXQPTPNII ALQDSGLEV PIQRETWOTW QDDHESVHSWW QDDHESVHSWW QDDHESVHSWW ALQDSGLEV ELIATVRI PAEPYQQL ALTELCYTL	Strong binder Hon binder E 20 Inferred (Scauented Hon binder Bring binder Ut die minimider regionen, secupie	1 1 1 1 1 1 1 1 1 1 1 1 0 0 0 0 0 1	
	pol vpr	A *02201 A *02201 A *02201 B *02201 B *02201 A *02201 A *02201 A *02201 A *02201 A *02201 A *02201 A *02201 A *02201 B *0402 C *0501 C *0501 A *02201 A *0200 A *0200 A *0200 A *02201 A *0201 A *0201 A *0201 A *0200 A *0000 A *000 A *000 A *0000 A *0000 A *0000 A *0000	SINTVATL TUANWOV MISNIPPIN VALAMAGU VALAMAGU VALAMAGU VALAMAGU VAVORTPANI AUVACTEM VAVORTPANI AUVACTEM VAVORTPANI AUVACTEM VAVORTPANI AUVACTEM VAVORTPANI AUVACTEM VAVORTPANI VAVORTPANI VAVORTPANI VAVORTPANI AUVACTEM VAVORTPANI VAVO	TUANWWV WISEMERY WISEMERY WISEMERY AGGCGGVWW AGGCGGF WVGPTPNI AUXGTTPNI AUXGTTPNI AUXGTTPNI VUGPTPNI V	Story binder ED interveldecumente ecope Rein binder Story binder Non Binder Non Binder Weis Dinder Weis Dinder	1 1 1 1 1 1 1 1 1 1 1 1 1 1	
RRM1.745A	pol vpr rev	A*02201 B*4402 C*05501 B*0201 B*02000 B*02000000000000000000000000000	SINTVATI TLANAWAY MARAMAY MARAMAY MARAMAY MARAMAY ALARAMA	TUANWOVU MISHPPPV VUALAMSQV AGCCTGUNWW VUALAMSQV AGCCTGUNWW VUGPTNI AGCCTGUNW VUGPTNI AGCCTGUNW VUGPTNI AGCCTGUNG AG	Strong binder E20 inflime document Strong binder Lid stronger Kan binder Kan binder Kan binder Kan binder Strong binder Strong binder Strong binder Strong binder Strong binder	1 1 1 1 1 1 1 1 1 1 1 1 1 1	
RRM1.785A	pol vpr rev	A*02201 A*0200 A*0200	SINTVATL TLANWOV MISSINPU MISSINA	TUANWWV WIAEMSQV ASCCGGVWW IMBGGGGI ASCCGGVWW MUGGGGI AMUGGGI WWW WIQYMGCW LLEPHIQY ALQSGLGV PAGTWOTW LLEPHIQY ALQSGLGV PAGTWOTW LLEPHIQ LLEPHIQ ALQSGLGV PAGTWOTW LLEPHIQ LLEPHIQ AMUGGGW AMUGGGW ALLEPHIQ LLEPHIQ AUGUGGW ALLEPHIQ LLEPHIQ LLEPHIQ AUGUGGW ALLEPHIQ LLEPHIQ LLEPHIQ ALLEPHIQ ALLEPHIQ LLEPHIQ ALLEP	Story binder ED interveldecumente ecope Rein binder Story binder Non Binder Non Binder Weis Dinder Weis Dinder	1 1 1 1 1 1 1 1 1 1 1 1 1 1	
total	pol vpr rev env nef	A*02:01 A*02:0	SUNTATI HUARAWAY NULARAWAY NULARAWAY HUARA	TUAAWWY MISMPPHY VLCCPCCOSM VLCCPCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	Brong binder Ein Inform Glocuranie Ein Inform Glocuranie Brong Binder Le diministrati response Rein Binder Nen Binder Werk binder Storg Binder H. 14. Ki docurretetter desspe Umber Binder Nen Binder		
RRM1.745A	pol vpr rev env	A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 A*02:01 B*44:02 C*05:01 B*44:02 C*05:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 A*02:01 B*44:02 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 B*42:04 C*07:04 D*07:0	SINTYATI TUAAWAYAY WAEMASGU AGGOODYNW AGGOODYNW AGGOODYNW AGGOODYNW AGGOODYNW AGGOODYNW AGURTHA WICHMORCH HURPHOR WICHMORCH AGURTHA HURPHOR HU	TUAAWWV WAAAAGU WSRPPHY WAAAAGU MAGGIGGT MAGGIGT MAGGIGGT MAGGIGG	Stong binder Han kinder Ein interrectidecument Eine binder Stong binder Bon binder Han binder Han binder Kan binder Alt, NAS dochersteilt scope Alt, NAS dochersteilt scope	1 1 1 1 1 1 1 1 1 1 1 1 1 1	
total	pol vpr rev env nef	A*02.01 A*02.01 A*02.01 B*02.01 A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 B*44.02 B*44.0	SINTYATI ILAAWWAY VALAMSOV ALAAWSOV VALAMSOV AGOGOVINW AGOGOVINW AGOGOVINW AGOGOVINW AGOGOVINW AGOGOVIN VICINGOVIN VICINGOVIN PRETURKI AGOGOVIN PRETURKI VICINGOVIN AGOGOVIN PRETURKI SILADOG VICINGOVIN AGOGOVIN PRETURKI SILADOG VICINGOVIN AGOGOVIN PRETURKI SILADOG VICINGOVIN AGOGOVIN PRETURKI VICINO VICINO VI	TULANOVOV MISAPPIPV VULANOSOW MISAPPIPV VULANOSOW VULANOV VULA	Storg boder Non binder 12 Dinfererä(documento eccape Non binder Storg binder Non Binder Weik binder Storg binder Storg binder Storg binder Ytjör binding unchanged <u>Non Binder</u> Ytjör binding unchanged	1 1 1 1 1 1 1 1 1 1 1 1 1 1	
total	pol vyr rev env nef region	A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 A*02.01 B*44.02 B*44.02 B*44.02 B*44.02 B*44.02 B*44.02 B*44.02 C*05.01 A*02.0	SUNTATI LIAAWAN SUNTATI LIAAWAN LIA	TLANSWOV TLANSWOV MISSIPPIN ARCGCC0VNW MISSIPPIN ARCGCC0VNW MISSIPPIN	Storg Boder Die Storg Boder 20 Inferred(decument Storig Boder Non Binder Storig Boder 14 diminister and storige Non Binder ALT, ISK Genemister Storig Non Binder 21 Zir Bindig unchanged Storig Boder		
total	pol vpr rev env nef region	A*0201 A*	SINTVATI ILLAWWORK WAEAKSQV AGOGOVINW MACGGOGI MACG	TLAARWOY TLAARWOY HISPAPPU ACCCCUVRW HACCCCUVRW HACCCCUVRW HACCCCUVRW HACCCCUVRW HACCCCUVRW HACCUVRW HA	Storg boder Non binder 12 Dinfererä(documento eccape Non binder Storg binder Non Binder Weik binder Storg binder Storg binder Storg binder Ytjör binding unchanged <u>Non Binder</u> Ytjör binding unchanged	1 1 1 1 1 1 1 1 1 1 1 1 1 1	
	pol vyr rev env nef region	A*02201 A*0200 A*0200 A*0200 A	SINTIATE ULAAWAYA SA	TLAAMWAY TLAAMAANA MAGACGONTONI AAGACGONTONI AAGACGONTONI AAGACGONTONI AAGACGONTONI HARANGA MAGAGAANA HARANGA	Song bode Do bode E20 infere document Song bode Mark Song Bode Song Bode Song Bode Song Bode Song Bode Song Bode		
total	pol rev env ned region gag	A*02201 A*0200 A*0200 A*0200 A	SINTATI HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HITONOON HITO	TULANOVOV MISINPIPU ALGOCIGONINU ALGOCIGONINU ALGOCIGONINU ALGOCIGONINU ALGOCIGONINU ALGOCIGONINU ALGOCIGONINU ALGOCIGONINU EINTERNO ALGOLINU ALGOL	Storg boder Non binder 12 Dinfererä(documento eccape Non binder Storg binder Non Binder Weik binder Storg binder Storg binder Storg binder Ytjör binding unchanged <u>Non Binder</u> Ytjör binding unchanged	1 1 1 1 1 1 1 1 1 1 1 1 1 1	
	pol vpr rev env nef region	A*12231 A*12331 A*1233	SINTVATU HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HITDHOORF HITHHOORF HITHHOORF HITHHOORF HITHHOORF HITHOORF HITHHOORF H	TULANOVOV TULANOVOV MISIOPPIN AGOCGOVINU AGOCGOVINU AGOCGOVINU AGOCGOVINU AGOCGOVINU AGOCGOVINU LIEPPINO EMBELIA AGOCGOVINU EMBELIA AGOCGOVINU EMBELIA AGOCGOVINU AGONECTION AGO	Bong boder Bon binder Bon binder Bong boder Bong boder Bon binder Bon binder Bon binder Bon binder Bon binder Bon binder Bon binder Bon binder Bon binder Bong boder Bong boder Bod	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
	pol ver rev env and region gag gag	A*02031 A*0203	SINTYATI ULAAWAWE MAL	TULANOVOV MISINPIPIV VLGCPTOVOV VLGCPTOVOV VLGCPTOVOV VLGCPTOVOV VLGCPTOVOV VLGCPTOVOV VLGCPTOVOV ULGCPTOVOVOV ULGCPTOVOV ULGCPTOVOV ULGCPTOVOV ULGCPTOVOVOV ULGCPTOVOVOV ULGCPTOVOVOVOVOVOVOVOVOVOVOVOVOVOVOVOVOVOVOV	Song Boder Din honder ED informédecurane Song Madrie Maria Madrie M	1 1 1 1 1 1 1 1 1 1 1 1 1 1	
	pol vpr rev env nef region seg seg seg sol	A*02231 A*02331 A*0233	SINTYATI ULAAWAYA MALAWAYA MALAWAYA ULAAWAYA ALAAMAYA ALAAMAYA WALAWAYA WALAWAYA WALAWAYA WALAWAYA WALAWAYA HIDBOORF HID	TULANOVOV TULANOVOV MISTAPPIPY NACOTONOV NACOTONOV NACOTONOV NACOTONO NACOTONICIONO NACOTONI	Song boder Din honder (2) Song boder Song boder Song boder Song boder Song boder Song boder Song boder Song boder 1/25 boding unchanget Song boder 1/25 boding unchanget Song boder Song boder EL documented ecope ED calculated ecope ED calculated ecope ED calculated ecope	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
	pol vpr rev env nef region seg seg seg sol	A*02031 A*0203	SINTYATI ILAAWWAY VALAMSOV AGOGOVIW AGOGOVIW AGOGOVIW AGOGOVIW AGOGOVIW AGOGOVIW AGOGOVIW AGOGOVI AGOGOVI PRETURATIV ELLANDIG ELLANDIG ELLANDIG BARDON ELLANDIG AGOGOVI AGOGOVI SILANDIG BARDON ELLANDIG BARDON ELLANDIG BARDON ELLANDIG BARDON ELLANDIG BARDON ELLANDIG BARDON BARDON ELLANDIG BARDON B	TLAARWOY TLAARWOY HISPAPPU ACCCCUVSWI ACCCCUVSWI ACCCCUVSWI ACCCCUVSWI ACCCCUVSWI ACCCCUVSWI ACCCCUVSWI ACCCCUVSWI ACCCUVSU ACC	Storg bioler Bon binder 12 bindered(documented eccape bon biolar Storg bioler Bon binder Bon binder Bon binder Bon binder Storg bioler A17, IAS documented scape V10F binder Storg bioler Storg bioler Biol Bioler Biol Bioler A12, Biol Bioler Storg bioler Biol B	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
	pol ver rec env recion recion recion pol env recion recion	A*12231 A*12231 A*12231 A*12231 A*12231 A*12331 A*12321 A*123231 A*12331 A*123	SINTYATI HAAAAAAA KAAAAAAAAAAAAAAAAAAAAAAAAAAAA	TLANSWORD TLANSWORD ADDCTOURSUE ADDCTOURSU	bong bode Do Interestico-cash Neo boder Do Interestico-cash Neo boder Dog Boder Li discolari Wash boder Wash boder Wash boder Wash boder Do Boder Neo boder Do Boder Neo boder Dog Boder Storege Boder Storege Boder Do Cashalated accept Li Di cashalated accept Di Dockalated accept Di Docka		1927 - Promot
	pol ver rec env recipion recipion recipion pol recipion r	A*10201 A*1020	SINTYATI U LAAAWAYA U LAAAAWAYA U LAAAWAYA U LAAAWAYAYA U LAAAWAYA U LAAAWAYAYA U LAAAWAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYA U LAAAWAYAYAYA U LAAAAWAYAYA U LAAAAWAYAYA U LAAAAWAYAYAYAYAYA U LAAAAWAYAYAYAYAYAYAYAYAYAYAYAYAYAYAYAYAY	TLAARWOY TLAARWOY MARGEGO ANGEGOVINNU ANGEGOVINNU ANGEGOVINNU ANGEGOVINNU ANGEGOVINNU ANGEGOVINU LIEPANGO DOGENITATION LIEPANGO ANGEGOVINU ANGE	Sing bide Sing bide District of countries		8*57 8*57 WTT
	pol ver rec env recion recion recion pol env recion recion	A 12223 1 A 1223 1 A 1233	SINTYATI HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HITDHOON HITTHOON HITHOON HITH	TULANOVOV TULANOVOV ALGCCGOVINU ALGCCGOVIN	bong boder Do binder 120 binder diekander Beng binder 14 dinnter de source Beng binder 14 dinnter de source 14 binder 14 binder 14 binder 14 binder 14 binder 14 binder 14 binder 14 binder 14 binder 15 bin	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1
	pol vpr rev anv anv nef region seg pol vpr rev anv region nef nef region	A*12231 A*12331 A*1233	SINTYATI HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HAAAWAYA HITDHODY HITCHOO H	TLAARWOVE TLAARWOVE MISSIPPIN ACCCCONSTRUCTION ACCCCCONSTRUCTION ACCCCCONSTRUCTION ACCCCCONSTRUCTION ACCCCCONSTRUCTION ACCCCCONSTRUCTION ACCCCCONSTRUCTION ACCCCCONSTRUCTION ACCCCCONSTRUCTION ACCCCCONSTRUCTION ACCCCCCONSTRUCTION ACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	Englisher Den beder Englisher den sone Steng beder He den beder Steng beder He den beder He beder Steng beder He beder	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1
	pol vyr cev env env region region god yr env env env region region god region region god	A*02031 A*0203	SUNTATIC HURAWOW VILLEAMANDY ALLANDON UNLEAMANDY ALLANDON UNLEAMANDY ALLANDON VILLEAMANDY	TULANOVOV TULANOVOV MISINPIPIV AGOCGOVINU AG	Elong boder Elong boder Elong boder Elong boder Ren bloder Ren bloder Re	1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1
	pol vpr rev anv anv nef region seg pol vpr rev anv region nef nef region	A*02201 A*02201 A*02201 A*02201 A*02201 A*02201 A*02201 A*02201 A*02201 A*02201 B*046201 A*02201 B*046201 A*02001 A*02001 A*02001 A*02001 A*02001 A*02001 A*02001 A*02000 A*02000 A*02000 A*02000 A*02000 A*02000 A*02000 A*02000 A*02	SUNTATI ILLAWAWE ALLAWAWE ALLAWAWE ALLAWAWE ALLAWAWE ALLAWAWE ALLAWAWE ALLAWAWE ALLAWAWE ALLAWAWE ALLAWAWE ALLAWAWE ALLAWAWE ALLAWAWE ALLAWA A	TLANSWOV TLANSTOPPIN ADDCTOURSE A	Song bioder Lind minister ingroom, except biologies and a song biologies Lind minister ingroom, except biologies biologies Lind minister ingroom, except biologies and biologies Stong biologies Lind konnented except Lind konnented konnented except Lind konnented konnented except Lind konnented konnente	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1
	pol vyr cev env env region region god yr env env env region region god region region god	A 12223 1 A 1223 1 A 1233 1 A	SINTYATI JALAWAYA MALAWAYA MALAWAYA JALAWAYA JALAWAYA MALAWAYAYA MALAWAYAYA MALAWAYA MALAWAYA MALAWAYA MALAWAYA MALAWAYA	TLAARWOY TLAARWOY MISSIPPIN AGCCCONSTONE AGCCCONSTONE AGCCCONSTONE AGCCCONSTONE AGCCCONSTONE AGCCCONSTONE AGCCCONSTONE AGCCONS	bong bode Do bonder Einimer de sege Seng bode He dentre de sege He dentre de sege H	1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1
1044 P3 27VVE3.745C	baq rayo rayo and rayo rayo rayo and rayo rayo rayo rayo rayo rayo rayo rayo	A*02201 A*0200 A*	SINTYATA GALAGOODANA ALAAONA A	TLAARWOY TLAARWOY MISSIPPIN AGCCGUVSWY AGCCUVSWY AGCCUVSWY AGCCUVSWY AGCCUVSWY AGCCUVSWY	ED calculated encoge and a second and a second a se	1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1
1044 P3 27VVE3.745C	vpr rev env env region region env env env env env env env env env en	A 12223 1 A 1223 1 A 1233 1 A	SUNTATI SUN	TULANOVOV TULANOVOV ALGCCGOVINU ALGCCGOVIN	bon binder Dan binder Dan binder Dan binder Dan binder Dan binder Bei binder Bei binder Wast binder Storg binder Mark binder Storg binder Mark binder Storg binder Mark binder Storg binder Mark binder Storg binder Mark binder Storg binder Mark binder Storg binde		
1044 P3 27VVE3.745C	baq rayo rayo and rayo rayo rayo and rayo rayo rayo rayo rayo rayo rayo rayo	A 10203 A 1020	SINTYATI JALAWAYAR MALAWAYA MALAWAYAYA MALAWAYA MALAW	TULANOVOV TULANOVOV ATTANDEPHY ARCCCOPYNNU ARCCCOPYNNU ARCCCOPYNNU ARCCCOPYNNU ARCCCOPYNNU ARCCCOPYNNU ARCCCOPYNU ARCCCOP	biog bode Discholarier Einenker Beng bode Beng bode	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
1044 P3 27VVE3.745C	bq rev env rev nef region pal env env env region for seg seg seg seg seg seg seg seg seg seg	A*02031 A*0203	SUNTATI SUN	TULANOVOV TULANOVOV ALGOCGOVINU ALGOCGOVINU ALGOCGOVINU ALGOCGOVINU ALGOCGOVINU ALGOCGOVINU ALGOCGOVINU ALGOCGOVINU ALGO	Bong boder Die henre diebenaams Bong boder Die henre diebenaams Bong boder Die henre Bong boder Die henre Bong boder Ant, Tick Goommet de scope Die henre Die henre Die henre Die henre Die henre Die henre Die henre Die henre Die henre Die die henre Die henre Die die henre Die henre Die henre Die die henre Die henre		
1044 P3 27VVE3.745C	bq rev env rev nef region pal env env env region for seg seg seg seg seg seg seg seg seg seg	A*12231 A*12231 A*12231 A*12231 A*12231 A*12231 A*12231 A*12231 A*12231 A*12231 A*12231 A*12231 B*146231 A*12231 B*146231 A*12331 A*12	SUNTATIC ILLANDONE ACADEDATIC ACADEDATICA	TULANOVOV TULANOVOV ANDERIONAL ANDERINAL ANDERIONAL ANO	Song boder Die henner die decape Die henner die decape Die henner die decape Song binder Song binder Song binder Song binder Song binder Song binder Song binder Song binder Song binder Song binder Die binder Song binder Binder Song binder Song binder Binder Song binder Song binder Die binder Song binder Die binder Song binder	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
1044 P3 27VVE3.745C	pat vyp rae anv rasion pat anv rasion pat anv rasion sag anv ragion sag anv ragion sag anv ragion sag anv anv sag anv anv anv anv anv anv anv anv anv anv	A 10203 II A 10203 II	SINTYATI JALAWAYAR JALAWAYAYAYA JALAWAYAYA JALAWAYAYAYA JALAWAYAYAYA JALAWAYAYAYA JALAWAYAYAYA JALAWAYAYAYAYA JALAWAYAYAYAYA JALAWAYAYAYAYA JALAWAYAYAYAYAYAYAYAYAYA JALAWAYAYAYAYAYAYAYAYAYAYAYAYAYAYAYAYAYAY	TLAARWOY TLAARWOY MISSIPPIN AGCCGOVENT MISSIPPIN AGCCGOVENT MISSIPPIN AGCCGOVENT MISSIPPIN MISSI	Exong body Exong		

Supplementary Table S4. Analysis of CTL escape

Assay	Delesson			le genome sequencing (SGS) PCR	
	Primer name u5gagSGS_fo	PCR reaction	Primer type forward	Primer sequence GTARCTAGAGATCCCTCAGAC	reference
u5-gag	u5gagSGS_ro	outer	reverse	TGACATGCTGTCATCATYTCYTC	Simonetti et al.
uo-gag	u5gagSGS_fn	nested	forward	AAATCTCTAGCAGTGGCGCC	(33301425)
	u5gagSGS_rn 1840+		reverse forward	CATCATTTCTTCTARTGTAGCTSCT GATGACAGCATGTCAGGGAG	
P6-RT	3500-	outer	reverse	CTATYAAGTCTTTTGATGGGTCATAA	Van Zyl et al.
	1870+ 3410-	nested	forward	GAGTGTTGGCTGAGGCAATGAG	(28891813)
	3410- envSGS_fo		reverse forward	CAGTTAGTGGTACTATGTCTGTTAGTGCTT GCCAGTAGTRTCAACYGAA	
env	envSGS_ro	outer	reverse	GCARATGAGTTTTCYAGAGCA	Simonetti et al.
	envSGS_fn envSGS_m	nested	forward reverse	CTGCTAAATGGCAGTCTAGC TTGCCTGGAGCTGYTTRATGC	(33301425)
	6110000_111	Primers used		hesis from plasma or cell-associated RNA	
Assay	Primer name	Reaction	Primer type	Primer sequence	reference
u5-gag P6-RT	u5gagSGS_ro 3500-	cDNA cDNA	reverse	AAATCTCTAGCAGTGGCGCC CTATYAAGTCTTTTGATGGGTCATAA	
env	Env.B3	cDNA	reverse	TTGCTACTTGTGATTGCTCCATGT	Bertagnolli et al.
-					(33239444)
Assay	Primer name	PCR reaction	Primers use Primer type	ed for Sanger sequencing Primer sequence	reference
	u5gagSGS_fn	Sanger	forward	AAATCTCTAGCAGTGGCGCC	Simonetti et al.
u5-gag	INT3A2 u5gagSGS_rn	Sanger Sanger	reverse reverse	AGCTTCCTCATTGATGGTCTCTTT CATCATTTCTTCTARTGTAGCTSCT	(33301425)
P6-RT	1870+	Sanger	forward	GAGTGTTGGCTGAGGCAATGAG	Van Zyl et al.
	3410- envSGS_fn	Sanger Sanger	reverse forward	CAGTTAGTGGTACTATGTCTGTTAGTGCTT CTGCTAAATGGCAGTCTAGC	(28891813) Simonetti et al.
env	envSGS_m	Sanger	reverse	TTGCCTGGAGCTGYTTRATGC	(33301425)
		Primer	s and probes (used for to confirm integration site	
Assay	Primer name	PCR reaction	Primer type	Primer sequence	reference
ADK	ADK_FO ADK_FN	outer nested	forward forward	AGCCGTATCTACGGGTTACTGG GTTACTGGAAGGGCTAATTTACTC	
AAK1	AAK1_FO	outer	forward	GAGAAATCCAGTAACCAATCATGAC	
	AAK1_FN DNAJB14_FO	nested outer	forward forward	CCTTGCAAGGACTGACTTTTGAG TACTTTGTGCTATACCCATATAATCAAG	
DNAJB14	DNAJB14_FN	nested	forward	TTGGTATTCATTGGAAGGGCTAATTC	
RRM1	RRM1_FO	outer	forward forward	TATCAATAATCTCTTCACTGCCTTG GGATTCTATCTATTGACTAGGTGT	
ZFYVE9	RRM1_FN ZFYVE9_FO	outer	forward forward	AAACATGCCAATGCCTCTGGC	
2FIVE9	ZFYVE9_FN	nested	forward	CTAAACACTTGGAAAAATGATTGAG	
CCND3	CCND3_FO CCND3_FN	outer nested	forward forward	CAAAGAAAAGATGGGAGTTCCC GAAGGCCTGGAGAAAACTAGC	
CCND3	CCND3_RO	outer	reverse	AAGACGTCTGTTTATTATTCGCAAAG	
001120	CCND3_RN	nested	reverse	CTCTGGTTTGTTAAGCTTTGCCA	
Assay	Primer name	PCR reaction	Primer type	s used for HIV RNA quantification Primer sequence	reference
	RU5-F	digital PCR	forward	CTTAAGCCTCAATAAAGCTTGCC	Anderson et al
U5-R	RU5-Probe RU5-R	digital PCR digital PCR	probe reverse	5fam/AGTAGTGTG/zen/TGCCCGTCTG/3iabk GGATCTCTAGTTACCAGAGTC	(30253074)
ADK.d22	ADK_d22_FWD	digital PCR	forward	CAGGACTCGGCTTGCTGAG	
deletion	ADK_d22_PRB	digital PCR	probe	5fam/CGCACGGCA/zen/AGAGTACGCCATAAA/3iabk	
	ADK_d22_REV DNAJB_d21_FWD	digital PCR digital PCR	reverse forward	GCACCCATCTCTCCTTCTAGC CGAAAGGGAAACCAGAGGAG	
DNAJB14.d21 deletion	DNAJB_d21_PRB	digital PCR	probe	5fam/CTCCGCTAG/zen/TCAAACGCCGC/3iabk	
	DNAJB_d21_REV ADK-U3_FWD	digital PCR digital PCR	reverse	CGCTTAATACCGACGCTCTC	
			forward	AGGCATTGTGCTAAGTGAGAA	
ADK.d22 read	ADK-U3_PRB	digital PCR	forward probe	AGGCATTGTGCTAAGTGAGAA 5hex/CTACGGGTT/zen/ACTGGAAGGGCTAATTTAC/3iabk	
through	ADK-U3_PRB ADK-U3_REV	digital PCR digital PCR	probe reverse	5hex/CTACGGGTT/zen/ACTGGAAGGGCTAATTTAC/3iabk GTGGTGAACCCACAGATCAA	
through DNAJB14.d21	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB	digital PCR digital PCR digital PCR digital PCR digital PCR	probe reverse forward probe	5hex/CTACGGGTT/zen/ACTGGAAGGGCTAATTTAC/3iabk GTGGTGAACCCACAGATCAA GTGGCCAGGGAAATTTTCATTCAT 5hex/ATTTGGTA/zen/TTCATTGGAAGGGCTAATTC/3iabk	
through	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR	probe reverse forward probe reverse	5hex/CTAC5GGGTI/zen/ACTGGAAGGGCTAATTTAC/3labk GTGGTGACCCACACATTACA GTGGCCAGAGTAAATTTCTATTCAT 5hex/ATTTGGTA/zen/TATTGGAAGGCTAATTC/3labk GTGGTA/Sen/TCATTGGAAGGCTAATTC/3labk	
through DNAJB14.d21 read through	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR	probe reverse forward probe reverse single genome	5hex/CTACGGGTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCAGAGTAAATTTCTATTCAT 5hex/ATTTGGTA/zen/TTCATTGGAAGGGCTAATTC/3labk GTGGTGACCCCACAGATCAAG g sequencing (SGS) of spliced HIV transcripts	reference
through DNAJB14.d21	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction	probe reverse forward probe reverse	5hex/CTAC5GGGTI/zen/ACTGGAAGGGCTAATTTAC/3labk GTGGTGACCCACACATTACA GTGGCCAGAGTAAATTTCTATTCAT 5hex/ATTTGGTA/zen/TATTGGAAGGCTAATTC/3labk GTGGTA/Sen/TCATTGGAAGGCTAATTC/3labk	reference
through DNAJB14.d21 read through	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV Primer name 625_FO 1.8_REV	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse	5hex/CTACGGGTI/zen/ACTGGAAGGGCTAATTTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCAGAGTAAATTTCTATTCAT Shex/ATTTGGTA/zen/TTCATTGGAAGGGCTAATTC/3labk GTGGTAGACCCACAGAGCGCTAATTC/3labk GTGGTAGACCCACAGAACTAAG sequencing (SGS) of spliced HIV transcripts Primer sequence ACTTGAAAGTGAAAGTAGAAACCAG CAGTTCGGGAATTGGGAGGGGTGC	reference
through DNAJB14.d21 read through Assay	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_REV DNAJB14-U3_REV Primer name 625_FO 1.8_REV 678_FN	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction	probe reverse forward probe reverse single genome Primer type forward reverse forward	5hex/CTAC5GGGTT/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGACCCACAGATCAA GTGGCCACAGAGTAAATTTCTATTCAT 5hex/ATTTGGTA/zen/TTCATTGGAAGGGCTAATTC/3labk GTGGTAAACCCACACATCAAG 9 sequencing (SGS) of spliced HIV transcripts Primer sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCCGGGATTGGCAGGGGGTGGC GAGCTCTCGACCGCAGGAC	Adapted from
through DNAJB14.d21 read through Assay	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV Primer name 625_FO 1.8_REV	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested	probe reverse forward probe reverse single genome Primer type forward reverse	5hex/CTACGGGTI/zen/ACTGGAAGGGCTAATTTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCAGAGTAAATTTCTATTCAT Shex/ATTTGGTA/zen/TTCATTGGAAGGGCTAATTC/3labk GTGGTAGACCCACAGAGCGCTAATTC/3labk GTGGTAGACCCACAGAACTAAG sequencing (SGS) of spliced HIV transcripts Primer sequence ACTTGAAAGTGAAAGTAGAAACCAG CAGTTCGGGAATTGGGAGGGGTGC	Adapted from Emery et al.
through DNAJB14.d21 read through Assay	ADK-U3_PRB ADK-U3_FWD DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV Primer name 625_FO 1.8_REV 625_FO 1.8_REV 625_FO 4_REV	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for : PCR reaction outer	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse	5hex/CTACGGGTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCAGAGTAAATTCTATTCAT 5hex/ATTTGGTA/zen/TTCATTCAT GTGGTACACCCACAGATCAAG CTGGTACACCCACAGATCAAG CAGTGCGACACCCACAGATCAAG Primer sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGGATTGGAAGGTGGGTTGC CAGTTCGGGATTGGAAGGTGGGTTGC CAGTTCGGGATTGGAAGGTGGGTTGC ACTTGAAAGTGAAAGTAGAACCAG GTACTATAGGTTGCAAGTAGACCAG GTACTATAGGTTGCAATGACACAG	Adapted from
through DNAJB14.d21 read through Assay 1.8Kb	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV Primer name 625_FO 18_REV 678_FN 1.8_REV 625_FO	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward	5hex/CTAC5GGGTT/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGACCCAACAGTACAA GTGGCCAAGAGTAAATTTCTATTCAA 5hex/ATTTGGTA/zen/TTCATTGGAAGGCCTAATTC/3labk GTGGTA/zen/TTCATTGGAAGGCCTAATTC/3labk GTGGTA/AACCCACACAACGAAGA Pimer sequence Pimer sequence ACTTGAAAGTGAAAGTAGAACCAA CAGTTCGGGATTGGGAGGTGGC GAGCTCTCTCGACGCAGGAC CAGTTCGGGATTGGAAGGTGGGTTGC ACTTGAAAGTGAAAGTGAAACCAG	Adapted from Emery et al.
through DNAJB14.d21 read through Assay 1.8Kb	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_FWD DNAJB14-U3_REV DNAJB14-U3_REV 678_FN 1.8_REV 678_FN 1.8_REV 625_FO 4_REV 678_FN	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested outer semi_nested Primers and	probe reverse forward probe reverse single genome forward reverse forward reverse forward reverse forward reverse	Shex/CTACGGGTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGACCCAACAGTCAAA GTGGCCACGAGTAAATTCTATTCAT Shex/ATTTGGTA/zen/TTCATTGATAGGCCACAGATCAAG CTGGTAACCCCACAGATCAAG CTGGTAACCCACAAGTCAAG Primer sequence ACTTGAAAGTGAAAGTGAAACCAG CAGTTCGGGATTGGAAGGTGGGTTGC GAGCTCTCTGAACGGAGGGGGTGC CAGTTCGGGATTGGAAGGTGGGTTGC GAGCTCTCTGAACGGAGGGGGTGC CAGTTGGAAAGTGAAAGTGAAAGTGACAG GTACTATAGGTTGCATTACATGACTACTTAC GAGCTCTCTCGACGCAGGAC GTACTATAGGTTGCATTACATGTACTACTTAC GTACTATAGGTTGCATTACATGTACTACTTAC	Adapted from Emery et al.
through DNAJB14.d21 read through Assay 1.8Kb	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV Primer name 025_FO 18_REV 678_FN 18_REV 678_FN 1.8_REV 675_FO 4_REV 678_FN 4_REV Primer name	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR Dermers used for PCR reaction outer semi_nested outer semi_nested PCR reaction PCR reaction PCR reaction	probe reverse forward probe single genome Primer type forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse	Shex/CTACGGGTT/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGACCCCACAGTACA GTGGCACACCCACAGTACA Shex/ATTTGGT/Zen/TCATTGGAAGGCCTAATTC/3labk GTGGTA/zen/TCATTGGAAGGCCTAATTC/3labk GTGGTA/AACCCACACAGATCAAG Pimer sequence Pimer sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGGATTGGGAGGGGGTGC GAGCTCTCTCGACGCAGGAC CAGTTCGGGATTGGGAGGTGGTGC ACTTGAAAGTGAAAGTAGAACCAG GTACTATAGGTTGCATTACATGTACTACTAC GTACTATAGGTTGCATTACATGTACTACTAC TACGTATAGGTTGCATTACATGTACTACTAC TACGTATAGGTTGCATTACATGTACTACTAC STUSPBS quantification (late RT product) Pimer sequence	Adapted from Emery et al.
through DNAJB14.d21 read through Assay 1.8Kb 4.0Kb Assay	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV DNAJB14-U3_REV 625_F0 18_REV 678_FN 18_REV 678_FN 4_REV 05PB5_FWD	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested outer semi_nested Primers and PCR reaction digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse forward reverse probes used for	Shex/CTACGGGTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGACCCAACAGTCAAA GTGGCCACGAGTAAATTCTATTCAT Shex/ATTTGGTA/zen/TTCATTGAAAGGGCTAATTC/3labk GTGGTAACCCCACAGATCAAG CAGTGCACCCACAGATCAAG Primer sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGGATTGGAAGGTGGGTGC CAGTTCGGGATTGGAAGGTGGGTGC CAGTTCGGCATTGGAAGGTGGGTTGC CAGTTCGGCATTGGAAGGTGGGTTGC CAGTTCGGCATACAGTGACACAG GTACTATAGGTTGCATACATGACAAGTAGACCAG GTACTATAGGTTGCATACATGTACTACTTAC GAGCTCTCTCGACGCAGGAC GTACTATAGGTTGCATACATGTACTACTTAC	Adapted from Emery et al. (28077653)
through DNAJB14.d21 read through Assay 1.8Kb 4.0Kb	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV Primer name 025_FO 18_REV 678_FN 18_REV 678_FN 1.8_REV 675_FO 4_REV 678_FN 4_REV Primer name	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR Dermers used for PCR reaction outer semi_nested outer semi_nested PCR reaction PCR reaction PCR reaction	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward	Shex/CTACGGGTT/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCAACAGTACTACA GTGGCAAGCCAACAGTACTACTACA Shex/ATTTGGTA/zen/TTCATTGGAAGGCTAATTC/3labk GTGGTAACCCCAACAATCAAG CAGTGCAGCCCAACAATCAAG Primer sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGGATTGGAAGGTGGGTTGC GAGTCTCTGAACGAGGGGGGTGC CAGTTCGGGATTGGAAGGTGGGTTGC ACGTTCGAAGGTGGAAGTGGAACCAG GTACTTGAAGGTGGAAGTGGAAGCCAG GTACTTGAAGGTGCAATACCATGTACTTAC GAGCTCTCTCGACCCAGGAC GTACTATAGGTTGCATTACATGTACTACTTAC GTACTTAAGGTGCATTACATGTACTACTTAC DFVBPBS quantification (late RT product) Primer sequence CTGTGTGTGGACTGGTACCT	Adapted from Emery et al. (28077653)
through DNAJB14.d21 read through Assay 1.8Kb 4.0Kb Assay U5-PBS	ADK-U3_PRB ADK-U3_PRB DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV DNAJB14-U3_REV 625_FO 1.8_REV 678_FN 1.8_REV 678_FN 1.8_REV 678_FN 4_REV 05PB5_FVD U5PB5_PRB U5PB5_REV	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested PCR reaction digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward so forward reverse forward so forward reverse forward so forward reverse forward reverse forward so forward so forward forward so forward forwar	Shex/CTACGGGTT/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCAACAGTACTACA GTGGCAAGCCAACAGTACTACAA Shex/ATTTGGTAZzen/TTCATTGAAGGCCAACAGTC/AATGC/3labk GTGGTAAACCCAACAATCAAG Pilmer sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGAAGTGGAAGGTGGGTTGC GAGCTGTCTGGAAGGTGGGTTGC CAGTTCGGGATTGGAAGGTGGGTTGC ACGTCTGAAGGTGGAAGTGGAACCAG GTACTTATAGGTTGGATACCAG GTACTATAGGTTGGATACATGTACTACTAC GTACTATAGGTTGGATACATGTACTACTAC DPIBers sequence CGTGTGTGGCATTACATGTACTACTTAC DPIBers sequence CTGTTGTGGCTTACATGTACTACTTAC DPIBers sequence CGTGTGTGGCATCACATGTACTGGTACT Sfam/CAGTGCCGC/zen/CCGAACACGGGCTC/3labk ACGTCGTCGGTCGAGGAT used for site directed mutagenesis	Adapted from Emery et al. (28077653) reference
through DNAJB14.d21 read through Assay 1.8Kb 4.0Kb Assay U5-PBS Assay	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV 2625_FO 18_REV 678_FN 18_REV 678_FN 18_REV 678_FN 4_REV 678_FN 4_REV Primer name USPBS_PRB USPBS_REV DSPSS_REV DSP	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR semi_nested outer semi_nested PCR reaction digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR	probe reverse forward probe single genome Primer type forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse som bes used for probe reverse s and probes i	Shex/CTACSGGTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGACCCAACAGTACAA GTGGCACAAGCTAAATTCTATACA Shex/ATTTGGAX:en/TATGAAGCCAACAGTAAGA Sequencing (SGS) of spliced HIV transcripts Prime sequence Prime sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGGATTGGAGGTGGGGTTGC CAGTTCGGGATTGGAGGGGGTGC GAGCTCTCCGACGCAGGGGTGC CAGTTCGGGATTGGAGGTGGGTGC CAGTTCGGGATTGGAGGGGGGTGC GACTTCAAGGTGGAAAGTGGAAACCAG GTACTATAGGTGCATTACATGTACTACTAC GTACTATAGGTGCATTACATGTACTACTAC STUPS quantification (tate RT product) Prime sequence CTGTTGTGGCC/CPACCCGGAACAGGGACTTG/3labk AGTCCTGCGCAGAGGAC	Adapted from Emery et al. (28077653)
through DNAJB14.d21 read through Assay 1.8Kb 4.0Kb Assay U5-PBS	ADK-U3_PRB ADK-U3_PRB DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV DNAJB14-U3_REV 625_FO 1.8_REV 678_FN 1.8_REV 678_FN 1.8_REV 678_FN 4_REV 05PB5_FVD U5PB5_PRB U5PB5_REV	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested PCR reaction digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward so forward reverse forward so forward reverse forward so forward reverse forward reverse forward so forward so forward forward so forward forwar	ShexiCTACGGGTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGACCCAACAGTACA GTGGCAGACTAAATTTCTATTCAT ShexiATTTGGTA/GGCCTAATTC/3labk GTGGTA/SACCCAACAGTCAAG 9 sequencing (SGS) of spliced HIV transcripts Prime sequence Prime sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGAATTGGAAGGGGGGTTGC CAGTCGGGATTGGGAAGGGGGGTTGC CAGTCGGGATTGGGAAGTGGGACCAG GAGCTCTCTCGACGCAGGGAC GTACTATAGGTGCATTACATGTACTACTAC GAGCTCTCGACGCAGGAC GTACTATAGGTGCATTACATGTACTACTAC DPimer sequence GTACTGTGAGTGGCG/CeA GTACTGCGC/CPA GTACTGCGCACAGGAC GTACTGCGCCAAAAGTGGAACTA GTACTGCGCCACAAGTGACT Sfam/CAGTGCCGCACAGGAC USPBS quantification (Iate RT product) Primer sequence CTGTTGTGCGCCACAGGGACTTG/3labk AGTCCTGCGCCAAAATTTGACTACGG GTACGCCCAAAATTTTGACTAGCGG GTACGCCCAAAATTTTGACTAGCGG TCTGCCCCACGGCCCGCC	Adapted from Emery et al. (28077653) reference
through DNAJB14.d21 read through Assay 1.8Kb 4.0Kb Assay U5-PBS	ADK-U3_PRB ADK-U3_PRB DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV DNAJB14-U3_REV 625_FO 1.8_REV 678_FN 1.8_REV 678_FN 1.8_REV 625_FO 4_REV 678_FN 4_REV USPBS_REV USPBS_PRB USPBS_REV USPBS_REV DYNE Primer name d22_SDM_F d22_SDM_F	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested outer semi_nested PCR reaction digital PCR digital PCR digital PCR digital PCR digital PCR DPCR reaction GS PCR QS PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse forward probe susd for Primer type forward probes s and probe s forward probes forward probes forward probes forward probes forward forward probes forward forward probes forward forward probes forward forward probes forward	Shex/CTAC5GGGTT/zen/ACTGGAAGGGCTAATTTAC/3labk GTGGTGACCCAACAGTAATTTCTATAC/3labk GTGGTGACCCAACAGTACATTCTATACA Shex/ATTTGGTA/zen/TCATTGGAAGGGCTAATTC/3labk GTGGTAAACCCACACATCAAG Primer sequence ACTTGAAGTGAAGTAGAAGTAGAACCAG CAGTTCGGGATTGGAAGGTAGGTGGC CAGTTCGGGATTGGAAGGTAGGTGG CAGTTCGGGATTGGAAGGTAGGTGC CAGTTCGGAGTGGAAGTGGAACCAG GTACTATAGGTTGCATACATGTACCATC GTACTATAGGTTGCATACATGTACTACTTAC GTACTATAGGTTGCATTACATGTACTACTTAC GTACTATAGGTTGCATTACATGTACTACTTAC GTACTATAGGTTGCATTACATGTACTACTTAC Sfam/CAGTGGCCC/zen/CCGACACAGGAC CTGTGGGCG/zen/CCGACACAGGACTTG/3labk AGTCCTGCGCTCGACGCAGAC Sfam/CAGTGGCCGCAAATTTGGACTAGCGG TCTCCCAAAAATTTGGACTGCGGG GTACGCCAAAAATTTGGACTGCGGG GTACGCCAAAAATTTGGACTGCGGG TCTGCCCGCGCGCCCCC CTTGCCATGCGCGCGCCCCCC	Adapted from Emery et al. (28077653) reference
through DNA,JB14,d21 read through Assay 1.8Kb 4.0Kb Assay U5-PBS Assay	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV 2625_FO 18_REV 678_FN 18_REV 678_FN 18_REV 678_FN 4_REV 678_FN 4_REV 978_FN 4_N	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested outer semi_nested PCR reaction digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse forward reverse forward reverse forward probe sesse for Primer type forward probe sesse for Primer type forward reverse forward forward reverse forward	Shex/CTACGGGTT/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCAACAGTACAA GTGGCAAGGTAAATTTCTATTCAT Shex/ATTTGGTAZzen/TTCATTGAAGGGCTAATTC/3labk GTGGTAAACCCAACAATCAAG Pilmer sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGGAATGGGAGGGGGGTGC GAGCTCTCGAACGAGGGGGGGTGC CAGTTCGGGATTGGAAGGTGGGTTGC ACGTCGGGATTGGAAGGTGGGTTGC GAGCTCTCGAACGCAGACCAG GTACTATAGGTTGGATACATGTACTACTAC GTACTATAGGTTGGATGCAAGTGAACCAG GTACTATAGGTTGGATACATGTACTACTTAC GAGCTCTCGACGCAGGAC GTACTATAGGTTGGATGCACGGGGT CTGTAAGGTGCGATACATGTACTGTAC	Adapted from Emery et al. (28077653) reference
through DNA,1814,d21 read through Assay 1.8Kb 4.0Kb Assay U5-PBS Assay NL4-3_d22 NL4-3_d21	ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV 2625_FO 18_REV 678_FN 18_REV 678_FN 18_REV 678_FN 4_REV 678_FN 4_REV 978_FN 4_REV 978_FN 4_REV 978_FN 978_FN 4_REV 978_FN 9	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested outer semi_nested PCR reaction digital PCR digital PCR	probe reverse forward probe single genome Primer type forward reverse forward forward reverse forward forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward forward reverse forward	Shex/CTACSGGTI/zen/ACTGGAAGGGCTAATTTAC/3labk GTGGTGACCCAACAGTAAA GTGGCCAGAGTAAATTTCTATTCAT Shex/ATTTGGTA/GCCAAGGCTAATTC/Jiabk GTGGTA/SACCCAACAGTCAAG Sequencing (SGS) of spliced HIV transcripts Prime sequence Prime sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGAATTGGCAGGGGGGTGC CAGTTCGGGATTGGCAGGGGGGTGC CAGTTCGGGATTGGCAGGGGGGTGC CAGTTCGGGATTGGCAGCAGGGGTGC CAGTTCGGGATTGGCAGCTGGC GTACTATAGGTGCATTACATGTACTACTTAC GTACTATAGGTGCATTACATGTACTACTTAC GTACTATAGGTGCATTACATGTACTACTTAC GTACTATAGGTGCATTACATGTACTACTTAC GTACTGCGC/CPCCCGAACAGGAC GTACTATAGGTGCATTCAGTGACT Sfam/CAGTGCGC/CPCC DPIME sequence CTGTTGCGCCCACAAGGGACTTG/3labk AGTCCTGCGCCCACAGGACTTG/3labk AGTCCTGCGCCCCACCGGCCCTTC TTGCGCCCAAAATTTTGACTACCGG TCTGCGCCCAAAATTTTGACTACCGG TCTGCGCCCAAAATTTTGACTACCGG TCTGCGCCCAAAATTTTGACTACCGG TCTGCGCCCCACAAATTTTGACTACCGG TCTGCCCCCCCCCC	Adapted from Emery et al. (28077653) reference reference
through DNA,1814,d21 read through Assay 1.8Kb 4.0Kb 4.	ADK-U3_PRB ADK-U3_PRB DNAJB14-U3_FWD DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_PRB DNAJB14-U3_REV 625_FO 18_REV 678_FN 18_REV 678_FN 18_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 978_FN 4_REV 978_FN 4_REV 978_FN 4_REV 978_FN 4_REV 978_FN 4_REV 978_FN 4_REV 678_FN 678_FN 77	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR outer semi_nested outer semi_nested Primers and PCR reaction digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward forward reverse forward forwar	Shex/CTACSGGTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGACCCAACAGTACA GTGGCAGACTAAATTCTATACA Shex/ATTTGGTA/GCCAAGGCTAATC/3labk GTGGTA/SACCCAACAGTCAAG Sequencing (SGS) of spliced HIV transcripts Prime sequence Prime sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGAATTGGAAGGGGGGTGC CAGTTCGGAATGGGAAGGGGGGTTGC CAGTTCGGGATTGGCAGGGGGTGC CAGTTCGGGATTGGCAGGGGGGTGC CAGTTCGGGATTGGCAGGGGGC GTACTATAGGTGCATTACATGTACTACTAC GTACTATAGGTGCATTACATGTACTACTAC GTACTATAGGTGCATTACATGTACTACTAC GTACTATAGGTGCATTACATGTACTACTAC SIGTACTATAGGTGCATTCACTGTACT OF USPBS quantification (Iate RT product) Primer sequence GTACGTCGCAAAAGTGGAACT GTACGCCCAAAATTTTGACTACGGG GTACGCCCAAAATTTTGACTACGGG GTACGCCCAAAATTTTGACTACGGG GTACGCCCAAAATTTTGACTACGGG TCTGCGCCCAAAATTTTGACTACGGG TCTGCCCCCACACGGACTC TTTGACTACGGGAGCCTGC HTTGACTACGGGAGCCTACAAGGAGAGAG CCCCCCAAAATGTGACT CTTGCCCCTGCCCCCTCT VI RNA quantification from transfected cells Primer sequence	Adapted from Emery et al. (28077653) reference reference
through DNA,1814,d21 read through Assay 1.8Kb 4.0Kb Assay U5-PBS Assay NL4-3_d22 NL4-3_d21	ADK-U3_PRB ADK-U3_PRB DNA,B14-U3_FKP DNA,B14-U3_PKP DNA,B14-U3_PKR DNA,B14-U3_REV Primer name 625_FO 1.8_REV 678_FN 1.8_REV 678_FN 4_REV 976_FN 976_FN	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested outer semi_nested PCR reaction digital PCR digital PCR digital PCR digital PCR digital PCR DPCR reaction GS PCR QS PCR SS PCR SS PCR SS PCR	probe reverse forward probe reverse forward Primer type forward reverse forward reverse forward reverse forward probe sused for Primer type forward reverse forward probe s and probes forward reverse forward probes s Primer type forward reverse forward primer type forward primer type fo	Shex/CTACSGGGTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCACAGGTAATTTCTATAC/3labk GTGGTAACCCACAGATCAA Shex/ATTTGGTA/zen/TTGATAGGAGGCCAATTC/3labk GTGGTAAACCCACAGTCAAG Pimer sequence ACTTGAAGTGAAAGTAGAAGCAG CAGTTCGGGGTTGGCAGGGTGC GAGCTCTCTGAACGCAGGGTGGC GAGCTCTCTGAACGCAGGAC CAGTTCGGGATTGGAAGGTGGGTTGC ACGTCGGGATTGGAAGGTGGGTTGC GAGCTCTCTGAACGCAGGAC GTACTATAGGTTGCATACATGTACCAG GTACTATAGGTTGCATTACATGTACTACTTAC GAGCTCTCTGGACGCAGGAC GTACTATAGGTTGCATTACATGTACTACTTAC Sfam/CAGTGGCCC/zen/CCGAACAGGGACTTG/3labk AGTCGCCCAAAATTTTGACAGGG GTACGCCCAAAATTTTGACTAGGGC GTACGCCAAAAATTTTGACTAGGGG GTACGCCCAAAAATTTGGACGCG TTTGCCGTGGGCGCCTC Y USPBS quanification (alte RT product) Pimer sequence GTACGCCAAAAATTTGGACTGGGACTG/3labk AGTCCTGGGTGGGAGAGA GTACGCCCAAAAATTTGACTAGGGG TTCTGCCGTGGGCGCCTC Y-I RNA quantification from transfected cells Pimer sequence CTCTGCCTGGGTCGCGTTACATGA CGCCCCCAGATGCTRACATAA Sfam/TGCCTGGGTCCTCGGTTACCT	Adapted from Emery et al. (28077653) reference reference
through DNAJB14.d21 read through Assay 1.8Kb 4.0	ADK-U3_PRB ADK-U3_PRB ADK-U3_REV DNA,B14-U3_FWD DNA,B14-U3_PRB DNA,B14-U3_PRB DNA,B14-U3_REV 625_FO 1.8_REV 678_FN 1.8_REV 625_FO 4_REV 678_FN 4_REV Primer name USPBS_REV USPBS_REV Primer name Primer name Primer name Freadth-2 Preadth-1 ST25 4kb_FWD	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR methods PCR reaction outer semi_nested outer semi_nested PCR reaction digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse forward probe reverse s and probes Primer type forward reverse forward reverse s everse Primer type forward reverse forward forward reverse forward forw	Shex/CTACSGGCTI/zen/ACTGGAAGGGCTATTTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCACGACGATAATTTCTATAC/3labk GTGGTAACCCACAGATCAA Shex/ATTTGGTA/zen/TTGATAGGAGGCCAATTC/3labk GTGGTAAAACCCACAGATCAAG Pimer sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGGATTGGAAGGTGGGTTGC GACTCTGAAGGTGGAAGTGGAGGGGGTTGC CAGTTCGGGGATTGGAAGGTGGGTTGC CAGTTCGGGATTGGAAGGTGGGTTGC CAGTTCGGGATTGGAAGGTGGGTTGC GACTCTGAAGGTGGAAGTGGAACCAG GTACTATAGGTTGCATTACATGTACTACTAC GTACTATAGGTTGCATTACATGTACTACTAC OF USPBS quantification (alte RT product) Pimer sequence CTGTTGTGTGGCC/CCGACAGGACGCTTG/3labk AGTCCTGCGGCAGGAC GTACCTGCGGCAGGAC GTACCTGGGCGCCAGGACGCGGGTTGC CTGTTGTGTGGCCCCACAGGACGCGTTG/3labk AGTCCTGCGGCGCCAGGACGCCTTG TCTGCCGGGCCCGCCTGGTAAGGAGGAGG CGCCCCCCCGCCTCGCTAGGAGGAGG CGCCCCCCCGCCTCGCTAGGAGGAGG CGCCCCCCGGCCTGGAGGAGG CGCCCCCCGGCTGGAGGAGG CGCCCCCCGGCTGGAGGAGG CGCCCCCCGCCTGCTGGTAGCT V-I RNA quantification from transfected cells Pimer sequence Sfam/GCGCTGGATGCATACATTAA Sfam/TGCCTGGGTCCTCGGTTAGCTGGGGAGGAGG CGCCCCCCGGAGGATGATAAGGAGGAGGAGG CGCCCCCGGCCTGGGTTGCGTGAGGAGGAGG CGCCCCCCGGGCTGGAGGAGGAGG CGCCCCCGGGCTGGAGGAGGAGG CGCCCCCCGGCTGCGGTTAGGAGGAGGAGG CGCCCCCCGGCCTCGCTTGC V-I RNA quantification from transfected cells Pimer sequence CGCCCCCCGGAGGATGCATAAGGAGGAGGAGG CGCCCCCCGGGCTCGGTTAGGTGGGTGCGTGGGTAGGAGGAGG CGCCCCCCGGGCTGGAGGAGGAGG CGCCCCCGGGCCTGGGTGGTGGGGTGG	Adapted from Emery et al. (28077653) reference reference reference
through DNA,1814,d21 read through Assay 1.8Kb 4.0Kb 4.	ADK-U3_PRB ADK-U3_PRB DNAJB14-U3_FWD DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_PRB DNAJB14-U3_REV 625_FO 18_REV 678_FN 625_FO 18_REV 678_FN 625_FO 4_REV 678_FN 678_FN 778	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR semi_nested outer semi_nested outer semi_nested Primers and PCR reaction digital PCR digital PCR digital PCR QS PCR QS PCR QS PCR QS PCR digital PCR	probe reverse forward probe reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward probe sand probe sand probe forward reverse forward probe reverse forward reverse forward reverse forward probe forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward probe	Shex/CTACSGGTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGACCCCACAGATCAA GTGGCCAGAGTAAATTICTATTCAT Shex/ATTTGGTA/GCCACAGATCAAG Shex/ATTGGTA/zen/CTATTGATAGGCCTAATC/3labk GTGGTA/GACCCACAGATCAAG Pimer sequence Pimer sequence CAGTCGGAATGGGAAGTGGGGTGC CAGTCGGAATGGGAAGTGGGGTGC CAGTCGGAATGGGAAGTGGGGTGC CAGTCGGAATGGGAAGTGGGATGC CAGTCGGAATGGGAAGTGGGATGC CAGTCGGAATGGGAAGTGGGATGC GACCTCTCGACCAGGAC GTACTATAGGTGCATTACATGTACTACTAC GTACTATAGGTGCATTACATGTACTACTAC GTACTATAGGTGCATTACATGTACTACTAC Sfam/CAGTGCGC/2en/CCCAACAGGACTG/3labk AGTCCTGCCGCACAGGAC CTGCGCCAAAATTTIGACTACGGG GTACGCCCAAAATTTIGACTACGGG CTTCGCCCAAAAATTTIGACTACGG GTACGCCCAAAAATTTIGACTACGG GTACGCCCAAAAATTTIGACTACGG TTTGGCGCCCAACAGGACTG/3labk AGTCCTGCCCCCCCCCCCCCCCTC TTTGACTACGGGACGCTGC/3labk CCCCCCCAAAATTTIGACTACGGG TCTCGCCCCCCAACAGGACAGG CCCCCCAAAATTTIGACTACGG TCTGCCCCCCCCCCCCCCCCCCCCCCC TTTGACTACGGGACGCAGGAGGAGGAGG GCCCCCAAAATTTIGACTACGGG TCTGCCCCCCCCCCCCCCCCCCCCCCCCCC	Adapted from Emery et al. (28077653) reference reference reference
through DNAJB14.d21 read through Assay 1.8Kb 4.0	ADK-U3_PRB ADK-U3_PRB ADK-U3_REV DNA,B14-U3_FWD DNA,B14-U3_PRB DNA,B14-U3_PRB DNA,B14-U3_REV 625_FO 1.8_REV 678_FN 1.8_REV 625_FO 4_REV 678_FN 4_REV Primer name USPBS_REV USPBS_REV Primer name Primer name Primer name Freadth-2 Preadth-1 ST25 4kb_FWD	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR methods PCR reaction outer semi_nested outer semi_nested PCR reaction digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse forward probe reverse s and probes Primer type forward reverse forward reverse s everse Primer type forward reverse forward forward reverse forward forw	Shex/CTACSGGCTI/zen/ACTGGAAGGGCTATTTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCACGACGATAATTTCTATAC/3labk GTGGTAACCCACAGATCAA Shex/ATTTGGTA/zen/TTGATAGGAGGCCAATTC/3labk GTGGTAAAACCCACAGATCAAG Pimer sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGGATTGGAAGGTGGGTTGC GACTCTGAAGGTGGAAGTGGAGGGGGTTGC CAGTTCGGGGATTGGAAGGTGGGTTGC CAGTTCGGGATTGGAAGGTGGGTTGC CAGTTCGGGATTGGAAGGTGGGTTGC GACTCTGAAGGTGGAAGTGGAACCAG GTACTATAGGTTGCATTACATGTACTACTAC GTACTATAGGTTGCATTACATGTACTACTAC OF USPBS quantification (alte RT product) Pimer sequence CTGTTGTGTGGCC/CCGACAGGACGCTTG/3labk AGTCCTGCGGCAGGAC GTACCTGCGGCAGGAC GTACCTGGGCGCCAGGACGCGGGTTGC CTGTTGTGTGGCCCCACAGGACGCGTTG/3labk AGTCCTGCGGCGCCAGGACGCCTTG TCTGCCGGGCCCGCCTGGTAAGGAGGAGG CGCCCCCCCGCCTCGCTAGGAGGAGG CGCCCCCCCGCCTCGCTAGGAGGAGG CGCCCCCCGGCCTGGAGGAGG CGCCCCCCGGCTGGAGGAGG CGCCCCCCGGCTGGAGGAGG CGCCCCCCGCCTGCTGGTAGCT V-I RNA quantification from transfected cells Pimer sequence Sfam/GCGCTGGATGCATACATTAA Sfam/TGCCTGGGTCCTCGGTTAGCTGGGGAGGAGG CGCCCCCCGGAGGATGATAAGGAGGAGGAGG CGCCCCCGGCCTGGGTTGCGTGAGGAGGAGG CGCCCCCCGGGCTGGAGGAGGAGG CGCCCCCGGGCTGGAGGAGGAGG CGCCCCCCGGCTGCGGTTAGGAGGAGGAGG CGCCCCCCGGCCTCGCTTGC V-I RNA quantification from transfected cells Pimer sequence CGCCCCCCGGAGGATGCATAAGGAGGAGGAGG CGCCCCCCGGGCTCGGTTAGGTGGGTGCGTGGGTAGGAGGAGG CGCCCCCCGGGCTGGAGGAGGAGG CGCCCCCGGGCCTGGGTGGTGGGGTGG	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188)
through DNAJB14.d21 read through Assay 1.8Kb 4.0	ADK-U3_PRB ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV Primer name 625_FO 1.8_REV 678_FN 1.8_REV 678_FN 4_REV 678_FN 4_REV Primer name VSPBS_REV USPBS_REV Primer name d22_SDM_F d22_SDM_R d21_SDM_R d22_SDM_R d21_SDM_R d21_SDM_R d21_SDM_R d21_SDM_R d22_SDM_R	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR semi_nested outer semi_nested PCR reaction digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse forward probe sused for Primer type forward probe forward reverse forward probe	Shex/CTACSGGCTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCAGAGCTAAATTCTATAC/3labk GTGGTAACCCACAGATCAA Shex/ATTTGGTA/SCACACAGCCTAATC/3labk GTGGTAAAACCCACAGATCAAG Pimer squence ACTTGAAAGTGAAGTAGAACCAG CAGTCGGGATTGGGAGGTGGGGGTTGC CAGTCGGGATTGGGAGGTGGGGGTTGC CAGTCGGGATTGGGAGGTGGGGTTGC CAGTCGGGATTGGGAGGTGGGGTTGC CAGTCGGGATTGGGAGGTGGGGTTGC CAGTCGGGATTGGGAGGTGGGGTGC GACCTCTCGAACGTGGGGTGC GTACTATAGGTTGCATTACATGTACTACTAC GTACTATAGGTTGCATTACATGTACTACTAC DPimer squence CTGTTGGGCC/2mCCCGAACAGGACTG/3labk AGTCCTGCGC/2mCCCGAACAGGACTG/3labk AGTCCTGCGC/2mCCCGAACAGGACTG/3labk AGTCCTGCGCCACAGGCCTTG/3labk AGTCCTGCGCCCACAGGCCCTTC TTTGACTAGCGGACAGGCCCGCTTC V1 RNA quantification from transfectid cells Pimer squence GCCCTCAAGTGCTCCAGATACATGATACA Sfam/TGCCTGTACATGACTACTACTAC Sfam/GCCCTGCAGCAGGACTTG/3labk TTTTTTTTTTTTTTTTTTTTTTGACTACGG GCCCCAAAGTAATCCCGGCCCTC V1 RNA quantification from transfectid cells TCTGCCCCAAGATGCAGGACTCGG3labk TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	Adapted from Emery et al. (28077653) reference reference reference
through DNAJB14.d21 read through Assay 1.8Kb 4.0	ADK-U3_PRB ADK-U3_PRB DNAJB14-U3_REV DNAJB14-U3_PRB DNAJB14-U3_REV DNAJB14-U3_REV 625_FO 1.8_REV 678_FN 1.8_REV 678_FN 1.8_REV 625_FO 4_REV 625_FO 4_REV 05PB5_REV USPBS_REV USPBS_REV USPBS_REV USPBS_REV DPrimer name Primer name	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested PCR reaction digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse s and probes used for Primer type forward reverse s and probe forward reverse Primer type forward reverse Primer type forward reverse Primer type forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward probe reverse forward probe	Shex/CTACSGGCTI/zen/ACTGGAAGGGCTATTTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCACGACGACTACATCAA GTGGCCACGACGCACAGTCAA Shex/ATTTGGTA/Zen/TTGGAAGGCCAAATC/3labk GTGGTAAAACCCACAGTCAAG Pimer sequence ACTTGAAAGTGAAAGTAGGACCAG CAGTTCGGGATTGGAAGGTGGGTTGC GACTCTGAAGGTGGAAGGTGGGTTGC CAGTTCGGGGATTGGAAGGTGGGTTGC CAGTTCGGGGTTGCAAGTGAAAGTAGAACCAG GTACTGAAGGTGGATGCAAGTAGAACCAG GTACTGAAGGTGGCATCACGGGC GTACTGAAGGTGGCATCACGGGC GTACTGAAGGTGGCATCACGGAC GTACTATAGGTTGCATTACATGTACTACTTAC GACCTCTCGACGCAGGAC GTACTATAGGTTGCATTACATGTACTGTAC	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188) Yuki et al.
through DNAJB14.d21 read through Assay 1.8Kb 4.0	ADK-U3_PRB ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV Primer name 625_FO 1.8_REV 678_FN 1.8_REV 678_FN 4_REV 678_FN 4_REV Primer name VSPBS_REV USPBS_REV Primer name d22_SDM_F d22_SDM_R d21_SDM_R d22_SDM_R d21_SDM_R d21_SDM_R d21_SDM_R d21_SDM_R d22_SDM_R	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested PCR reaction digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse s and probes used for Primer type forward reverse s and probe forward reverse Primer type forward reverse Primer type forward reverse Primer type forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward probe reverse forward probe	Shex/CTACSGGTT/zen/ACTGGAAGGGCTATTTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCAGAGTAAATTTCTATAC/3labk GTGGTAACCCACAGATCAA Shex/ATTTGGTA/Zen/TTGGAAGGGCAAATC/3labk GTGGTAAACCCACAGATCAAS sequencing (SGS) of spliced HIV transcripts Pimer sequence ACTTGAAAGTGAAGTGGAGGTGGCGGGGTTGC GACTTCGGGATTGGGAGGTGGCGGGGGTTGC CAGTTCGGGGATTGGGAGGGGGGTTGC CAGTTCGGGGATTGGGAGGGGGGTTGC CAGTTCGGGGTTGCGACGCAGGAC GTACTGAAGTGGAAGTGGAAGCTGGAACCAG GTACTGAAGTGGAAGTGGAACCAG GTACTTGAAGTGGAAGTGGAACCAG GTACTGAAGTGGAAGTGGAACCAG GTACTGAAGTGGAAGTGGAACCAG GTACTGAAGTGGAAGTGGAACCAG GTACTGGGCC/CCACAGGAC GTACTGGGCC/CCACAGGAC GTACTGGGCC/CCACAGGAC CCTGTTGGGCC/CCCGACAGGACTG/3labk AGTCCTGCGCC/CCGACAGGACTTG/3labk AGTCCTGGGCCCCACAGGGACGCTG/3labk AGTCCTGGGCCCCCCTC VI PNS the directed mutagenesis Pimer sequence CCTGTGGCGCCCACAGGGCCTGC TTTGGCTGGCGCCCCCTC VI RNA quantification from transfectid cells Pimer sequence GCCCTCCAGATGCGCGAGAGCTGG/3labk TTTTTTTTTTTTTTTTGGCTAGGGAC GGCCCTCGAGTGGCCCGACAGGAGAGAG CCCCTCCGGCCCGCCTC VI RNA Quantification for uncontactor Sfam/AGGTCTCCTCCTTGGCTAGATAA Sfam/ACGACTCTCC/CCAGAGGAGAGAG GCCCTCCCAGAGGCTGGGGGCCTCC Sibm/AGGTCTCC/CCAGAGGAGAGAGAGCCCGCCCCCGAGGACTCGG/3labk TTTTTTTTTTTTTTTTTGGCTAGGAGAGAGAGAGCCCGCCC	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188) Yuki et al.
through DNAJB14.d21 read through Assay 1.8Kb 4.0Kb 4.0Kb ML4-3_d22 NL4-3_d22 NL4-3_d21 Assay PolyA 4kb-class tat/rev	ADK-U3_PRB ADK-U3_PRB ADK-U3_FWD DNAJB14-U3_FWD DNAJB14-U3_FWB DNAJB14-U3_PRB DNAJB14-U3_REV 625_FO 18_REV 678_FN 625_FO 18_REV 678_FN 625_FO 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 7 7 8 7 8 7 8 7 8 7 8 7 8 7 8 7 8 7 8	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR semi_nested outer semi_nested outer semi_nested Primers and PCR reaction digital PCR digital PCR	probe reverse forward probe reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward probe sused for Primer type forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward probe reverse forward probe forward reverse forward probe forward probe reverse forward prob	Shex/CTACSGGTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGACCCCACAGATCAA GTGGCCAGAGTAAATTICTATTCAT Shex/ATTTGGTA/GCCCACAGATCAAG 9 sequencing (SGS) of spliced HIV transcripts Primer sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTCGGGATTGGGAAGTGGGGTTGC CAGTCGGGATTGGGAAGTGGGGTTGC CAGTCGGGATTGGGAAGTGGGGTTGC CAGTCGGGATTGGGAAGTGGGGTTGC CAGTCGGGATTGGGAAGTGGGATGC GAGCTCTCCGACGCAGGAC GTACTATAGGTGCATTACATGTACTACTAC GTACTATAGGTGCATTACATGTACTACTAC GTACTATAGGTGCATTACATGTACTACTAC 51mi/CAGTGGCG/2en/CCCAACAGGACTG/3labk AGTCCTGCCGCACAGGAC CTGTAGTGGCG/2en/CCCAACAGGACTG/3labk AGTCCTGCGCGCAGAGAT Us9BS quantification (Iate RT product) Primer sequence GTACGCCCAAAAATTITGACTACGTAC CTGTTGGGCG/2en/CCCGAACAGGACTG/3labk AGTCCTGCCGCCGCAGGACT US9BS te directed mutagenesis Primer sequence GTACGCCCAAAAATTITGACTACGGG TCTGGCCCCTGCCCCTC TTTGACTAGCGGAGGCGTGC US9CSCCCGCAAAGTAAAGCCAGGAGGAGG GCCCCCAAAATTTTGACTACGGG TCTGCGCCCCACAGGACTG/ US4BACGCGAAGTGCTCCTGGTACCT VI RNA quantification from transfected cells Primer sequence GCCCCCAAAGTGCTCCTGGTCACGGG GTGCATTACATGCTCCTGCTCTC VI RNA quantification from transfected cells CTTGACAGCGCCCTCCCTT UTAGCCTACCTCCTCTCCCAACAGGA Sfami/AGCCGAACAGCAAGGCAGGAG GGCCCCCAAAGTAAAGCCAGGG GGTCCAATTACATGCACGGGGCCGGGAGAG GGTCGCATTACATGCTCCTCGGTTGCGGGAGGAG GGTGCATTACCATGCACGGGAGCAGGA Sfami/AGCCGAACGCAGGCCGGAGAG GGTCGCAAGCCGCGCCGGGCCGG	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188) Yuki et al. (29491188)
through DNAJB14.d21 read through Assay 1.8Kb 4.0Kb A.0Kb A.0Kb US-PBS NL4-3_d22 NL4-3_d21 Assay PolyA 4kb-class tat/rev	ADK-U3_PRB ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV Primer name 625_FO 18_REV 678_FN 18_REV 678_FN 4_REV 678_FN 4_REV Primer name VSPBS_REV USPBS_REV USPBS_REV Primer name 722_SDM_F d22_SDM_R d21_SDM_R d32_SDM_R d3	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR semi_nested outer semi_nested outer semi_nested PCR reaction digital PCR digital PCR digital PCR digital PCR digital PCR R digital PCR digital PCR dig	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse s and probes used fo Primer type forward reverse s and probes used fo Primer type forward reverse s and probes used fo Primer type forward reverse s and probes s used for Market forward reverse forward reverse forward reverse forward probe forward probe reverse forward probe forward probe reverse forward probe forward forward forward forward forward forward fo	Shex/CTACSGGTT/zen/ACTGGAAGGGCTATTTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCAGAGTAAATTTCTATAC/3labk GTGGTAACCCACAGATCAA Shex/ATTTGGTA/Zen/TTGGAAGGGCAAATC/3labk GTGGTAAACCCACAGATCAAS sequencing (SGS) of spliced HIV transcripts Pimer sequence ACTTGAAAGTGAAGTGGAGGTGGCGGGGTTGC GACTTCGGGATTGGGAGGTGGCGGGGGTTGC CAGTTCGGGGATTGGGAGGGGGGTTGC CAGTTCGGGGATTGGGAGGGGGGTTGC CAGTTCGGGGTTGCGACGCAGGAC GTACTGAAGTGGAAGTGGAAGCTGGAACCAG GTACTGAAGTGGAAGTGGAACCAG GTACTTGAAGTGGAAGTGGAACCAG GTACTGAAGTGGAAGTGGAACCAG GTACTGAAGTGGAAGTGGAACCAG GTACTGAAGTGGAAGTGGAACCAG GTACTGGGCC/CCACAGGAC GTACTGGGCC/CCACAGGAC GTACTGGGCC/CCACAGGAC CCTGTTGGGCC/CCCGACAGGACTG/3labk AGTCCTGCGCC/CCGACAGGACTTG/3labk AGTCCTGGGCCCCACAGGGACGCTG/3labk AGTCCTGGGCCCCCCTC VI PNS the directed mutagenesis Pimer sequence CCTGTGGCGCCCACAGGGCCTGC TTTGGCTGGCGCCCCCTC VI RNA quantification from transfectid cells Pimer sequence GCCCTCCAGATGCGCGAGAGCTGG/3labk TTTTTTTTTTTTTTTTGGCTAGGGAC GGCCCTCGAGTGGCCCGACAGGAGAGAG CCCCTCCGGCCCGCCTC VI RNA Quantification for uncontactor Sfam/AGGTCTCCTCCTTGGCTAGATAA Sfam/ACGACTCTCC/CCAGAGGAGAGAG GCCCTCCCAGAGGCTGGGGGCCTCC Sibm/AGGTCTCC/CCAGAGGAGAGAGAGCCCGCCCCCGAGGACTCGG/3labk TTTTTTTTTTTTTTTTTGGCTAGGAGAGAGAGAGCCCGCCC	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188) Yuki et al. (29491188)
through DNA,JB14,d21 read through Assay 1.8Kb 4.0Kb 4.	ADK-U3_PRB ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_REV Primer name 625_FO 18_REV 678_FN 1.8_REV 678_FN 4_REV 678_FN 4_REV Primer name VSPBS_REV USPBS_REV Primer name 722_SDM F 622_SDM F 622_SDM R 622_SDM R 6	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR semi_nested outer semi_nested outer semi_nested PCR reaction digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse forward probe sused for terverse s and probes forward probe reverse forward probe forward probe forward reverse forward probe forward forward probe forward	Shex/CTACSGGTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCACAGATCAA GTGGCCAGAGTAAATTTCTATTCAT Shex/ATTTGGTA/SCACCCACAGTCAA GTGGCAGACCACACGATCAAG Sequencing (GSS) of spliced HIV transcripts Primer sequence ACTTGAAAGTGGAAGTGGAGGGGGTGC GAGCTCTCTCGACGCAGGAC CAGTTCGGGATTGGGAGGTGGGGTTGC CAGTTCGGGATTGGGAGGTGGGGTTGC CAGTTCGGGATTGGGAGGTGGGGTTGC CAGTTCGGGGATCGGAAGTGGAACCAG GTACTATAGGTTGCATTACATGTACTACTAC GACTCTCTCGACGCAGGAC GTACTATAGGTTGCATTACATGTACTACTAC GTGCTATAGGTTGCATTACATGTACTACTAC TAGGTGCGC/CARCCCGACAGGAC GTACTATAGGTTGCATTACATGTACTACTAC STUSPBS quantification (late RT product) Primer sequence CTGTTGGCGC/CARCCGGACAGGAC GTACGTGCGCC/CARCAGGGACTTG/3labk AGTCCTGCGCGCACAGGGACTTG/3labk AGTCCTGCGCCGCACAGGGACTTG/3labk AGTCCTGCGCCGCCCCTTC TTTGACTAGCGGAGGCTGGGAGGAGGAGGAGG CGCCTCAGATGCTGCCGCCTTC V1 RNA quantification from transfectid cells Primer sequence GCCCTCGACGAGGCTGCAGGAGAGG GGCCTCCGACGCGGCCCCAGGAGCTCG/3labk GTTGCGCCCAAAGTGATAAAGCTGCG CTTAGGCGCCAAAGTGCTGCAGGAGGAGG GGCCTCCGAAGGCTGCTGCTCCT CTTGGCTGCCCCCACCAGGACTCG/3labk GTGACGCCCAAAGTGCTGCATATAA Sfam/ACGCTGCTGCCCCCTC V1 RNA quantification from transfectid cells Primer sequence GCCTCCAGACGCGCCCCCGGAGGCTGG/3labk GTTGCATTACATGTACTACTACTGC CTTAGGCAACGTGCTCCTGGGGAGGA Sfam/ACGATGCTCCTGTGCCCCCCCCCCCCCCCCCCCCCCCCC	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188) Yuki et al. (29491188) reference
through DNAJB14.d21 read through Assay 1.8Kb 4.0Kb 4.0Kb ML4-3_d22 NL4-3_d22 NL4-3_d21 Assay PolyA 4kb-class tat/rev	ADK-U3_PRB ADK-U3_PRB DNA,B14-U3_REV DNA,B14-U3_PRB DNA,B14-U3_PRB DNA,B14-U3_REV 625_F0 18_REV 678_FN 18_REV 678_FN 18_REV 678_FN 4_REV Primer name USPBS_PRB USPBS_REV Primer name C22_SDM_F d22_SDM_F d22_SDM_F d22_SDM_F d22_SDM_F d22_SDM_F d22_SDM_F d21_SM_F d21_SM_F d21_SM_F d21_SM_F d21_SM_F d21_SM_F d21_SM_F d21_SM_F d21_SM_F d21_SM_F d21_SM_F d21_SM_F	digital PCR digital PCR digital PCR digital PCR digital PCR Primers used for PCR reaction outer semi_nested 0 PCR reaction PCR reaction digital PCR digital PCR digital PCR digital PCR QS PCR QS PCR QS PCR QS PCR QS PCR QS PCR QS PCR digital PCR	probe reverse forward probe reverse forward Primer type forward reverse forward reverse forward reverse forward probe sused for Primer type forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse forward probe reverse forward probe forward fo	Shex/CTACSGGCTIZen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCAACAGTAAATTGTATAC/3labk GTGGTGAACCCAACAGTACAT Shex/ATTTGGTA/Zen/TTCATTGGAAGGGCTAATTC/3labk GTGGTAAACCCAACAGTCAAG Pimer sequence ACTTGAAAGTGAAAGTAGAACCAG CAGTTCGGGGTTGGAAGGTGGGTGC CAGTTCGGGATTGGAAGGTGGGTGC CAGTTCGGGGTTGGAAGGTGGGTGC CAGTTCGGGGTTGGAAGGTGGGTGC CAGTTCGGGGTGGCAACTGGAAGTGGAACCAG GTACTATAGGTTGCATTACATGTACCATCA GTGCTATAGGTTGCATACATGTACTACTTAC GAGCTCTCTGAACGCAGGAC GTACTATAGGTTGCATTACATGTACTACTTAC GAGCTCTCTGGACGCAGGAC GTACTATAGGTTGCATTACATGTACTGTAC	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188) Yuki et al. (29491188) reference
through DNAJB14.d21 read through Assay 1.8Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0KJ 4.0Kb 4.0	ADK-U3_PRB ADK-U3_PRB ADK-U3_REV DNAJB14-U3_REV DNAJB14-U3_PRB DNAJB14-U3_PRB DNAJB14-U3_REV 625_F0 1.8_REV 678_FN 1.8_REV 678_FN 1.8_REV 678_FN 4_REV 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 7678_FN 4_REV 105PBS_PRB USPBS_REV 105PBS_REV 10	digital PCR digital PCR digital PCR digital PCR digital PCR semi_nested outer semi_nested Primers and prob PCR reaction digital PCR digital PCR digita	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse forward probe sead for HI Primer type forward reverse forward reverse forward reverse forward reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe forward probe reverse forward probe forward forward probe forward forward probe forward	Shex/CTACSGGCTIZen/ACTGGAAGGGCTATTTAC/3labk GTGGTGAACCCAACAGTAAATTCTATAC/3labk GTGGTGAACCCAACAGTAAATTCTATACA Shex/ATTTGGTA/Zen/TTGGAAGGGCAAATGC/3labk GTGGTAAACCCAACAGTCAAG Pimer sequence ACTTGAAAGTGAAAGTAGAAGCAG CAGTTCGGGATTGGAAGGTGGGTGC CAGTTCGGGATTGGAAGGTGGGTGC CAGTTCGGGATTGGAAGGTGGGTGC CAGTTCGGGATTGGAAGGTGGGTGC CAGTTCGGGATTGGAAGGTGGGTGC CAGTTCGGGATTGGAAGGTGGGTGC CAGTTCGGGATGGAAGGTGGATCC GTGCTGAAGGTGGAAGTGGAACCAG GTACTATAGGTTGCATTACATGTACTACTTAC GAGCTCTCTGAACGCAGGAC GTACTATAGGTTGCATTACATGTACTACTTAC SPUSPBS quanification (alte RT product) Pimer sequence CTGTTGGCGC/zen/CCGAACAGGGACTTG/3labk AGTCCTGGCGCGCGCTC 5fam/CAGTGGCGC/zen/CCGAACAGGGACTTG/3labk AGTCCTGGCGTGGAGGCGCGCTT TTGGCGCGCGCCCCCCGCGCTT VJ RNA quantification from transfected cells Pimer sequence GTCGCCCAAAGTGTACATGACAGGA GCCCCCCCAGATGCTRACTACTAC Sfam/CAGTCCTC/Zen/GAGCAGGAGAGG GCCCCCCAGATGCTRACATAA Sfam/CAGTCCTCTCTCTCGGGTCCTCCGTTAG/3labk GTGCGCAAGGCACAAGTACAGGACAGGGGGAGA Sfam/AGTACTCC/Zen/GACCAGGGCTGG/3labk GTGCCCCAAGTGCTRACATAA Sfam/AGTACTCCCCACAGGGACTCGG/3labk GTGCCCTGAAGTGAAGTAAGCTGGGCCC CTTAGCCCTGAAGTACATACAGCAGGAGAGG Sfam/AGTACTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188) Yuki et al. (29491188) reference
through DNA,JB14,d21 read through Assay 1.8Kb 4.0Kb 4.	ADK-U3_PRB ADK-U3_PRB ADK-U3_REV DNAJB14-U3_REV DNAJB14-U3_PRB DNAJB14-U3_PRB DNAJB14-U3_PRB AREV 625_FO 18_REV 678_FN 625_FO 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 4_REV 678_FN 622_SDM_F 622_SDM_F 622_SDM_F 622_SDM_F 622_SDM_R 62	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR semi_nested outer semi_nested PCR reaction digital PCR digital PCR	probe reverse single genome Primer type forward reverse forward reverse forward reverse forward reverse forward reverse forward reverse s and probes I Primer type forward probe reverse s and probes I Primer type forward probe reverse forward probe forw	Shex/CTACSGGCT/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCAACAGTCAA GTGGCCAGAGTAAATTTCTATTCAT Shex/ATTTGGTA/SGCCAAGACTAAG GTGGTA/SCCCAACAGTCAAG Shex/ATTGGA/ZGCAAGCCAAG CAGTGA/Zen/TGGCA/GAGGGTGC ACTGAAAGTGAAAGTGAAACCAG CAGTCGGAATGGCAAGGGGGTGC GAGCTCTCCGACGCAGGGTGC CAGTCGGGATGGCAAGGGGGTGC CAGTCGGGATGGCAGGGGTGC GAGCTCTCCGACGCAGGGC GTACTATAGGTGCATTACATGTACTACTAC GTGCTATAGGTGCATTACATGTACTACTAC GTACTGCAGTGGCAC/2en/ GTACTGCAGCAGGGCG GTACTATAGGTGCATTACATGTACTACTAC GTACTGCGC/2en/ CGTGTGCGC/2en/ GTACGCCGCAAAGTGAAACCAG GTACTGCGC/2en/ GTACGCCGCAAAGTGAAACCAG GTACTGCGC/2en/ GTACGCCGCAAAGTGAACCAG GTACTGCGC/2en/ GTACGCCGCAAAGTGAACCAG GTACGCCGCAAAGTGAACCAG GTACGCCGCAAAGTGAACCAG GTACGCCGCAAAGTGAACCAG GTACGCCGCAAAGTGAACCAG GTACGCCGCAAAGTGAACCAG GTACGCCCAAAATTTTGACTACCGG GTACGCCCAAAATTTTGACTACGCG GTACGCCCAAAATTTTGACTACGCG TCTGCCCCCCCCCC	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188) Yuki et al. (29491188) reference
through DNAJB14.d21 read Hrough Assay 1.8Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0KJ 4.0Kb 4.0K	ADK-U3_PRB ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_PRB DNAJB14-U3_PRB DNAJB14-U3_PRB 4 8 2 2 2 5 7 8 7 7 8 7 7 8 7 7 8 7 7 8 7 7 7 8 7 7 7 7 7 8 7 7 7 7 7 7 7 7 7 7 7 7 7	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR semi_nested outer semi_nested PCR reaction digital PCR digital PCR	probe reverse forward probe reverse single genome Primer type forward reverse forward reverse forward reverse forward probe sead for HI Primer type forward reverse forward reverse forward reverse forward reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe reverse forward probe forward probe reverse forward probe forward forward probe forward forward probe forward	Shex/CTACSGGCTI/zen/ACTGGAAGGGCTAATTTAC/3labk GTGGTGAACCCAACAGTAAATTCTATAC/3labk GTGGTGAACCCAACAGTCAA Shex/ATTTGGTA/zen/TTCATTGGAAGGGCTAATTC/3labk GTGGTAAACCCAACAGTCAAG Pimer sequence ACTTGAAAGTGAAAGTAGGACGAGG CAGTTCGGGATTGGAAGGTGGGTTGC GACTCTGAAGGTGAAAGTAGAACCAG CAGTTCGGGATTGGAAGGTGGGTTGC GACTCTCGACGCAGGAC CAGTTCGGGATTGGAAGGTGGGTTGC GACTCTCGACGCAGGAC GTACTATAGGTTGCATTACATGTACTACTTAC GACTCTCTGAACGTGGAACCAG GTACTATAGGTTGCATTACATGTACTACTTAC GACTCTCTGACGCAGGAC GTACTATAGGTTGCATTACATGTACTGTAC	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188) Yuki et al. (29491188) reference
through DNAJB14.d21 read Hrough Assay 1.8Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0Kb 4.0KJ 4.0Kb 4.0K	ADK-U3_PRB ADK-U3_PRB ADK-U3_REV DNAJB14-U3_FWD DNAJB14-U3_PRB DNAJB14-U3_PRB DNAJB14-U3_REV 625_FO 1.8_REV 678_FN 1.8_REV 678_FN 1.8_REV 678_FN 4_REV 778_FN 4_REV Primer name USPBS_REV USPBS_REV Primer name C2_SDM_F d22_SDM_F d22_SDM_R d22_SDM_R d22_SDM_R d22_SDM_R d22_SDM_R d21_SMR_R d21_SMR_R	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR semi_nested outer semi_nested PCR reaction digital PCR digital PCR	probe reverse forward probe reverse forward Primer type forward reverse forward reverse forward reverse s and probes used fo Primer type forward reverse s and probes s mortune Primer type forward reverse s and probes s and probes s and probes s and probes forward reverse forward probe	Shex/CTACSGGCTI/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCAACAGTAAATTCTATAC/3labk GTGGTGAACCCAACAGTCAA Shex/ATTTGGTA/zen/TTCATTGGAAGGGCTAATTC/3labk GTGGTAAACCCAACAGTCAAG Pimer sequence ACTTGAAAGTGAAAGTAGAACCAG ACTTCAGAGGGATGGGAGGGGGTGC GACTCCGGGATTGGGAGGGGGGTGC GACTCCGGGATTGGGAGGGGGGTGC GACTCTCGACGCAGGAC CAGTTCGGGATTGGGAGGGGGGTGC GACTCTCGACGCAGGAC GTACTATAGGTTGCATTACATGTACTACTAC GTGCTAAAGTGAAAGTAGAACCAG GTACTATAGGTTGCATTACATGTACTACTTAC GACCTCTCGACGCAGGAC GTACTTGAAGTGGAAGTGGGAC GTACTATAGGTTGCATTACATGTACTACTTAC GACCTCTCGACGCAGGAC GTACTATAGGTTGCATTACATGTACTACTTAC JPImer sequence CTGTTGGCGC/ENCCCGAACAGGACGACTTG/3labk AGTCCTGGCGC/ENCCCGAACAGGACGACTG/3labk AGTCCTGGCGCCAACAGGACGACTTG/3labk AGTCCTGGCGCCACAGGACGCTTG/3labk AGTCCTGGCGCCACAGGACGACTGG/3labk AGTCCTGGCGCCACAGGACGACTGG/3labk AGTCCTGGCGCCACAGGACGACTGG/3labk AGTCCTGGCGCCACAGGACGACGACG CCGCCCCCCGCCTCCTC V-I RNA quantification Pimer sequence GTACGCCCCCAGAGGACTAGAGGAGAGG CGCCCCCCGCAGGACTTGG7Jabk AGTCCTGCCTGGGCCCTCCTC V-I RNA quantification Pimer sequence GTACGCCCCCAGAGGACTGGG7Jabk GTTGCTTCCCTGGGTCCTCGGTTAGG3labk TTTTTTTTTTTTTTTTGACTAGGGAGGAGGAG GGACCTGCTCCTATGGCAGGACTCGG7Jabk GGATCGTCCTCATGAGCGAGGACTCGG7Jabk GTTGCACTGCCTGGAGGACTCGG7Jabk GGATCGTGCTCCTGTTGCCTGGCTGCCTGGJabk GGATCGTGCTCCTGTTGCCTGGGAGGCTGG7Jabk GGATCGTGCCGAGAGGCCAGGACTCGCG3labk GGATCGTGCTCCTGTTGCCTGCCTGGJabk GGATCGTGCCGAGAGGCTAATTACCTGC CTTAGGCCGAGAGGCTAATTGGCAGGA Sfam/AGACTGCCCACAGGCCAGAGCCCGCGJabk GGATCGTGCTAGTGCCGCCCGCCCTGGJabk GGATCGTGCTAGTGCCGCCGCGCTAGTTGCJabk GGATCGTGCAGAGGCCAAAGCTCGGCTAATTACJ3labk GTGGTAACCGCACAGGCCTAATTACJ3labk GTGGTAACCGCACAGGCCTAATTACJ3labk GTGGTAACCGCACAGGCCTAATTACJ3labk GTGGTAACCGCACAGGCCTAATTACJ3labk GTGGTAACCGCACAGGCTAATTTCCJ3labk GTGGTGAACCCACAGGCCTAATTACJ3labk GTGGCGAAGCTCGCCTGGCAGGCTAATTGCJ3labk GTGGTAACCCACAGGCCTAATGCCACAG Shex/CTACGGCCTGGCTGGCTGGCTGGCTGGAGGGCTAATTGCJ3labk GTGGTGAACCCACAGGCTAATTGCJ3labk GTGGTGAACCCCACAGGCTAATTGCJ3labk	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188) Yuki et al. (29491188) reference
through DNAJB14.d21 read through Assay 1.8Kb 4.0	ADK-U3_PRB ADK-U3_PRB ADK-U3_REV DNAJB14-U3_REV DNAJB14-U3_PRB DNAJB14-U3_PRB DNAJB14-U3_PRB DNAJB14-U3_PRB 4_REV 678_FN 625_FO 4_REV 678_FN 622_SDM F 622_SDM F 622_SDM R 622_SDM R	digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR digital PCR outer semi_nested outer semi_nested PCR reaction digital PCR digital PCR digit	probe reverse single genome Primer type forward reverse forward reverse forward reverse forward reverse s and probes i Primer type forward reverse s and probes i Primer type forward reverse s and probes i Primer type forward probe reverse s and probes i Primer type forward probe reverse forward probe forward forward probe forward	Shex/CTACSGGCT/zen/ACTGGAAGGGCTAATTAC/3labk GTGGTGAACCCAACAGTCAA GTGGCCAGAGTAAATTTCTATTCAT Shex/ATTTGGTA/SGCCAAGACTAAG GTGGTA/SCCCAACAGTCAAG Prime sequence Prime sequence ACTTGAAAGTGAAAGTGGAAGCAG CAGTTCGGAATTGGCAGGGGGTGC GAGCTCTCTGACGCAGGGTGC GAGCTCTCTGACGCAGGGTGC GAGCTCTCTGACGCAGGGTGC GAGCTCTCTGACGCAGGGTGC GAGCTCTCTGACGCAGGGTGC GAGCTCTCTGACGCAGGGTGC GTGCTATAGGTGCATTACATGTACTACTAC GTGCTATAGGTGCATTACATGTACTACTAC GTGCTATAGGTGCATTACATGTACTGTAC	Adapted from Emery et al. (28077653) reference reference Yuki et al. (29491188) Yuki et al. (29491188) reference Anderson et al

Supplementary Table S5. Oligos used in this study. Numbers in parenthesis represent reference PMID.