**Supplementary information**

**Supplementary Table 1. Latency promoting agents: overview of indication, clinical trial status and potential effect on HIV transcription and/or reactivation.**

| Compound | Target | Indication and usage (status) | Clinical trials | Effect on HIV transcription and/or reactivation | Refs |
|---|---|---|---|---|---|
| ruxolitinib | Jak/STAT | Rheumatoid arthritis (RA) and myelofibrosis (FDA approved) | Phase II to study inflammation and immune activation in people with HIV (PWH) on ART | Downregulation of immunomodulatory cytokines (IL6, IL2, TNFalpha) that contribute to chronic inflammation, chronic infection, and HIV disease progression | (1–3) |
| aspirin | NF-κB inhibitor | Non-steroidal anti-inflammatory drug (FDA approved) | Phase I to reduce cardiovascular disease in ART-treated PWH; tested in combination with HCQ as HIV prevention strategy | Inhibition of NF-κB-dependent HIV transcription | (4) |
| mesalamine | NF-κB | Inflammatory bowel disease (FDA approved) | Phase I/II to reduce immune activation and inflammation in ART-treated PWH | Inhibition of NF-κB-dependent HIV transcription | |
| spironolactone | TFIIH inhibitor | Potassium-sparing diuretic for hypertension and cardiovascular disease (FDA approved) | Phase III trial for its anti-inflammatory properties in RA | Inhibition of HIV transcriptional initiation by proteasomal degradation of the XPB subunit of TFIIH | (5, 6) |
| resveratrol | SIRT-1 activator | Supplement with antioxidant, anti-inflammatory, immunomodulator, lipid regulatory, | Ongoing trials for treatment of several cancers; for its neuroprotective | Inhibition of Tat-dependent HIV transcription via SIRT1 activation | (7) |

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| | | and neuroprotective effects | effects and for the treatment of diabetes type 2 | | |
| triptolide | Inhibitor of NF-κB, RNA Polymerase (RNA P), and Tat | Used in Chinese traditional medicine for immunosuppressive (RA), anti-inflammatory and anti-cancer properties | Phase I/II/III to reduce immune activation, inflammation, and the HIV reservoir in PWH; for small cell lung cancer; polycystic kidney disease; and Crohn's disease | Proteasomal degradation of Tat; reduction of enhancer mediated transcription by reducing H3K27ac and BRD4 occupancy; Inhibition of NF-κB and NFAT signaling; Inhibitor of RNAP I, II, III | (8–12) |
| digoxin | vRNA processing inhibitor | Cardiac glycoside used to treat heart failure and atrial fibrillation (FDA approved) | Several trials for various cancers | Inhibition of HIV protein synthesis by inducing oversplicing of the HIV mRNA | (13, 14) |
| 8-azaguanine | vRNA processing inhibitor | Purine analogue | - | Induces oversplicing of HIV mRNA and inhibits Rev function | (15) |
| alvocidib | CDK1,2,4,9,6 inhibitor | Orphan status for B-CLL, AML | Phase I completed for advanced solid tumors with TP-1287, a prodrug of alvocidib | Inhibition of CDK-induced RNAPII phosphorylation | (16) |
| dinaciclib | CDK2,5,1,9 inhibitor | - | Phase I/II for solid tumors and melanoma | Inhibition of CDK-induced RNAPII phosphorylation | (16) |
| AZD4573 | Selective CDK9 inhibitor | - | Phase II for T-cell lymphoma, classical Hodgkin’s lymphoma and hematologic malignancies | Inhibition of CDK-induced RNAPII phosphorylation | (16) |
| atuveciclib | PTEF-b/CDK9 inhibitor | - | Phase I for hematologic malignancies (completed) | | (16) |
| staurosporine | PKC a,g,n,d,e inhibitor | - | Phase I/II for 7-hydroxy-staurosorine and midostaurin to | Proapoptotic effects triggered by inhibition of PKC- | (17) |

| | | | | | |
|---|---|---|---|---|---|
| | | | treat various cancers | induced gene expression | |
| sotrastaurin | PKC pan-inhibitor | - | Phase II for B-cell lymphoma and uveal melanoma; Phase II for kidney transplant rejection | Inhibition of PKC-induced gene expression inhibits T cell activation | (17) |
| enzastaurin | PKC b inhibitor | Glioblastoma (FDA orphan status) | Phase I/II/III for various cancers; Phase I for central nervous system malignancies | Inhibition of NF-κB-mediated transcription induces antiproliferative and proapoptotic effects | (17) |
| quercetin | SIRT1 activator and PI3K inhibitor | Flavonoid known for its antioxidant, antiviral, antimicrobial, and anti-inflammatory, and neuroprotective effects (FDA GRAS status as dietary supplement) | Ongoing trials for its use in inflammatory, cardiovascular, metabolic diseases and aging | SIRT-1 deacetylation reduces Tat activity and downregulates induction factors of T cells: NF-κB, activator protein 1 (AP-1) and IL-2; Inhibition of PI3K/Akt pathway interferes with latency reactivation | (18–21) |
| SPV-106 | HAT inhibitor | - | - | Inhibition of p300-HAT reduces Tat transactivation | (22) |
| cordycepin | polyA inhibitor | Used in Chinese traditional medicine for its potent anti-cancer, antioxidant and anti-inflammatory effects | Phase I/II for leukemia, solid tumors, and lymphoma | Adenosine analog that functions as chain terminator for mRNA polyadenylation by poly(A) polymerases | (23) |
| pladienolide B | splicing inhibitor | - | Phase I with synthetic analogues (H3B-8800; E7107) for myelodysplastic syndromes and solid tumors | Inhibits the function of splicing factors SAP130, SF3b1, and U2 snRNP | (24, 25) |
| isoginkgetin | splicing inhibitor | - | - | Pre-mRNA splicing inhibition by | (26) |

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|---|---|---|---|---|---|
| | | | | preventing stable recruitment of the U4/U5/U6 tri-small nuclear ribonucleoprotein | |
| herboxidiene | splicing inhibitor | - | - | Inhibits the function of splicing factor SF3b1 subunit SAP145 | (27) |
| KRIBB11 | HSF1/PTEF-B | - | - | Inhibits HSF-1 from recruiting P-TEFb to HSP promotors | (28) |
| tanespimycin | HSP90 inhibitor | - | Phase II for relapsed lymphomas, solid tumors, and leukemia | HSP90 localizes to the HIV LTR and upregulates NF-κB, NFAT and STAT5 induced gene expression | (29–32) |
| mithramycin A | SP-1 inhibitor | - | In vitro studies for cervical cancer | Inhibits the function of Sp1 that drives viral gene expression | (33, 34) |
| C26 | NR2F1 agonist | - | In vitro and mice studies to induce dormancy in cancer cell lines | NR2F1 recruitment to HIV promotor activates downstream target genes SOX9, RARβ, and p27 that induce global chromatin repression | (35, 36) |
| 5342191 | MAPK/ERK modulator | - | - | Activation of MEK1/2-ERK1/2 signalling reduces HIV expression by interfering with RNA splicing | (37, 38) |

**Abbreviations: PWH, People with HIV; RA, Rheumatoid arthritis; RNA P, RNA polymerase; HQC, Hydroxychloroquine; B-CLL, B cell chronic lymphocytic leukemia; AML, acute myeloid leukemia.**

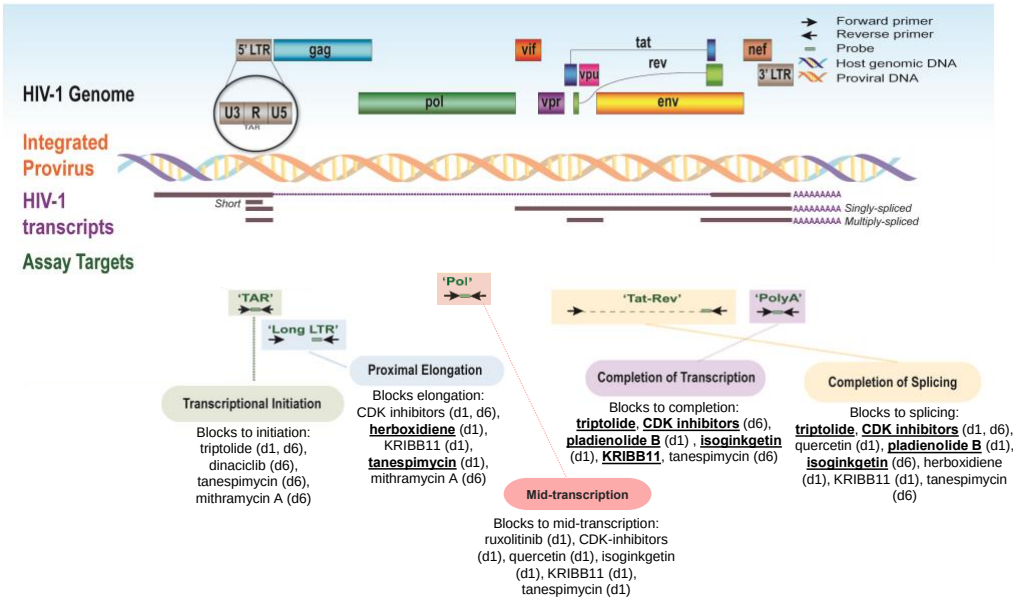



**Figure S1. LPAs acting through different mechanisms to induce blocks at various stages of HIV transcription.** This schematic shows the genetic organization of proviral HIV DNA and the HIV 'transcription profiling' assays targeting specific RNA sequence regions that provide insight into blocks to HIV transcription. Promising LPAs that induce specific blocks at different steps of transcriptional reactivation are listed. d1 indicates LPAs that significantly reduced the respective HIV transcript relative to activated DMSO at day 1 (24h), while d6 indicates LPAs that significantly reduced the HIV transcript relative to activated DMSO on day 6. Drugs that significantly altered the ratios at day 1 and/or day 6 are shown in bold and are underlined. Figure adapted from (39).

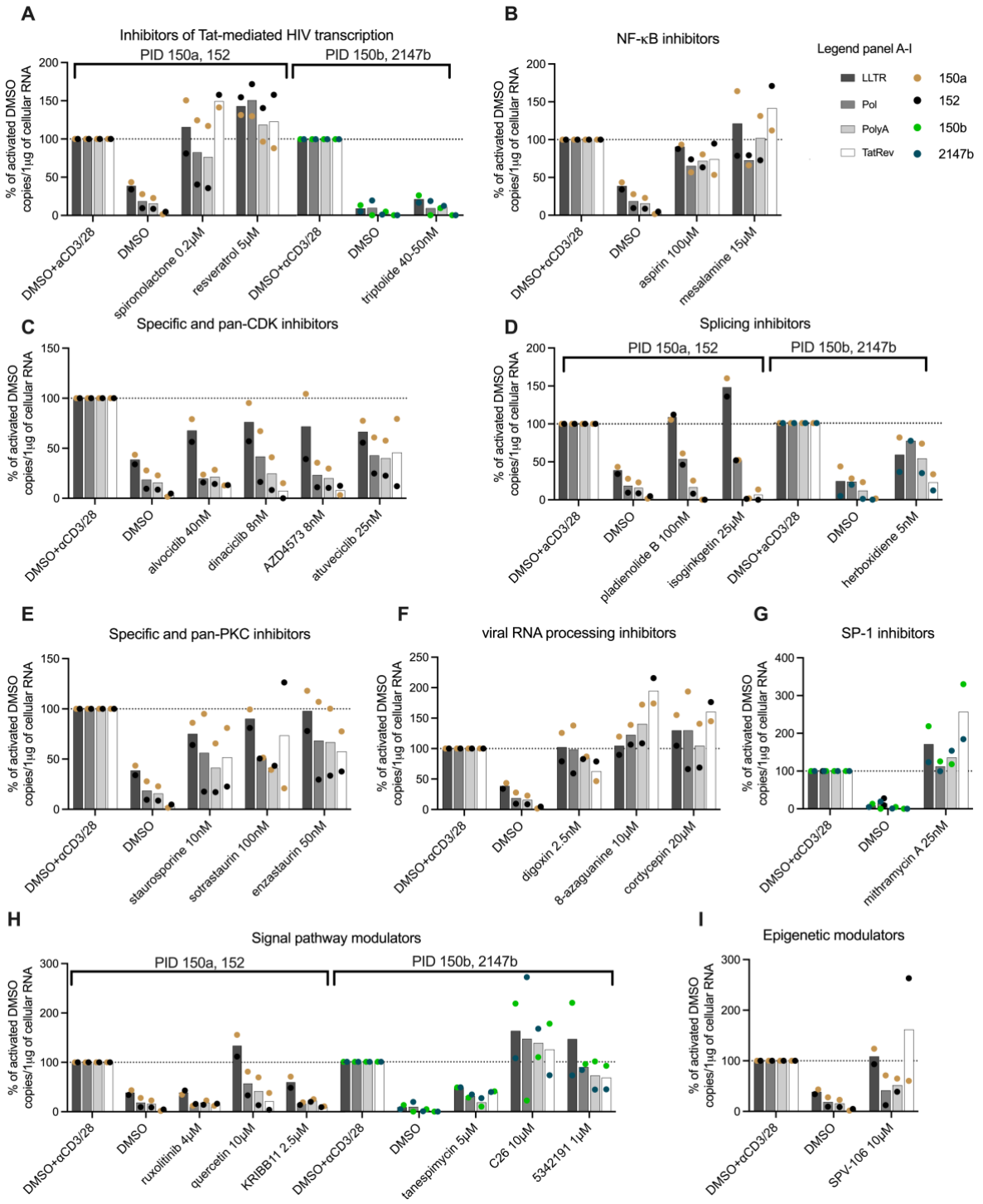



**Figure S2. Screening of candidate LPAs by measuring changes in 5' elongated, mid-transcribed, completed, and multiple spliced HIV transcripts after activation. (A-I)**

Each drug was tested in PBMCs from two ART-suppressed study participants and the PBMCs were aliquoted into wells at $6x10^6$ cells/well. After activation, the cells were cultured with antiretrovirals and individual drugs or controls (DMSO and activated DMSO). All drugs

were tested in the presence of CD3/28 T cell activating beads, except for the unactivated 'DMSO' condition. Total cellular RNA was extracted after 24h and 5' elongated (LLTR), Pol (unspliced, mid-transcribed), completed (PolyA), and multiply spliced (TatRev) HIV transcripts were quantified by RT-ddPCR. The levels of each transcript were quantified, normalized to 1 µg of total cellular RNA, and expressed as a percent of the activated DMSO control (% of activated DMSO). Medians are shown, as well as the values of the individual study participants in different colors.

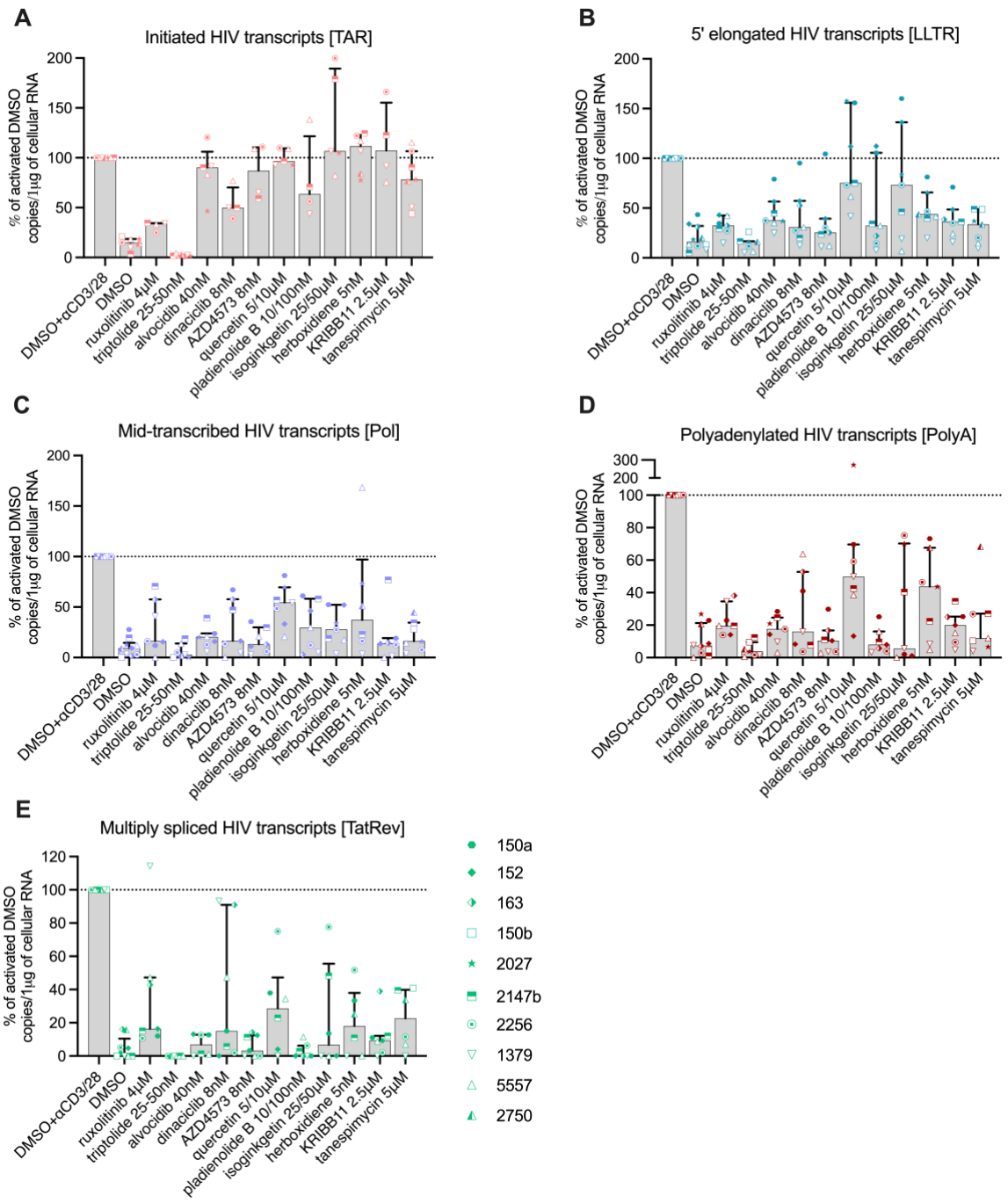



**Figure S3. Various LPAs impact distinct phases of HIV transcription upon activation.**

**A-E)** Each drug was tested in PBMCs from seven ART-suppressed study participants and the PBMCs were aliquoted into wells at $6x10^6$ cells/well. After activation, the cells were cultured with antiretrovirals in the presence of individual drugs in DMSO or DMSO alone as control. After 24h, total RNA was extracted and the progression through different stages of HIV transcription was quantified by measuring the levels of **A)** initiated (TAR), **B)** 5' elongated

(LLTR), **C)** mid-transcribed/unspliced (Pol), **D)** completed (PolyA) and **E)** multiply spliced (TatRev) HIV transcripts. The levels of all HIV transcripts were quantified, normalized to 1 µg of total cellular RNA, and expressed as a percent of the activated DMSO control (% of activated DMSO). Medians with IQR are shown, and varying symbol shapes denote individual study participants.

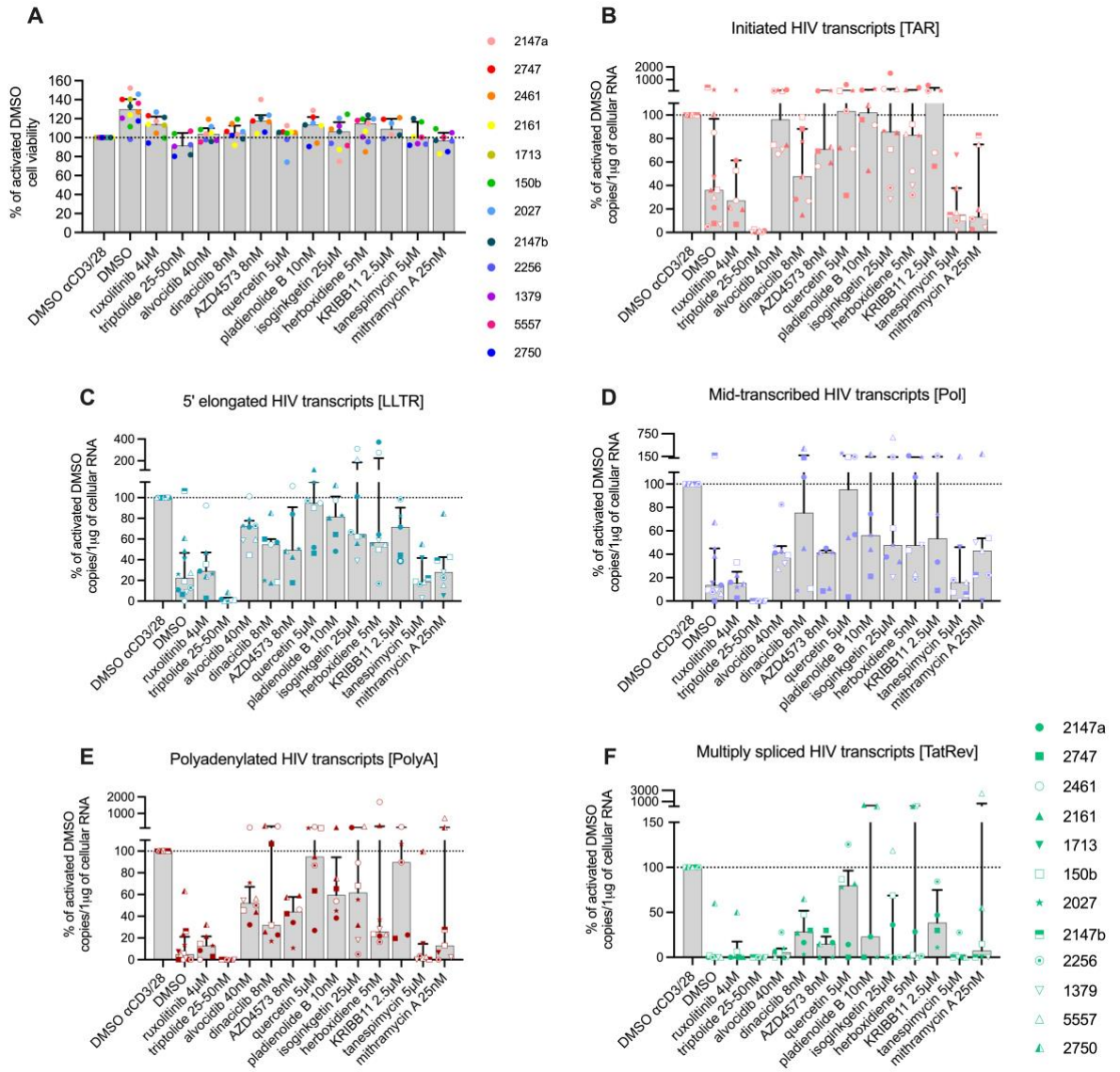
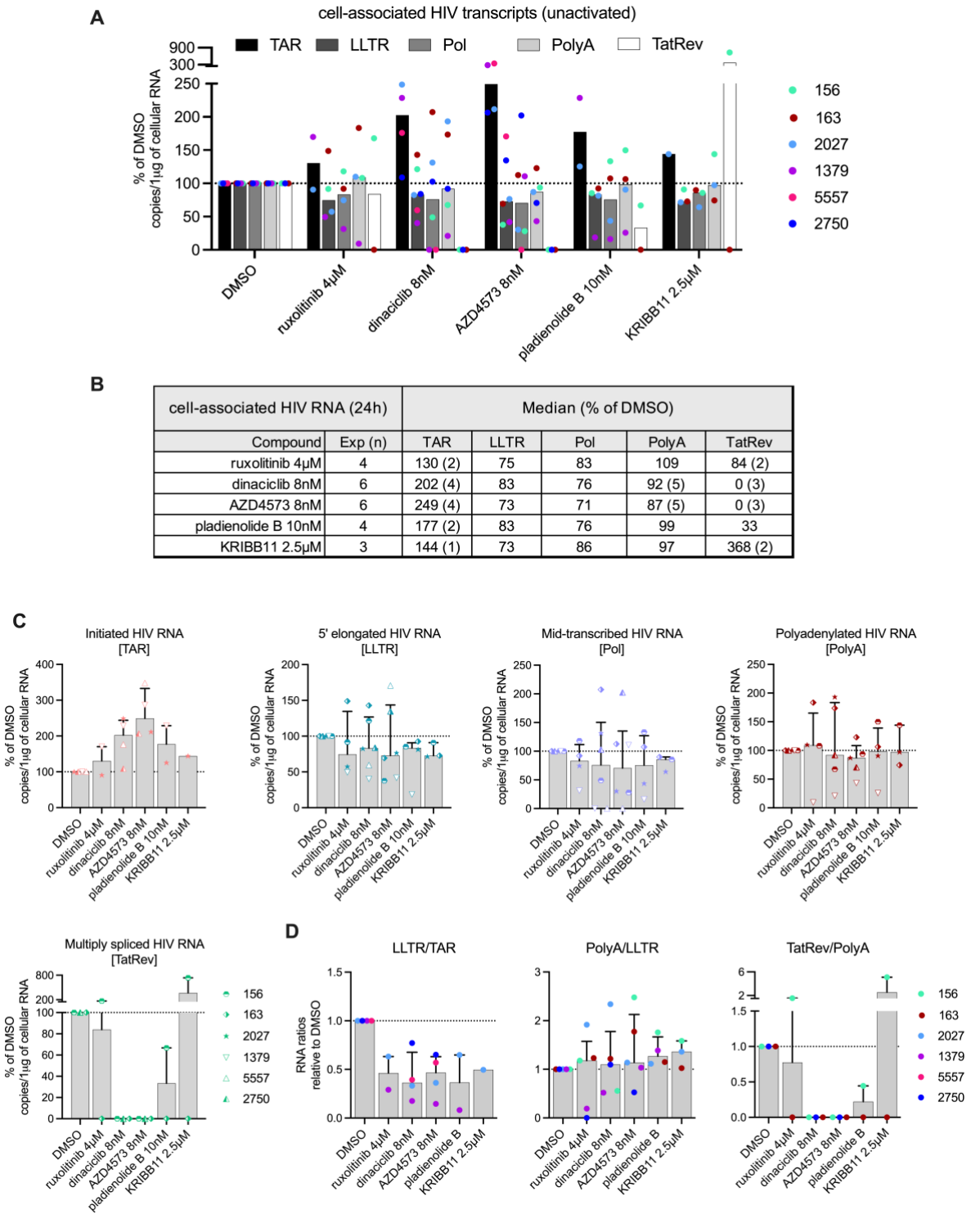



**Figure S4. Some LPAs sustain reduced HIV transcription for six days depending on the study participant but without effect on cellular viability. A)** After six days, the viability was measured by trypan blue staining and then normalized to the levels of the activated DMSO (% of activated DMSO). Bars indicate medians and different colors indicate individual study participants. **B-F)** Each drug was tested in PBMCs from at least six ART-suppressed study participants (except for KRIBB11, n=5) and the PBMCs were aliquoted into wells at $6x10^6$ cells/well. After activation, the cells were cultured with antiretrovirals in the presence of individual drugs in DMSO or DMSO alone as control. After six days, total RNA was extracted and the progression through different stages of HIV transcription was

quantified by measuring the levels of **B)** initiated (TAR), **C)** 5' elongated (LLTR), **D)** mid-transcribed/unspliced (Pol), **E)** completed (PolyA) and **F)** multiply spliced (TatRev) HIV transcripts. The levels of all HIV transcripts were quantified, normalized to 1 µg of total cellular RNA, and expressed as a percent of the activated DMSO control (% of activated DMSO). Medians with IQR are shown, and varying symbol shapes denote individual study participants.




| cell-associated HIV RNA (24h) | | Median (% of DMSO) | | | | |
|---|---|---|---|---|---|---|
| Compound | Exp (n) | TAR | LLTR | Pol | PolyA | TatRev |
| ruxolitinib 4µM | 4 | 130 (2) | 75 | 83 | 109 | 84 (2) |
| dinaciclib 8nM | 6 | 202 (4) | 83 | 76 | 92 (5) | 0 (3) |
| AZD4573 8nM | 6 | 249 (4) | 73 | 71 | 87 (5) | 0 (3) |
| pladienolide B 10nM | 4 | 177 (2) | 83 | 76 | 99 | 33 |
| KRIBB11 2.5µM | 3 | 144 (1) | 73 | 86 | 97 | 368 (2) |

**Figure S5. CDK-inhibitors appear to decrease baseline HIV splicing even in the absence of T cell activation.** Each drug was tested in PBMCs from at least three ART-suppressed study participants and PBMCs were aliquoted into wells at $6x10^6$ cells/well. The cells were cultured with antiretrovirals but without activation in the presence of individual drugs in

DMSO or DMSO alone as control. After 24h, total RNA was extracted and the progression through different stages of HIV transcription was quantified by measuring the levels of initiated (TAR), 5' elongated (LLTR), mid-transcribed/unspliced (Pol), completed (PolyA) and multiply spliced (TatRev) HIV transcripts. **A**-**B)** The levels of all HIV transcripts were quantified, normalized to 1 µg of total cellular RNA, and expressed as a percent of the DMSO control (% of DMSO). **A)** Medians are shown, as well as the values of the individual study participants in different colors. **B)** The medians from panel A) are presented. The effect of each drug on the different transcripts was studied in three to six study participants, as indicated in the second column, unless specified otherwise in parentheses. **C)** The levels of initiated (TAR), 5' elongated (LLTR), mid-transcribed/unspliced (Pol), completed (PolyA) and multiply spliced (TatRev) HIV transcripts relative to the DMSO control (% of DMSO). Medians with IQR are shown, with varying symbol shapes indicating individual study participants. **D)** The effect of each drug on HIV transcriptional progression was analyzed by the ratio of one HIV transcript to another. Ratios are independent of HIV infection frequency or normalization to cell numbers. Shown are the proportion of i) all HIV transcripts that were elongated [LLTR/TAR]; ii) elongated HIV transcripts that were completed [PolyA/LLTR]; and iii) completed transcripts that were multiply spliced [TatRev/PolyA]. Medians and IQR are presented, as well as the individual values per study participant in different colors.

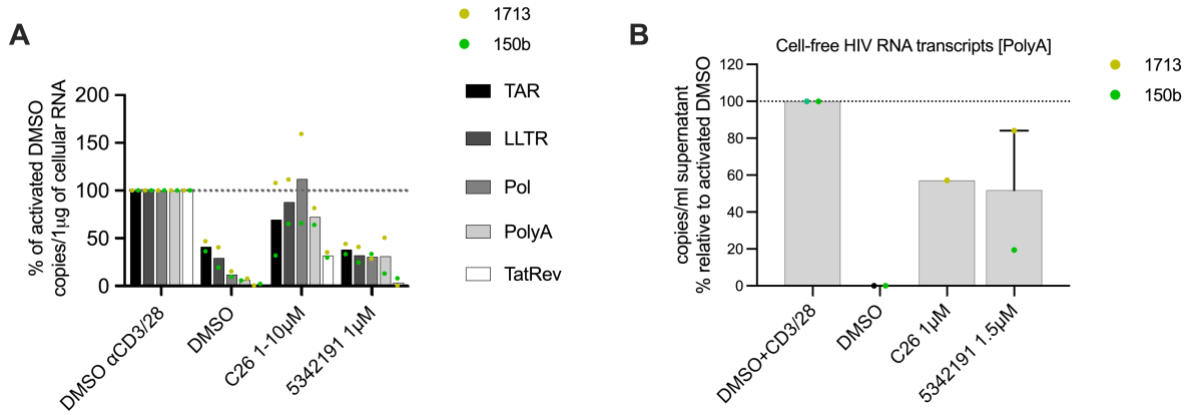



**Figure S6. The effects of 5342191 and C26 on HIV transcriptional reactivation and viral RNA supernatant release at six days.** PBMCs were isolated from ART-suppressed study participants and aliquoted into wells at $6x10^6$ cells/well. After activation, the cells were cultured with antiretrovirals in the presence of individual drugs in DMSO or DMSO alone as control. **A)** After six days, total RNA was extracted and the progression through different stages of HIV transcription was quantified by measuring the levels of initiated (TAR), 5' elongated (LLTR), mid-transcribed/unspliced (Pol), completed (PolyA) and multiply spliced (TatRev) HIV transcripts. The levels of all HIV transcripts were quantified, normalized to 1 µg of total cellular RNA, and expressed as a percent of the activated DMSO control (% of activated DMSO). Medians with IQR are shown, and different colours denote individual study participants. **B)** After six days, RNA was extracted from the culture supernatant and polyadenylated HIV RNA was quantified by RT-ddPCR as a measure of viral RNA in the supernatant and then normalized to the levels of the activated DMSO (% of activated DMSO). Medians and IQR are presented, as well as the individual values per study participant in different colors.

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