Methods

Data Reporting

No statistical methods were used to predetermine sample size. The experiments were not randomized and the Investigators were not blinded to allocation during experiments and outcome assessment.

Data Availability Statement

All data that support the findings of this study are available in the manuscript, Figures and Extended Data Figures. The gene counts and code for the analysis and visualization of the bulk RNA-seq data can be found at https://github.com/abcwcm/CovaxxHIV DOI: 10.5281/zenodo.6360535. Additional information is available from the corresponding authors upon request.

Code Availability Statement

All custom algorithm or software central to this paper have been reporter in published research papers, as cited in the references.

Statistics

Statistical analyses were performed in Prism Graphpad or Statistical Analysis Software (SAS). Statistical tests used are indicated in figure legends.

Study Approval and Participant Recruitment

Study participants with HIV were recruited at Weill Cornell Medicine's Uptown or Chelsea Clinical Research Site. The Institutional Review Board at Weill Cornell Medicine approved this study (IRB# 21-02023358). Informed consent was obtained from all participants. Inclusion criteria were 18-89 years of age, people living with HIV with sustained HIV suppression for at least one year, HIV viral load <50 copies/mL within 12 months prior to baseline visit and planned receipt of vaccination with an mRNA-based SARS-CoV-2 vaccine. Exclusion criteria included contraindication to receipt of SARS-CoV-2 vaccination, plasma HIV RNA >200 copies/mL within one year prior to the baseline visit, known anemia with a hemoglobin < 10 gm/dL, prior receipt of SARS-CoV-2 vaccination,

and discontinuation of ART for 7 or more consecutive days within the prior. Blood samples used in this study were collected at the baseline visit up to 6 weeks prior to planned vaccination, 2 weeks after the first vaccine dose, and 2 weeks after the second vaccine dose. Participants completed a post-vaccine side effects survey after receipt of each dose. Results were recorded and stored in REDCap. Blood was collected via phlebotomy in gold top serum separator tubes (SST) for anti-S SARS-CoV-2 serology, pearl top plasma preparation tubes (PPT) for HIV viral load, or ethylenediaminetetraacetic acid (EDTA) tubes for PBMC processing. HIV viral load and anti-S SARS-CoV-2 serology were performed by the New York-Presbyterian Hospital clinical laboratory.

Deidentified samples from adults without HIV were obtained from the Rockefeller University (IRB protocol DRO-1006). Eligible participants were healthy adults with no history of infection with SARS-CoV-2, as determined by clinical history and confirmed through serology testing, receiving one of the two Moderna (mRNA-1273) or Pfizer-BioNTech (BNT162b2).

Our independent validation cohort consisted of a subset (n=15) of study participants from a longitudinal study based in Vancouver, Canada [1]. Of the 100 PLWH participants in the Vancouver cohort, 15 were selected based on sample availability and sufficient PBMC count at the time for validation purposes in this study. Ethical approval was granted through the University of British Columbia/ Providence Health Care and Simon Fraser University Research Ethics Boards. Informed consent was obtained from all participants. Vancouver participants had samples collected from baseline (pre-vaccine, V1), one month after SARS CoV-2 mRNA vaccine dose 1 (V2; median 31, range 28-37, Q1-Q3 30-32.5), and one month after vaccine dose 2 (V3; median 30, range 27-32, Q3-Q3 29-30) (**Table 1**). The time between doses 1 and 2 were median 54, range 49-61, Q1-Q3 51.5-57.5).

PBMC were isolated by density gradient centrifugation using Ficoll-Paque PLUS (Cytiva) and SepMate-50 tubes (StemCell Technologies). Whole blood was centrifuged at room temperature for 10min at 400g to separate cellular fraction and plasma. Plasma was centrifuged again at 1500g for 10min and then stored at -80C. Blood was diluted and centrifuged for 10min at 1200g. PBMC were collected and washed twice with 1X DPBS (Gibco). After the second centrifugation, cells were resuspended in 1X DPBS and counted using the Countess FL II

(Invitrogen). Isolated PBMC were cryopreserved in cell recovery media containing 10% DMSO (Corning), supplemented with 90% heat-inactivated fetal bovine serum (Gibco), and stored in liquid nitrogen.

Ex vivo latency reversal assay

Cryopreserved PBMCs were thawed and washed twice with warm R-10 medium (RPMI 1640 supplemented with 10% FBS, L-glutamine, and PenStrep, 10mM Hepes), resuspended at $2x10^6$ cells/ml, in R-10 medium with 10uM T20 (NIH AIDS Reagent Program), then plated at 3 ml/well in 6-well plates. Putative latency reversing agents (LRAs) or vaccines, and controls were added at the indicated concentrations, and cells were incubated for 24 hours at 37 °C 5% CO_2. LRAs include 2% volume/volume Fluzone™ Quadrivalent influenza vaccine (Sanofi-Pasteur Inc., 2021-2022), 25 nM bryostatin-1 (Sigma-Aldrich, B7431), 40 nM romidepsin (Selleck Chemicals, S3020) and phytohemagglutinin-L (PHA) (Thermo Scientific/Remel, R30852801), 100 μL of resuspended cells were removed from each well and stained for flow cytometry to assess viability and activation (see below). Cells were then centrifuged at 400 x g for 10 minutes and 2 ml media was discarded from each sample. Cells were resuspended in the remaining 1 ml media, transferred to 3 ml polystyrene tubes, and incubated for an additional 24 hours. 100 μL of resuspended cells were removed from each well and stained for flow cytometry. Cultures were then centrifuged at 400 x g for 10 minutes and supernatants were transferred to a 1.5 ml tube. Cell fractions: $CD4^+$ T-cells were enriched from PBMCs using the EasySep Human $CD4^+$ T Cell Isolation Kit (Cat. No. 19052). DNA and RNA were co-extracted using the AllPrep Mini (Cat. No. 80204) from the same $CD4^+$ cell sample. RNA was used for RNA-sequencing (see below). Supernatant fractions: The tubes containing supernatants were spun at 10,000 x g for an additional 10 minutes and supernatants were transferred to clean 1.5 ml tubes then frozen at -80°C. *Flow cytometry*: At each timepoint sampled, cells were stained with 1/100 dilutions of the following antibodies in PBS with 2% FBS, 2mM EDTA, fixable viability dye (aqua; Thermo Fisher), anti-human CD3 (clone SK7; BioLegend), anti-human CD4 (clone A161A1; BioLegend), anti-human CD8 (clone RPA-T8; BioLegend), anti-human CD69 (clone FN50; Invitrogen), anti-human CD25 (clone BC96, BioLegend). BD Cytofix/Cytoperm (BD Biosciences; 554722) was used to fix and permeabilize cells, and 1x Perm Wash buffer was used to dilute anti-HIV-1 core antigen p24 (clone KC57; Beckman Coulter, 1:100). *Viral RNA quantification:* Method 1 – Chun Lab, Fig. 1A – Viral RNA was quantified using Cobas Ampliprep/Cobas Taqman HIV-1 Test, version 2.0 (Roche Diagnostics), in quadruplicate. Method 2 – Jones Lab, Fig. 1B - Viral RNA was extracted from plasma using the

QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's recommendations and eluted in 60 µl nuclease-free water. HIV infection in mice was monitored weekly by measuring HIV RNA concentrations in plasma using the previously described integrase single-copy assay protocol[2]. Samples were analyzed on an ABI Viia7 Real-Time PCR System using the following cycling parameters: 50°C for 10 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Cycle threshold values were compared with a validated HIV RNA standard run on each plate to determine HIV RNA concentration.

RNA-Sequencing

RNA was extracted using the AllPrep DNA/RNA Mini kit (Qiagen; 80204). 2-mercaptoethanol, (Bio-Rad; 1610710) was used as directed in the lysis buffer. RQ1 DNase (Promega, M6101) treatment was performed according to the manufacturer. RNA integrity was assessed by Agilent Bioanalyzer 2100, using a Total Eukaryote RNA Pico (v2.6) kit. SMART-Seq v4 Ultra Low Input RNA plus Nextera XT DNA Sample Preparation was performed. The DNA library, QC and sequencing were all performed by the Genomics Core Facility at Weill Cornell Medicine. Illumina NovaSeq 6000 was used for sequencing, using an S2 flow cell and PE 2x50 cycles.

RNA-Sequencing Analysis

Raw reads were quality checked with FastQC v0.11.7 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and adapters were trimmed using Trim Galore v0.6.7 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Reads were aligned to the human reference genome (GRCh38.p12) using STAR v2.6.0c[3] with default parameters. Gene abundances were calculated with featureCounts v1.6.2[4] using composite gene models from Gencode release 28[5]. Principle component analysis was performed using the plotPCA function from DESeq2 v1.32.0[6], after removing patient specific effects with limma v3.48.0 removeBatchEffect[7]. Differentially expressed genes were determined with DESeq2 v1.32.0 using Wald tests ($q < 0.05$) with a two-factor model incorporating patient. Gene set enrichment analysis was performed using fgsea v1.18.0[8]; genes were ordered between treated and untreated cells by the DESeq2 Wald statistic. Gene sets were retrieved from the Broad Institute's MSigDB collections[9,10]. Only pathways with an adjusted P value < 0.05 were considered enriched. The expression heatmap of the leading-

edge genes for the MSigDB Hallmark TNFA Signaling via NFKB pathway was generated using variance-stabilized data after removing patient specific effects, with the values centered and scaled by row.

Activation induced marker (AIM) assay

$10x10^6$ PBMCs from each study participant collected at visits one, two, and three were thawed and rested for 3 hours at 37 °C 5% CO2 in R-10 medium (RPMI 1640 supplemented with 10% FBS, L-glutamine, and PenStrep). PBMCs from each timepoint and participant were divided into seven conditions in duplicate at a concentration of 700,000-1,000,000 cells/condition and stimulated for 24 hours with the following whole gene product peptide pools from the NIH HIV Reagent Program at 1ug/mL: HIV-Gag (cat # ARP-12425), and HIV-Nef (cat # ARP-12545); and CMVpp65 peptide pool (cat # PM-PP65-2), PepMix™ SARS-CoV-2 Spike Glycoprotein (cat # PM-WCPV-S-1) from JPT. Phytohemagglutinin was added at 2ug/mL as a positive control, and 0.5% DMSO in PBS and R-10 media was used as a negative control. 1/200 of anti-CD107a PE ((LAMP-1) Antibody Clone H4A3 Biolegend cat# 328608) from biolegend was added to each well. Post-stimulation, PBMCs were washed in 2% FBS 2mM EDTA- PBS and surface stained with the following fluorochrome-conjugated antibodies from Biolegend: anti-CD3-Brilliant Violet 785 clone SK7 (cat# 344842), anti-CD8-BV605 clone RPA-T8 (cat# 301040), anti-CD137-APC (4-1BB) clone 4B4-1 (cat # 309810), anti-OX40-Brilliant Violet 711 clone Ber-ACT35 (ACT35) (cat # 350030), anti-CXCR5-AF488 clone J252D4 (cat # 356912), and anti-CD4-AF700 clone A161A1 (cat # 357418); and the following from Invitrogen: Anti-CD69-APC-eFluor 780 clone FN50 (cat# 47-0699-42), as well as fixable aqua viability dye (cat # L34966). All antibodies were added at a concentration of 1/100. Cells were fixed using 4% paraformaldehyde and then analyzed on an Attune NxT flow cytometer. Data were analyzed using Flowjo software, TreeStar.

IFN-γ and Granzyme B ELISPOT Assays

Mabtech Interferon-γ (cat # 3420-2A) and Granzyme B (cat # 3486-2A) enzyme-linked immune absorbent spot (ELISPOT) assays against HIV-Gag (cat # ARP-12425), HIV-Env (cat # ARP-12540), HIV-Pol (cat # ARP-12438), HIV-Nef (cat # ARP-12545), HIV-Tat (cat # ARP-12706), HIV-Rev (cat # ARP-6445), and HIV-Vpr/Vpu/Vif peptide pool (cat #s ARP-6447, ARP-6444, ARP-6446) all from the NIH HIV Reagent Program ; and CMVpp65 peptide pool (cat # PM-PP65-2), PepMix™ SARS-CoV-2 VME1 (cat # PM-WCPV-VME), and

PepMix™ SARS-CoV-2 Spike Glycoprotein (cat # PM-WCPV-S-1) from JPT gene product peptide pools were performed in duplicate as previously described[11]. In brief, Multiscreen IP 96-well plates (Millipore) were coated with 0.5 ug/mL of anti-IFN-γ antibody clone 1-D1K or 7.5ug/mL of anti-Granzyme B antibody clone MT28 in phosphate-buffered saline and incubated overnight. Plates were washed, PBMCs were added at 100,000-200,000 cells/well and stimulated with peptide pools and 0.5% DMSO and phytohemagglutinin at 2ug/mL as negative and positive controls, respectively. Plates were incubated overnight, washed and biotinylated antibody was added (anti-IFN-γ antibody clone 7-B6-1 and anti-Granzyme B antibody clone MT8610 from Mabtech and incubated for 1 hour for IFN-γ and 2 hours for Granzyme B plates. Plates were developed with Streptavidin-ALP from Mabtech and with Color Development Buffer (Bio-Rad, Hercules, CA). Plates were washed, dried overnight and spots were counted. Responses against whole gene product peptide pools were background subtracted (thus, nonzero responses were more than 1 time background), but no other ad hoc empirical cutoff was applied — consistent with other studies examining correlations with objectively reported T-cell responses as assessed by ELISPOT assay[11,12] .

Duplex Digital Droplet PCR (Intact Proviral DNA Assay)

Genomic DNA was isolated from CD4+ T-cells using the AllPrep DNA/RNA Mini Kit (Qiagen) with precautions to minimize DNA shearing. Intact HIV copies/million CD4 + T-cells were determined by droplet digital PCR (ddPCR) using the Intact Proviral DNA Assay (IPDA), where HIV and human RPP30 reactions were conducted independently in parallel and copies were normalized to the quantity of input DNA. In each ddPCR reaction, a median 7.5 ng (IQR 7– 7.5 ng) (RPP30) or a median 347 ng (IQR 274– 484 ng) (HIV) of genomic DNA was combined with ddPCR Supermix for Probes (no dUTPs, BioRad), primers (final concentration 900 nM, Integrated DNA Technologies), probe(s) (final concentration 250 nM, ThermoFisher Scientific) and nuclease free water. Primer and probe sequences (5′–>3′) were: RPP30 Forward Primer- GATTTGGACCTGCGA GCG, RPP30 Probe- VIC-CTGACCTGAAGGCTCT- MGBNFQ, RPP30 Reverse Primer- GCGGCTGTCTCCACAAGT; RPP30-Shear Forward Primer CCATTTGCTGCTCCTTGGG, RPP30-Shear Probe- FAM- AAGGAGCA AGGTTCTATTGTAG- MGBNFQ, RPP30-Shear Reverse Primer- CATGCA AAGGAGGAAGCCG; HIV Ψ Forward Primer- CAGGACTCGGCTTGCTGA AG, HIV Ψ Probe- FAM- TTTTGGCGTACTCACCAGT-MGBNFQ, HIV Ψ Reverse Primer- GCACCCATCTCTCTCCTTCTAGC; HIV env Forward Primer-

AGTGGTGCAGAGAGAAAAAAGAGC, HIV env Probe- VIC-CCTTGGGTTCTTGGGA- MGBNFQ, anti-Hypermutant env Probe CCTTAGGTTCTTAGGAGC- MGBNFQ, HIV env Reverse Primer GTCTGGCCTGTACCGTCAGC. Droplets were prepared using the QX200 Droplet Generator (BioRad) and cycled at 95 °C for 10 min; 45 cycles of (94 °C for 30 sec, 59 °C for 1 min) and 98 °C for 10 min. Droplets were analyzed on a QX200 Droplet Reader (BioRad) using QuantaSoft software (BioRad, version 1.7.4). Four technical replicates were performed for each participant sample. Intact HIV copies (Ψ and env-RRE double-positive droplets) were corrected for DNA shearing based on the frequency of RPP30 and RPP30-Shear double-positive droplets.

Cell-Associated HIV RNA

PBMCs collected from study participants were enriched for CD4+ cells using EasySep Human CD4+ T Cell Isolation Kit (Cat. No. 19052). DNA and RNA were co-extracted using the AllPrep Mini (Cat. No. 80204) from the same CD4+ cell sample. RNA was used for total polyadenylated cDNA generation using dT20 primer and reverse transcription was performed with Thermo Scientific SuperScript IV First-Strand Synthesis (Cat. No. 18091150). Resulting HIV-cDNA levels were quantified using BIO-RAD QX200 droplet digital PCR using the following HIV-specific primer and probe sets targeting two regions of the viral transcriptome: HXB2 coordinates 684-810 for unspliced HIV mRNA (Forward primer: 5'-TCTCGACGCAGGACTCG-3', reverse primer 5'-TACTGACGCTCTCGCACC-3', and probe 5'-/56-FAM/CTCTCTCCT/ZEN/TCTAGCCTC/31ABkFQ/-3'); HXB2 coordinates 9435-9525 for total polyadenylated viral RNA with forward primer 5'-GGGACTTTCCGCTGGG-3', reverse primer 5'-AGCAGCTGCTTATATGCAG-3', and probe 5'-/56-FAM/TGAGGGCTC/ZEN/GCCACTCC/3IABkFQ/-3'. DdPCR data analyses were performed using the BIO-RAD QuantaSoft software suite.

Tat/Rev Induced Limiting Dilution Assay.

To estimate the size of the latent reservoir capable of reactivation, we performed the Tat/Rev Induced Limiting Dilution Assay (TILDA) as previously described[13]. 10M PBMCs from study participants were thawed, enriched

for CD4+ T cells, resuspended at 2×10^6 cells/ml in R10 medium containing antiretrovirals (200nM emtricitabine and 200nM dolutegravir) and rested for 2-6 hours at 37 °C, 5% CO2 in a 24-well plate (1mL/plate). CD4+ T cells were stimulated for 12h with 100ng/mL PMA and 1 µg/ml ionomycin (both from Sigma). After stimulation, cells were washed in R10, counted and serially diluted to 18×10^6 cells/mL, 9×10^6 cells/ml, 3×10^6 cells/mL and 1×10^6 cells/mL in PBS/FBS 10%. 1µl from each cell suspension was distributed to 24 wells of a 384-well plate containing 5µl of 2 × reaction buffer from the SuperScript III Platinum One-Step qRT-PCR Kit (Life Technologies) corresponding to 18,000, 9000, 3000 and 1000 cells per well. Pre-amplification was carried out by adding 5µl of a PCR mix containing 0.2 µl Superscript III Platinum Taq (Life Technologies), 0.1 µl RNase inhibitor (Life Technologies), 0.125 µl of each primer (tat1.4 and rev both at 20 µM), 2.2 µl Tris–EDTA (TE) buffer and 2.25 µl H2O to each well (final reaction volume = 11 µl). The sequences of the oligonucleotides are as follows: tat1.4: 5′-TGG CAG GAA GAA GCG GAG A-3′; rev: 5′-GGA TCT GTC TCT GTC TCT CTC TCC ACC-3′. Pre-amplification was carried out using the following steps: reverse transcription at 50 °C for 15 min, denaturation at 95 °C for 2 min, 24 cycles of amplification (95 °C 15 s, 60 °C 4 min) on a C1000 Touch PCR instrument (BioRad). This reaction was performed by adding 5 µl of the TaqMan Fast Advanced Master Mix (Invitrogen), 0.2 µl of each primer (tat2 and rev, both at a working concentration of 20 µM), 0.2 µl of the probe MSHIV FamZen at 5 µM and 3.4 µl H2O to each well (final reaction volume = 10 µl). Sequence of tat2 and the HIV probe are as follows: tat2: 5′- ACA GTC AGA CTC ATC AAG TTT CTC TAT CAA AGC A -3′. Probe HIV: 5′-/56-FAM/TTC CTT CGG /ZEN/GCC TGT CGG GTC CC/3IABkFQ/-3′. All primers and probes were synthesized by IDT. The real-time PCR reaction was carried out in a QuantStudio 6 Flex (ThermoFisher) using the following program: Preincubation 95 °C for 20s, 45 cycles of 95 °C 1s and 60 °C 20 s. Positive wells at each dilution were counted and the maximum likelihood method was used to calculate the frequency of cells with inducible HIV msRNA (http://bioinf.wehi.edu.au/software/elda).

HIV gp120 Enzyme Linked Immunosorbent Assay (ELISA)

96 well EIA/RIA clear flat bottom polystyrene high bind microplates (Corning) were coated with 1 mg/mL of recombinant YU-2 gp120 provided by Dr. Mascola (VRC, NIH) protein in phosphate-buffered saline (PBS) overnight at 4°C. Plates were blocked with B3T buffer (30 mM NaCl, 10 mM Tris-HCl, 0.2 mM EDTA, 0.66% fetal bovine serum, 0.4% bovine albumin, 0.014% Tween 20, 0.004% thimersol) and incubated with 5-fold serial

dilutions of heat-inactivated plasma starting at a dilution of 1:100. After incubation with peroxidase-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch), SureBlue TMB substrate (Kirkegaard & Perry Laboratories) was added, and plates were read at 450 nm. All incubations were for 1 h at 37°C, and all volumes were 100 ml, except for blocking, which was 200 ml. The plates were washed 6 times between incubations with PBS-T (PBS with 0.1% Tween 20). Each plasma sample was run one time. After background subtraction, results were plotted and fit by nonlinear regression using the sigmoidal dose-response (variable slope) model in GraphPad Prism.

Data Presentation

Figures were arranged in Adobe Illustrator 2020.

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13 Mehta, K. *et al.* An Improved Tat/Rev Induced Limiting Dilution Assay With Enhanced Sensitivity and Breadth of Detection. *Front Immunol* **12**, 715644, doi:10.3389/fimmu.2021.715644 (2021).

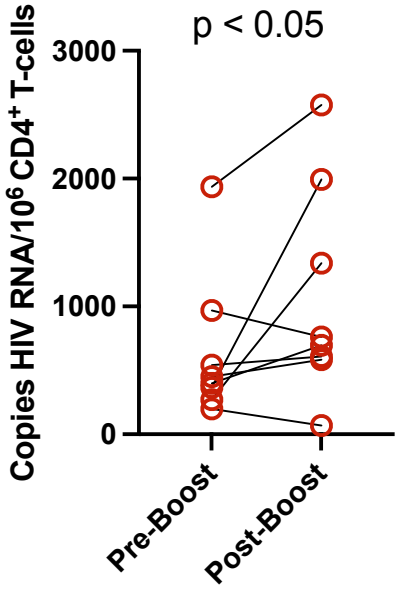
Extended Data

Extended Data Table 1. Participant demographic and clinical data, ex vivo latency reversal.

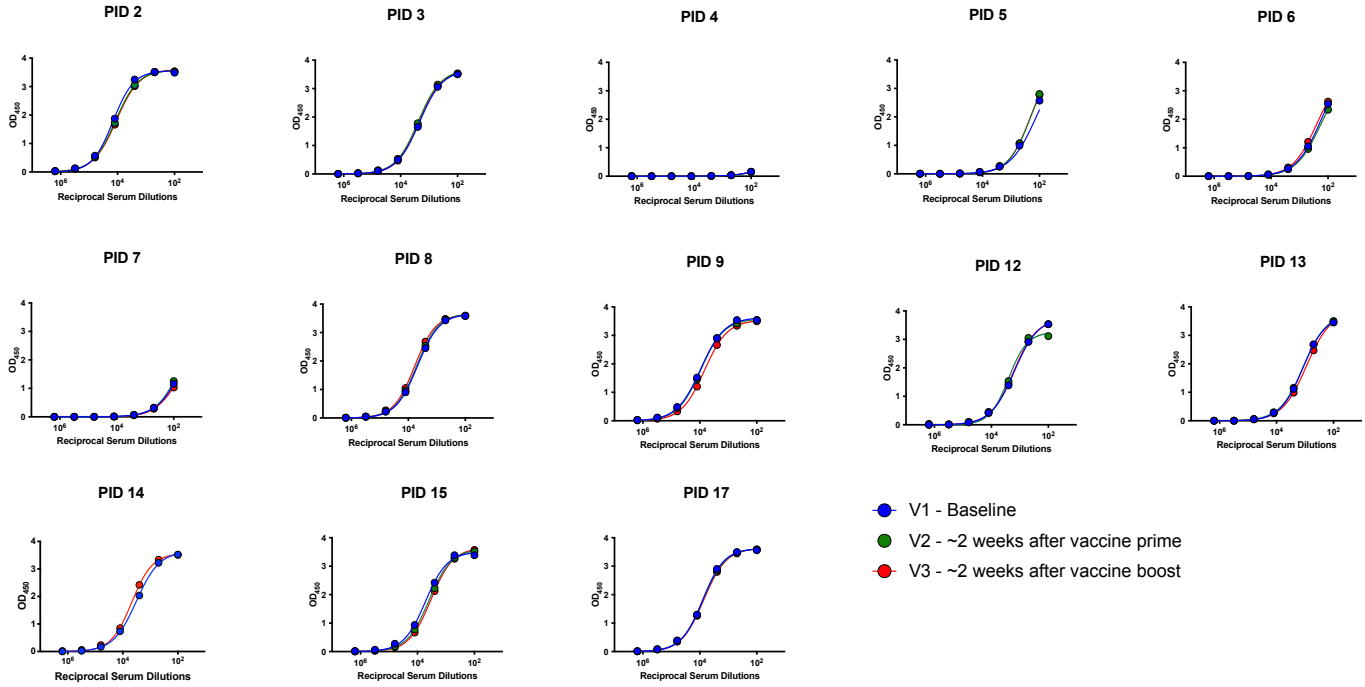
Participant ID	Age at Enrollment (years)	Race	Ethnicity	Sex	Gender Identity	COVID Vaccine Received	Anti-Spike Serology at Entry	Year of HIV Diagnosis	Year started multi-agent ART	Most recent CD4 (cells/ul)	Nadir CD4 if known (cells/uL)	Current ART	Sample Date
OM5011	46	White	Caucasian	Male	Man	NA	NA	2005	2008	602	340	ABC/DTG/3TC	10/7/21
OM5258	62	White	Caucasian	Male	Man	NA	NA	1989	2002	240	581	ABC/DTG/3TC	8/1/17
OM5334	29	White	NA	Male	Man	NA	NA	2013	2013	840	460	TAF/FTC/c/EVG/RPV	4/18/18
WWH-B005	51	White	Caucasian	Male	Man	NA	NA	1995	2005	176	311	FTC/TAF/RIL/DRV/c/DTG	12/4/17
WWH-B025	28	African-American	Not Hispanic or Latino	Male	Man	NA	NA	2018	2018	398	408	TAF/FTC/c/EVG	13/8/18
WWH-B029	35	White	Caucasian	Male	Man	NA	NA	2018	2018	287	287	TAF/FTC/BIC	4/18/18
WWH-B032	62	African-American	Not Hispanic or Latino	Male	Man	NA	NA	1983	2005	212	181	TDF/FTC/DRV/r/ETR	4/30/18

Extended Data Table 2. Participant demographic and clinical data, booster cohort.

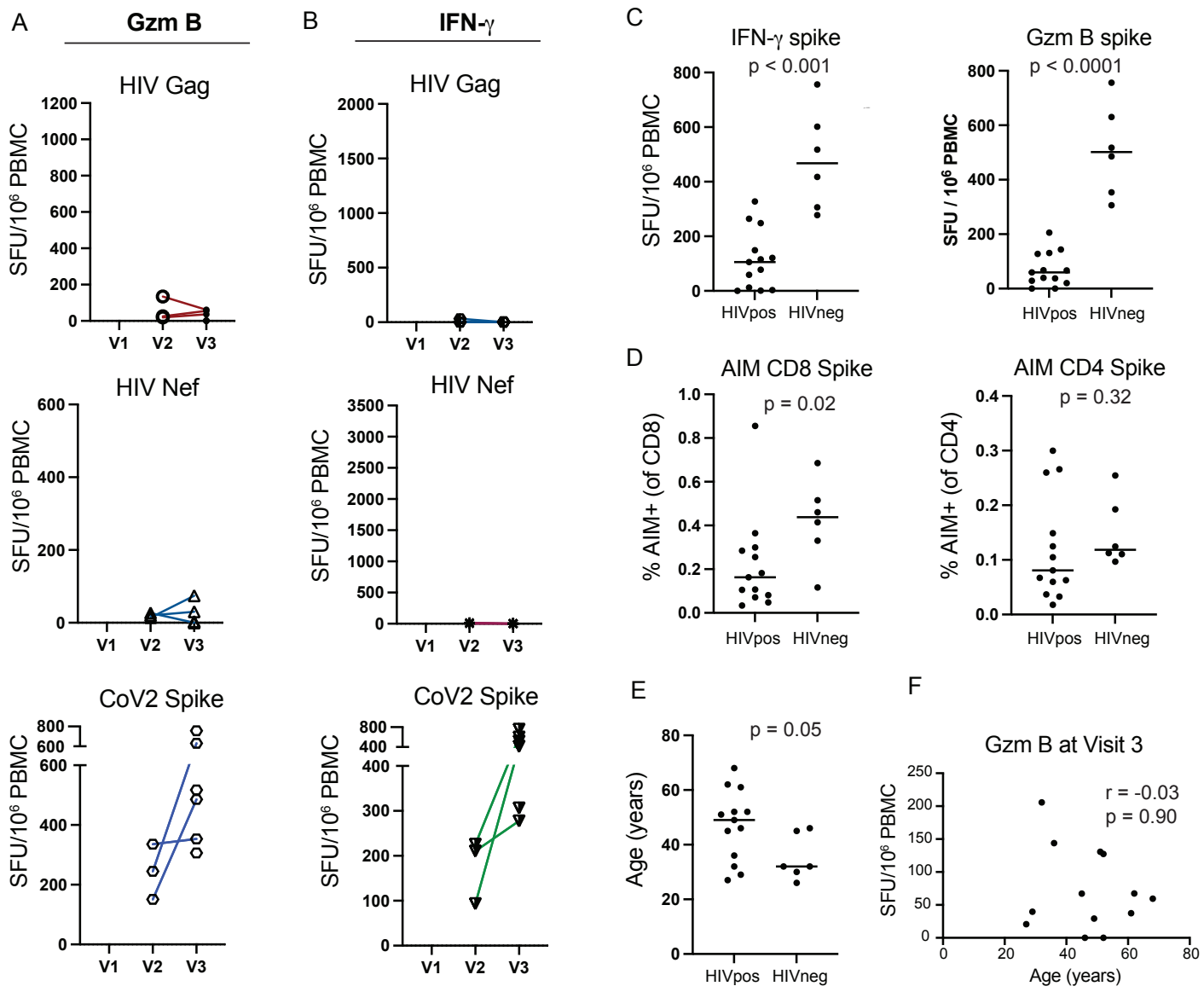
Participant ID	Age at Enrollment (years)	Race	Ethnicity	Sex	Gender Identity	COVID Booster Vaccine Received	Year of HIV Diagnosis	Year started multi-agent ART	Most recent CD4 (cells/ul)	Nadir CD4 (cells/uL)	Current ART
12	29	White	Not Hispanic or Latinx	Male	Man	mRNA 1273	2016	2016	760	640	TAF/FTC/c/EVG
20	34	White	Not Hispanic or Latinx	Male	Man	BNT162b2	2010	2012	1066	480	RPV/DTG
21	57	White	Hispanic or Latinx	Female	Woman	mRNA 1273	1988	1996	493	30	TAF/FTC/BIC
23	41	unknown	Hispanic or Latinx	Male	Man	BNT162b2	2000	2005	620	300	ABC/3TC/DTG
24	40	White	Hispanic or Latinx	Male	Man	BNT162b2	2006	2018	720	unknown	TAF/FTC/BIC
25	43	White	Hispanic or Latinx	Male	Man	BNT162b2	2017	2017	864	584	RPV/DTG
27	58	Black or African American	Not Hispanic or Latinx	Male	Man	mRNA 1273	1997	2005	699	300	BIC/FTC/TAF
28	63	Black or African American	Not Hispanic or Latinx	Female	Woman	BNT162b2	1991	2002	1895	94	RPV/DTG



Extended Data Fig. 1. Increases in cell-associated HIV RNA following second SARS CoV-2 mRNA vaccine boosters. Shown are mean levels of unspliced cell-associated HIV RNA (from technical triplicates), assessed prior to and within days (median 4, range 3-5 days) of receiving mRNA vaccine boosts. The p value was calculated by Wilcoxon matched pairs signed rank test (one-tailed).



Extended Data Fig. 2. No detectable changes in anti-HIV gp120 antibody titers following COVID vaccine dose 1 or dose 2. Shown are antibody binding results to rgp120 YU-2 graphing optical density (OD_{450}) at the indicated plasma dilutions for each participant. Timepoints for graphed samples are baseline (V1), and ~2 weeks after vaccine dose 1 (V2) or vaccine dose 2 (V3). After background subtraction, results were plotted and fit by nonlinear regression using the sigmoidal dose-response (variable slope) model in GraphPad Prism.



Extended Data Fig. 3. Comparisons with T-cell responses in an HIV-negative cohort. A – Gzm B, and **B** – IFN-γ ELISPOT responses, plotted on same axes as HIV-positive cohort in **Fig. 3**. The lack of induction of HIV-specific responses rules out CoV-2 cross-reactivity as a driver of the increases in Nef- and Rev-specific T-cell responses observed at the first vaccine dose in the HIV-positive cohort. **C & D**. Comparisons of CoV-2-Spike-specific T-cell responses at V3 (~2 weeks post second dose) between an HIV-positive versus HIV-negative cohort. **C** – ELISPOT results, **D** – Activation induced marker (AIM) results, Mann-Whitney tests. **E**. Comparisons of ages across HIV-positive versus HIV-negative cohorts show that the former tended to be older, approaching statistical significance (Mann-Whitney test). **F**. No significant correlations were observed within the HIV-positive cohort between age and CoV-2-specific T-cell responses by any measure tested, at V3; shown are results for granzyme B ELISPOT – Spearman's correlation test. For each of **A – E**: n = 13 for HIV-positive cohort at V1 and

V3 and n = 12 at V2 (PID 14 missed V2); n = 3 at V2 and n = 6 at V3 for HIV-negative cohort (three of the V3 donors did not have a visit 2).

Our study provided the additional opportunity to compare vaccine-induced Spike-specific T-cell responses with a cohort of HIV-negative donors collected at similar timepoints following vaccine dose 1 (n = 3) and dose 2 (n = 6) (median, range: 22, 18-27 days after dose 2). Following second vaccine doses, we observed significantly higher magnitudes of SARS-CoV2-Spike-specific T-cells by both IFN-γ and Gzm B ELISPOT (IFN-γ mean, 114 SFU/10^6 PBMCs in HIVpos versus 480 SFU/10^6 PBMCs in HIVneg – $p < 0.001$; Gzm B mean, 71 SFU/10^6 PBMCs in HIVpos versus 508 SFU/10^6 PBMCs in HIVneg – $p < 0.0001$, **Extended Data Fig. 3C**). By AIM assay, we also observed higher frequency CD8+ T-cell responses in HIVneg versus HIVpos individuals (mean 0.42 % AIM+ versus 0.11 % AIM+, $p = 0.02$), whereas a significant difference was not observed in CD4+ T-cell responses ($p = 0.32$) (**Extended Data Fig. 3D**). A caveat to this side-observation of our study is that the HIVpos and HIVneg cohorts were not age matched, with a younger HIVneg cohort ($p = 0.05$, **Extended Data I Fig. 3E**). However, we note that we observed a lack of correlations between age and any of our T-cell measures following second vaccine doses – shown for gzm-B ELISPOT in **Extended Data Fig. 3F**. Additionally, the cohorts were not vaccine matched. The HIVneg cohort had more individuals that received the mRNA1273 vaccine (**Table 1 and Extended Data Table 5).**

Extended Data Table 3. Spearman correlations between changes in HIV RNA (V3 / V1) and T-cell responses measured by ELISPOT

				Gag	Env	Pol	Nef	Tat	Rev	Vif/Vpr/Vpu	CMV-pp65	CoV2-Spike
Granzyme B	5' RNA (spliced)	**V1**	Spearman r	-0.09	-0.25	-0.22	-0.22	0.07	-0.05	0.34	-0.05	0.08
			Spearman p	0.78	0.41	0.46	0.47	0.82	0.87	0.25	0.87	0.80
		V2	Spearman r	-0.33	-0.01	-0.26	-0.51	-0.12	-0.27	-0.09	-0.11	-0.32
			Spearman p	0.29	0.97	0.41	0.09	0.71	0.39	0.78	0.73	0.27
		V3	Spearman r	0.04	-0.08	-0.37	-0.37	-0.21	-0.09	-0.26	-0.20	0.42
			Spearman p	0.89	0.79	0.21	0.21	0.48	0.76	0.38	0.53	0.15
	3' RNA (total)	**V1**	Spearman r	-0.06	-0.24	-0.35	-0.43	-0.06	-0.14	-0.50	-0.22	0.07
			Spearman p	0.85	0.45	0.27	0.16	0.34	0.65	0.10	0.47	0.83
		V2	Spearman r	-0.05	-0.31	-0.29	**-0.73**	-0.40	-0.27	-0.17	-0.31	-0.02
			Spearman p	0.87	0.33	0.35	**0.006**	0.19	0.39	0.60	0.32	0.97
		V3	Spearman r	-0.12	-0.24	-0.20	-0.54	-0.27	**-0.61**	-0.34	-0.31	-0.01
			Spearman p	0.70	0.45	0.53	0.07	0.39	**0.04**	0.27	0.32	0.97
IFN-γ	5' RNA (spliced)	**V1**	Spearman r	-0.02	-0.29	0.07	-0.12	-0.40	-0.17	-0.17	-0.19	0.55
			Spearman p	0.96	0.35	0.83	0.72	0.20	0.59	0.03	0.54	0.31
		V2	Spearman r	0.10	-0.30	-0.01	0.00	-0.44	0.00	-0.15	-0.28	0.11
			Spearman p	0.77	0.34	0.97	1.00	0.15	1.00	0.65	0.38	0.74
		V3	Spearman r	0.15	-0.15	0.15	0.01	-0.35	-0.39	0.00	-0.17	0.50
			Spearman p	0.65	0.63	0.65	0.97	0.26	0.21	0.99	0.57	0.10
	3' RNA (total)	**V1**	Spearman r	-0.02	-0.30	0.07	-0.01	-0.40	-0.17	-0.09	0.40	0.12
			Spearman p	0.96	0.35	0.83	0.72	0.20	0.59	0.78	0.21	0.74
		V2	Spearman r	0.10	-0.30	-0.01	0.00	-0.44	0.00	-0.02	0.22	0.28
			Spearman p	0.77	0.34	0.97	1.00	0.15	1.00	0.96	0.50	0.37
		V3	Spearman r	0.15	-0.15	0.15	0.01	-0.35	-0.39	0.34	0.16	0.50
			Spearman p	0.65	0.63	0.65	0.97	0.26	0.21	0.27	0.62	0.10

Extended Data Table 4. Spearman correlations between fold changes in HIV RNA (V3 / V1) and T-cell responses measured by AIM assay.

			CD8				**CD4**			
			HIV-Gag	HIV-Nef	CMV-pp65	CoV2 Spike	HIV-Gag	HIV-Nef	CMV-pp65	CoV2 Spike
5' RNA (unspliced)	**V1**	Spearman r	0.08	-0.39	-0.67	-0.66	-0.25	-0.59	-0.36	-0.26
		Spearman p	0.79	0.19	**0.02**	**0.02**	0.40	**0.04**	0.23	0.38
	V2	Spearman r	0.00	-0.51	-0.17	-0.38	0.24	-0.22	-0.20	0.24
		Spearman p	1.00	0.09	0.60	0.22	0.45	0.50	0.52	0.44
	V3	Spearman r	0.20	0.00	-0.25	0.19	0.11	-0.20	-0.32	0.20
		Spearman p	0.52	1.00	0.41	0.53	0.73	0.52	0.29	0.52
3' RNA (total)	**V1**	Spearman r	-0.30	-0.36	-0.17	-0.02	-0.53	-0.50	0.15	0.24
		Spearman p	0.34	0.24	0.59	0.96	0.08	0.10	0.65	0.46
	V2	Spearman r	-0.36	-0.76	-0.17	-0.48	-0.35	-0.66	-0.11	-0.31
		Spearman p	0.25	**0.006**	0.60	0.11	0.26	**0.02**	0.74	0.33
	V3	Spearman r	-0.17	-0.54	-0.27	-0.46	-0.43	-0.43	-0.04	0.02
		Spearman p	0.60	0.07	0.39	0.13	0.16	0.16	0.91	0.96

Extended Data Table 5. Participant demographic and clinical data in the Rockefeller University cohort of people without HIV.

Record ID	Age at Enrollment (years)	Race	Ethnicity	Sex	COVID Vaccine Received
C012	30	White	non-Hispanic	F	mRNA 1273
C016	32	White	Hispanic	M	mRNA 1273
C037	46	White	Hispanic	F	BNT162b2
C043	26	White	non-Hispanic	F	mRNA 1273
C044	32	White	non-Hispanic	F	BNT162b2
C049	45	White	Hispanic	M	BNT162b2