Supplemental Material

Supplemental Methods

Enrollment definition of post-menopausal

Eligible women were 18-65 years old and postmenopausal status was defined as either (1) age ≥40 years, amenorrhea ≥12 months and follicle stimulating hormone(FSH) >40mIU/mL, negative pregnancy test and no oral/injected exogenous hormones in the 12 months prior OR (2) age ≥18 years with a documented history of surgical removal of ovaries >6 months prior to study entry and no oral/injected exogenous hormones in the 12 months prior.

Virological Assays

$CD4^+$ T cells were isolated from cryopreserved mononuclear cells (PBMCs) using negative immunoselection (EasySep Human Memory $CD4^+$ T Cell Enrichment Kit, Stemcell Technologies). Genomic DNA and total RNA were isolated from approximately $5x10^6$ $CD4^+$ T cells (AllPrep DNA/RNA kit, Qiagen). Total HIV-1 DNA and unspliced cell-associated RNA (caRNA) levels were quantified in triplicate by real-time PCR targeting a region in *gag*, with methods modified to include Taqman Universal Mastermix (DNA) or Taqman Fast Virus 1-Step Mastermix (RNA)[24] as follows.

HIV DNA

Primers for HIV amplification included forward primer 5'-TACTGACGCTCTCGCACC-3', reverse primer 5'-TCTCGACGCAGGACTCG-3', and probe 5' FAM-CTCTCTCCTTCTAGCCTC-MGB 3' (ThermoFisher Scientific). DNA cycling conditions in a total reaction volume of 25µL were 95°C

for 15 min followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. Input for genomic DNA was approximately 500ng per well and cell input numbers were quantified by measuring human genome equivalents of CCR5 DNA using forward primer 5'-ATGATTCCTGGGAGAGACGC-3' , reverse primer 5'-AGCCAGGACGGTCACCTT-3', and probe 5' FAM-CTCTCTCCTTCTAGCCTC-MGB 3' (ThermoFisher Scientific) as described[1]. The limit of quantification for the HIV-1 DNA assay was 1 copy per reaction.

Cell-associated HIV RNA

For quantification of caRNA, from the RNA extracted as described above and using the identical primers, RNA cycling conditions in a total reaction volume of 20µL were 55°C for 15 min, 95°C for 20 sec, followed by 40 cycles of 95°C for 3 sec and 60°C for 30 sec. RNA integrity was assessed with quantification of the human reference gene *IPO8*. To generate standards, we cloned a fragment corresponding to nucleotides 3636-3707 of IPO8 mRNA (Sequence ID NM_006390.4) into a pCR4-TOPO vector. IPO8 RNA was synthesized (T3 Megascript Kit, ThermoFisher), cleaned (Qiagen MinElute kit, Qiagen), and used across a range of 10^6 – 10^3 copies per well. The Taqman assays used the following primers and probe: IPO8-FOR CCTTTGTACAACAGAAGGCAC; IPO-REV TGCACGTCTCAGGTTTTTGC; probe 5' FAM-TCCGCATAAATCCATTGATTCTGC -MGB 3' (Life Technologies). IPO8 RNA cycling conditions, in a total reaction volume of 20µL, were 55°C for 5 min, 95°C for 20 sec, and then 40 cycles of 95°C for 3 sec and 55°C for 30 sec. All extracted RNA was used in the assay, split across the triplicate wells and the limit of quantification for the HIV-1 caRNA assay is 3 copies per reaction.

EDITS assay

Spliced envelope transcripts were measured from primary $CD4^+$ T cells using the EDITS assay [2]. $CD4^+$ T cells were isolated from cryopreserved PBMCs as above(EasySep^{TM}Human Memory $CD4^+$ T-cell Enrichment Kit(Stemcell)) and RNA isolated with the Qiagen RNeasy kit(Qiagen). The entire sample was used as template for a one-step RT-PCR reaction with primers spanning the spliced region of Env; this excludes proviral amplification due to the length spanned by the primers on an unspliced template. Subsequent nested amplification using a synthetic GEX tag on the original reverse primer and an internal sequence was followed by library preparation and sequencing on the Ion Torrent platform. Reads were filtered the GEX tag and short reads excluded. Remaining mapped reads were quantified with a standard curve spanning a frequency of 1 to 300 primary memory $CD4^+$ infected with replication competent GFP-tagged HIV-1 NL4-3 in a total pool of 1.25×10^6 uninfected cells. Total input was ~1.25×10^6 purified memory cells/sample and the output measurement is frequency of cells spontaneously producing spliced envelope RNA[2].

Single copy assay

Residual plasma HIV-1 levels were measured using single copy assay with primers targeting the integrase region of the *pol* gene and a spike-in of a known quantity of internal standard (replication-competent avian leukosis virus [ALV] long terminal repeat [LTR] with a splice adaptor [RCAS]) and HIV-1 and RCAS recovery quantified all precisely as previously described [3]. A plasma volume of 4.5ml per sample was used and the limit of quantification for the assay was 0.38 copies/mL.

Histone acetylation measurement

Bulk histone 4 (H4) acetylation status was assessed in PBMC lysates as previously described [4]. Cryopreserved PBMCs were resuspended in 1% Triton X-100 (#X100, Sigma-Aldrich, St Louis, MO) in PBS, then diluted at $1e10^6$ cells/mL with 3% bovine serum albumin in PBS. ELISA plates were coated with 2μg/mL of a monoclonal anti-H4 antibody (MBL, Woburn, MA) in 100μL of coating buffer (Sigma-Aldrich, St Louis, MO) in PBS, and then blocked with 3% BSA/PBS for 2 hours. 100μL of the PBMC lysates were added with 50μL of a 1:500 dilution of anti-H4K5/8/12/16 monoclonal antibody (Millipore, Billerica, MA) conjugated to alkaline phosphatase. After overnight incubation at 4°C with shaking, plates were washed (5 time in 0.05% Tween 20 in PBS) with shaking. 100μL of Tropix CDP-Star Sapphire II substrate (Applied Biosystems, Carlsbad, CA) were added, incubated at room temperature for 20 minutes and luminescence quantified with an EnVision plate reader (Perkin Elmer, Waltham, MA). Results are quantified as pmol per million cells.

Pharmacologic assays

Vorinostat concentrations were quantified from serum using a validated liquid chromatography/mass spectrometry (LC-MS/MS) method developed at the Clinical Pharmacology and Analytical Chemistry Core, University of North Carolina Chapel Hill. Briefly, serum samples underwent protein precipitation prior to injection into the LC-MS/MS (SCIEX,

Framingham, MA). Chromatographic separation of analytes and internal standard compounds from matrix components was achieved using reverse-phase chromatography on a Waters Atlantis T3 (50 x 2.1mm, 3µm) analytical column under gradient conditions. The analytes were detected on an AB Sciex API-5000 triple quadrupole mass spectrometer using electrospray ionization in the positive ion mode. The lower limit of quantitation was 1.0 ng/ml.

Tamoxifen concentrations were measured with ultra-performance liquid chromatography (UPLC)-MS/MS (TQD Triple Quadrupole Mass Spectrometry, Waters Milford, MA) method developed at the Translational Pharmacology Research Core, University at Buffalo. Briefly, a protein precipitation was performed on all plasma samples followed by injection into the UPLC-MS/MS. Chromatographic separation of analytes and internal standards was achieved using UPLC on a Waters Acquity BEH C18 (50 x 2.1mm, 1.7µm) column. The analytes were detected on a Waters Acquity triple quadrupole mass spectrometer using electrospray ionization in the positive ion mode. The lower limit of quantitation was 5.0 ng/ml.

All assays used to measure the drug concentrations were validated according to US FDA Guidelines for Bioanalytical Method Validation and the Assay Validation Reports and were peer reviewed by the NIAID Clinical Pharmacology Quality Assurance Program assay review process.

Methods References

1. Malnati MS, Scarlatti G, Gatto F, et al. A universal real-time PCR assay for the quantification of group-M HIV-1 proviral load. Nat Protoc **2008**; 3(7): 1240-8.
2. Das B, Dobrowolski C, Luttge B, et al. Estrogen receptor-1 is a key regulator of HIV-1 latency that imparts gender-specific restrictions on the latent reservoir. Proc Natl Acad Sci U S A **2018**; 115(33): E7795-E804.
3. Cillo AR, Vagratian D, Bedison MA, et al. Improved single-copy assays for quantification of persistent HIV-1 viremia in patients on suppressive antiretroviral therapy. J Clin Microbiol **2014**; 52(11): 3944-51.
4. Fidler S, Stohr W, Pace M, et al. Antiretroviral therapy alone versus antiretroviral therapy with a kick and kill approach, on measures of the HIV reservoir in participants with recent HIV infection (the RIVER trial): a phase 2, randomised trial. Lancet **2020**.

Supplemental Table 1: Detailed ART regimens

Antiretroviral regimen	Overall (N=31)	Arm A (VOR+TAMOX) (N=21)	Arm B (VOR) (N=10)
Integrase inhibitor+NRTIs	**24(77%)**	**18(86%)**	**6(60%)**
BIC/FTC/TAF	4(13%)	2(10%)	2(20%)
DTG/ABC/3TC	3(10%)	2(10%)	1(10%)
DTG+FTC/TAF	8(26%)	6(29%)	2(20%)
DTG+FTC/TDF	1(3%)	1(5%)	0(0%)
ELVI/c/FTC/TAF	7(23%)	6(29%)	1(10%)
RAL+ABC/3TC	1(3%)	1(5%)	0(0%)
NNRTI+NRTIs	**3(10%)**	**1(5%)**	**2(20%)**
EFV/FTC/TDF	1(3%)	0(0%)	1(10%)
RPV/FTC/TDF	1(3%)	0(0%)	1(10%)
RPV/FTC/TAF	1(3%)	1(5%)	0(0%)
PI+NRTIs	**1(3%)**	**1(5%)**	**0(0%)**
DRV/r+FTC/TDF	1(3%)	1(5%)	0(0%)
Other combination	**3(10%)**	**1(5%)**	**2(20%)**
DTG+DRV/r	1(3%)	0(0%)	1(10%)
DTG+DRV/r+TDF	1(3%)	1(5%)	0(0%)
DTG+FTC/TAF+MVC	1(3%)	0(0%)	1(10%)

NRTI=nucleoside/nucleotide reverse transcriptase inhibitor; NNRTI=non-nucleoside reverse transcriptase inhibitor; PI=protease inhibitor; BIC=bictegravir; FTC=emtricitabine; TAF=tenofovir alafenamide; DTG=dolutegravir; ABC=abacavir; 3TC=lamivudine; TDF=tenofovir disproxil; ELVI/c=elivitegravir-cobicistat; RAL=raltegravir; EFV=efavirenz; RPV=rilpivirine; DRV/r=darunavir-ritonavir; MVC=maraviroc.

Supplemental Table 2. Adverse events reported during the study. Adverse events were reported in two participants as detailed below with event, grading and relationship to study drugs as determined by the protocol team.

Participant	Study Arm	Study Day	Event Description	Event Grade	Related to Study Drug		Comment
					Tamoxifen	Vorinostat	
A5366-XXX	Arm A	28	Hyperglycemia	Severe	No	No	Prior diabetes diagnosis
		35	Hyperglycemia	Moderate	No	No	
		43	Hyperglycemia	Severe	No	No	
A5366-YYY	Arm B	35	Dysgeusia	Mild	No	Yes	
		35	Thirst	Moderate	No	Yes	

Vorinostat concentration		**Arm A** **Tamoxifen/Vorinostat**	**Arm B** **Vorinostat**
Day 38 post dose vorinostat concentration (ng/mL)	Median [Q1, Q3]	75 [60, 111]	76 [61, 81]
	Min, Max	10, 284	11, 145
	N	19	8

Supplemental Table 3. Vorinostat concentration at the post-vorinostat dose 2 timepoint on study day 38. Data is presented as the median concentration with the Q1 and Q3 ranges as well as the minimum and maximum values for each group. For all participants, pre-dose concentration on day 38 was <1 ng/mL.

Timepoint	Total N=27	Arm A Tamoxifen/Vorinostat N=19	Arm B Vorinostat N=8
Pre-entry	16 (59%)	10 (53%)	6 (75%)
Entry	12 (44%)	9 (47%)	3 (38%)
Day 38	12 (44%)	7 (37%)	5 (63%)

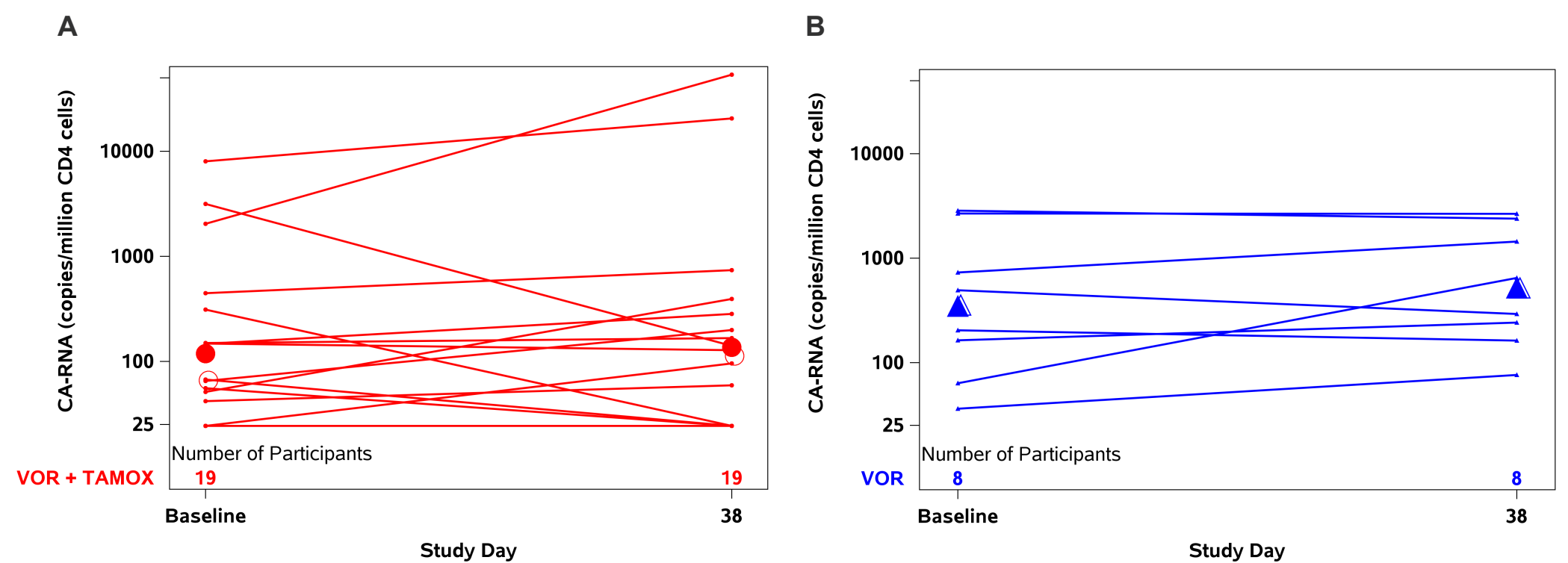
Supplemental Table 4. Proportion of participants with plasma viremia detectable by single copy assay. The number and percentage of participants with detectable viremia by single copy assay at the two timepoints prior to any intervention (pre-entry and entry) and after the second dose of vorinostat for all participants and separated by study arm. The lower limit of quantification for the assay was <0.47 copies HIV RNA/mL of plasma.

Study Arm	N	Mean fold change (log_{10} copies HIV DNA/10^6 CD4 cells	95% Confidence interval
Arm A: Tamoxifen/Vorinostat	19	0.00	[-0.12, 0.13]
Arm B: Vorinostat	8	-0.04	[-0.33, 0.25]

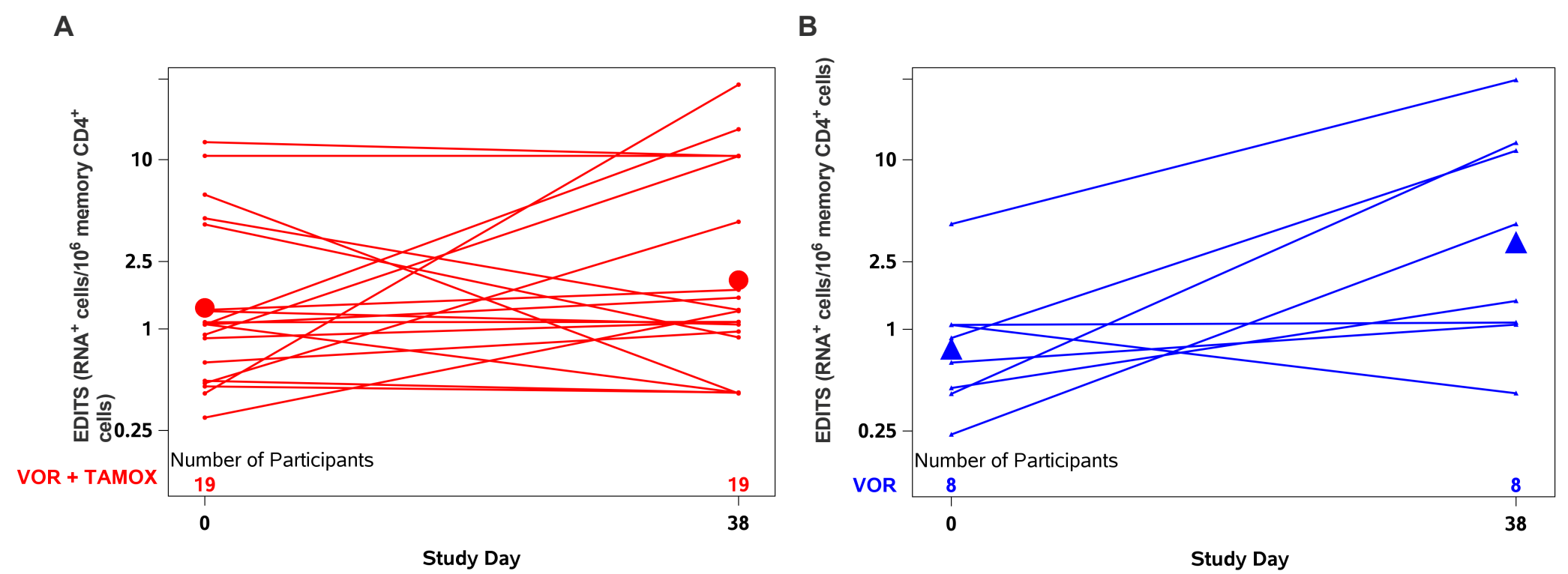
Supplemental Table 5. Change in cell-associated HIV proviral DNA between baseline and after the second dose of vorinostat. Proviral DNA values were compared between the baseline, an averaged for the two pre-intervention timepoints (pre-entry and entry), and after the second dose of vorinostat (study Day 38). Mean fold change between the baseline and post-intervention measure with the 95% confidence interval are shown.

Tamoxifen Concentrations		**Arm A** **Tamox/Vor**
Average of Day 35 and 38 tamoxifen concentration (ng/mL)	Median [Q1, Q3]	121 [92, 166]
	Min, Max	71, 223
	N	19

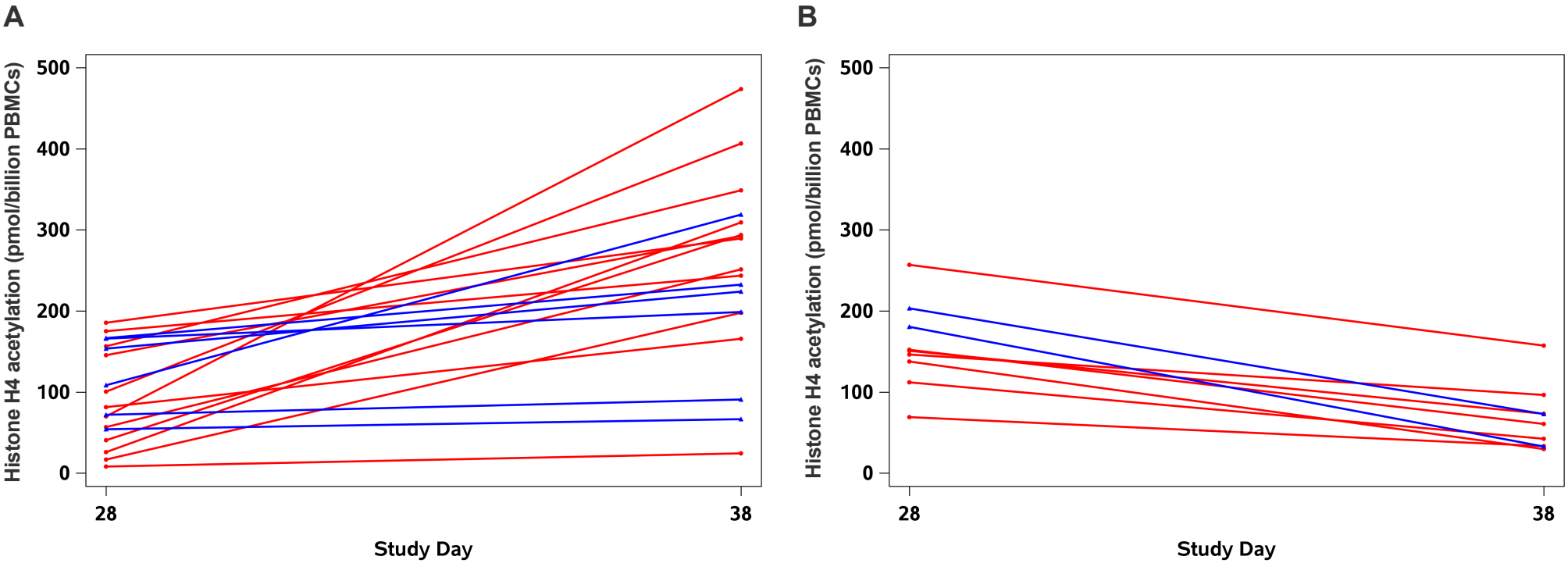
Supplemental Table 6. Tamoxifen concentration values averaged across two samples from Day 35 and Day 38, both timepoints would be expected to reflect steady-state levels of drug. All participants had detectable tamoxifen and were within range of therapeutic dosing.



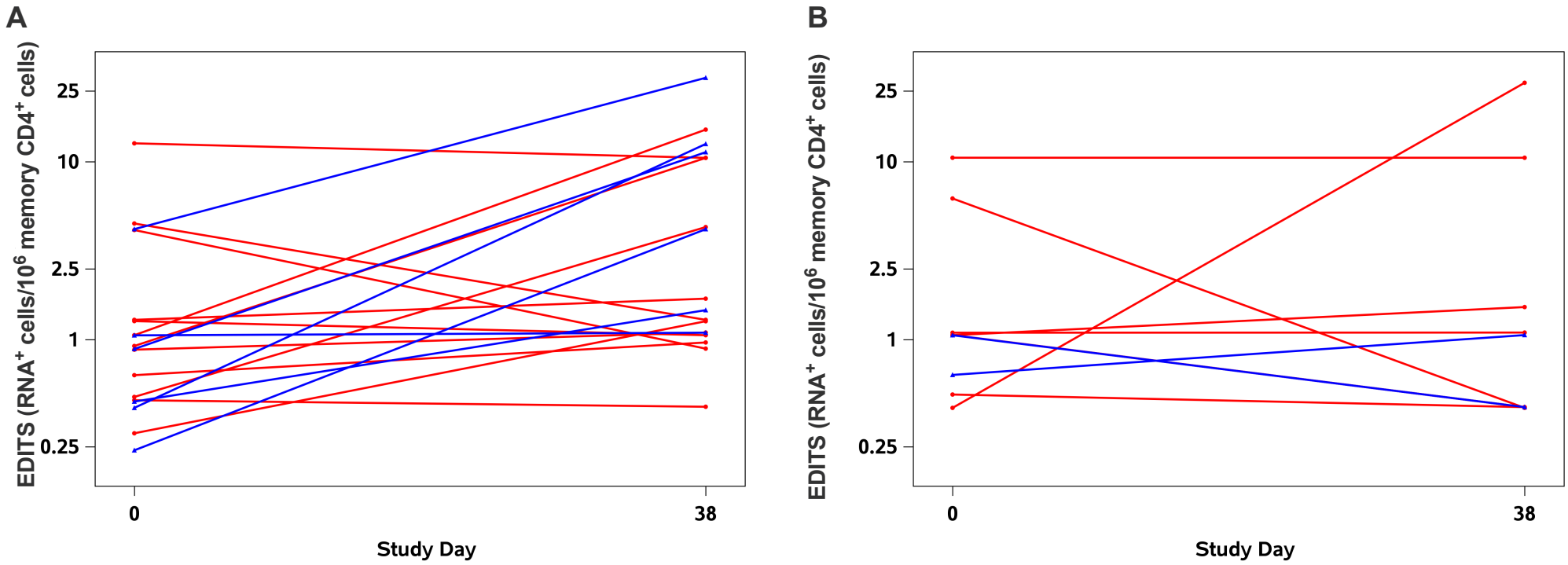
Supplemental Figure 1. Participant level data for cell associated HIV RNA. Change from baseline (an average of two measures from pre-entry and entry) to the primary endpoint timepoint at 5 hours after the second dose of vorinostat on day 38 are shown for (A) Arm A with the combination of vorinostat and tamoxifen and (B) Arm B with vorinostat alone. Solid symbols are the geometric mean, open symbols are the geometric means when calculated using a participant specific lower limit of quantification.



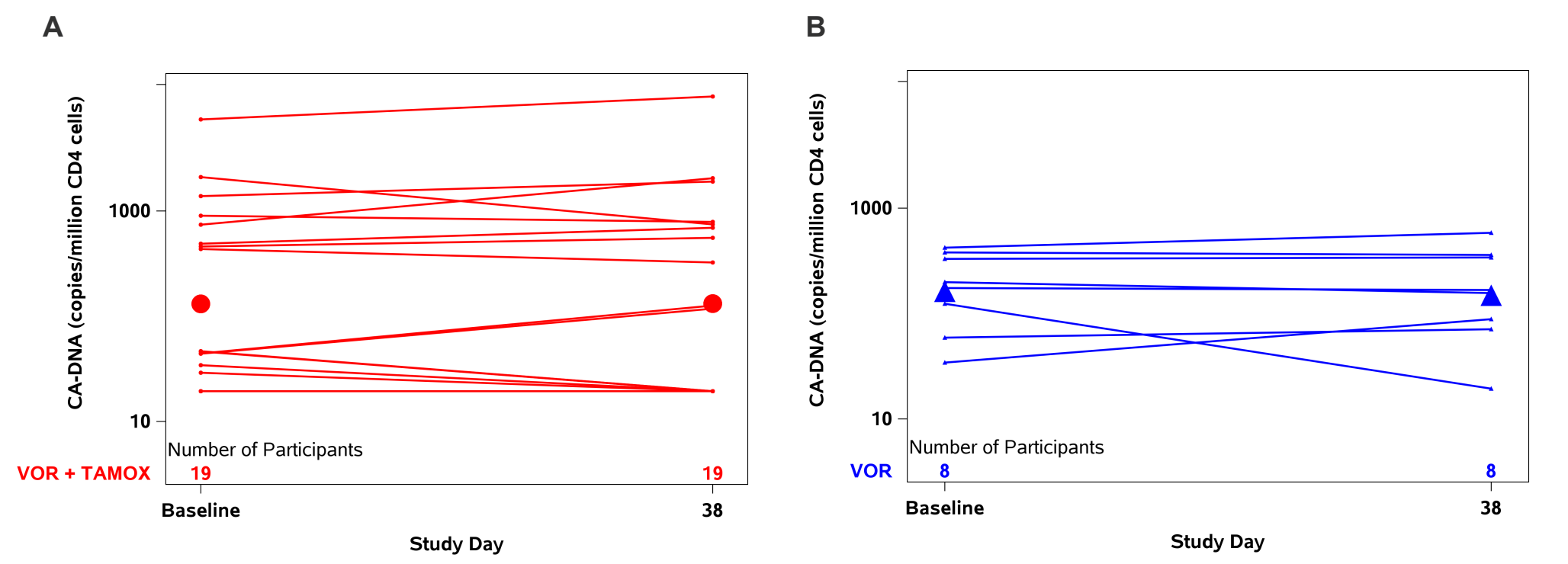
Supplemental Figure 2. Participant level data for EDITS. Change from baseline to the primary endpoint timepoint at 5 hours after the second dose of vorinostat on day 38 are shown for (A) Arm A with the combination of vorinostat and tamoxifen and (B) Arm B with vorinostat alone. Solid symbols are the geometric mean.



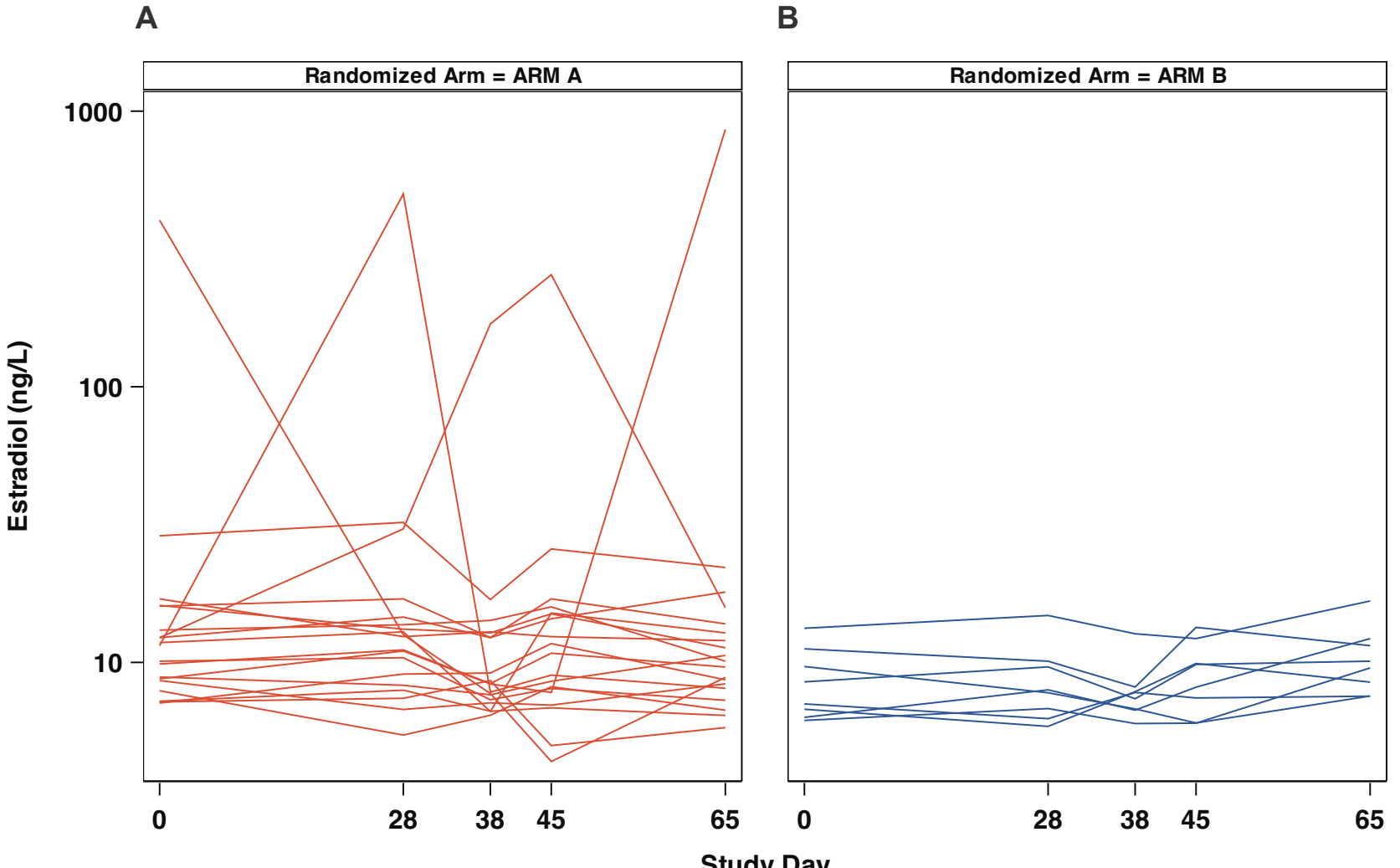
Supplemental Figure 3. Participant level data for change in histone H4 acetylation. Change in histone H4 acetylation as measured by immunoassay on PBMCs between day 28 and the primary endpoint timepoint at 5 hours after the second vorinostat dose on study day 38. The participants are stratified into those with an increase in histone acetylation (panel A) and those with a decrease in histone acetylation (panel B). The red lines indicate participants from Arm A (combination vorinostat and tamoxifen) and the blue lines indicate participants from Arm B (vorinostat alone).



Supplemental Figure 4. Participant level data for change in HIV RNA production stratified by histone H4 acetylaton increase or decrease. Change in HIV RNA production as quantified by EDITS among participants with an increase in histone H4 acetylation (panel A) after two doses of vorinostat, and in those with a decrease in histone H4 acetylation (panel B). Participants with red lines are from Arm A (vorinostat/ tamoxifen) and with blue lines are from Arm B (vorinostat alone).



Supplemental Figure 5. Participant level data for cell associated HIV proviral DNA. Change from baseline to the primary endpoint timepoint at 5 hours after the second dose of vorinostat on day 38 are shown for (A) Arm A with the combination of vorinostat and tamoxifen and (B) Arm B with vorinostat alone. Solid symbols are the geometric mean.



Supplemental Figure 6. 17β estradiol levels over the study period. Plasma levels of 17β estradiol levels were measured at baseline, day 28, 38, 45 and 65 in all participants and trajectories over the course of the study are shown for the combination Arm A (vorinostat + tamoxifen) and for the vorinostat alone are (Arm B, blue lines panel B).