Supplemental Materials

Methods

VRC01 serum concentration modeling

The final population pharmacokinetics model developed to describe the serum concentration data collected in the parent HVTN104 study for IV VRC01 (1) was used to estimate serum concentrations at designated time-points for participants in the current substudy. Participants' body weight, infusion time, dose levels and sampling time were accounted for in the estimation. Mean, 2.5th and 97.5th percentiles of the estimated concentrations for each participant based on 1000 Monte Carlo runs were reported.

Neutralization assay

A previously described neutralization assay (2, 3) was modified to measure neutralization of VRC01 against the sNLuc.HIV-1Bal26 and sNLucHIV-1.Du422.1 as a function of reductions in sNLuc activity in virus-infected TZM-BL cells. Briefly, about 2000 infectious units of virus were incubated in duplicate with threefold serial dilutions of VRC01 or b12 (a kind gift from Dr. Dennis Burton, The Scripps Research Institute) or with growth medium only in a total volume of 50 µl at 37°C for 1 h. To account for sNLuc activity in the Bal26 and Du422.1 viral stocks derived from 293T cell transfection supernatants, Env-deficient sNLuc.HIV-1mssD (delta env) (4) was added to the cells only control at a dilution resulting in equivalent snLuc activity as either the Du422.1 or Bal26 dose being tested. TZM-bl cell suspension (10,000 cells) in 100 µl of growth medium containing 15 µg/ml diethylaminoethyl-dextran was added to the virus-antibody conjugates. To minimize background from input virus sNLuc, the growth media was replaced after 24 h and infection levels were measured 24 h thereafter in the culture supernatant by Nano-Glo® assay (Promega), as described above. The IC_{50} was defined as the antibody concentration that reduces RLUs by 50% relative to the RLUs in virus control wells (cells + virus only) after

subtraction of background RLU (cells + delta env only) and calculated using the log(inhibitor) vs. response (four parameter) function in Prism (GraphPad).

Viral sequencing

HIV RNA was extracted from ~700 µl of previously frozen explant culture supernatant using ZR Viral RNA Kit (Zymo Research) per the manufacturer's instructions. Extracted RNA was eluted in 15 µl elution buffer.

Extracted RNA was reverse transcribed using the SuperScript III Reverse Transcriptase - Platinum Taq DNA Polymerase High Fidelity one-step RT-PCR kit (Life Technologies); 2 µl of extracted RNA was amplified using 2 µl of each 10 µM primer, env OR (5'-ACTGGTACTAGCTTGAAGCACC-3') and env OF (5'-GATCCTAGACTAGAGCCCTGG-3'), in a 50 µl total reaction volume. Each replicate was amplified in a second nested PCR reaction using 2 µl of 1st round product, 3 µl each 10 µM primer, env IR (5'-CCTTGTAAGTCATTGGTCTTAAAGGTACC-3') and env IF (5'-TATGGCAGGAAGAAGCGGAGACAG-3'), in a 50 µl total reaction volume. RT PCR reaction 1 was 50°C for 30 min, melting at 94°C (2 min), and 30 cycles of 94°C (15s), 55°C (30s),and 68°C (3.5 min), with a final extension at 68°C (10 min). PCR reaction 2 had a melting step at 94°C (2 min), followed by 30 cycles (94°C (30s), 60°C (30s), 72°C (3.5 min)), and a final extension at 72°C (10 min).

Amplicons were gel purified and diluted to 1.0 ng/µl and indexed using a single indexing strategy (Illumina Nextera XT) and normalized and pooled according to the manufacturer's instructions (Illumina). Following library preparation samples were denatured and loaded on a 600 cycle version3 reagent chemistry MiSeq flowcell (Illumina), with a 10% Phi-X (Illumina) spike in.

The resulting reads were processed using a previously published VirAmp pipeline which is a series of connected analytical methods for optimal assembly of viral genomes (5). Briefly, sequences were pre-filtered for low-quality PHRED scores (sequences with less than 20 were

filtered) and the remaining paired-end reads were initially mapped to a standard reference (Accession#DQ318211: Bal26 Env). The overlapping portions of paired-end reads were merged and consensus sequences were built using reference guided genome assembly.

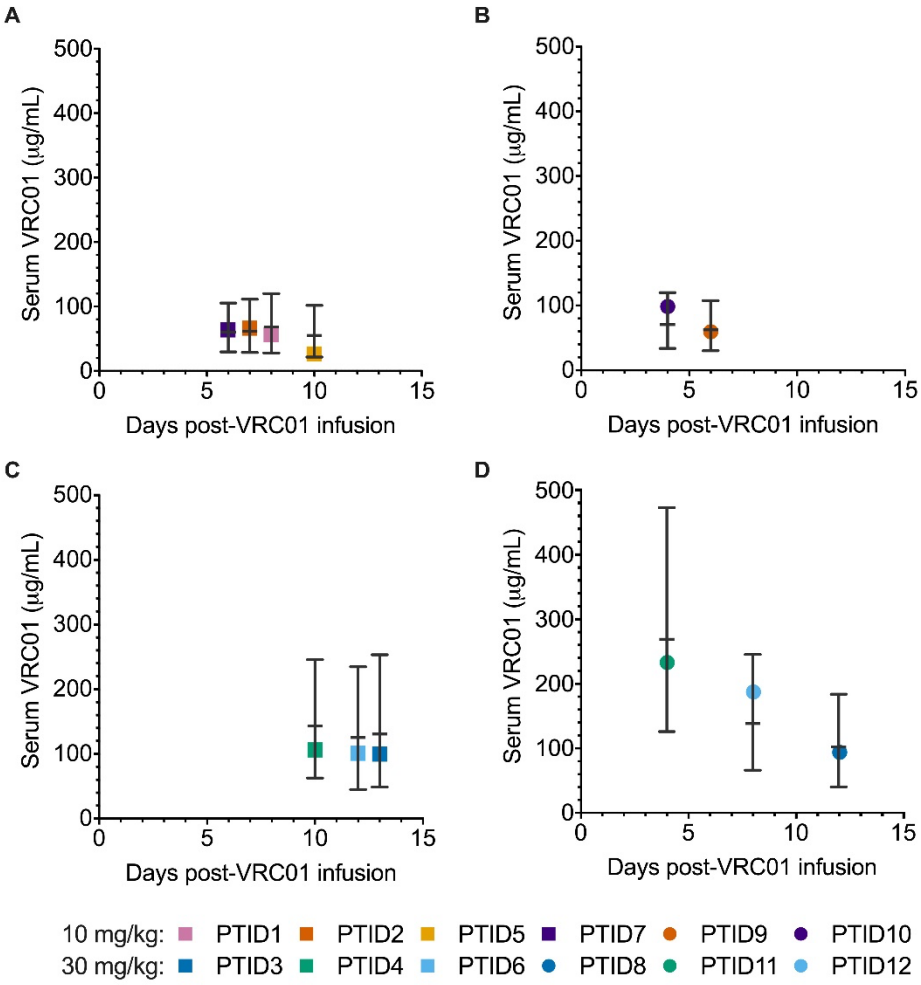
Binding Antibody Multiplex Assay (BAMA)

Serum IgG was purified as previously described (6, 7). VRC01 levels in purified IgG from serum and in mucosal secretions and tissue homogenates were assessed by BAMA using a Bioplex 200 instrument (BioRad, Hercules, CA) as previously described (8-10). VRC01 sample concentration was measured by binding to Con S gp140 (11) and RSC3 (12), supplied by the Duke Protein Production Facility (Drs. Barton Haynes and James Peacock, Duke Human Vaccine Institute). VRC01 concentration was calculated for samples meeting a positivity threshold of 100 MFI using 5-PL logistic regression from a VRC01 standard curve run on the same plate in the assay (BioPlex Manager, BioRad, Hercules, CA).

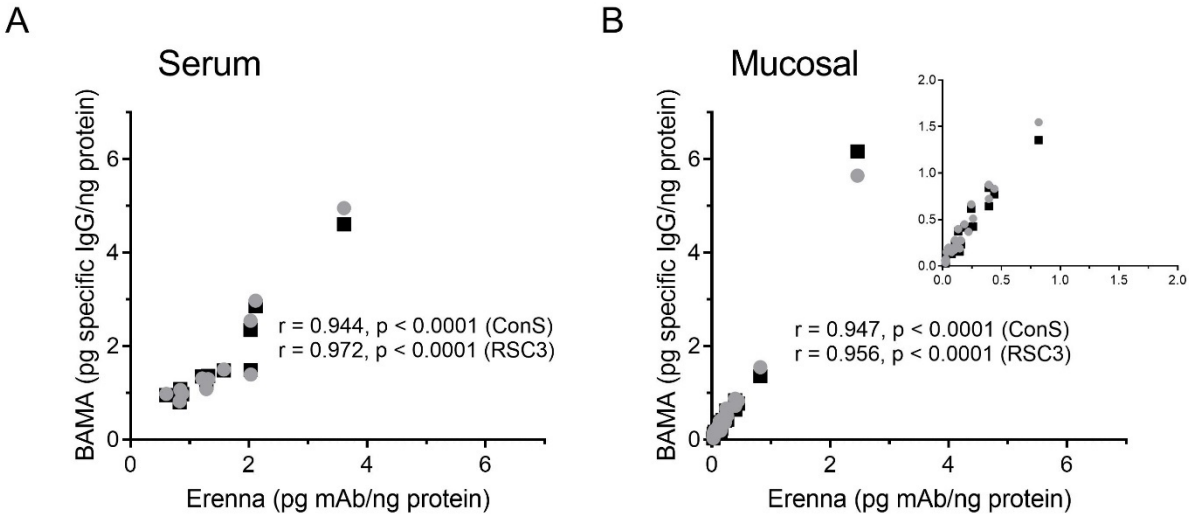
Rectal Challenges with Seminal Fluid

Semen was collected in the context of 2.5 ml of RPMI containing Pennicilin, Streptomycin and Nyastatin (all from Gibco), centrifuged at 800 × g to remove cells, and the supernatant stored frozen in the context of Protease Inhibitor Cocktail I. Before use in explants, seminal fluid from 4 HIV negative donors was dyalized for 18h at 4°C to remove protease inhibitors and pooled for addition in rectal explant assays. Differences between rectal explant conditions were calculated using Mann-Whitney tests.

Supplemental Figures and Tables



Supplemental Figure 1. Measured serum VRC01 concentrations in relation to predicted serum VRC01 ranges. Measured values (symbols) are overlayed onto predicted value ranges (lines depicting mean, 2.5% and 97.5% quartiles) for Group 4 males (a) and females (b) and Group 5 males (c) and females (d).



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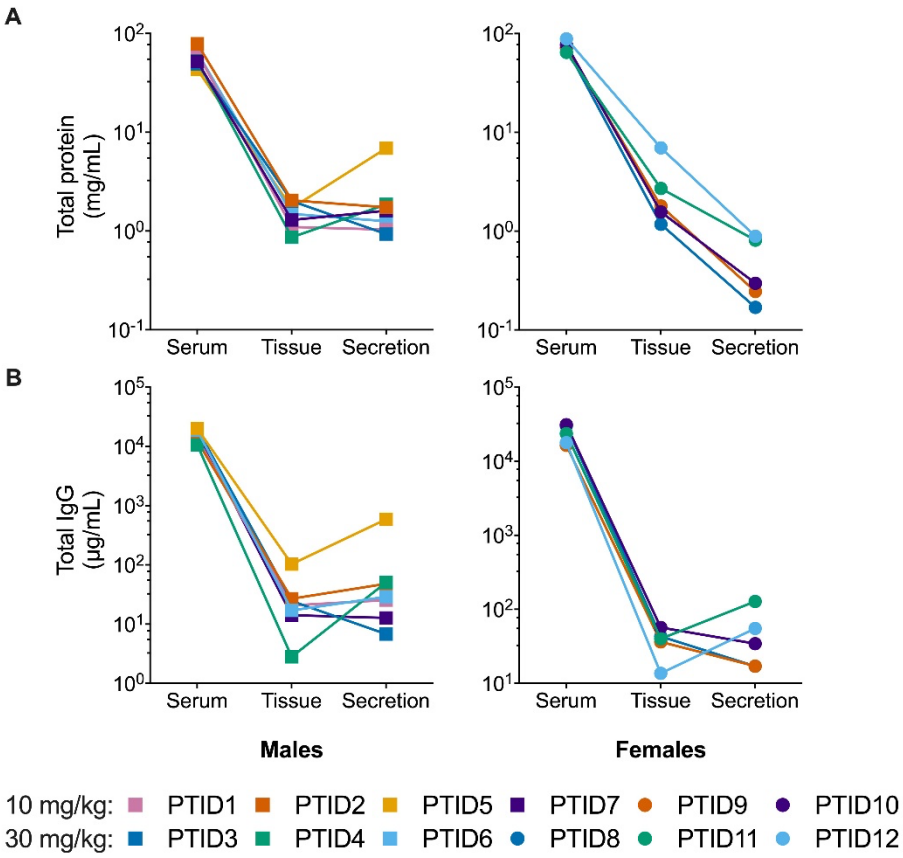
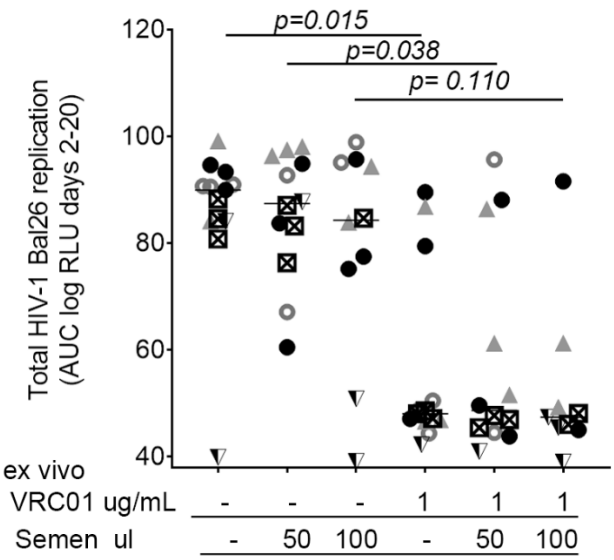
79 **Supplemental Figure 2. Comparison of VRC01 levels measured by BAMA or Erenna**
80 **assays.** Total-protein-normalized VRC01 levels measured by BAMA using Con S gp140 (gray
81 circle) or RSC3 (black squares) probes compared to those measured by Erenna using 5C9 are
82 shown for serum samples (**A**) and all mucosal secretion and tissue homogenate samples from
83 VRC01-infused participants (**B**). The inset for B enlarges the data excluding the high
84 concentration outlier. Correlations determined by Spearman's rank coefficients, n = 12 (sera)
85 and n = 24 (mucosal). With the exception of one cervical secretion sample (0.0361 pg Con S
86 gp140 specific IgG/ng protein), all samples from control participants were below the positivity
87 cutoff for both BAMA assays.



Supplemental Figure 3. Total protein (a) and total IgG (b) concentrations measured across serum, tissue homogenates and secretion eluates.



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93 **Supplemental Figure 4. No effect of seminal proteins in HIV-1Bal26 replication and**
94 **VRCO1-mediated inhibition in rectal explant challenges**

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Supplemental Table 1. Unnormalized VRC01 concentrations (ng/ml)[a] measured by Erenna.

	VRC01-infused			Control		
Sample Type[b]	N	Median	Range	N	Median	Range
Serum (all)	12	96211.6	2610.8-23255.9	11	<LLOQ	<LLOQ-1381.9[c]
Serum (male[e])	7	66177.5	2610.8-10690.4	6	<LLOQ	<LLOQ-1381.9[c]
Serum (female[e])	5	98796.9	5976.1-23255.9	5	<LLOQ	n/a
Diluted rectal secretion	7	190.7	25.6-1282.5	6	<LLOQ	<LLOQ-11.2[c]
Rectal tissue lysate	7	94.4	38.9-260.8	5	<LLOQ	n/a
Diluted cervical secretion	5	116.9	53.9-1990.6[d]	4	<LLOQ	n/a
Vaginal tissue lysate	5	171.6	139.7-422.4	5	<LLOQ	n/a

[a]Assay LLOQ is specific to each plate (see methods): median, 68.8 pg/mL, range, 33.5-134.5.

[b]To measure VRC01 concentrations, serum and processed mucosal samples were diluted 1:1000 and 1:100, respectively, in the assay. All mucosal samples were also diluted during sample processing, and therefore, the estimates are not the actual in vivo concentrations.

[c]One sample above the LLOQ.

[d]One sample above the ULOQ.

[e] Sex assigned at birth

Supplemental Table 2. VRC01 neutralization sensitivities of sNLuc HIV-1 reporter viruses used for ex vivo challenge assay measured by TZM-bl neutralization assay.

	IC_{50} (µg/ml) in TZM-bl Cells[a]	
Virus Name	**VRC01**	**b12**
vNL-SNLuc.T2A.Du422.1.ecto	>50	0.25
vNL-secNanoLuc.T2A-BAL26.ecto	0.05	n/a

[a]Values are the antibody concentration (µg/ml) at which relative luminescence units (RLUs) were reduced 50% compared to virus control wells (no test sample).

References

1. Huang Y, Zhang L, Ledgerwood J, Grunenberg N, Bailer R, Isaacs A, et al. Population pharmacokinetics analysis of VRC01, an HIV-1 broadly neutralizing monoclonal antibody, in healthy adults. *MAbs.* 2017;9(5):792-800.
2. Wu X, Parast AB, Richardson BA, Nduati R, John-Stewart G, Mbori-Ngacha D, et al. Neutralization escape variants of human immunodeficiency virus type 1 are transmitted from mother to infant. *J Virol.* 2006;80(2):835-44.
3. Montefiori DC. Measuring HIV neutralization in a luciferase reporter gene assay. *Methods Mol Biol.* 2009;485:395-405.
4. Astronomo RD, Santra S, Ballweber-Fleming L, Westerberg KG, Mach L, Hensley-McBain T, et al. Neutralization Takes Precedence Over IgG or IgA Isotype-related Functions in Mucosal HIV-1 Antibody-mediated Protection. *EBioMedicine.* 2016;14:97-111.
5. Wan Y, Renner DW, Albert I, and Szpara ML. VirAmp: a galaxy-based viral genome assembly pipeline. *Gigascience.* 2015;4:19.
6. Fouda GG, Yates NL, Pollara J, Shen X, Overman GR, Mahlokozera T, et al. HIV-specific functional antibody responses in breast milk mirror those in plasma and are primarily mediated by IgG antibodies. *J Virol.* 2011;85(18):9555-67.
7. Mansour RG, Stamper L, Jaeger F, McGuire E, Fouda G, Amos J, et al. The Presence and Anti-HIV-1 Function of Tenascin C in Breast Milk and Genital Fluids. *PLoS One.* 2016;11(5):e0155261.
8. Archary D, Seaton KE, Passmore JS, Werner L, Deal A, Dunphy LJ, et al. Distinct genital tract HIV-specific antibody profiles associated with tenofovir gel. *Mucosal Immunol.* 2016;9(3):821-33.
9. Mkhize NN, Durgiah R, Ashley V, Archary D, Garrett NJ, Karim QA, et al. Broadly neutralizing antibody specificities detected in the genital tract of HIV-1 infected women. *AIDS.* 2016;30(7):1005-14.
10. Tomaras GD, Yates NL, Liu P, Qin L, Fouda GG, Chavez LL, et al. Initial B-cell responses to transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia. *J Virol.* 2008;82(24):12449-63.
11. Liao HX, Sutherland LL, Xia SM, Brock ME, Scearce RM, Vanleeuwen S, et al. A group M consensus envelope glycoprotein induces antibodies that neutralize subsets of subtype B and C HIV-1 primary viruses. *Virology.* 2006;353(2):268-82.
12. Lynch RM, Tran L, Louder MK, Schmidt SD, Cohen M, Members CCT, et al. The development of CD4 binding site antibodies during HIV-1 infection. *J Virol.* 2012;86(14):7588-95.