Enhanced and sustained biodistribution of HIV-1 neutralizing antibody VRC01LS in human genital and rectal mucosa

Supplemental Information

Quality control and Quantitation for IgG, Total Protein and VRC01/VRC01LS levels

Exclusions for failed quality control were pre-established. All samples above the plate-specific maximum upper limit, (ULomax, the larger value of the upper limit of detection [ULOD] and ULOQ), or both below the %CV threshold (%CV of signals ≤20% for IgG and VRC01/VRC01LS levels, %CV of estimated concentration ≤21% for protein levels) and above the plate-specific minimum lower limit (LLomin, the smaller value of lower limit of detection [LLOD] and LLOQ) are excluded from analysis and rerun at dilutions that supported quantitation. When data are available from multiple runs and/or dilutions, among samples above the plate-specific LLOQ and below the %CV threshold (%CV of signals ≤ 20% for IgG and VRC01/VRC01LS levels, %CV of estimated concentration ≤ 21% for protein levels), estimated concentration levels with the smallest standard error are used for downstream analyses. If multiple results have the same standard error, the geometric mean of those tied results was reported. For total IgG levels and total protein levels, if all the runs for that sample are below the LLomin, or a sample had a result above the LLomin but its %CV is greater than the threshold (%CV of signals for IgG levels > 20%, %CV of estimated concentration for protein levels > 21), and the rerun of this sample and all the other runs are all valid (%CV not above the threshold, estimated concentration above the LLomin) except for being below the LLomin, the run with the smallest dilution factor (DF) is selected. If there are multiple records with the same DF for the study sample, to be conservative, pick the one with larger LLomin and the value is replaced by half of the dilution-adjusted LLomin determined in each run.

For VRC01/VRC01LS levels, all standard curve dilutions used for extrapolation had a recovery in the range of 80-120%. If all the runs for that sample are below the LLomin, or a sample had a

result above the LLomin but its %CV of signals was greater than the threshold, 21%, and the rerun of this sample and all the other runs are all valid (%CV not above the threshold) except for being below the LLomin, the run with the smallest dilution factor (DF) is selected. If there are multiple records with the same DF for the study sample, to be conservative, pick the one with larger LLomin and the value is replaced by half of the dilution adjusted LLomin determined in each run.

Supplementary Tables

Supplementary Table 1. Local reactogenicity 0-3 days post-infusions

Local Reactogenicity (0-3 days post-infusion)	VRC01 30 mg/kg	VRC01LS 30 mg/kg	Total
Number (n)	16	10	26
Pain[a]			
None	15 (93.8%)	10 (100.0%)	25 (96.2%)
Mild	1 (6.3%)	0 (0.0%)	1 (3.8%)
Tenderness[a]			
None	13 (81.3%)	9 (90.0%)	22 (84.6%)
Mild	3 (18.8%)	1 (10.0%)	4 (15.4%)
Pain and/or Tenderness[a]			
None	13 (81.3%)	9 (90.0%)	22 (84.6%)
Mild	3 (18.8%)	1 (10.0%)	4 (15.4%)
Erythema/Redness[b]			
None	16 (100.0%)	10 (100.0%)	26 (100.0%)
Not gradable	0 (0.0%)	0 (0.0%)	0 (0.0%)
Induration/Swelling[b]			
None	16 (100.0%)	10 (100.0%)	26 (100.0%)
Not gradable	0 (0.0%)	0 (0.0%)	0 (0.0%)
Erythema and/or Induration[b]			
None	16 (100.0%)	10 (100.0%)	26 (100.0%)
Not gradable	0(0.0%)	0(0.0%)	0 (0.0%)

[a] Moderate, Severe and Potentially Life-threatening events were 0 (0.0%) for all groups; 0 (0.0%) in total.

[b] There were no gradable local reactions in the study (Gr 1: 2.5 - <5 cm dim.; Gr 1: 6.25 - <25 cm^2 area; Gr 2: 5 - <1 0cm dim.; Gr 2: 25 - <100 cm^2 area; Gr 3: ≥ 10 cm dim.; Gr 4: ≥ 100 cm^2 area)

Supplementary Table 2. Systemic reactogenicity.

Systemic Reactogenicity (0-3 days post-infusion)	VRC01 30 mg/kg	VRC01LS 30 mg/kg	Total
Number (n)	16	10	26
Malaise and/or fatigue[a]			
None	12 (75.0%)	9 (90.0%)	21 (80.8%)
Mild	3 (18.8%)	1 (10.0%)	4 (15.4%)
Moderate	1 (6.3%)	0 (0.0%)	1 (3.8%)
Myalgia[a]			
None	15 (93.8%)	9 (90.0%)	24 (92.3%)
Mild	1 (6.3%)	1 (10.0%)	2 (7.7%)
Moderate	0 (0.0%)	0 (0.0%)	0 (0.0%)
Headache[a]			
None	15 (93.8%)	10 (100.0%)	25 (96.2%)
Mild	0 (0.0%)	0 (0.0%)	0 (0.0%)
Moderate	1 (6.3%)	0 (0.0%)	1 (3.8%)
Nausea[a]			
None	14 (87.5%)	10 (100.0%)	24 (92.3%)
Mild	2 (12.5%)	0 (0.0%)	2 (7.7%)
Moderate	0 (0.0%)	0 (0.0%)	0 (0.0%)
Vomiting[a]			
None	16 (100.0%)	10 (100.0%)	26 (100.0%)
Mild	0 (0.0%)	0 (0.0%)	0 (0.0%)
Moderate	0 (0.0%)	0 (0.0%)	0 (0.0%)
Chills[a]			
None	16 (100.0%)	10 (100.0%)	26 (100.0%)
Mild	0 (0.0%)	0 (0.0%)	0 (0.0%)
Moderate	0 (0.0%)	0 (0.0%)	0 (0.0%)
Arthralgia[a]			
None	15 (93.8%)	10 (100.0%)	25 (96.2%)
Mild	1 (6.3%)	0 (0.0%)	1 (3.8%)
Moderate	0 (0.0%)	0 (0.0%)	0 (0.0%)
Diarrhea[a]			
None	15 (93.8%)	10 (100.0%)	25 (96.2%)
Mild	1 (6.3%)	0 (0.0%)	1 (3.8%)
Moderate	0 (0.0%)	0 (0.0%)	0 (0.0%)
Max Systemic Symptoms[a]			
None	11 (68.8%)	8 (80.0%)	19 (73.1%)
Mild	4 (25.0%)	2 (20.0%)	6 (23.1%)
Moderate	1 (6.3%)	0 (0.0%)	1 (3.8%)
Temperature[b]			
No Fever<38.0°C	16 (100.0%)	10 (100.0%)	26 (100.0%)

[a]Severe and potentially life-threatening events were 0 (0%) for all groups; 0 (0%) in total.
[b]Grade 1: 38.0-<38.6°C; Grade 2: 38.6-<39.3°C; Grade 3: 39.3-<40.0°C; Grade 4: ≥40.0°C, were 0 (0.0%) for all groups; 0 (0.0%) in total.

Supplementary Table 3. Study related and unrelated adverse events.

Adverse Events			Treatment Group		Total
			VRC01 30 mg/kg	VRC01LS 30 mg/kg	
Study related AEs	Severity	Symptoms	Number of participants with AEs (% of total)		
	Mild[a]	hot flush	0 (0%)	1 (10%)	1 (3.4%)
	Moderate[a]	infusion reaction	1 (6.3%)	0 (0%)	1 (3.4%)
Study unrelated AEs[a,b]			6 (37.5%)	2 (20.0%)	8 (30.8%)
Total AEs			7 (43.8%)	3 (30.0%)	10 (38.5%)

a. There were no severe, potentially life-threatening AEs or deaths.

b. The following AEs were also assessed and determined to be not study-related: Upper respiratory tract infection, Increased blood pressure, increased blood creatinine, increased alanine aminotransferase, headache, increased aspartate aminotransferase, presyncope, viral upper respiratory tract infection, anxiety, arthropod bite, bacterial vaginosis, diarrhea, genitourinary chlamydia infection, myalgia, urinary tract infection, vulvovaginal candidiasis, abdominal pain, anorectal discomfort, arthralgia, arthropod sting, back pain, increased blood alkaline phosphatase, constipation, dermatitis, eczema, folliculitis, gastritis, decreased hemoglobin, influenza-like illness, insomnia, musculoskeletal pain, nausea, decreased neutrophil count, pain in extremity, papule, decreased platelet count, procedural pain, rash pruritic, rectal hemorrhage, rhinitis, sinusitis, subcutaneous abscess, tension headache, tinea versicolor, toothache, vaginal hemorrhage, vulvovaginal discomfort, vulvovaginal mycotic infection, vulvovaginal pruritus, decreased white blood cell count, upper abdominal pain, limb abscess, acarodermatitis, adenomyosis, anal hemorrhage, anal pruritus, ankle fracture, appendicitis, blood loss anemia, body tinea, breast mass, bronchitis, *Candida* cervicitis, *Candida* infection, cellulitis, conjunctivitis, cough, cystadenofibroma of fallopian tube, decreased appetite, dental caries, dry skin, dyschezia, dysentery, dyspepsia, ectopic pregnancy, epistaxis, fatigue, flank pain, food poisoning, gastroenteritis, norovirus gastroenteritis, gingivitis, glycosuria, hemorrhoid infection, hemorrhoids, impetigo, injury associated with device, irritable bowel syndrome, laryngitis, localized infection, lower respiratory tract infection, macule, irregular menstruation, miliaria, muscle strain, neck injury, neck pain, night sweats, oral herpes, paranasal sinus discomfort, pharyngitis, post-procedural infection, post-traumatic stress disorder, procedural dizziness, procedural nausea, proctalgia, popular rash, restless legs syndrome, skin laceration, skin ulcer, tendonitis, thrombophlebitis, tinea capitis, tinea pedis, tinnitus, transient ischemic attack, decreased vitamin D, vitreous floaters, and vulva cyst.

Supplementary Table 4. Anti-Drug Antibody testing in serum pre- and post-mAb infusion.

	VRC01 30 mg/kg Positive / Total Tested			VRC01LS 30 mg/kg Positive / Total Tested		
Time post infusion	**Tier 1**	**Tier 2**	**Tier 3**	**Tier 1**	**Tier 2**	**Tier 3**
Pre-infusion	1/16[#]	1/1[#]	0/1	0/10		
25-26 weeks	1/15[#]	0/1[#]				
51-52 weeks				0/8		

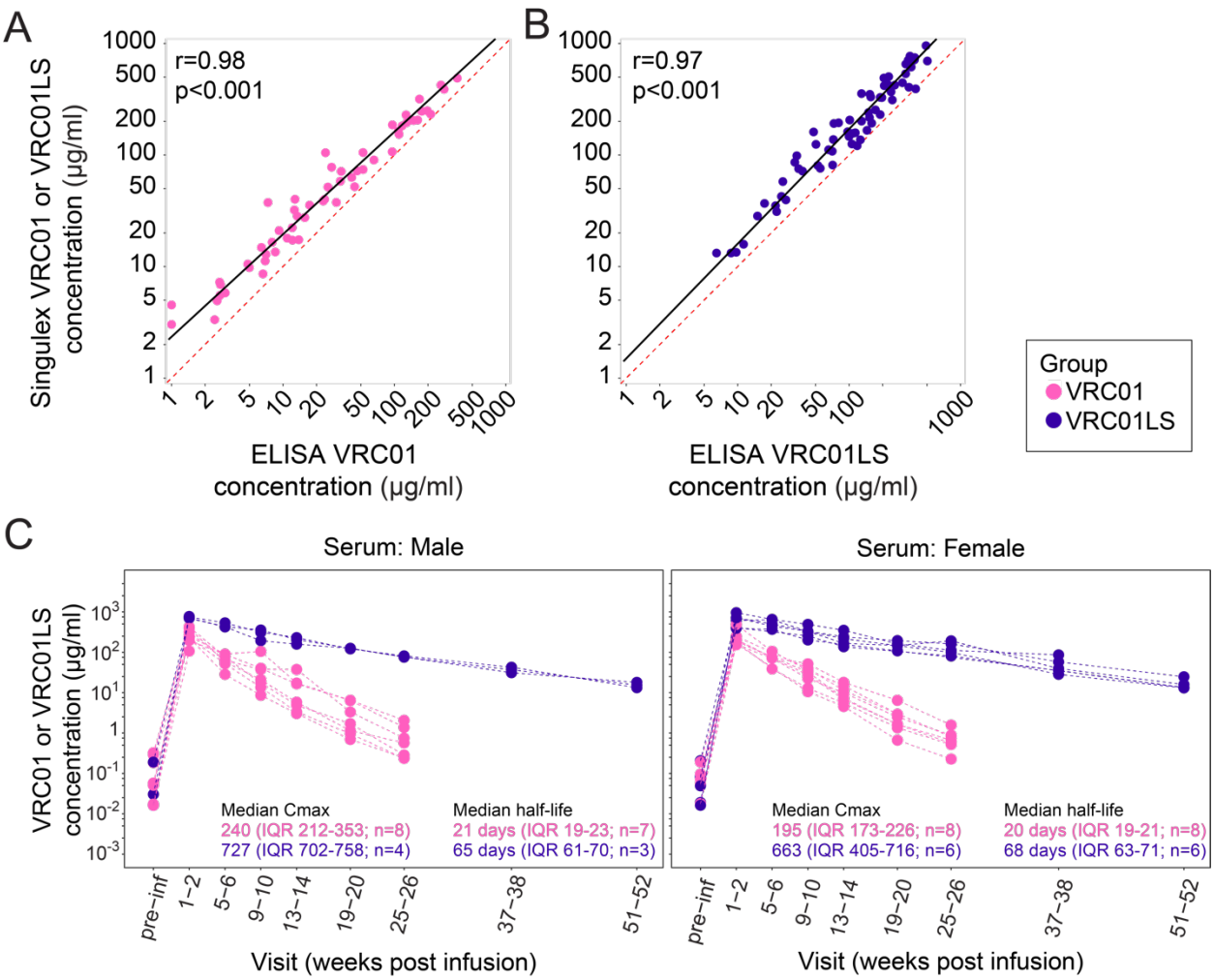
[#] All participants were screened for Tier 1 serum anti-drug antibodies. The single participant with tier 1 positive serum anti-drug antibodies pre-infusion and at 25-26 weeks was assayed for tier 2 serum antibodies. This participant had tier 2 responses at pre-infusion, but was negative for tier 3 serum antibodies. No tier 2 serum antibodies were detected at their 25-26 week visit.

Supplementary Table 5. Levels of infused mAbs at 1-2 weeks post infusion and half-life after adjustment for body weight and total protein.

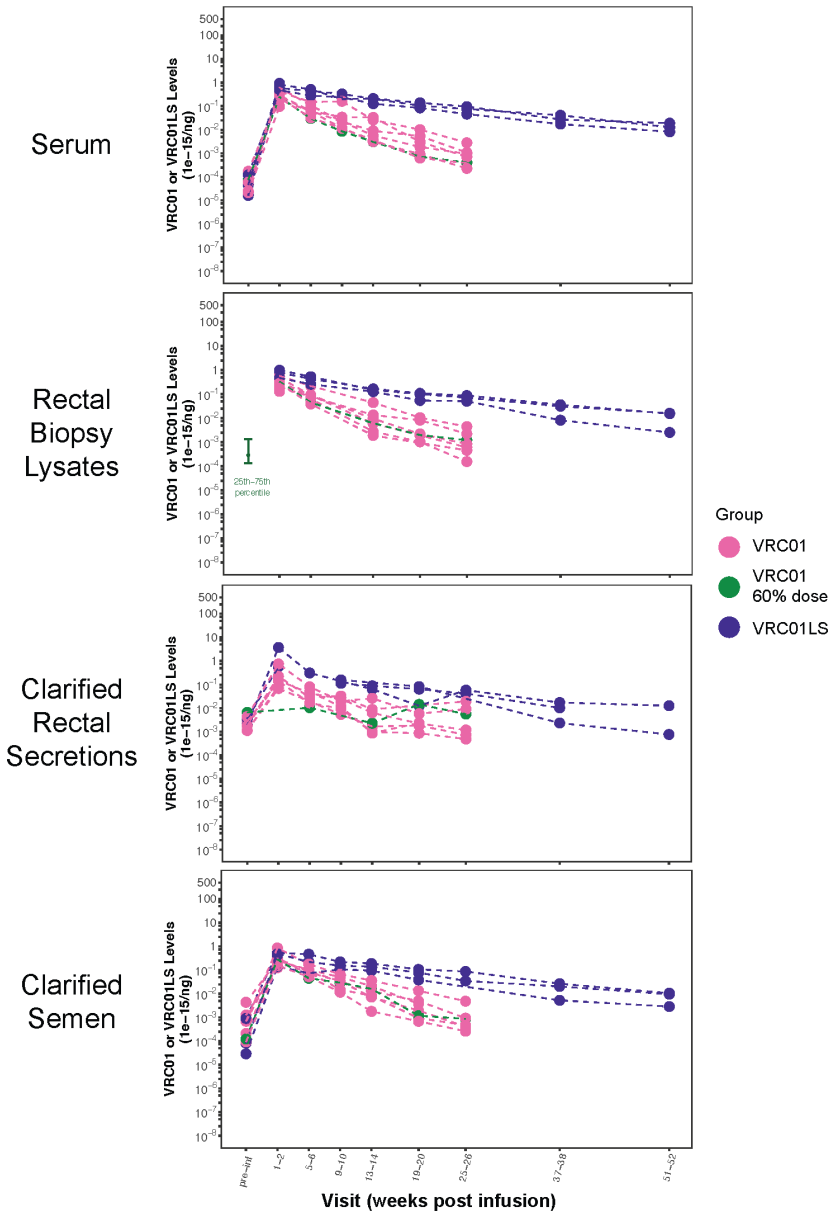
Sex assigned at birth	Male			Female		
Infusion	VRC01 30 mg/kg	VRC01LS 30 mg/kg	Adjusted p value	VRC01 30 mg/kg	VRC01LS 30 mg/kg	Adjusted p value
Blood Serum [n] 1-2 weeks Median pg of mAb/ng of protein per kg body weight (IQR)	[8] 0.036 (0.027-0.043)	[4] 0.081 (0.075-0.088)	0.012	[8] 0.034 (0.029-0.039)	[6] 0.132 (0.103-0.168)	0.003
Half-life median days **[n]** (IQR)	[7] 22 (21-23)	[3] 62 (61-64)	0.050	[8] 20 (19-21)	[6] 67 (61-70)	0.003
Rectal Tissue Lysates [n] 1-2 weeks Median pg of mAb/ng of protein per kg of body weight (IQR)	[8] 0.005 (0.004-0.007)	[4] 0.010 (0.008-0.012)	0.032	NA	NA	
Half-life median days **[n]** (IQR)	[7] 24 (21-28)	[3] 59 (52-70)	0.050	NA	NA	
Clarified Rectal Secretions [n] 1-2 weeks Median pg of mAb/ng of protein per kg of body weight (IQR)	[6] 0.0002 (0.0002-0.0005)	[2] 0.0004 (0.0000-0.0008)	NC	NA	NA	
Half-life median days **[n]** (IQR)	[7] 29 (25-43)	[2] 84 (61-107)	NC	NA	NA	
Clarified Seminal Fluid [n] 1-2 weeks Median pg of mAb/ ng of protein per kg of body weight (IQR)	[8] 0.0003 (0.0002-0.0003)	[4} 0.0005 (0.0004-0.0025)	0.032	NA	NA	
Half-life median days **[n]** (IQR)	[7] 21 (19-25)	[3] 104 (73-112)	0.050	NA	NA	
Vaginal Tissue Lysates [n] 1-2 weeks Median pg of mAb/ ng of protein per kg of body weight (IQR)	NA	NA		[8] 0.011 (0.008-0.015)	[6] 0.024 (0.013-0.034)	0.003
Half-life median days **[n]** (IQR)	NA	NA		[8] 25 (22-31)	[6] 72 (64-81)	0.003
Cervical Tissue Lysates [n] 1-2 weeks Median pg of mAb/ ng of protein per kg of body weight (IQR)	NA	NA		[8] 0.016 (0.012-0.017)	[6] 0.030 (0.029-0.037)	0.059
Half-life median days **[n]** (IQR)	NA	NA		[8] 25 (21-33)	[6] 67 (63-73)	0.003
Clarified Cervicovaginal Secretions [n] 1-2 weeks Median pg of mAb/ ng of protein per kg of body weight (IQR)	NA	NA		[5] 0.012 (0.006-0.013)	[3] 0.004 (0.002-0.005)	0.250
Half-life median days **[n]** (IQR)	NA	NA		[5] 22 (17-66)	[6] 144 (101-184)	0.030

NA, not applicable
Individual concentrations were body weight and total protein adjusted to facilitate comparisons.
NC, Not calculated because n <4
All p values are two sided Wilcoxon rank sum tests adjusted using the Holm-Bonferroni method

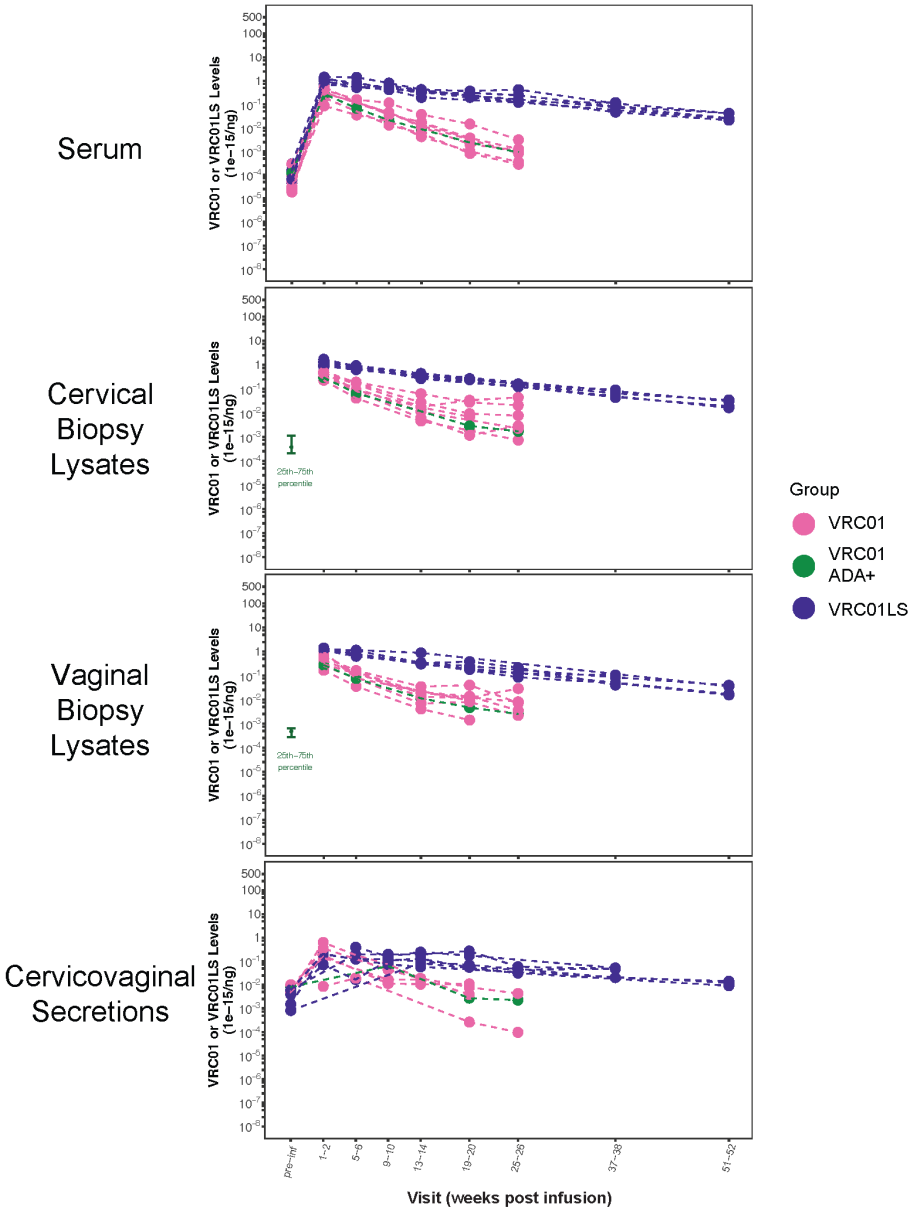
Supplementary Figures



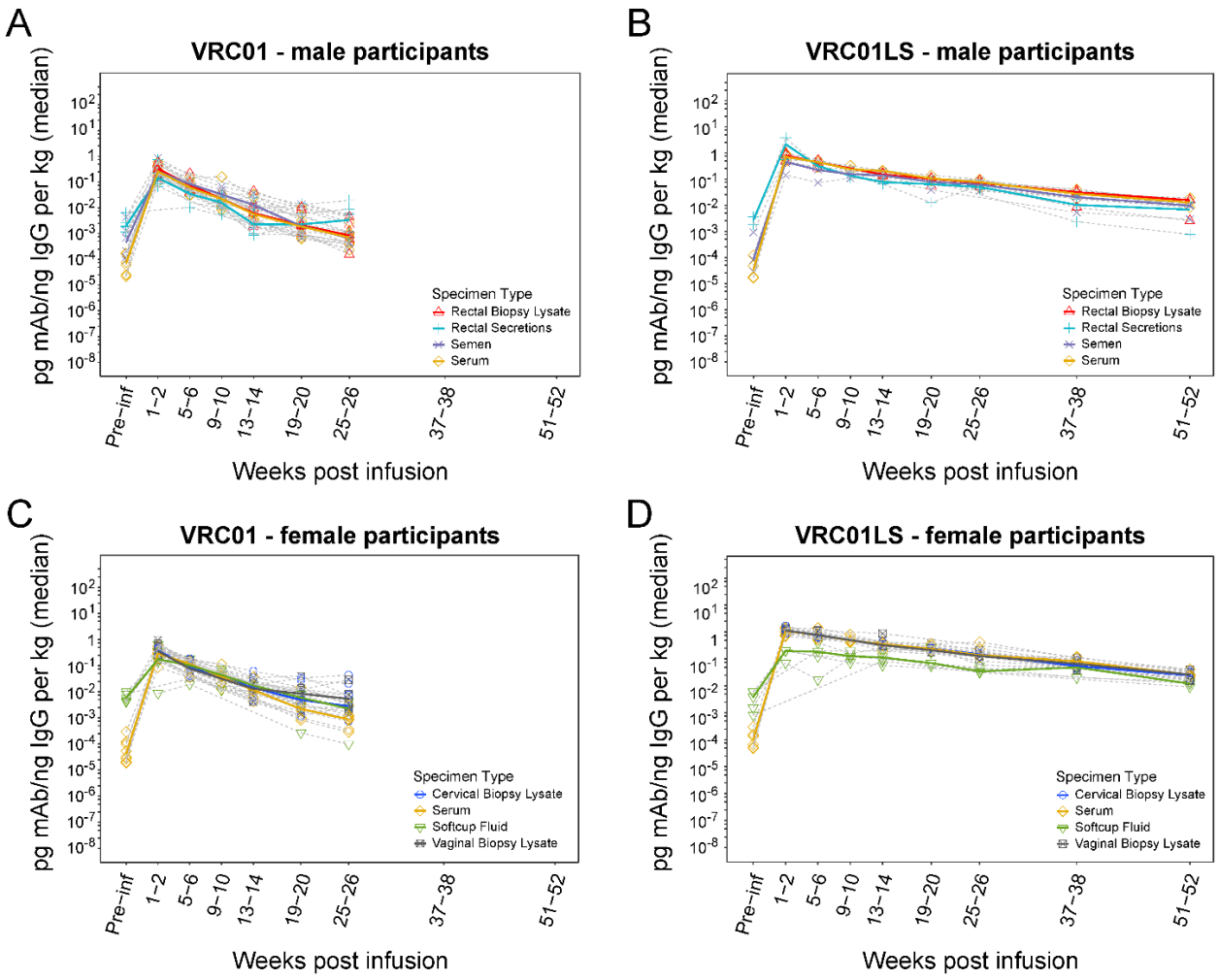
Supplementary Figure 1. Singulex-based measurements of VRC01 and VRC01LS. Correlation between Singulex and ELISA Quantitation of (A) VRC01 (n=57; pink) and (B) VRC01LS (n=63; purple). Serum samples from all timepoints were measured in parallel in the Singulex assay and a validated ELISA. Pearson correlation coefficients and p-values are depicted (upper left). Points away from red dashed lines (perfect concordance) indicate that ELISA estimates were conservative compared to Singulex. (C) Pharmacokinetic profiles of absolute serum VRC01 (pink) and VRC01LS (purple) concentrations (µg/ml) in the males and females enrolled in the study. Ns for each calculation in B) and C) are depicted in the graph.



Supplementary Figure 2. Pharmacokinetic profile of the participant who received a partial dose of VRC01. The data is identical to Figure 2, except that the participant who received a partial dose (VRC01 60% dose; green) to allow comparisons other VRC01 recipients (pink) and VRC01LS recipients (purple) who received full dose. The Y axis depicts weight-adjusted, IgG normalized VRC01 or VRC01LS; the X axis weeks post infusion. A) Serum, B) Rectal biopsy lysates, C) Clarified rectal secretions, and D) Clarified serum. The ns for each regimen of the graph are depicted in Figure 2B.

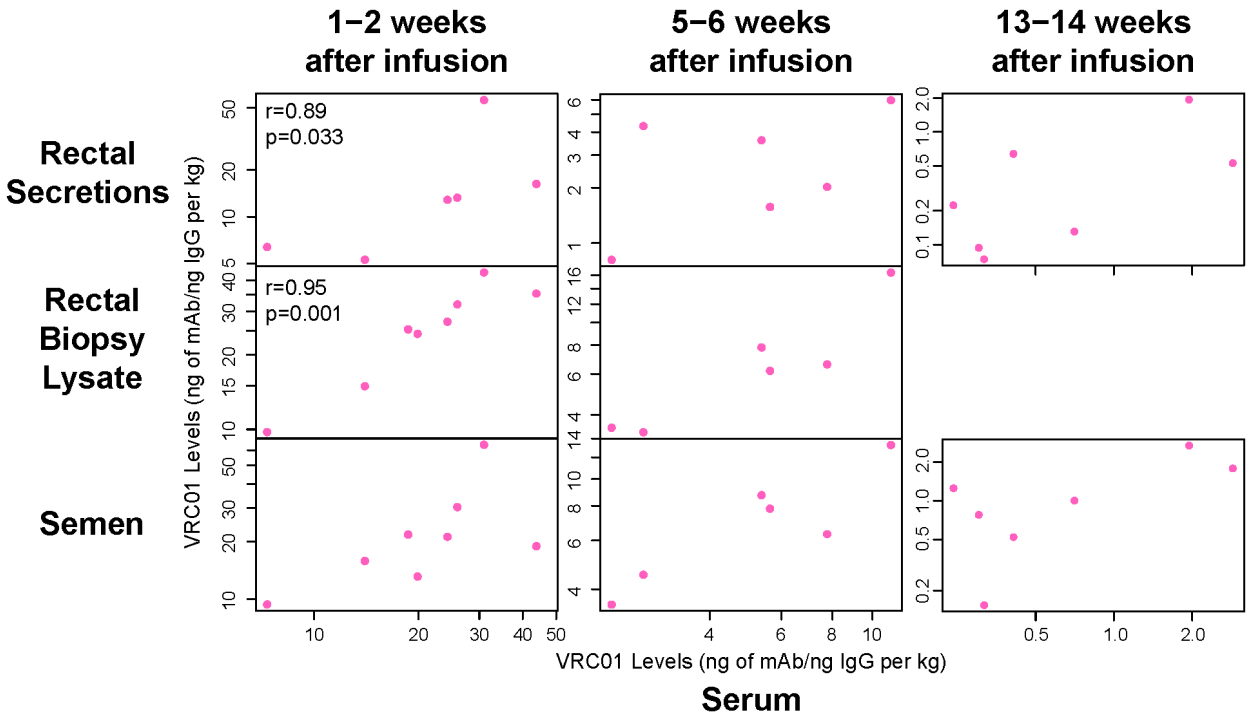


Supplementary Figure 3. Pharmacokinetic profile of the participant who had tier 2 ADA (but not tier 3) at baseline. The participant with positive ADA was color coded (VRC01 ADA+ green) to allow comparisons other VRC01 recipients (pink) and VRC01LS recipients (purple) who received full dose. The Y axis depicts weight-adjusted, IgG normalized VRC01 or VRC01LS; the X axis weeks post infusion. A) Serum, B) Cervical biopsy lysates, C) Vaginal Biopsy Lysates, and D) Clarified Cervicovaginal secretions. The ns for each regimen of the graph are depicted in Figure 2C.

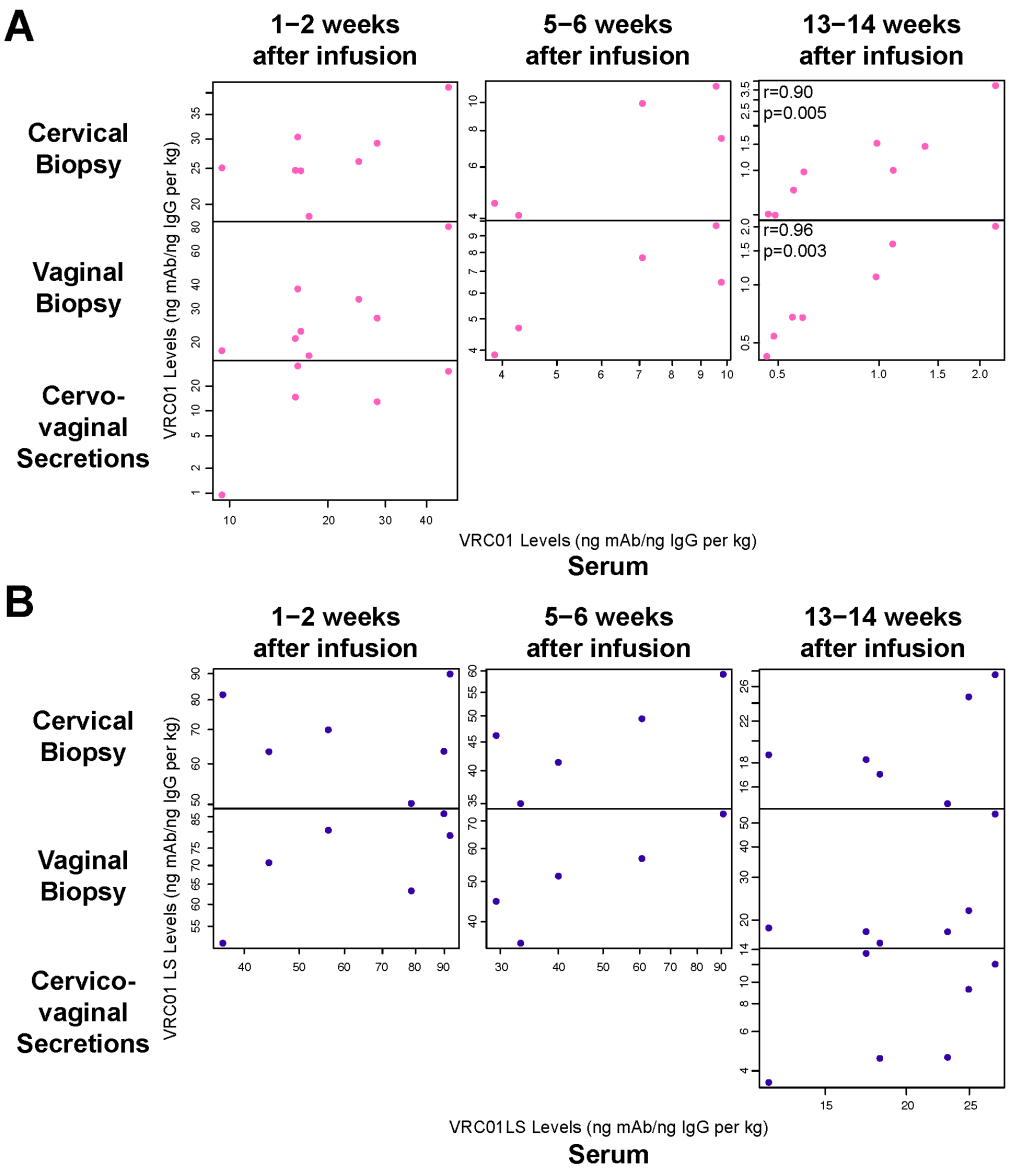


Supplementary Figure 4. IgG-normalized mAb levels of VRC01 and VRC01LS in participants who received a single IV infusion at 30 mg/kg. A, B) IgG-normalized mAb concentrations of A) VRC01 and B) VRC01LS in male participants. Blood serum (yellow diamonds), rectal tissue lysates (red triangles), clarified seminal fluid (purple asterisks) and clarified rectal secretions (blue crosses) are depicted. Gray dashed lines denote each individual participant, colored bold lines highlight the median of each group. Fourteen of the rectal secretions (n=4 males; 21.5% of collections) were excluded due to high hemoglobin levels, indicating blood contamination. Three additional rectal secretion samples did not have sufficient IgG for quantitation at 1:5 dilution, so their denominator was replaced by ½ the LLOQ of their IgG ELISA runs. C,D) IgG-normalized mAb concentrations of C) VRC01 and D) VRC01LS in female

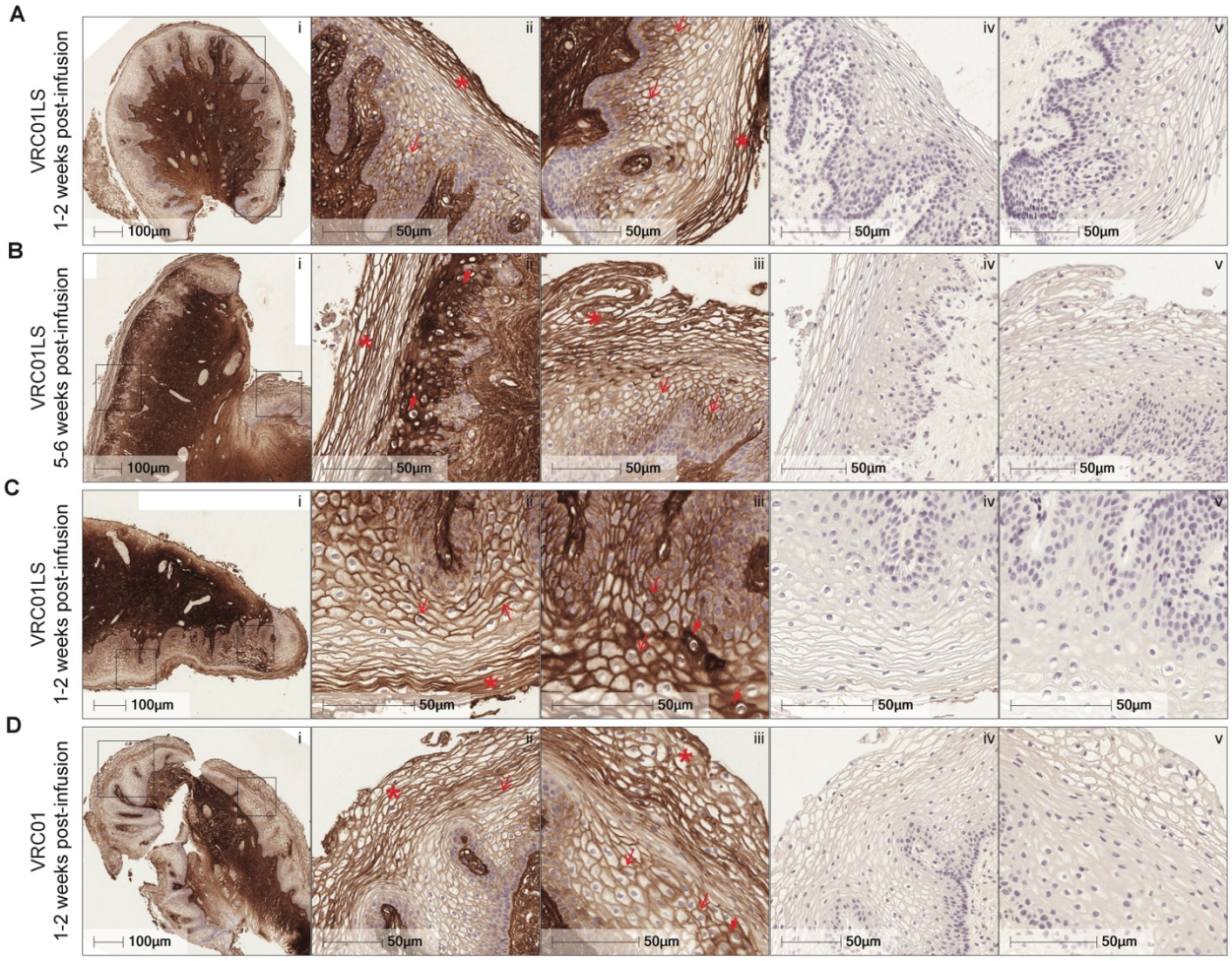
participants. Median serum (yellow diamonds), cervical tissue lysates (blue circles), vaginal tissue lysates (black squares) and cervicovaginal secretions (green triangles) are depicted. Gray dashed lines denote each individual participant, colored bold lines highlight the median of each group. Seven softcup collections (n=6 females; 8.9% of collections) did not contain sufficient fluid to assay and were not included in these estimates. An additional 7 softcups from multiple visits (n=5 females; 8.9% collections) had evidence of hemoglobin contamination and were also excluded from further analysis. The ns for each specific compartment are depicted in Figures 3C (for Supplemental Figure 4 A and B) and 3F (for Supplemental Figure 4C and 4D).



Supplementary Figure 5. Correlations of mAbs in mucosal tissue and blood in male participants IV infused with VRC01 at 30 mg/kg. IgG-normalized serum VRC01 levels (pink) correlated at 1-2 weeks with rectal tissue lysates but not correlated at 5-6 or 13-14 weeks. All VRC01 quantitation was conducted via Singulex; total IgG used ELISA. Correlations were assessed only at timepoints and in sample types with at least 5 datapoints, so sample size was insufficient for VRC01LS. The ns for each specific mucosal compartment are depicted in Figure 3C.

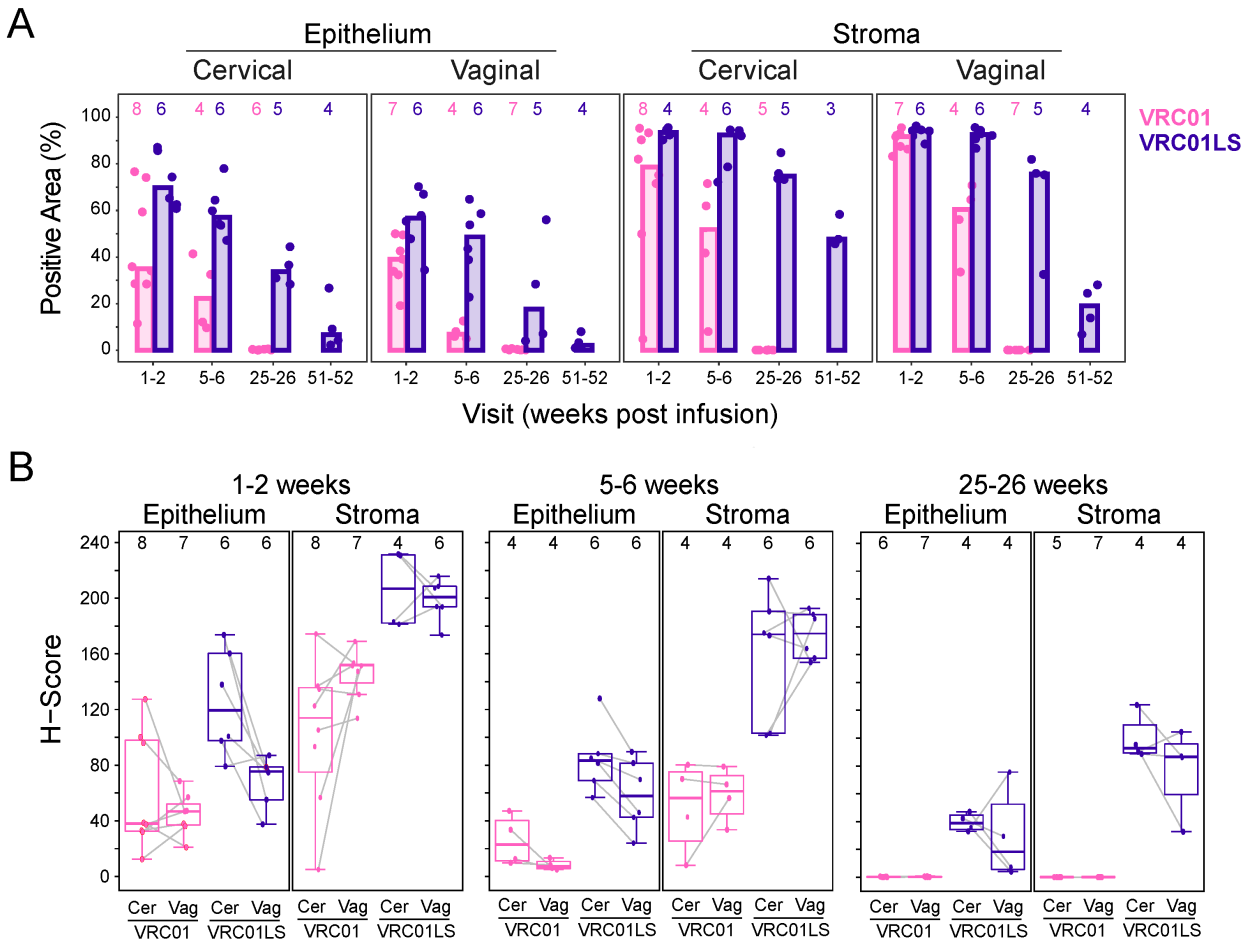


Supplementary Figure 6. Correlations of mAbs in mucosal tissue and blood in females participants IV infused with VRC01 or VRC01LS Correlations were assessed only at timepoints and in sample types with at least 5 datapoints. A) IgG-normalized serum VRC01 levels (pink) correlated at 13-14 weeks with cervicovaginal tissue lysates but were not correlated at 1-2 or 5-6 weeks. B) IgG- normalized serum VRC01LS levels (purple) did not correlate with cervicovaginal tissue lysates at 1-2, 5-6 or 13-14 weeks. All VRC01 and VRC01LS quantitation was conducted via Singulex; total IgG used ELISA. The ns for each specific mucosal compartment are depicted in Figure 3F.



Supplementary Figure 7. Microanatomical and cellular localization of VRC01 and VRC01LS in in vaginal epithelium, extension of Figure 5. Panels A-D show representative examples of mAb localization patterns in vaginal epithelium from four different study participants at 1-2 weeks (out of n=13 total evaluated) or 5-6 weeks post-infusion (out of n=10 total evaluated). From left to right, column one (i) shows overviews of the tissue section stained with 5C9 and reference frames corresponding to the higher magnification views in columns two (ii) and three (iii). Columns four (iv) and five (v) show the matched isotype controls for the same reference frames. Examples of pericellular localization and pericellular plus cytoplasmic localization, which are less common in vaginal specimens, are indicated with open and closed arrowheads, respectively, in the 5C9 higher magnification views. The stratum corneum in all samples shows prominent regions of

pericellular localization; examples are labeled with red asterisks in the higher magnification views. Intermediate layers and deeper parabasal/basal layers show variable degrees of pericellular localization. For example, panels B (ii,iii), C (ii,iii) and D (iii) show prominent pericellular localization in intermediate layers, unlike in panel A (ii,iii) where mAb localizes more in the parabasal and basal layers than the intermediate layers. Cytoplasmic (plus pericellular) localization is most evident in the intermediate layers in panel B (ii). Note, panel C shows higher magnification views of the same VRC01LS vaginal sample depicted in Figure 5C and D.



Supplementary Figure 8. Spatial distribution of VRC01 and VRC01LS into the epithelium and stroma of cervical and vaginal tissues. A) Percent area of stroma or epithelium in cervical and vaginal tissues that stained positive for VRC01 (pink) or VRC01LS (purple) by 5C9 IHC, shown over time post-infusion. B) VRC01 (pink) and VRC01LS (purple) IHC H-scores in cervical compared to vaginal tissues over time. For each mAb, paired H-scores for cervical and vaginal tissues, grouped by stroma and epithelium, are shown over time post-infusion. Individual ns at each timepoint are depicted at the top of each chart.