Science Translational Medicine
AAAS

www.sciencetranslationalmedicine.org/cgi/content/full/7/319/319ra206/DC1

Supplementary Materials for

Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection

Rebecca M. Lynch, Eli Boritz, Emily E. Coates, Adam DeZure, Patrick Madden, Pamela Costner, Mary E. Enama, Sarah Plummer, Lasonji Holman, Cynthia S. Hendel, Ingelise Gordon, Joseph Casazza, Michelle Conan-Cibotti, Stephen A. Migueles, Randall Tressler, Robert T. Bailer, Adrian McDermott, Sandeep Narpala, Sijy O'Dell, Gideon Wolf, Jeffrey D. Lifson, Brandie A. Freemire, Robert J. Gorelick, Janardan P. Pandey, Sarumathi Mohan, Nicolas Chomont, Remi Fromentin, Tae-Wook Chun, Anthony S. Fauci, Richard M. Schwartz, Richard A. Koup, Daniel C. Douek, Zonghui Hu, Edmund Capparelli, Barney S. Graham, John R. Mascola,* Julie E. Ledgerwood,* VRC 601 Study Team

*Corresponding author. E-mail: jmascola@mail.nih.gov (J.R.M.); ledgerwood@mail.nih.gov (J.E.L.)

Published 23 December 2015, *Sci. Transl. Med.* **7**, 319ra206 (2015)
DOI: 10.1126/scitranslmed.aad5752

The PDF file includes:

Supplementary Materials and Methods:

The VRC 601 Study Team: Galina Yamshchikov, Floreliz Mendoza, Jamie G. Saunders, Laura Novik, Kathy Zephir, William Whalen, Brenda Larkin, Olga Vasilenko, Sandra Sitar, Nina Berkowitz, Brandon Wilson, Iris Pittman, Gretchen Schieber, Hope Decederfelt, Judith Starling, John Gilly, Srinivas Rao, Florence Kaltovich, Phyllis Renehan, Meghan Kunchai, Sarah Romano, Katie Menard, Ly Diep, and Randall Tressler.

Anti-drug antibody assay

Antidrug antibody analysis was performed as previously described (*45*). Briefly, a Meso Scale Discovery (MSD) electrochemiluminescence (ECL) bridging assay was used to screen for the presence of VRC01 anti-idiotypic antibodies in serum. Detection of VRC01 anti-drug antibodies (ADA) was achieved by a homogenous solution phase overnight incubation of diluted serum sample along with biotinylated and SULFO-TAG labeled drug (VRC01). Any ADA present in the serum bound to biotinylated and SULFO-TAG labeled drug and formed a complex. Biotin-labeled VRC01 served as a capture molecule on to a streptavidin pre-coated MSD plate and the SUFO-TAG labeled VRC01 was the reporter used for detection.

Pharmacokinetics Analysis

Individual subject non-compartmental pharmacokinetic (PK) analysis was performed as previously described (*45*). Briefly, a population PK analysis was also performed across the dosing arms. A two-compartment model was developed using the computer program NONMEM 7.2 (ICON, Dublin) using both serum VRC01 concentrations from both HIV-uninfected subjects (*45*), n=27) and HIV- infected subjects (VRC 601, n=28). Due to the small number of subjects covariate assessment was limited to IgG1 GM allotype, baseline pre-infusion HIV virus load (virus load considered 0 for HIV-uninfected subjects and HIV-infected subjects with undetectable virus load) and dose level for their potential impact on VRC01 terminal half-life. Pharmacokinetic parameters were assumed to be proportional to body weight, which was incorporated into the population PK model before the covariate assessment. The covariate assessment included a univariate screen followed by a multivariate assessment. In the univariate screen, covariates that improved the overall goodness-of-fit of the data, as indicated by a reduction in the objective function by at least 3.84 (equivalent to $p<0.05$), were included in the multivariate analysis. The multivariate analysis was performed using a backwards elimination of the

covariates discovered in the univariate screen. Covariates resulting in an objective function change of greater 7.8 during the multivariate analysis were considered to be significant.

Subject IgG1 GM (gamma marker) Allotyping

Subjects were evaluated for the GM3/17 IgG1 allotypes to assess potential allotype-specific effects on VRC01 (GM3) pharmacokinetics as previously described (*45*). IgG1 markers GM 3 and 17 (arginine to lysine), were determined by a pre-designed TaqMan® genotyping assay from Applied Biosystems Inc., employing the following primers and probes: Forward primer: 5` CCCAGACCTACATCTGCAACGTGA-3,` Reverse primer: 5` CTGCCCTGGACTGGGACTGCAT-3, `Reporter 1 (GM 17-specific): VIC-CTCTCACCAACTTTCTTGT-NFQ, and Reporter 2 (GM 3-specific): FAM-CTCTCACCAACTCTCTTGT-NFQ, as previously described (*68*).

Maximum likelihood trees of pre-infusion and longitudinal *env* sequences

233 pre-infusion amplicons derived from SGA and 39 heterologous subtype B sequences or longitudinal SGA amplicons from pre-infusion, day 2, day 7 and day 28 or day 35 post-infusion for each subject were codon aligned with MUSCLE using the Geneious suite version (8.1.7) (http://www.geneious.com, (*67*)). Maximum likelihood trees were generated from alignments with RAxML version 8 run on the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway.

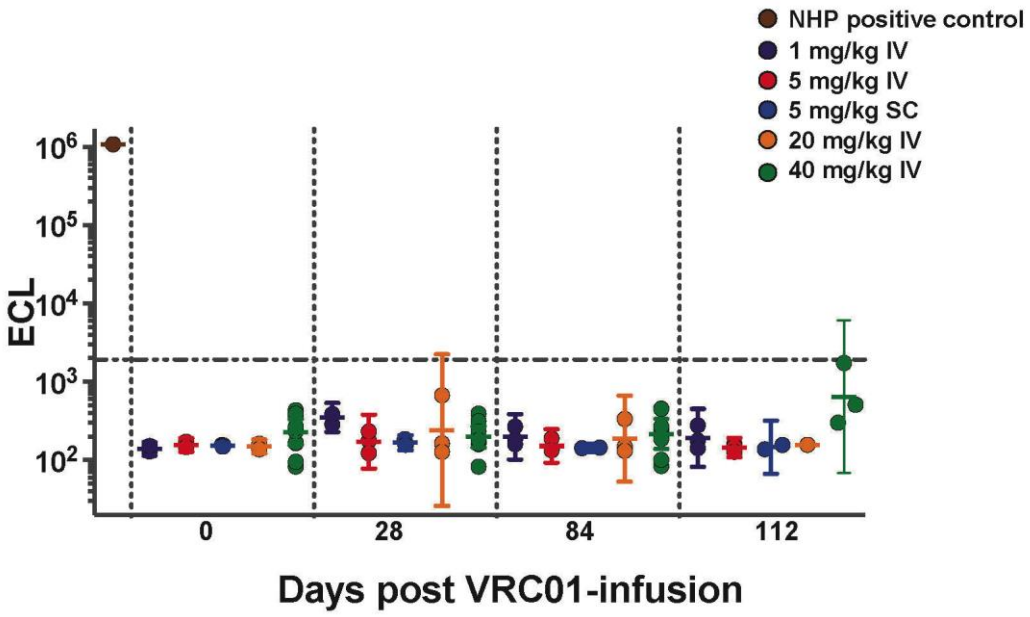

Fig. S1. Assessment of anti-VRC01 response after infusion with monoclonal VRC01. The anti-drug antibody (ADA) response is measured by homogenous bridging electrochemiluminescence (ECL) format with biotin and sulfo-tag labeled VRC01 being bound together by anti-VRC01antibodies present in the serum to create a positive signal. Sera from a non-human primate (NHP),where an anti-VRC01 antibody signal was detected 56 days after infusion with VRC01, was used as positive assay control. The horizontal line represents the upper bound of all known negative ADA responses from subjects never exposed to VRC01. Error bars indicate geometric means with 95% confidence intervals.

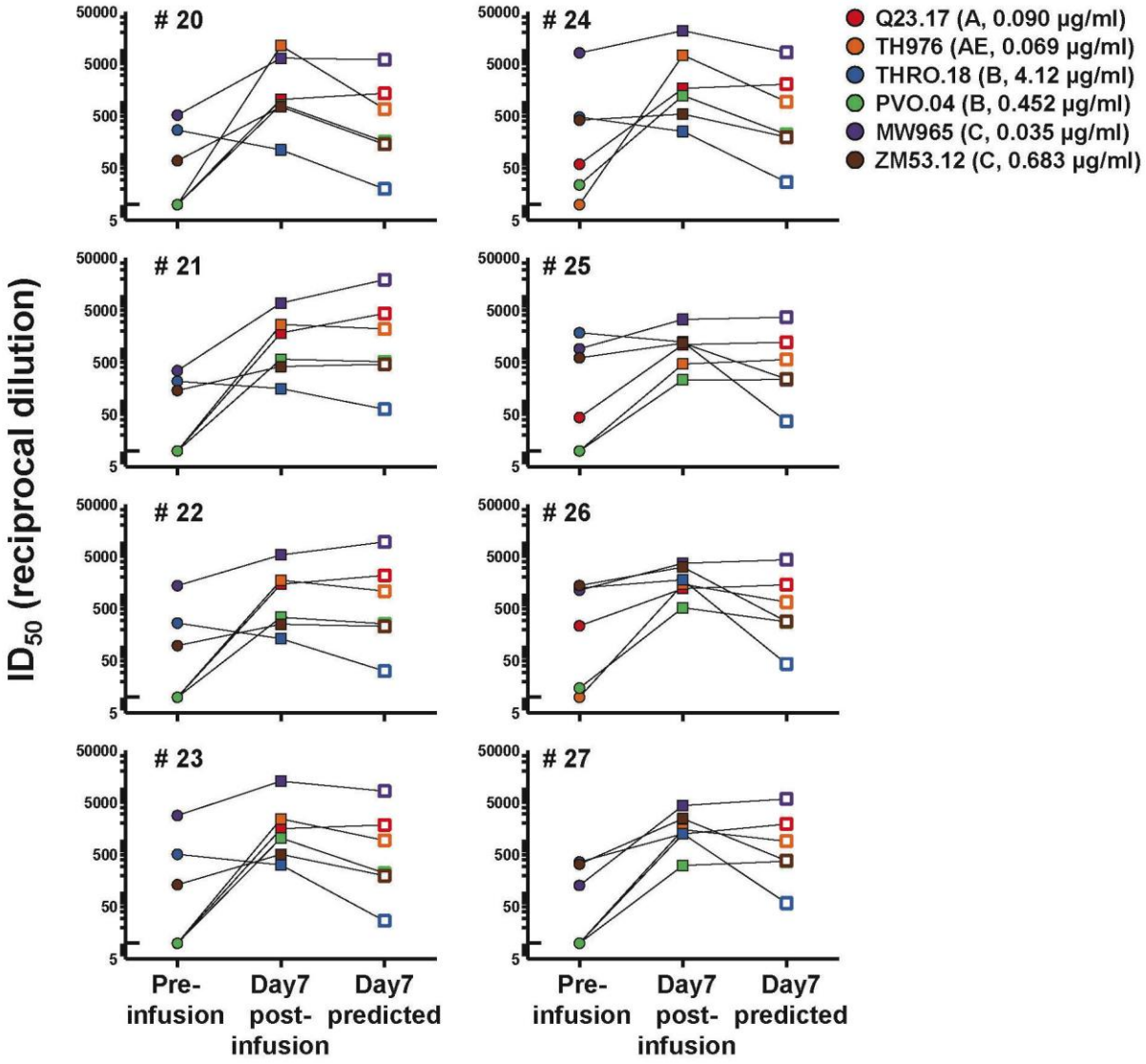

Fig. S2. Characteristics of serum neutralization after VRC01 infusion. Sera neutralization pre and post-infusion with VRC01 on a multi-subtype six virus panel is plotted by reciprocal dilution ID_{50}. The subtype and VRC01 IC_{50} of each virus is indicated in the legend. The pre-infusion serum time point is from a screening visit between 63-11 days before infusion. Limit of detection of the assay (LOD) is a dilution of 10 and indicated by a tick. The predicted ID_{50} was calculated based on the serum concentration of VRC01 for each subject on day 7 post-infusion and the indicated IC_{50} of VRC01.

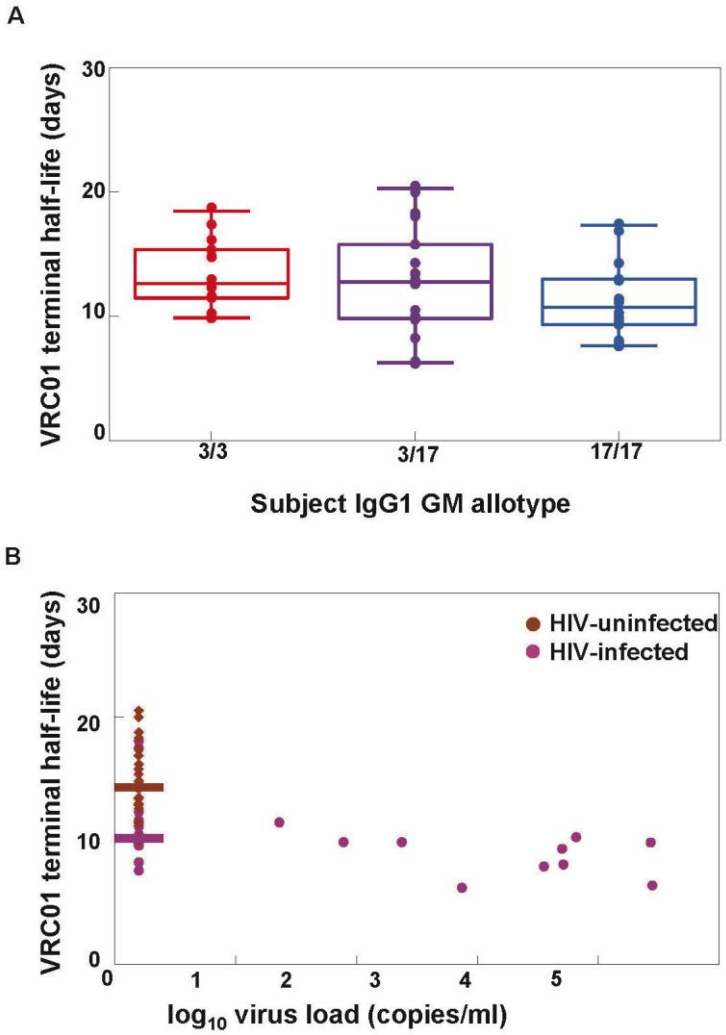

Fig. S3. Population PK analysis of VRC01 infusion. VRC01 terminal half-life was assessed for association with GM allotype (A) and baseline HIV virus load (B) of both HIV-infected (n=27) and HIV-uninfected (n=28) subjects. Box plots indicate upper and lower quartiles. Horizontal colored lines indicate medians. No statistically significant correlations were observed for either GM allotype or virus load as population model covariants.

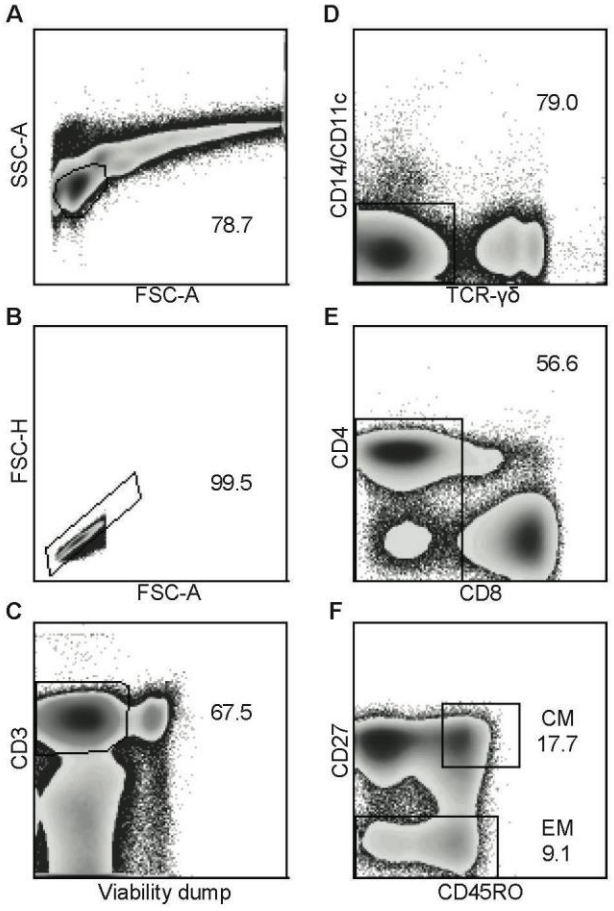

Fig. S4. Gating tree for flow cytometric sorting of cellular subsets from blood. PBMCs from ART-treated study participants were stained with a viability marker and monoclonal antibodies against cell surface proteins to allow sorting of central memory (CM) and effector memory (EM) CD4 T cell subsets by gating as above. Leukocytes (A) not part of multi-cell conjugates (B) that were viable (C) were separated according to staining for the T cell marker CD3. Viable CD3+ cells (C) not staining for myeloid markers or gamma-delta T cell receptor (D) and not staining for CD8 (E) were divided by CD27 and CD45RO expression and collected as CM (F, top gate) and EM (F, bottom gate) subsets. Numbers on plots represent percentages of plotted cells falling within the gates shown.

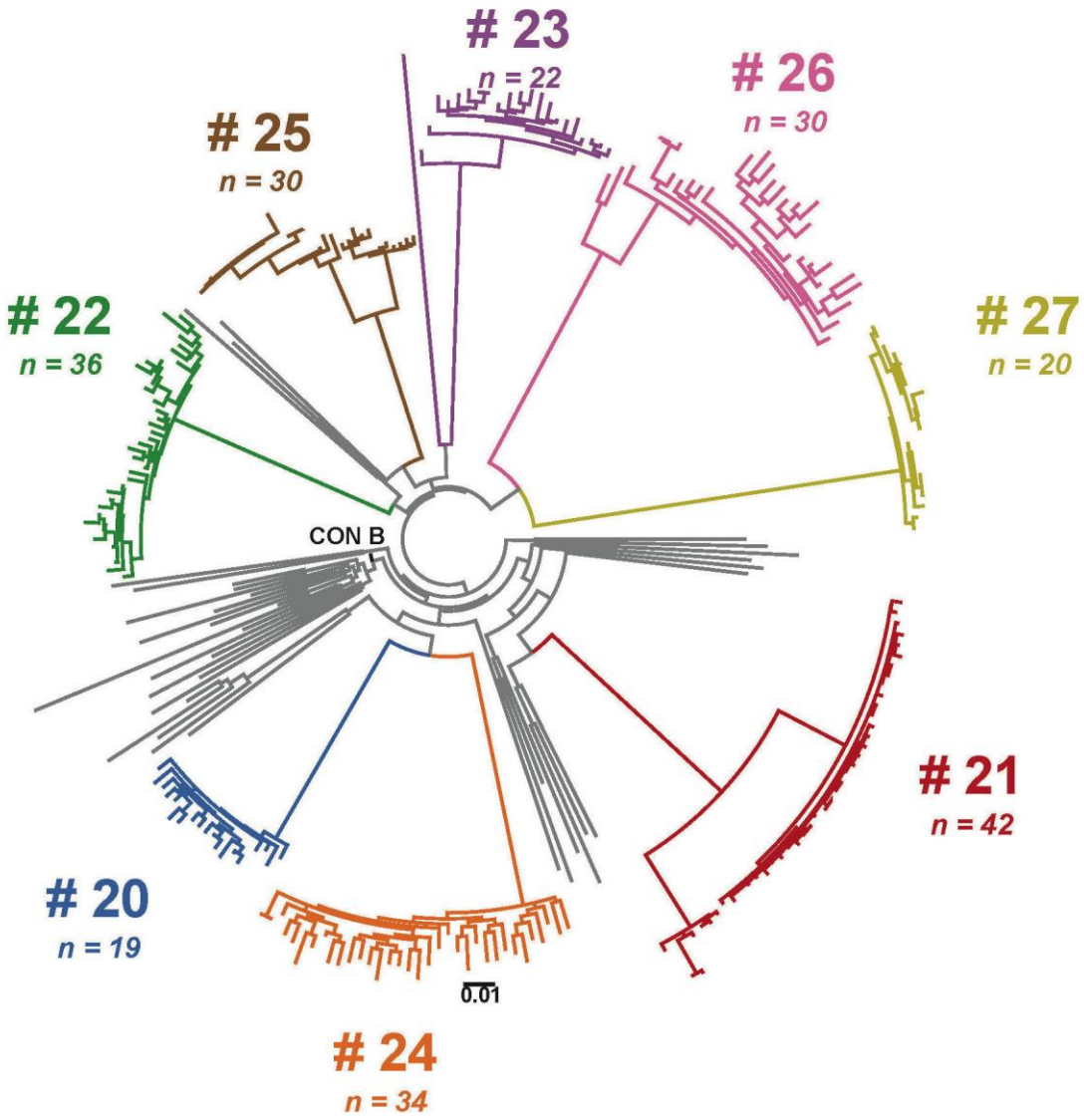

Fig. S5. Genetic diversity of preinfusion autologous virus in viremic subjects. Maximum likelihood tree of pre-infusion virus from 8 viremic subjects codon aligned to 40 heterologous subtype B viruses (gray), including consensus B virus highlighted in black. Viremic sequences are colored according to subject and number of sequences is indicated. The tree is rooted at midpoint for visualization.

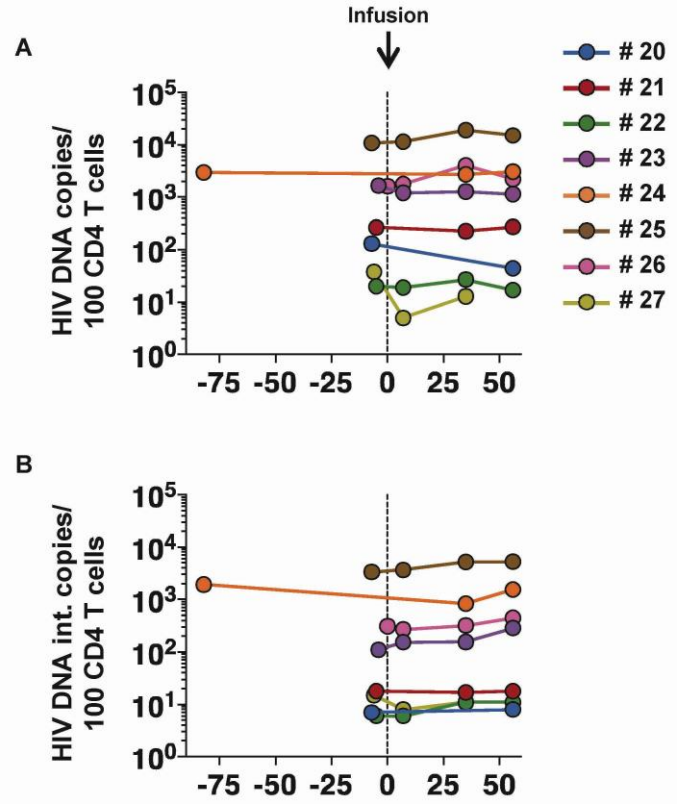

Fig. S6. The effect of VRC01 infusion on CD4 T cell–associated virus DNA levels in viremic individuals. The percentage of all CD4 T cells in each sample containing total HIV DNA (A) or integrated HIV DNA (B) was measured by qPCR (n=8).

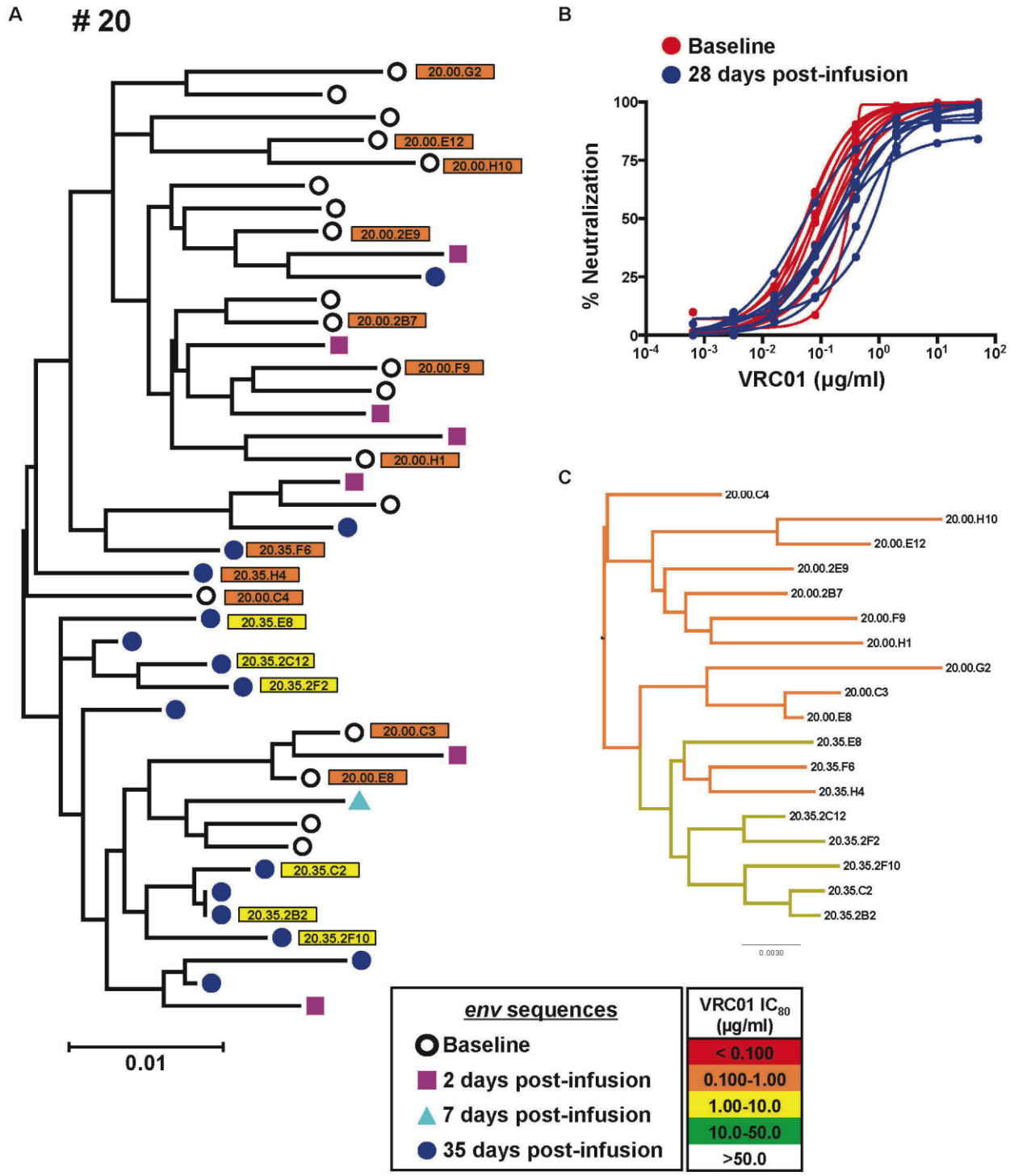

Fig. S7. Selection pressure on autologous virus from subject 20 after infusion with VRC01. (A) Longitudinal sequences of full-length *env* genes from pre and post infusion time points were amplified, aligned and used to generate a maximum likelihood tree. The tree is midpoint rooted for visualization and each colored symbol indicates an amplicon from the corresponding time point according to the legend. Amplicons that were cloned and tested for sensitivity to VRC01 in an Env-pseudovirus neutralization assay are indicated by squares with the sequence name and colored by IC_{80} sensitivity. (B) Neutralization curves of all cloned Envs from subject 20 are colored by time point and are from pre-VRC01 infusion (red; n = 10) and post-infusion (blue; n = 8). (C) Maximum likelihood tree of only cloned *envs* with branches colored according to IC_{80} as indicated in the legend.

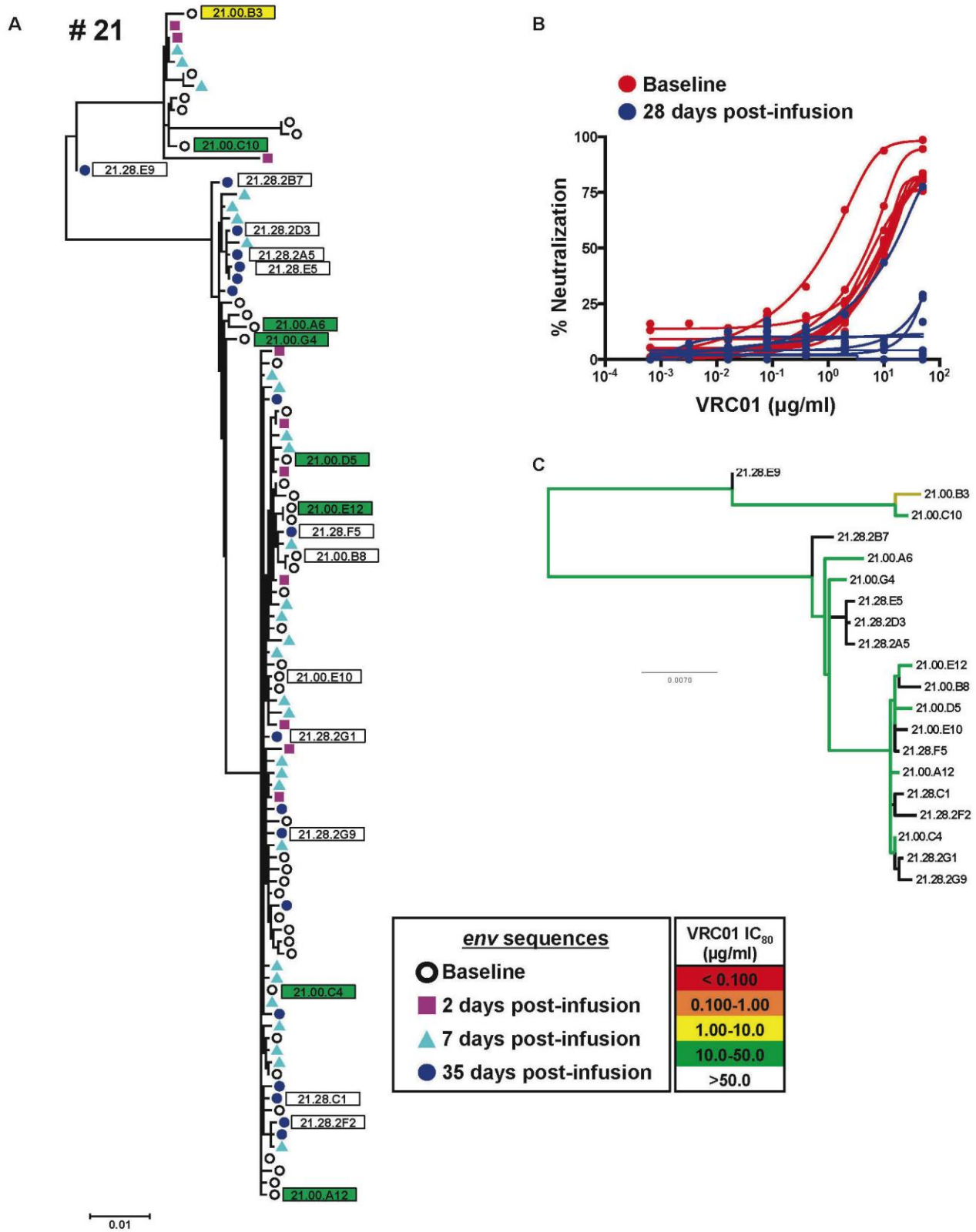

Fig. S8. Selection pressure on autologous virus from subject 21 after infusion with VRC01. (A) Longitudinal sequences of full-length *env* genes from pre and post infusion time points were amplified, aligned and used to generate a maximum likelihood tree. The tree is midpoint rooted for visualization and each colored symbol indicates an amplicon from the corresponding time point according to the legend. Amplicons that were cloned and tested for sensitivity to VRC01 in an Env-pseudovirus neutralization assay are indicated by squares with the sequence name and colored by IC_{80} sensitivity. (B) Neutralization curves of all cloned Envs from subject 21 are colored by time point and are from pre-VRC01 infusion (red; n = 10) and post-infusion (blue; n = 10). (C) Maximum likelihood tree of only cloned *envs* colored according to IC_{80} as indicated in the legend.

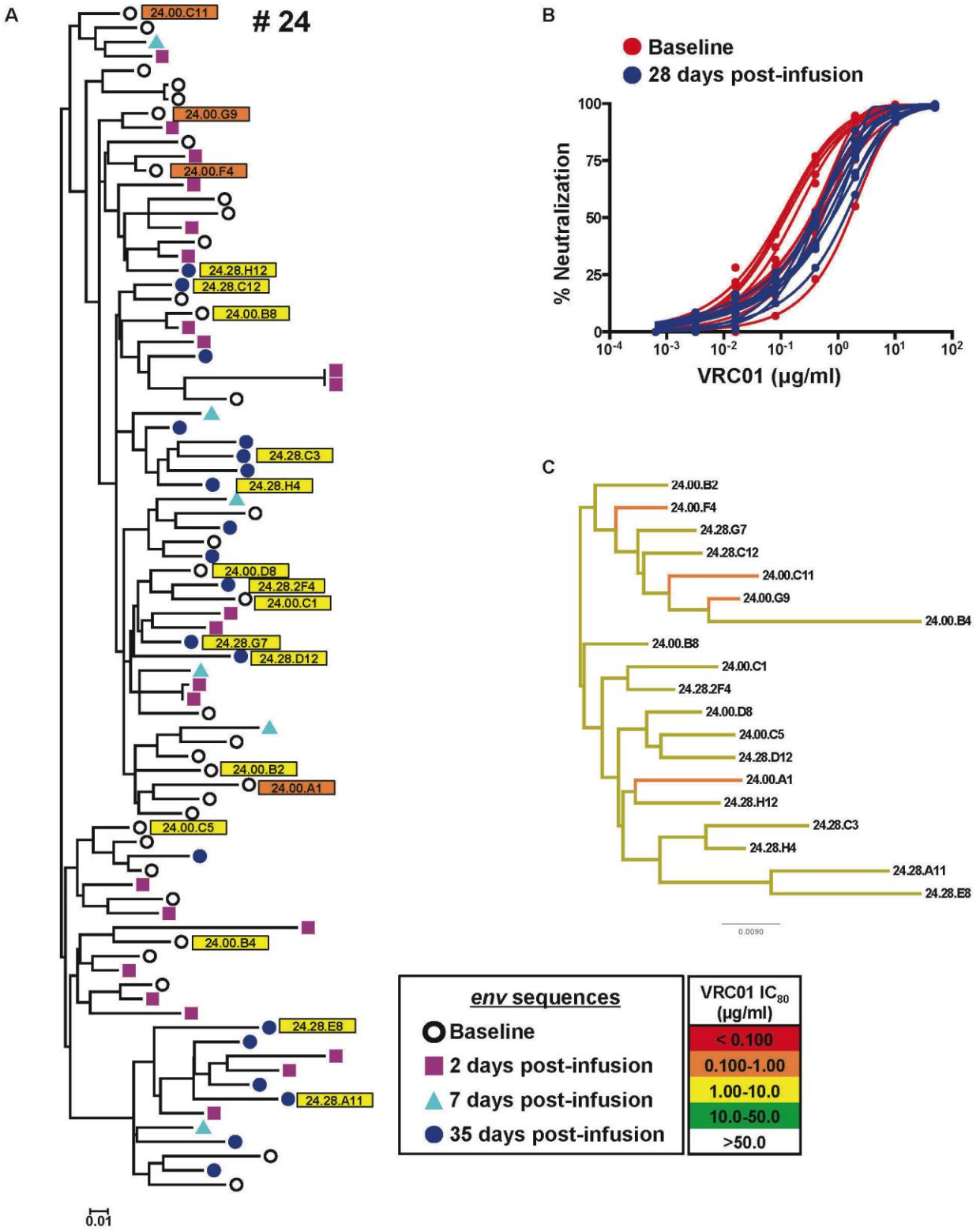

Fig. S9. Selection pressure on autologous virus from subject 24 after infusion with VRC01. (A) Longitudinal sequences of full-length *env* genes from pre and post infusion time points were amplified, aligned and used to generate a maximum likelihood tree. The tree is midpoint rooted for visualization and each colored symbol indicates an amplicon from the corresponding time point according to the legend. Amplicons that were cloned and tested for sensitivity to VRC01 in an Env-pseudovirus neutralization assay are indicated by squares with the sequence name and colored by IC_{80} sensitivity. (B) Neutralization curves of all cloned Envs from subject 24 are colored by time point and are from pre-VRC01 infusion (red; n = 10) and post-infusion (blue; n = 9). (C) Maximum likelihood tree of only cloned *envs* with branches colored according to IC_{80} as indicated in the legend.

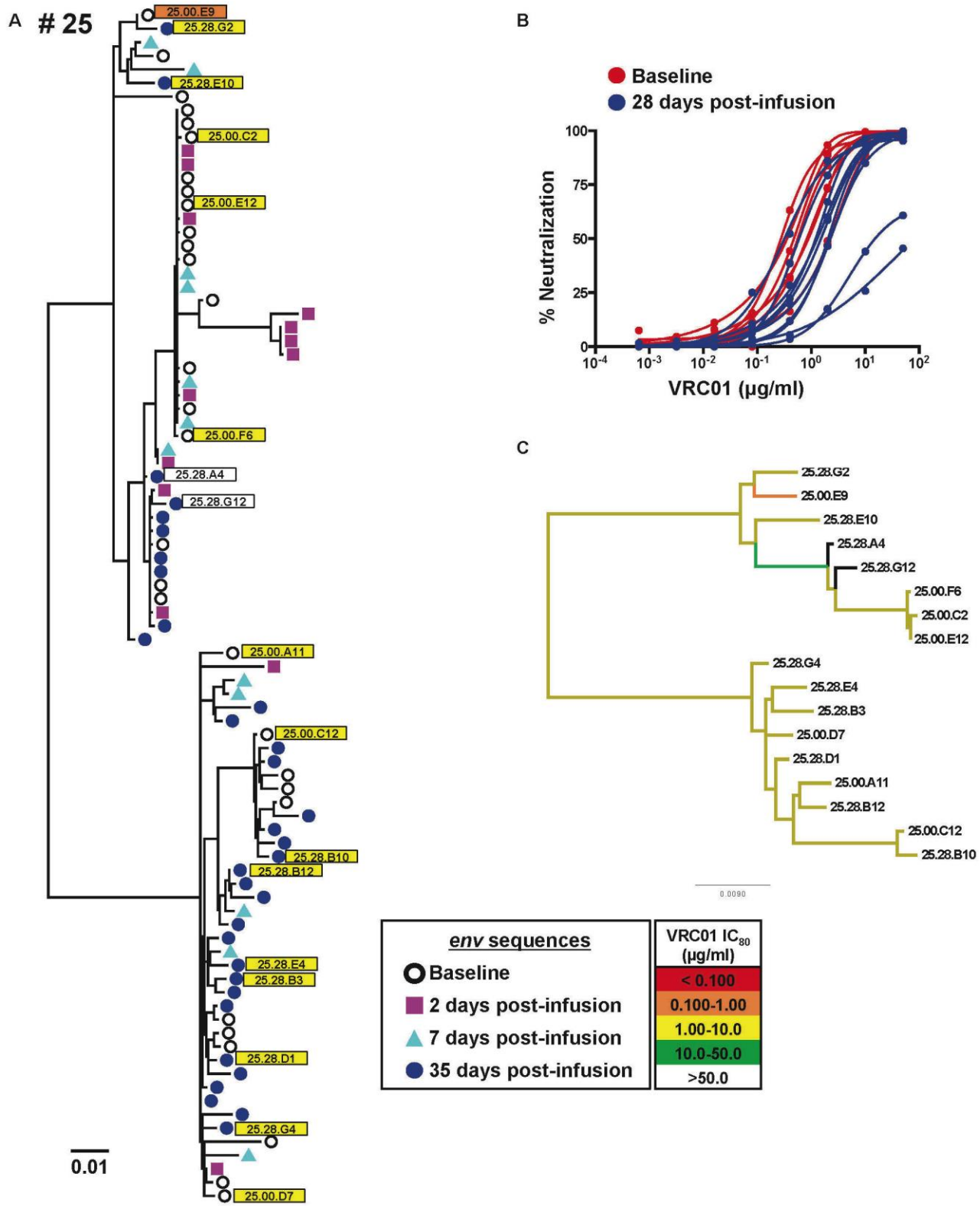

Fig. S10. Selection pressure on autologous virus from subject 25 after infusion with VRC01. (A) Longitudinal sequences of full-length *env* genes from pre and post infusion time points were amplified, aligned and used to generate a maximum likelihood tree. The tree is midpoint rooted for visualization and each colored symbol indicates an amplicon from the corresponding time point according to the legend. Amplicons that were cloned and tested for sensitivity to VRC01 in an Env-pseudovirus neutralization assay are indicated by squares with the sequence name and colored by IC_{80} sensitivity. (B) Neutralization curves of all cloned Envs from subject 25 are colored by time point and are from pre-VRC01 infusion (red; n = 7) and post-infusion (blue; n = 10). (C) Maximum likelihood tree of only cloned *envs* with branches colored according to IC_{80} as indicated in the legend.

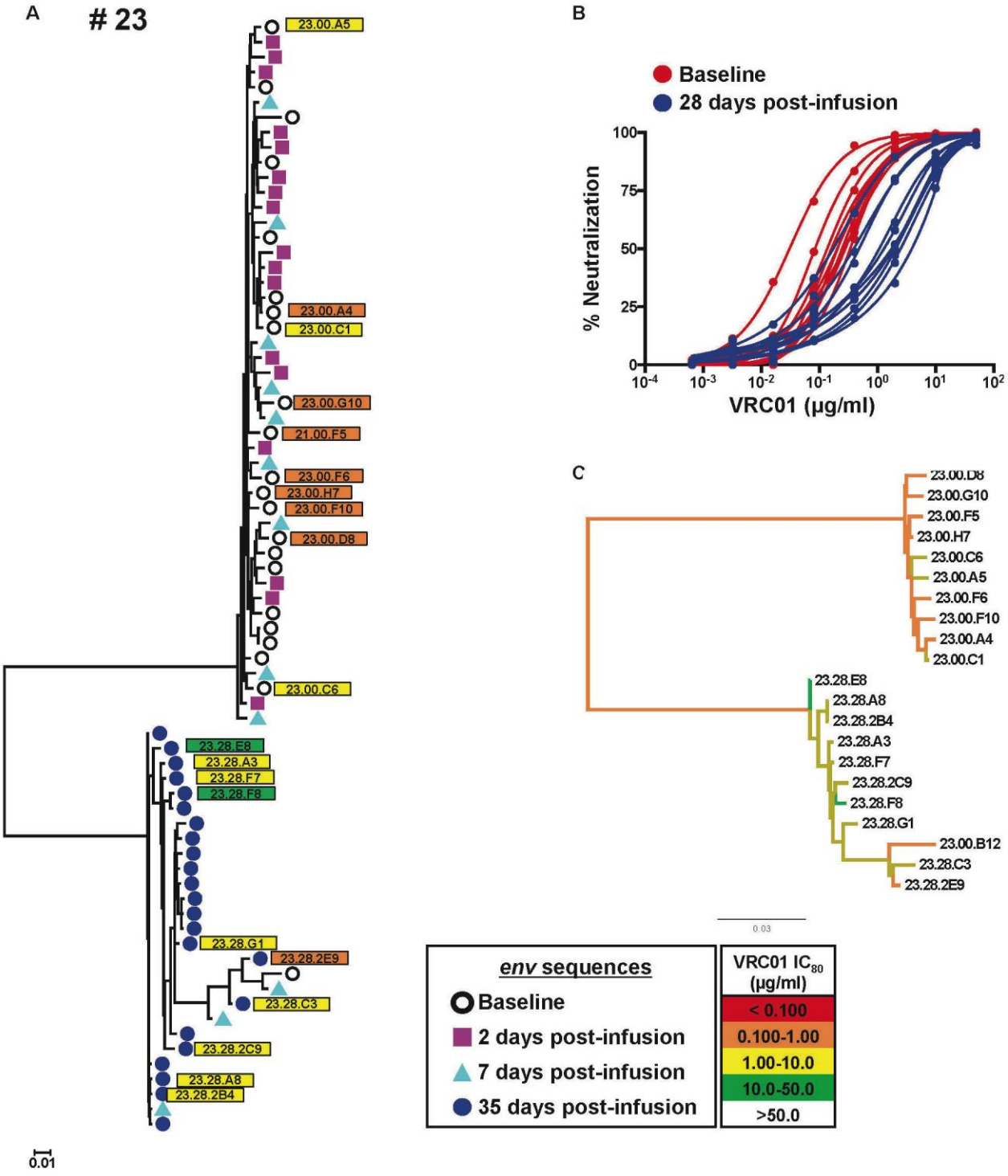

Fig. S11. Selection pressure on autologous virus from subject 23 after infusion with VRC01. (A) Longitudinal sequences of full-length *env* genes from pre and post infusion time points were amplified, aligned and used to generate a maximum likelihood tree. The tree is midpoint rooted for visualization and each colored symbol indicates an amplicon from the corresponding time point according to the legend. Amplicons that were cloned and tested for sensitivity to VRC01 in an Env-pseudovirus neutralization assay are indicated by squares with the sequence name and colored by IC_{80} sensitivity. (B) Neutralization curves of all cloned Envs from subject 23 are colored by time point and are from pre-VRC01 infusion (red; n = 10) and post-infusion (blue; n = 10). (C) Maximum likelihood tree of only cloned *envs* colored according to IC_{80} as indicated in the legend.

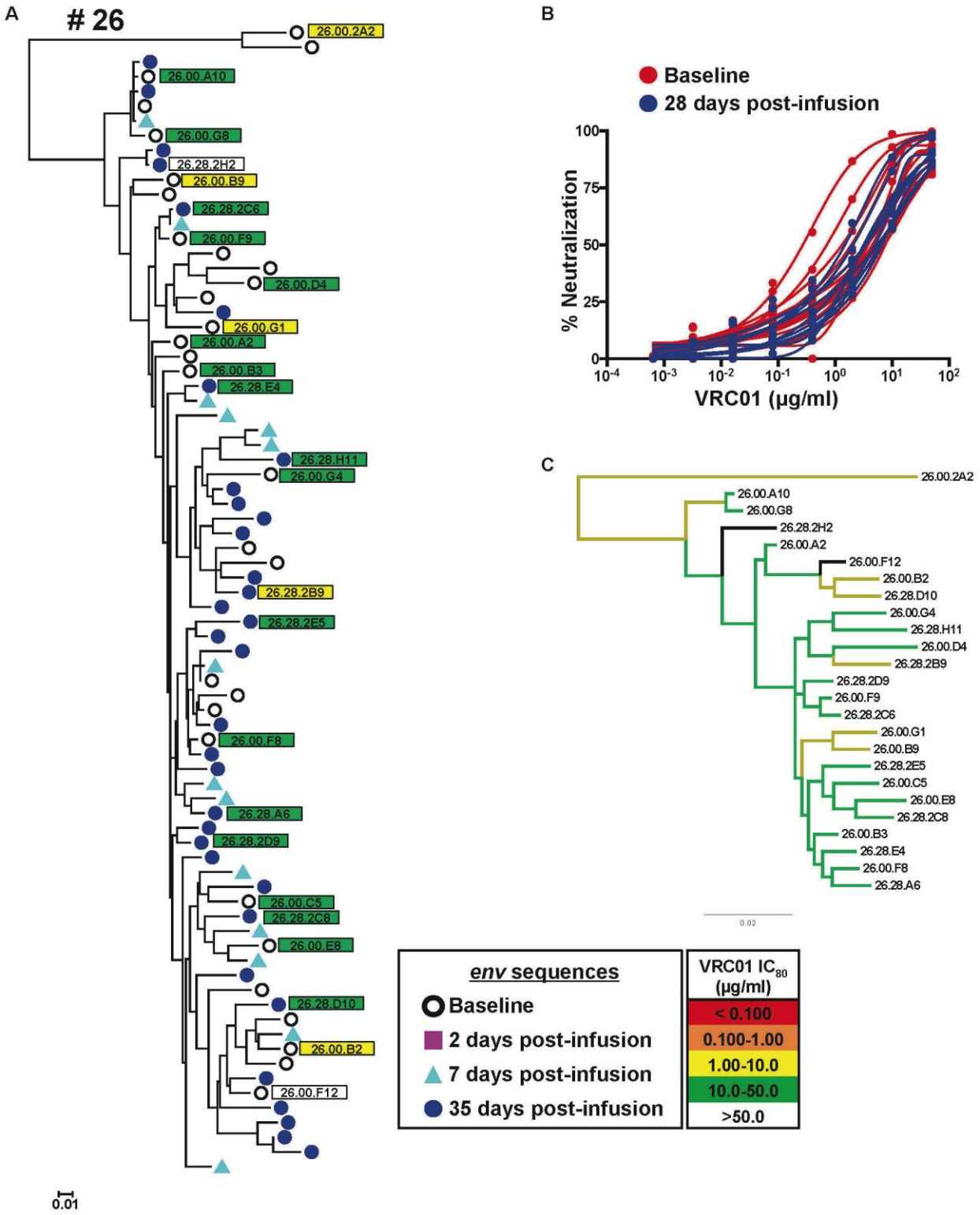

Fig. S12. Selection pressure on autologous virus from subject 26 after infusion with VRC01. (A) Longitudinal sequences of full-length *env* genes from pre and post infusion time points were amplified, aligned and used to generate a maximum likelihood tree. The tree is midpoint rooted for visualization and each colored symbol indicates an amplicon from the corresponding time point according to the legend. Amplicons that were cloned and tested for sensitivity to VRC01 in an Env-pseudovirus neutralization assay are indicated by squares with the sequence name and colored by IC_{80} sensitivity. (B) Neutralization curves of all cloned Envs from subject 26 are colored by time point and are from pre-VRC01 infusion (red; n = 15) and post-infusion (blue; n = 10). (C) Maximum likelihood tree of cloned *envs* colored according to IC_{80} as indicated in the legend.

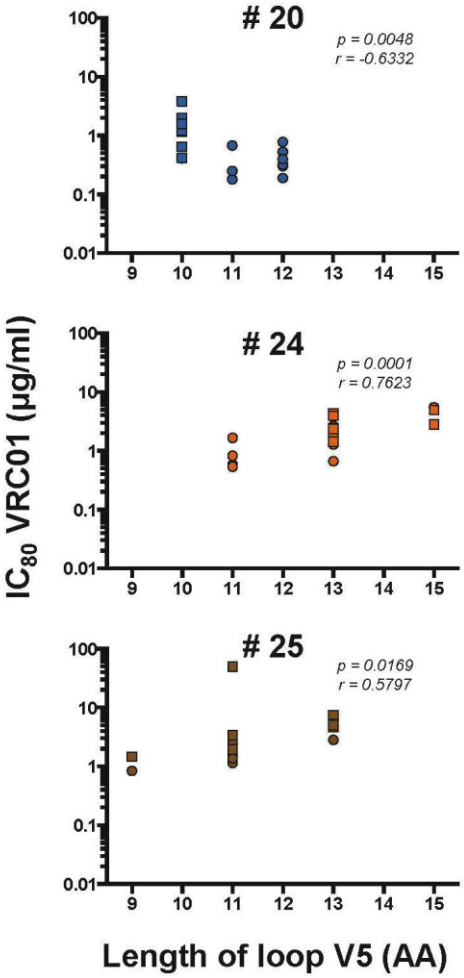

Fig. S13. Correlation of virus neutralization sensitivity to VRC01 and V5 loop length. VRC01 IC_{80}s for autologous Envs cloned from baseline and 1 month post-infusion were correlated to the length of their V5 loop for three subjects whose V5 length changed post-infusion (subjects 20, 24 and 25). Pre-infusion clones are indicated by circles and post-infusion clones by squares. Spearman coefficient r and p-value for each correlation are indicated.

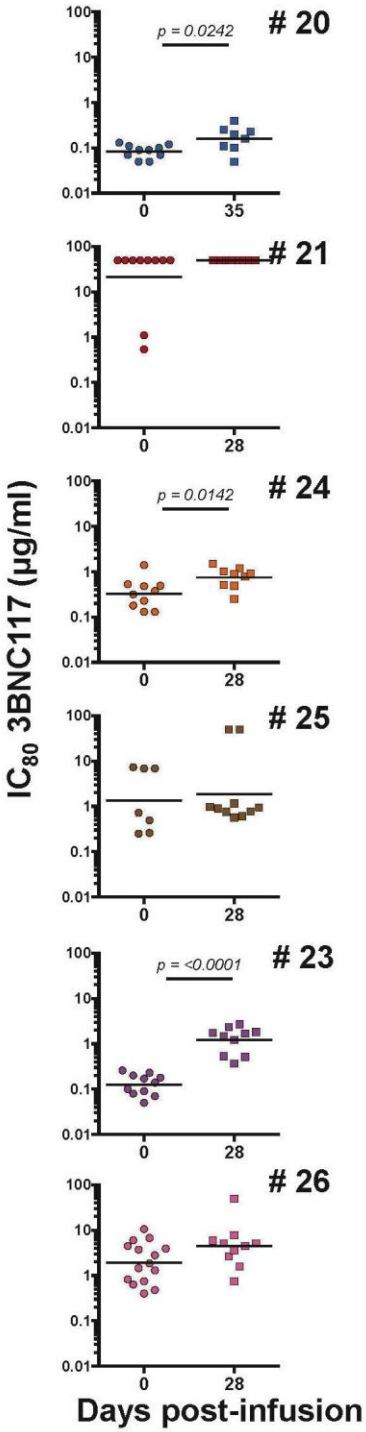

Fig. S14. Selection for reduced sensitivity to 3BNC117 in postinfusion virus. Envs cloned from baseline and 1 month post-infusion for each subject were tested for neutralization sensitivity to 3BNC117. IC_{80}s of each Env clone are plotted for two time points (pre-infusion in circles and post-infusion in squares) for subjects who had detectable virus (>20 copies/ml). Black line indicates geometric mean IC_{80} and groups were compared by Mann-Whitney. Significant p-values (<0.05 are shown).

Table S1. Clinical characteristics of HIV-1–infected subjects.

Subject	*Age (years)*	*Race*	*Years since HIV diagnosis*	*ART[a] status at baseline*	*Baseline[b] HIV viral load (copies/mL)*	*Baseline[b] CD4 (cells/mcL)*	*Subject IgG GM Allotype*	*VRC01 dose Day 0 (mg/kg)*	*VRC01 dose Week 4 (mg/kg)*	*Route[c]*
1	50	Black/ African American	23	ABC, 3TC, EFV	<20	503	17/17	1	1	IV
2	29	White	2	FTC, TDF, RPV	<20	626	3/17	1	1	IV
3	46	White	14	FTC, TDF, EFV	<20	751	3/17	1	1	IV
4	29	Black/ African American	6	FTC, TDF, RPV	<20	969	17/17	5	5	IV
5	31	Black/ African American	3	FTC, TDF, RPV	<20	429	17/17	5	5	IV
6	30	Multiracial	5	FTC, TDF, RPV	<20	533	3/3	5	5	IV
8	26	Multiracial	6	FTC, TDF, EFV	78	650	3/17	5	ND[d]	SC
9	44	White	9	FTC, TDF, RAL	<20	505	3/3	5	5	SC
10	47	Black/ African American	15	ABC, 3TC, ATV, RTV, RAL	<20	1157	3/17	5	5	SC
11	26	White	1	FTC, TDF, RPV	23	632	3/3	20	20	IV
14	28	Black/ African American	5	FTC, TDF, EFV	<20	415	3/17	20	20	IV
15	44	Black/ African American	4	FTC, TDF, RAL	<20	949	17/17	20	20	IV
16	46	Black/ African American	29	FTC, TDF, ATV, RTV	<20	409	3/17	40	40	IV
18	40	Black/ African American	6	FTC, TDF, EFV	<20	1008	17/17	40	ND	IV
19[e]	30	Black/ African American	7	FTC, TDF, RPV	<20	613	3/17	40	40	IV
20	27	Multiracial	1.5	naive	3547	313	17/17	40	ND	IV
21	21	Black/ African American	0.33	naive	6551	614	17/17	40	ND	IV
22	64	Black/ African American	19	naive	745	935	17/17	40	ND	IV
23	24	Black/ African American	2	naive	27894	406	3/17	40	ND	IV
24	37	Black/ African American	19	Off ART	5019	229	3/17	40	ND	IV
25	53	White	23	Off ART	27090	228	3/3	40	ND	IV
26	36	Multiracial	10	naive	5141	891	17/17	40	ND	IV
27	27	Multiracial	2	naive	237	1190	17/17	40	ND	IV

[a] Anti-retroviral (ART), Abacavir (ABC), Lamivudine (3TC), Efavirenz (EFV), Emtricitabine (FTC), Tenofovir (TDF), Rilpivirine (RPV), Amprenavir (ATV), Ritonavir (RTV), Raltegravir (RAL).

[b]Baseline was defined as average virus load of 2-3 timepoints within 100 days of infusion (except subject 25 who had only one).

[c] Intravenous (IV) or sub-cutaneous (SC).

[d]Not done (ND).

[e]Subjects 7,12,13&17 withdrew prior to infusion.

Table S2. Demographic characteristics of study participants.

Category	***Characteristic***	***Overall (N=27[a])***
GENDER N(%)	Male	22 (81.5)
	Female	5 (18.5)
AGE (years)	Mean [S.D.]	35.4 [11]
	Range	[21, 64]
RACE N(%)	Black or African American	16 (59.3)
	White	6 (22.2)
	Multiracial	5 (18.5)
ETHNICITY N(%)	Non-Hispanic/Latino	26 (96.3)
	Hispanic/Latino	1 (3.7)
WEIGHT (kilograms)	Mean [S.D.]	79.8 [16]
	Range	[58.1, 115]
HIV STATUS N(%)	Not on ARV Treatment	9 (33.3)
	On ARV Treatment	18 (66.7)
EDUCATION N(%)	Less than high school graduate	3 (11.1)
	High school graduate/GED	3 (11.1)
	College/University	15 (55.6)
	Advanced degree	6 (22.2)

[a] Includes 4 subjects who enrolled and withdrew prior to infusion.

Table S3. Reactogenicity after infusions with VRC01.

	Reactogenicity		*5mg/kg SC[b] (N=3)*	*1 mg/kg IV[b] (N=3)*	*5 mg/kg IV[b] (N=3)*	*20 mg/kg IV[b] (N=3)*	*40 mg/kg IV[b] (N=11)*
	Parameters	***Intensity[a]***			***N (%)***		
Maximum Local (through Day 7 post-infusion)	PAIN/ TENDERNESS	None	3 (100.0)	3 (100.0)	2 (66.7)	3 (100.0)	11 (100.0)
		Mild	0 (0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)
	BRUISING	None	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	11 (100.0)
		Mild	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0
	SWELLING	None	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	11 (100.0)
		Mild	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	REDNESS	None	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	11 (100.0)
		Mild	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Maximum Systemic (through Day 3 post-infusion)	MALAISE	None	3 (100.0)	2 (66.7)	2 (66.7)	1 (33.3)	11 (100.0)
		Mild	0 (0)	1 (33.3)	1 (33.3)	2 (66.7)	0 (0.0)
	MYALGIA	None	3 (100.0)	3 (100.0)	2 (66.7)	2 (66.7)	7 (63.6)
		Mild	0 (0)	0 (0.0)	1 (33.3)	1 (33.3)	4 (36.4)
	HEADACHE	None	2 (66.7)	2 (66.7)	2 (66.7)	1 (33.3)	9 (81.8)
		Mild	1 (33.3)	1 (33.3)	1 (33.3)	2 (66.7)	2 (18.2)
	CHILLS	None	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	10 (90.9)
		Mild	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	NAUSEA	None	1 (33.3)	3 (100.0)	3 (100.0)	3 (100.0)	9 (81.8)
		Mild	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (18.2)
	TEMPERATURE	None	3 (100.0)	3 (100.0)	2 (66.7)	3 (100.0)	11 (100.0)
		Mild	0 (0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)
	JOINT PAIN	None	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	8 (72.7)
		Mild	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (27.3)

[a]There were no moderate or severe reactions.
[b]Intravenous (IV) or sub-cutaneous (SC).

Table S4. VRC01 mean PK parameter values.

Group	Route[a]; VRC01 Dose (mg/kg)	Infusion #	Maximum concentration (µg/mL)	Time to maximum (hours)	Clearance (L/hour)	Terminal half-life (days)	AUC[b] 0-inf (µg*h/mL)	28 day Trough (µg/mL)
				Mean (Std Dev)				
Group 1 (n=3)	***IV;1***	***1***	27 (7.7)	1.8 (0.6)	0.024 (0.007)	13 (5.7)	6,500 (1,300)	1.0 (1.7)
		2	27 (9.7)	2.9 (2.2)				1.4 (1.3)
Group 2 (n=3)	***IV;5***	***1***	240 (42)	1.6 (0.62)	0.02 (0.003)	14 (0.82)	42,000 (7,500)	7.3(2.2)
		2	190 (29)	1.5 (0.5)				7.7 (0.64)
Group 4 (n=3)	***IV;20***	***1***	1000 (340)	2.4 (0.96)	0.018 (0.003)	18 (5.2)	200,000 (90,000)	33 (15)
		2	1000 (510)	1.7 (0.57)				46 (27)
Group 5A[c]	***IV;40***	***1 (n=3)***	1600 (220)	2.1 (0.5)	0.027 (0.01)[d]	8.6 (0.78[d]	- 250,000 (140,000)	34 (18)
		2 (n=2)	1700 (460)	2.6 (0.042)				65 (57)
Group 5B[e] (n=8)	***IV;40***	***1***	1400 (390)	1.6 (0.64)	0.025 (0.005)	9.1 (2.0)	130,000 (29,000)	28 (16)
Group 5	***IV;40***	***1 (n=11)***	1500 (340)	1.8 (0.63)	0.0026 (.006)[d]	9.0 (1.7)[d]	160,000 (37,000) [d]	30 (16)
		2 (n=2)	1700 (460)	2.6 (0.042)				65 (57)
Overall (n=20)	***IV***				0.024 (0.006)	12 (4.5)		
Group 3	***SC;5***	***1 (n=3)***	34 (5.0)	62 (15)	0.04 (0.01)	11 (5)[d]	20,000 (11,000) [d]	4.2 (2.9)
		2 (n=2)	44 (10)	32 (19)				5.6 (4.7)

[a]Intravenous (IV) or sub-cutaneous (SC).
[b]Area-under-curve.
[c]Aviremic subjects.
[d]Includes PK parameters from subjects that received one or two doses of VRC01.
[e]Viremic subjects.

Table S5. Source data for cell-associated virus in aviremic subjects (Fig. 3).

		Infusion dose; route subject #					
		Group 4: 20mg/kg; IV			**Group 5A: 40mg/kg; IV**		
		#11	**#14**	**#15**	**#16**	**#18**	**#19**
		Plasma virus load (copies/ml)					
	Days post-infusion					**0.168**	
Baseline	-52	**ND[a]**			**3.43**		
	-39						**[0.161][b]**
	-34			**1.34**			
	-27						
	-10		**[0.170]**				
	-1					**0.167**	
Post-infusion	35		**[0.161]**				**[0.161]**
	49			**1.29**			
	54				**11.7**		
	56						
		% Total HIV DNA (copies/100 CD4 T cells)					
Baseline	-52					**0.036**	
	-39				**0.193**		
	-34						**0.019**
	-27			**0.006**			
	-10	**0.056**					
	-1		**0.007**				
Post-infusion	35					**0.041**	
	49		**0.010**				**0.016**
	54			**0.010**			
	56				**0.245**		
	63	**0.049**					
		% Integrated HIV DNA (copies/100 CD4 T cells)					
Baseline	-52					**0.058**	
	-39				**0.085**		
	-34						**0.024**
	-27			**0.003**			
	-10	**0.013**					
	-1		**0.006**				
Post-infusion	35					**0.067**	
	49		**0.006**				**0.020**
	54			**0.005**			
	56				**0.105**		
	63	**0.007**					
		% Cells expressing tat/rev (copies/100 CD4 T cells)					
Baseline	-52					**0.00051**	
	-39				**0.00328**		
	-34						**[0.00014**
	-27			**0.00079**			
	-10	**0.0006**					
	-1		**[0.00016**				
Post-infusion	35					**0.00061**	
	49		**0.00032**				**[0.00015**
	54			**0.00068**			
	56				**0.00164**		
	63	**0.00042**					

		Infusion dose; route subject #					
		Group 4: 20mg/kg; IV			**Group 5A: 40mg/kg; IV**		
		#11	**#14**	**#15**	**#16**	**#18**	**#19**
		% Replication-competent infected cells (Infectious units/100 CD4 T cells)					
Baseline	-52		**ND[c]**	**ND[c]**		**0.00005**	
	-39				**0.00081**		
	-34						**0.00011**
	-27						
	-10	**0.00405**					
	-1						
Post-infusion	35					**0.00011**	
	49						**0.00005**
	54						
	56				**0.00087**		
	63	**0.00081**					
		% Total HIV DNA (copies/100 CM CD4 T cells)					
Baseline	-52					**0.170**	
	-39				**0.458**		
	-34						
	-27			**0.081**			
	-21				**0.460**		
	-13						**0.088**
	-10	**0.056**					
	-6					**0.331**	
	-3			**0.072**			
	-1		**0.006**				
Post-infusion	0	**0.068**					
	7	**0.031**	**0.023**	**0.103**	**0.613**	**0.263**	**0.069**
	35	**0.098**	**0.013**	**0.035**	**0.303**	**0.269**	**0.051**
	49		**0.026**				**0.176**
	54			**0.066**			
	56				**0.690**		
	63	**0.089**					
	168	**0.056**	**0.029**	**0.026**	**0.359**	**0.199**	**0.099**
		% Total HIV DNA (copies/100 EM CD4 T cells)					
Baseline	-52					**0.025**	
	-39				**0.248**		
	-34						
	-27			**0.078**			
	-21				**0.562**		
	-13						**3.23**
	-10	**0.018**					
	-6					**0.141**	
	-3			**0.086**			
	-1		**0.073**				
Post-infusion	0	**0.174**					
	7	**0.094**	**0.048**	**0.125**	**0.376**	**0.236**	**3.08**
	35	**0.013**	**0.016**	**0.112**	**0.742**	**0.180**	**3.71**
	49		**0.025**				**4.22**
	54			**0.104**			
	56				**0.117**		
	63	**0.121**					
	168	**0.060**	**0.042**	**0.084**	**0.555**	**0.180**	**3.17**

		Infusion dose; route					
		subject #					
		Group 4: 20mg/kg; IV			**Group 5A: 40mg/kg; IV**		
		#11	**#14**	**#15**	**#16**	**#18**	**#19**
		% Unspliced gag RNA in CM (copies gag/HIV DNA copy)					
Baseline	-52					**6.70**	
	-39				**3.36**		
	-34						
	-27			**0.203**			
	-21				**5.93**		
	-13						**6.55**
	-10	**1.13**					
	-6					**5.38**	
	-3			**0.798**			
	-1		**[2.13]**				
Post-infusion	0	**[0.174]**					
	7	**1.59**	**0.87**	**0.158**	**3.60**	**2.78**	**12.6**
	35	**0.167**	**3.66**	**0.466**	**5.61**	**6.51**	**0.248**
	49		**[0.450]**				**0.072**
	54			**1.09**			
	56				**3.03**		
	63	**1.11**					
	168	**0.226**	**[0.314]**	**[0.348]**	**4.65**	**17.9**	**2.32**
		% Unspliced gag RNA in EM (copies gag/HIV DNA copy)					
Baseline	-52					**33.8**	
	-39				**11.5**		
	-34						
	-27			**1.26**			
	-21				**6.46**		
	-13						**2.69**
	-10	**2.45**					
	-6					**6.05**	
	-3			**1.20**			
	-1		**[0.225]**				
Post-infusion	0	**0.243**					
	7	**[0.126]**	**0.339**	**0.93**	**7.03**	**3.04**	**3.30**
	35	**2.37**	**1.03**	**0.95**	**3.82**	**4.75**	**3.50**
	49		**0.646**				**2.12**
	54			**1.14**			
	56				**42.6**		
	63	**1.09**					
	168	**1.30**	**0.308**	**12.4**	**5.53**	**7.89**	**3.47**
		% Spliced rev RNA in CM (copies rev/HIV DNA copy)					
Baseline	-52					**1.62**	
	-39				**[0.0201]**		
	-34						
	-27			**[0.141]**			
	-21				**0.607**		
	-13						**0.146**
	-10	**0.293**					
	-6					**6.92**	
	-3			**[0.164]**			
	-1		**[2.13]**				

		Infusion dose; route *subject #*					
		Group 4: 20mg/kg; IV			**Group 5A: 40mg/kg; IV**		
		#11	**#14**	**#15**	**#16**	**#18**	**#19**
Post-infusion	0	**0.241**					
	7	**[0.383]**	**[0.507]**	**[0.114]**	**0.021**	**[0.035]**	**0.184**
	35	**0.167**	**[0.933]**	**[0.336]**	**0.042**	**[0.047]**	**0.248**
	49		**[0.450]**				**[0.052]**
	54			**[0.178]**			
	56				**0.510**		
	63	**[0.132]**					
	168	**[0.163]**	**[0.314]**	**[0.348]**	**[0.026]**	**[0.046]**	**[0.093]**
		% Spliced rev RNA in EM (copies rev/HIV DNA copy)					
Baseline	-52					**[0.373]**	
	-39				**0.791**		
	-34						
	-27			**[0.151]**			
	-21				**0.647**		
	-13						**0.089**
	-10	**0.907**					
	-6					**0.831**	
	-3			**0.190**			
	-1		**[0.162]**				
Post-infusion	0	**[0.068]**					
	7	**[0.126]**	**[0.245]**	**[0.094]**	**0.622**	**[0.039]**	**0.328**
	35	**[0.907]**	**[0.741]**	**[0.105]**	**0.254**	**0.061**	**0.063**
	49		**[0.466]**				**0.023**
	54			**[0.113]**			
	56				**0.937**		
	63	**[0.097]**					
	168	**[0.153]**	**[0.222]**	**[0.073]**	**0.023**	**[0.051]**	

[a]Not done because of sample quality.

[b] Where samples yielded values below the assay detection limit, the detection limit is shown in brackets.

[c] Not done because sample not optimal for assay sensitivity.

Table S6. Characteristics of plasma virus kinetics in relation to serum antibody concentration.

	Day 0			Day 7						Day 28[a]			
Subject	**Geometric mean IC_{80} (µg/ml)[b]**	**VRC01 serum conc (µg/ml)**	**Fold change D0 serum conc. to D0 geomean IC_{80}**	**VRC01 serum conc (µg/ml)**	**Fold change D7 serum conc. to D0 geomean IC_{80}**	**Nadir VL (copies /ml)**	**Day of nadir**	**Decline in VL (Δ baseline to nadir)**	**Fold change baseline VL to nadir VL**	**Geometric mean IC_{80} (µg/ml) [b]**	**VRC01 serum conc (µg/ml)**	**Fold change D28 serum conc. to D28 geomean IC_{80}**	**Day of return to baseline[d]**
21	30.3	1252.0	41	365.6	12	3627	5	2924	2	>50.0	58.2	1	N/A[e]
26	20.6	1202.8	58	184.9	9	3321	7	1820	2	21.2	27.1	1	N/A
22	0.815	1120.3	1375	182.4	224	<20	9	>725	37	ND[c]	17.2	ND	56
27	2.24	1382.5	617	249.3	111	<20	7	>217	12	ND	43.7	ND	42
20	0.361	825.6	2287	114.4	317	258	9	3289	14	1.24	8.92	7	35
23	0.617	987.2	1600	157.1	255	1289	7	26605	22	5.31	12.0	2	16
24	1.32	1052.6	797	171.5	130	340	5	4679	15	2.65	28.1	11	16
25	1.93	999.9	518	155.9	81	457	5	26633	59	6.66	27.8	4	21

[a]Day 35 for subject 20.
[b]Autologous Envs in pseudovirus neutralization assay.
[c]Not done (ND) because virus was undetectable.
[d]First time point post-nadir where virus load is < 0.5 log of pre-infusion average baseline.
[e]Not applicable (N/A) because virus load never declines >0.5 log.

Table S7. Sequence changes in Env protein sequences after infusion with VRC01.

Subject	Rank[a]	HXB2 Residue #	Region of Env	Score[b]	p value[c]
20	1	429[d]	B20/B21	0.6137	0.00
20	2	460	loop V5	0.4554	0.00
20	3	465	loop V5	0.3065	0.00
20	4	465b	loop V5	0.2996	0.00
20	5	394	loop V4	0.2675	0.00
21	1	280	loop D	0.4265	0.00
21	2	412	loop V4	0.3341	0.00
21	3	306	loop V3	0.3074	0.18
21	4	455	loop V5	0.2470	0.00
21	5	339	C3	0.2414	0.03
24	1	462	loop V5	0.2175	0.00
24	2	463a	loop V5	0.2162	0.00
24	3	268	C2	0.1835	0.02
24	4	148	loop V1	0.1794	0.00
24	5	360	C3	0.1631	0.04
25	1	462	loop V5	0.3283	0.00
25	2	187	loop V2	0.2290	0.00
25	3	171	loop V1	0.1779	0.00
25	4	465	loop V5	0.1659	0.14
25	5	463a	loop V5	0.1538	0.00

[a]Top five residues with the highest score.
[b]Score based on mutual information calculation normalized to the entropy of the input sequences.
[c]p-value of the mutual information score.
[d]VRC01 contact sites are shaded in gray (*35*).

Table S8. Sensitivity of pre- and postinfusion autologous virus clones from viremic subjects to mAbs.

Autologous virus clone[a]	Antibody				Antibody			
	VRC01	VRC07 -523LS	3BNC117	10E8	VRC01	VRC07 -523LS	3BNC117	10E8
	IC_{50} (µg/ml)				IC_{80} (µg/ml)			
20.00.C4	0.229	0.074	0.025	0.092	0.400	0.244	0.067	0.349
20.00.F9	0.229	0.081	0.045	0.086	0.779	0.316	0.122	0.444
20.00.C3	0.075	0.026	0.022	0.033	0.322	0.101	0.068	0.206
20.00.G2	0.144	0.060	0.030	0.049	0.678	0.261	0.127	0.336
20.00.H10	0.074	0.038	0.014	0.033	0.252	0.131	0.045	0.183
20.00.E8	0.128	0.037	0.027	0.069	0.526	0.139	0.092	0.364
20.00.E12	0.570	0.035	0.033	0.034	0.177	0.121	0.108	0.262
20.00.2B7	0.119	0.109	0.033	0.070	0.414	0.314	0.090	0.332
20.00.2E9	0.560	0.023	0.033	0.103	0.190	0.066	0.098	0.505
20.00.H1	0.089	0.036	0.016	0.026	0.299	0.140	0.049	0.149
20.35.C2	0.258	0.079	0.065	0.079	1.19	0.282	0.198	0.390
20.35.F6	0.055	0.010	0.014	0.043	0.415	0.039	0.046	0.250
20.35.H4	0.170	0.054	0.036	0.066	0.650	0.182	0.112	0.339
20.35.2F10	0.165	0.046	0.141	0.153	1.15	0.221	0.396	1.18
20.35.E8	0.216	0.013	0.025	0.040	3.83	0.051	0.095	0.271
20.35.2B2	0.163	0.039	0.040	0.016	1.23	0.167	0.155	0.112
20.35.2C12	0.447	0.072	0.072	0.024	2.01	0.246	0.231	0.142
20.35.2F2	0.609	0.076	0.081	0.025	1.58	0.253	0.247	0.173
21.00.A6	8.47	0.651	>50.0	0.363	36.8	2.16	>50.0	1.61
21.00.A12	8.99	0.939	>50.0	0.585	40.1	2.48	>50.0	2.53
21.00.B3	0.820	0.319	0.162	0.020	3.73	1.39	0.540	0.118
21.00.C10	5.09	0.493	0.331	0.277	14.7	2.00	1.10	1.41
21.00.E12	6.96	0.953	>50.0	0.801	41.0	2.85	>50.0	3.49
21.00.G4	10.8	1.20	>50.0	1.83	33.8	4.23	>50.0	6.17
21.00.B8	12.3	1.26	>50.0	0.865	>50.0	6.08	>50.0	4.68
21.00.C4	11.4	1.56	>50.0	1.51	47.5	7.41	>50.0	6.02
21.00.D5	8.47	1.25	>50.0	1.57	49.1	5.50	>50.0	6.89
21.00.E10	10.0	1.75	>50.0	1.69	>50.0	7.79	>50.0	8.40
21.28.C1	>50.0	0.610	>50.0	0.626	>50.0	2.82	>50.0	3.14
21.28.E5	>50.0	2.32	>50.0	0.805	>50.0	10.7	>50.0	3.00
21.28.E9	>50.0	2.18	>50.0	2.67	>50.0	7.23	>50.0	8.40
21.28.F5	15.1	1.28	>50.0	2.42	>50.0	4.88	>50.0	7.75
21.28.2F2	>50.0	0.730	>50.0	0.915	>50.0	3.58	>50.0	4.01
21.28.2G1	>50.0	3.05	>50.0	1.32	>50.0	13.4	>50.0	4.21
21.28.2G9	>50.0	3.02	>50.0	1.41	>50.0	14.0	>50.0	4.70
21.28.2A5	>50.0	0.800	>50.0	0.598	>50.0	5.94	>50.0	3.94
21.28.2B7	>50.0	1.76	>50.0	2.63	>50.0	14.5	>50.0	6.20
21.28.2D3	>50.0	1.55	>50.0	1.89	>50.0	3.73	>50.0	5.49

Autologous virus clone[a]	Antibody				Antibody			
	VRC01	VRC07 -523LS	3BNC117	10E8	VRC01	VRC07 -523LS	3BNC117	10E8
	IC_{50} (µg/ml)				IC_{80} (µg/ml)			
22.00.A1	0.676	0.078	0.067	0.147	2.02	0.264	0.283	0.974
22.00.B3	0.234	0.031	0.037	0.083	0.880	0.092	0.118	0.458
22.00.B7	0.087	0.018	0.039	0.129	0.266	0.051	0.103	0.567
22.00.B12	0.163	0.025	0.034	0.080	1.12	0.089	0.145	0.894
22.00.E7	0.378	0.049	0.095	0.117	1.33	0.154	0.244	0.530
22.00.F12	0.096	0.017	0.031	0.053	0.325	0.060	0.084	0.243
22.00.H10	0.300	0.036	0.116	0.250	1.11	0.11	0.31	1.08
22.00.F10	0.366	0.033	0.049	0.242	1.21	0.13	0.18	1.10
22.00.G10	0.124	0.014	0.021	0.213	0.453	0.051	0.081	1.19
22.00.H4	0.250	0.025	0.047	0.094	0.924	0.085	0.152	0.424
23.00.A4	0.186	0.037	0.053	0.137	0.688	0.113	0.167	0.930
23.00.A5	0.240	0.031	0.044	0.060	1.21	0.122	0.199	0.673
23.00.C1	0.260	0.037	0.061	0.148	1.01	0.141	0.230	1.16
23.00.F6	0.194	0.037	0.032	0.066	0.830	0.124	0.139	0.477
23.00.G10	0.088	0.019	0.013	0.125	0.291	0.073	0.046	0.749
23.00.H7	0.222	0.034	0.031	0.485	0.718	0.119	0.101	2.76
23.00.D8	0.137	0.032	0.027	0.074	0.493	0.098	0.080	0.515
23.00.F5	0.252	0.034	0.030	0.167	0.855	0.110	0.091	0.940
23.00.F10	0.031	0.004	0.020	0.621	0.126	0.015	0.074	3.42
23.00.C6	0.339	0.045	0.054	0.140	1.03	0.138	0.177	0.960
23.00.B12	0.143	0.024	0.056	0.143	0.853	0.130	0.255	1.36
23.28.A3	0.985	0.107	0.392	0.175	6.85	1.04	1.50	1.22
23.28.A8	1.41	0.267	0.430	0.180	9.22	1.45	1.69	1.19
23.28.C3	0.338	0.071	0.144	0.061	2.25	0.355	0.534	0.301
23.28.E8	2.08	0.365	0.714	0.334	11.2	1.80	2.32	1.89
23.28.F7	1.45	0.224	0.521	0.287	8.57	1.27	1.73	1.69
23.28.F8	3.74	0.479	0.774	0.476	12.0	2.49	2.70	1.96
23.28.G1	0.433	0.084	0.160	0.235	2.19	0.370	0.516	1.43
23.28.2B4	1.69	0.255	0.612	0.239	9.66	1.36	1.84	1.66
23.28.2C9	0.904	0.162	0.312	0.229	5.29	0.963	1.23	1.64
23.28.2E9	0.155	0.033	0.088	0.103	0.966	0.193	0.365	0.750
24.00.A1	0.103	0.016	0.024	0.046	0.582	0.087	0.132	1.77
24.00.B2	0.471	0.083	0.121	0.029	1.76	0.298	0.480	0.172
24.00.B4	1.44	0.227	0.474	1.72	5.46	0.784	1.40	5.33
24.00.B8	0.244	0.045	0.102	0.381	1.28	0.202	0.376	2.74
24.00.C1	0.319	0.058	0.118	0.427	1.72	0.267	0.488	3.73
24.00.C5	0.289	0.039	0.061	0.057	1.67	0.240	0.324	1.24
24.00.C11	0.156	0.032	0.059	0.154	0.823	0.128	0.226	1.58
24.00.D8	0.397	0.059	0.102	0.109	2.64	0.348	0.525	2.18
24.00.F4	0.087	0.018	0.022	0.049	0.536	0.083	0.134	0.771
24.00.G9	0.118	0.030	0.048	0.191	0.674	0.121	0.184	1.71

Autologous virus clone[a]	Antibody				Antibody			
	VRC01	VRC07-523LS	3BNC117	10E8	VRC01	VRC07-523LS	3BNC117	10E8
	IC_{50} (µg/ml)				IC_{80} (µg/ml)			
24.28.A11	0.649	0.068	0.212	0.114	4.29	0.514	1.03	1.53
24.28.C3	0.663	0.054	0.208	0.109	3.95	0.440	1.20	1.86
24.28.C12	0.326	0.034	0.097	0.064	1.71	0.258	0.510	0.837
24.28.D12	0.403	0.044	0.044	0.030	1.92	0.315	0.254	0.352
24.28.G7	0.612	0.119	0.265	0.284	2.42	0.517	0.783	1.42
24.28.H4	0.392	0.060	0.245	0.111	2.36	0.432	0.899	1.58
24.28.H12	0.518	0.075	0.268	0.489	2.81	0.405	0.907	3.83
24.28.2F4	0.397	0.082	0.139	0.969	1.43	0.287	0.491	4.18
24.28.E8	1.12	0.145	0.374	0.565	4.97	0.755	1.50	3.61
25.00.A11	0.826	0.110	2.24	0.143	2.65	0.356	7.32	0.534
25.00.C12	2.17	0.159	2.15	0.201	6.29	0.407	6.86	0.678
25.00.F6	0.559	0.094	2.03	0.102	1.63	0.294	6.91	0.300
25.00.E9	0.271	0.056	0.083	0.768	0.839	0.293	0.49	4.32
25.00.C2	0.296	0.063	0.090	2.37	1.14	0.193	0.26	8.80
25.00.D7	0.756	0.256	0.243	1.63	2.83	0.934	0.72	5.32
25.00.E12	0.445	0.068	0.097	2.43	1.36	0.207	0.25	7.56
25.28.B12	2.01	0.183	0.295	1.51	5.73	0.529	0.78	4.95
25.28.D1	2.01	0.168	0.267	1.35	6.91	0.543	0.95	5.43
25.28.E4	1.12	0.129	0.220	1.14	4.69	0.377	0.61	5.81
25.28.G2	0.320	0.083	0.177	0.714	1.46	0.322	1.16	3.26
25.28.G4	2.25	0.134	0.192	0.750	7.46	0.576	0.76	3.93
25.28.G12	>50.0	1.28	8.40	1.22	>50.0	14.9	>50.0	4.90
25.28.A4	14.9	0.891	13.3	1.29	>50.0	5.05	>50.0	5.73
25.28.B3	1.08	0.145	0.320	1.42	3.41	0.471	0.972	4.90
25.28.B10	1.43	0.150	0.288	1.28	5.05	0.446	0.895	4.84
25.28.E10	0.613	0.157	0.191	1.05	1.97	0.570	0.567	4.55
26.00.B3	7.71	0.746	0.261	1.50	24.7	3.65	1.46	6.48
26.00.B9	2.04	0.162	0.247	0.160	8.20	0.900	1.30	0.818
26.00.D4	4.73	0.234	0.054	0.455	19.8	2.07	0.399	2.82
26.00.F8	6.50	0.279	0.082	0.813	37.3	4.08	0.630	6.09
26.00.F9	7.28	0.768	0.129	1.52	44.0	5.80	0.751	8.15
26.00.G1	0.728	0.088	0.131	0.180	3.45	0.538	0.820	0.880
26.00.E8	3.02	0.152	0.432	0.033	16.9	2.21	2.80	0.351
26.00.A2	5.42	0.515	2.65	0.077	43.7	4.63	10.6	0.680
26.00.A10	3.50	0.306	0.820	0.098	13.5	3.01	4.48	0.872
26.00.B2	0.898	0.063	0.331	0.029	8.67	0.609	1.85	0.255
26.00.C5	4.44	0.364	1.05	0.099	22.6	2.59	3.94	0.745
26.00.F12	4.34	0.224	1.43	0.052	>50.0	3.41	6.72	0.455
26.00.G4	2.61	0.139	1.04	0.045	45.9	2.15	5.98	0.380
26.00.G8	3.81	0.362	0.779	0.145	23.6	3.21	3.73	1.15
26.00.2A2	0.238	0.034	0.116	0.005	1.35	0.183	0.478	0.045

Autologous virus clone[a]	Antibody				Antibody			
	VRC01	VRC07-523LS	3BNC117	10E8	VRC01	VRC07-523LS	3BNC117	10E8
	IC_{50} (µg/ml)				IC_{80} (µg/ml)			
26.28.2H2	4.17	0.404	1.09	0.128	>50.0	3.15	4.45	0.734
26.28.2C6	5.84	0.691	1.27	0.111	36.6	4.97	7.76	0.660
26.28.2B9	1.34	0.210	0.207	0.174	6.12	1.36	0.748	1.04
26.28.2E5	4.21	0.646	1.87	0.175	21.6	4.46	5.92	1.10
26.28.2D9	3.62	0.328	1.44	0.078	19.4	2.85	5.10	0.541
26.28.2C8	3.29	0.267	0.805	0.098	22.3	2.45	2.69	0.605
26.28.E4	2.52	0.195	0.972	0.075	30.0	2.13	3.57	0.648
26.28.H11	6.12	0.541	8.52	0.312	35.2	2.90	>50.0	1.61
26.28.A6	3.47	0.674	1.63	0.186	17.5	3.33	5.11	0.957
26.28.D10	1.70	0.220	0.427	0.196	9.63	1.31	1.57	1.36
27.00.2G1	1.17	0.439	0.557	0.129	2.51	1.21	1.46	0.641
27.00.B11	1.02	0.473	0.419	0.343	3.42	1.42	1.65	1.34
27.00.2G7	0.732	0.231	0.326	0.112	2.49	0.822	1.22	0.707
27.00.B4	0.288	0.101	0.123	0.044	1.70	0.473	0.477	0.322
27.00.2G10	0.323	0.121	0.140	0.185	1.19	0.381	0.449	1.00
27.00.H2	0.518	0.176	0.213	0.050	2.14	0.687	0.834	0.347
27.00.B12	0.477	0.152	0.177	0.151	1.64	0.462	0.518	0.657
27.00.G9	0.484	0.138	0.157	0.121	1.67	0.527	0.516	0.646
27.00.C10	0.914	0.358	0.299	0.164	2.98	1.27	1.10	0.722
27.00.2G4	0.866	0.232	0.293	0.120	4.14	0.990	1.23	0.488

[a]Clones are named by the convention “subject.time point.clone number”.