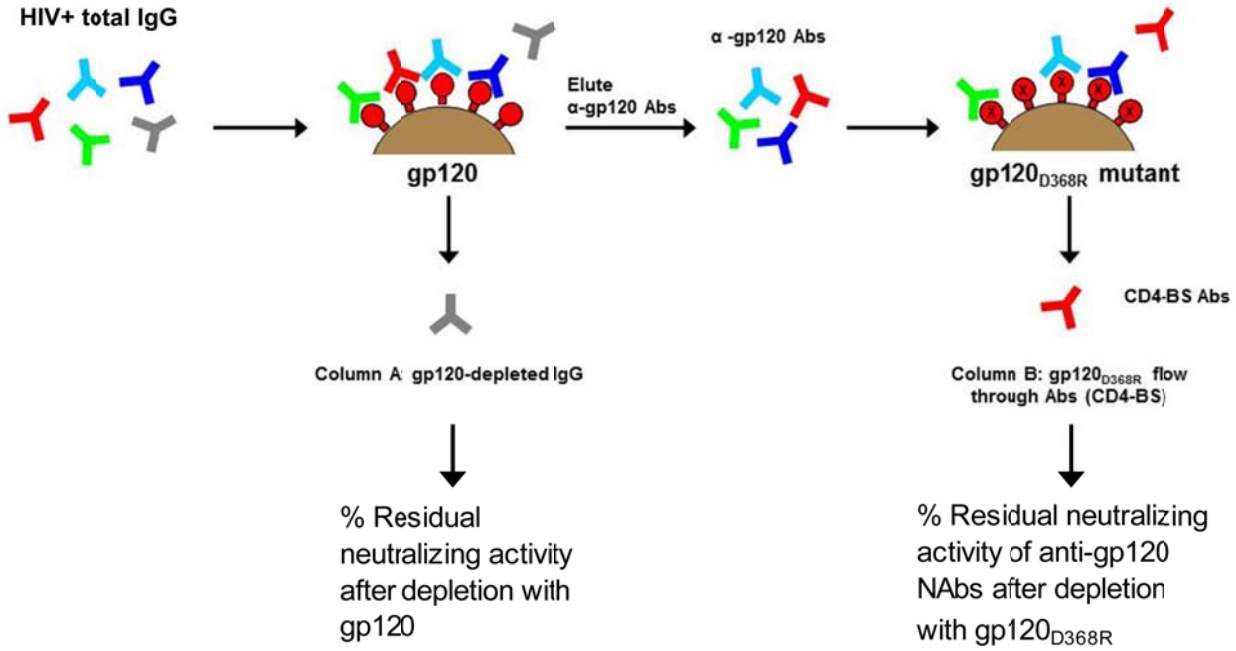
Supporting Information

SI Materials and Methods

Plasma IgG adsorptions on SF162 gp120- and SF162 gp120$_{D368R}$-coated beads. Plasma adsorptions were performed as previously described (1, 3), with some modifications, and the flow of the adsorptions is outlined in Figure S1. Briefly, total IgG was isolated by protein A chromatography (Pierce, Rockford Il, USA) from VC10042 plasma drawn 22 years post-infection. The purified total IgG was serially adsorbed onto SF162 gp120-coated beads (MyOne Tosylactivated Dynabeads, Invitrogen, Carlsbad, CA, USA), and the flow through collected. The antibodies bound to the gp120 coated beads were eluted by vortexing in increasingly acidic 0.1M glycine solutions, followed by buffer exchange into PBS. The anti-gp120 Ab fraction was then serially adsorbed onto SF162 gp120$_{D368R}$-coupled beads to remove Abs that do not bind the CD4-BS. Each fraction described above was tested for residual neutralizing activity against 4 clade B, 3 clade C, and 2 clade A isolates in the TZM-bl neutralization assay. The depleted total IgG and the gp120$_{D368R}$ depleted anti-gp120 fractions were tested for the presence of anti-CD4-BS antibodies, and the absence of non-CD4-BS gp120 Abs by Luminex assay (Luminex Corporation, Austin, TX, USA) against both wild type SF162 gp120 and SF162 gp120$_{D368R}$.

Figure S1



Clade	Isolate		
B	YU2	8.22	97.43
	QH0692	21.11	94.68
	TRO.11	14.08	96.48
	JR-FL	11.09	98.85
	SF162.LS	7.86	97.39
	ADA	13.35	86.61
	5768.4	22.22	94.97
	REJO4541.67	31.05	94.96
C	ZM249M.PL1	13.26	74.28
	Du156.12	27.41	97.81
	Du422.1	7.48	80.44
A	Q461.d1	16.94	88.04
	Q769.h5	30.16	95.29

Figure S1. Contribution of anti-CD4-BS NAbs to the overall neutralizing potential of plasma isolated from subject VC10042. IgG was first isolated from heat-treated plasma, and then antibodies that bind to gp120 were adsorbed onto SF162 gp120-coated magnetic beads. After 6 rounds of serial adsorption with gp120-coated beads, the flow through was tested for residual neutralizing activity against 4 clade B, 3 clade C, and 2 clade A isolates. The values in the first column represent the percent residual neutralizing activity against each isolate after depletion with gp120. Anti-gp120 antibodies were eluted from the SF162 gp120-coated magnetic beads, and then serially adsorbed onto magnetic beads coated with SF162 $gp120_{D368R}$ to remove antibodies that do not bind to the CD4-BS. The anti-gp120 antibodies and the $gp120_{D368R}$ flow through (containing the anti-CD4-BS NAbs) were tested against the 9 isolates described above. The percent residual neutralizing activity of the anti-gp120 antibody fraction after depletion with $gp120_{D368R}$ (the neutralizing activity due to anti-CD4-BS NAbs) is shown in the second column. All depletions were confirmed by Luminex assay against SF162 gp120 and SF162 $gp120_{D368R}$.

Figure S2

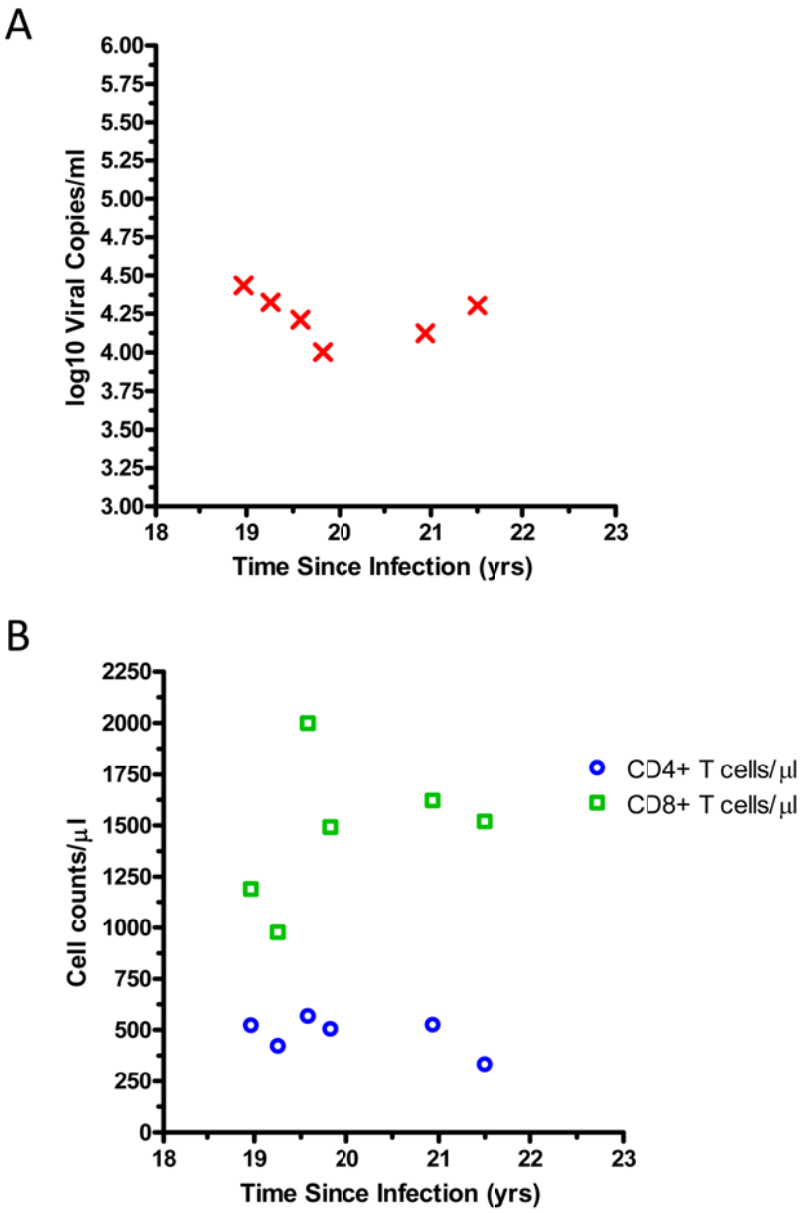


Figure S2. A) Log10 viral RNA copies per ml of blood during the time of observation. B) The number of CD4+ and CD8+ T cells per microliter of blood during observation.

Figure S3

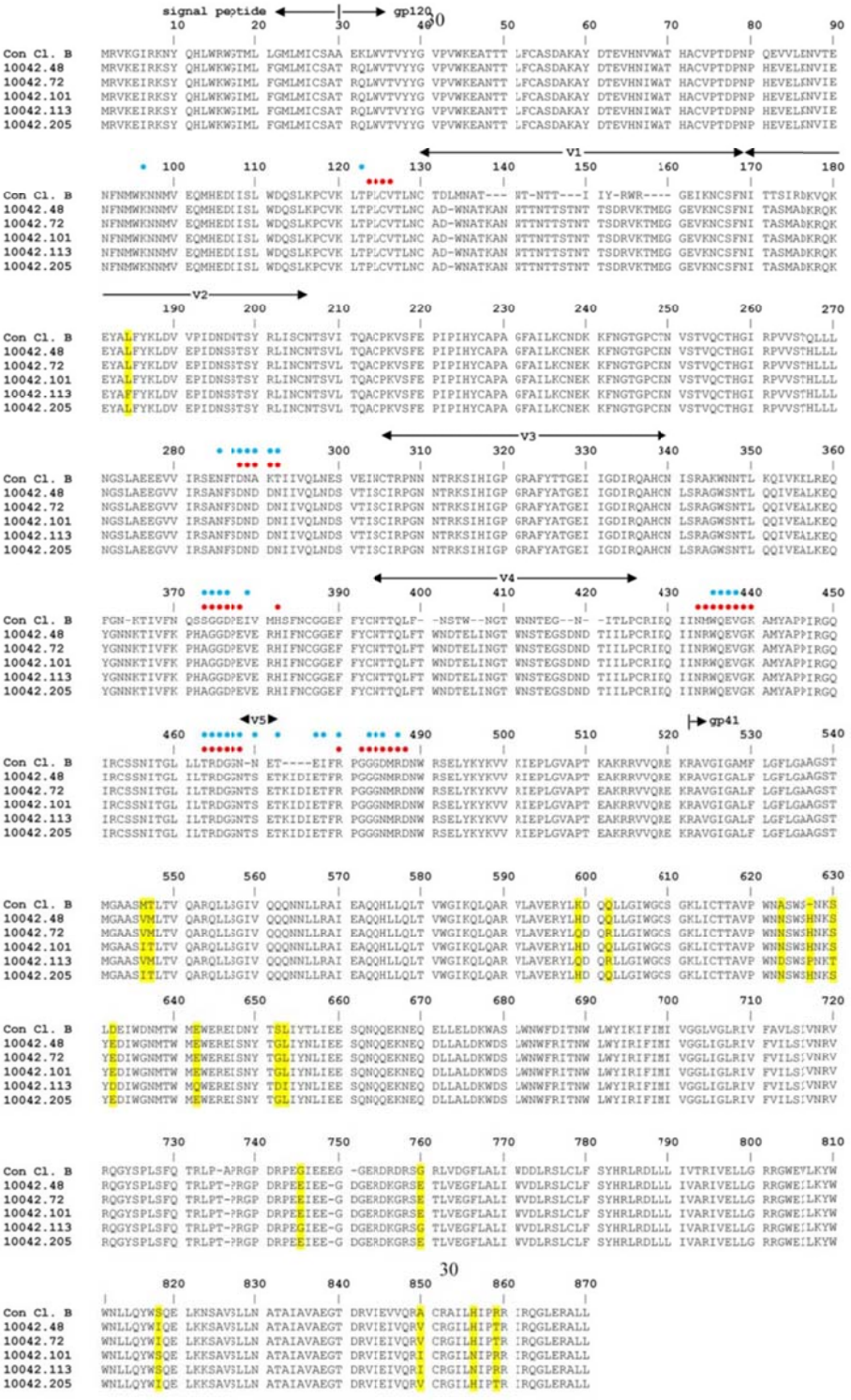


Figure S3. Full length gp160 amino acid alignment of the 'early' clones isolated from plasma that was collected approximately 19 years post infection. Representative Env clones were aligned with the consensus clade B sequence using Clustal W. The gp120, gp41 and signal peptide portions of Env are labeled, as are the variable loops V1-V5. Sites that are variable among the VC10042 clones are highlighted in yellow. Amino acid residues that are known to contact CD4 are marked with red circles and VRC01 are marked with blue circles.

Figure S4

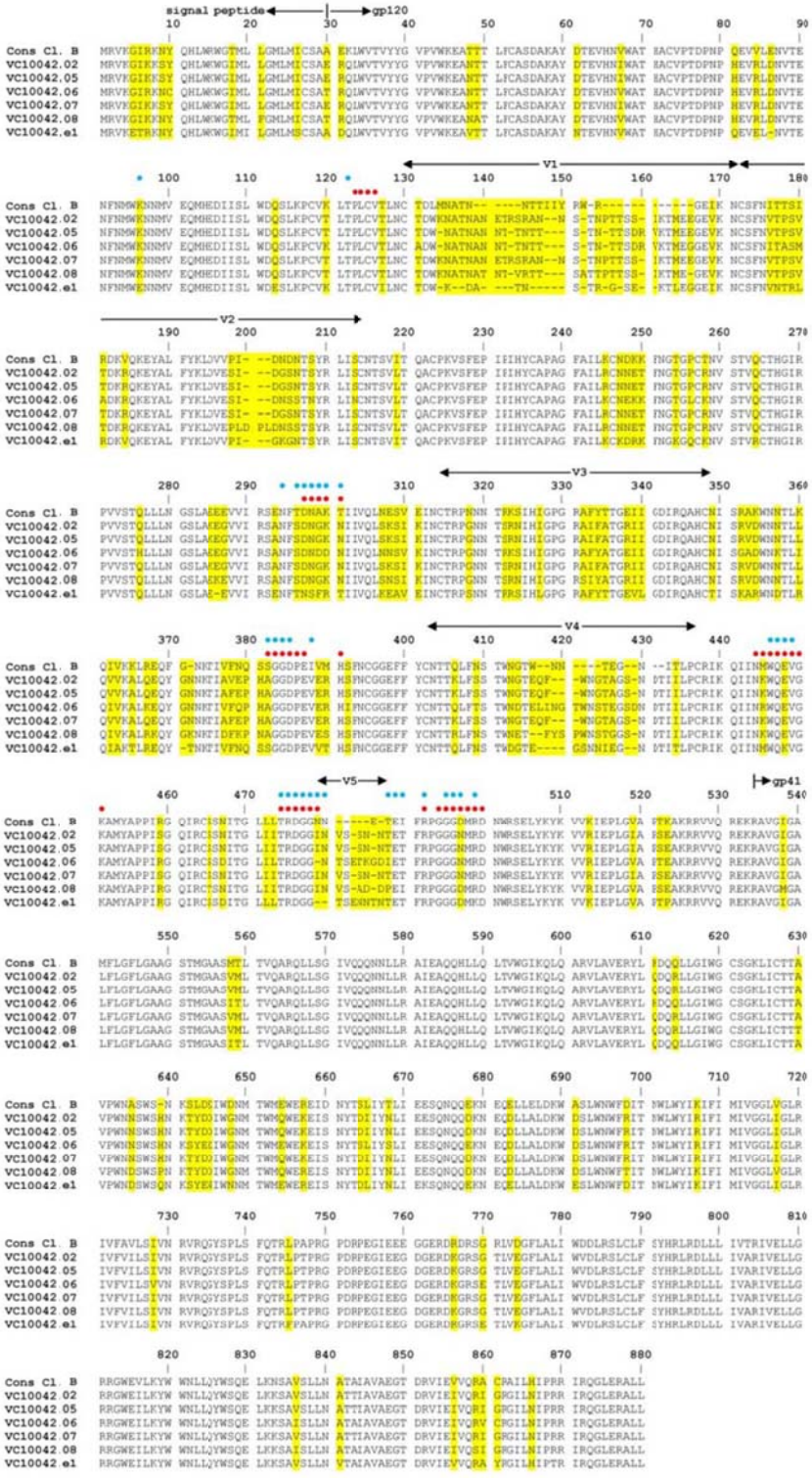


Figure S4. Full length gp160 amino acid alignment of the 'late' clones isolated from plasma that was collected approximately 22 years post infection. Representative Env clones were aligned with the consensus clade B sequence using Clustal W. The gp120, gp41 and signal peptide portions of Env are labeled, as are the variable loops V1-V5. Sites that are variable among the clones from VC10042 are highlighted in yellow. Amino acid residues that are known to contact CD4 are marked with red circles and VRC01 are marked with blue circles.

Figure S5

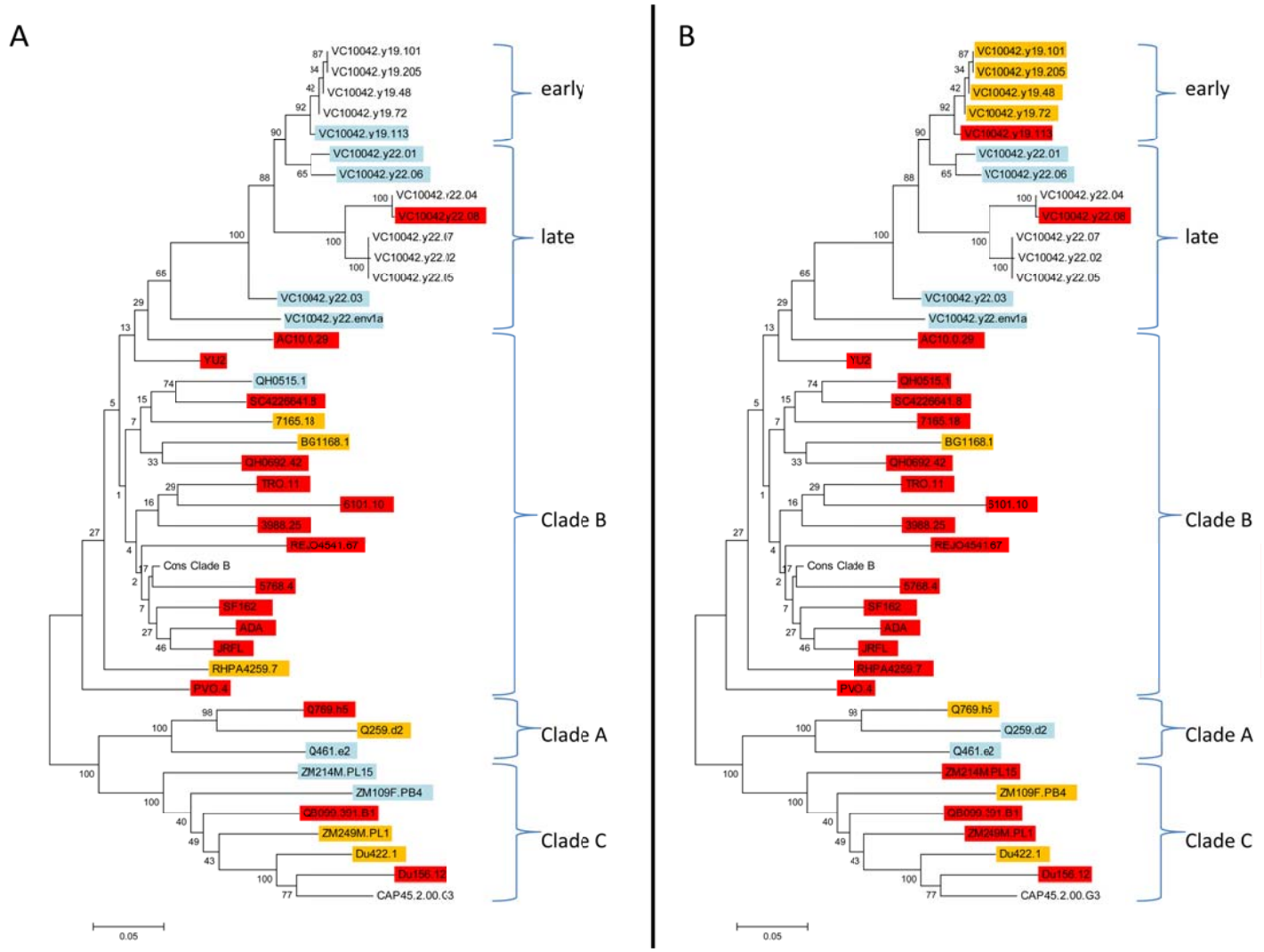


Figure S5. Phylogenetic tree showing the relationship between the autologous *env* clones circulating in subject VC10042 at 19 years (A) and 22 years (B) post infection and *env* clones from heterologous isolates that were tested for their neutralization susceptibility of VC10042 plasma. Phylogenies were reconstructed using MEGA5.1(4) using Maximum Likelihood with 100 bootstrap replications, utilizing the Jones-Taylor-Thornton (JTT) substitution model and utilizing uniform rates. Bootstrap values are listed at each node and the scale bar is located in the bottom left. The potency with which the plasma is able to neutralize a given isolate is heat mapped, with non-neutralized isolates left uncolored, IC50 titers below 100 colored blue, IC50 titers between 100-250 colored in orange, and IC50 titers above 250 colored in red. (A) Neutralization with plasma isolated at 19 years post-infection. (B) Neutralization with plasma isolated at 22 years post-infection.

Figure S6

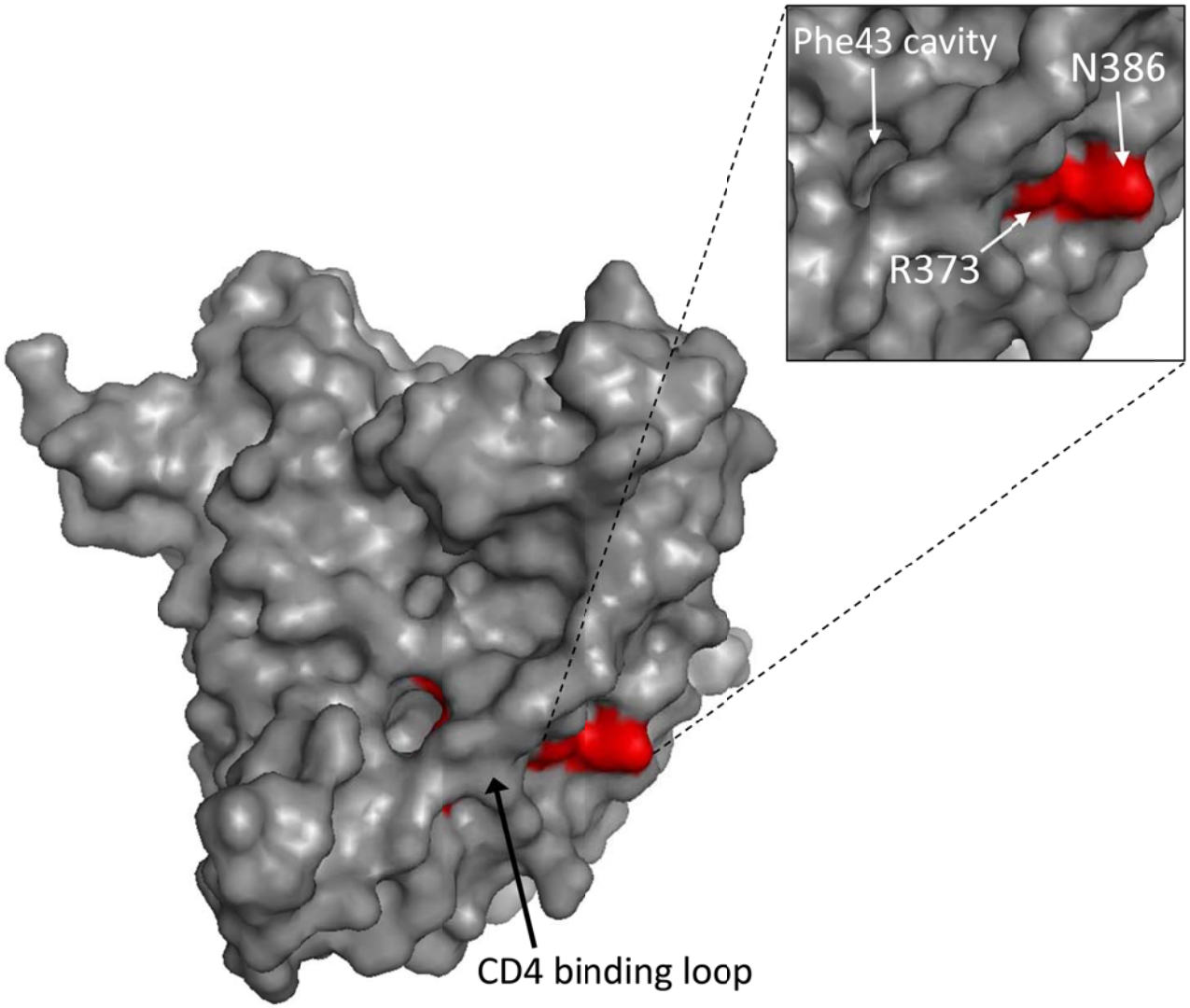


Figure S6. The regions on gp120 that mediate escape from the neutralizing activity of the anti-CD4-BS NAbs in VC10042. Residues R373 and N386 reside outside the CD4 binding pocket (inset). All residues were mapped on to the HXB2 core molecule in the b12-bound state (2). Inset is slightly rotated to better view relevant features.

Figure S7

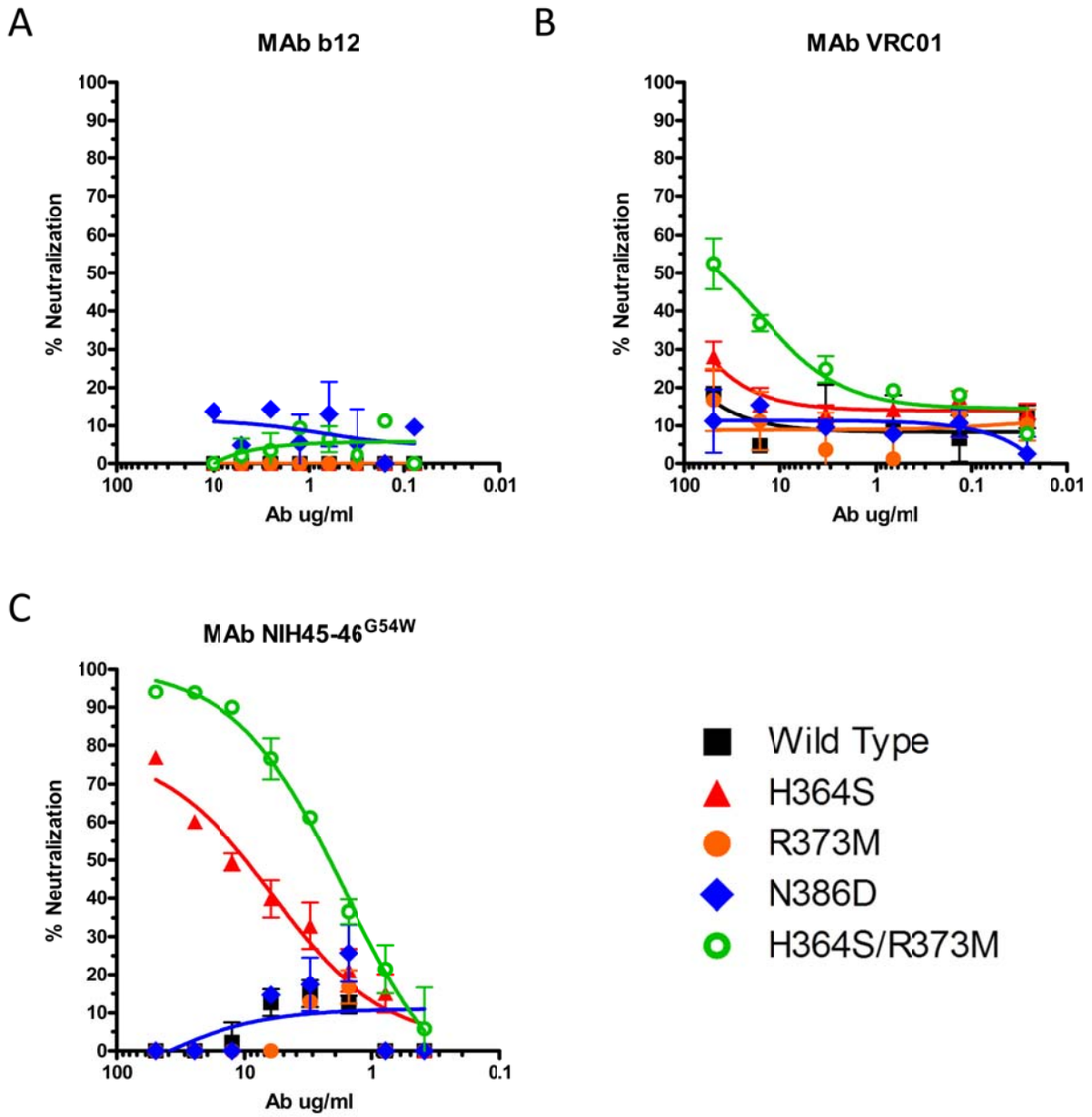


Figure S7. Neutralization sensitivity of the clone VC10042.y22.05 bearing reversion mutations to the monoclonal broadly neutralizing antibodies b12 (A), VRC01 (B), and NIH45-46^{G54W} (C). Three reversion mutations were introduced into the wild type VC10042.y22.05 (■) clone: H364S(▲) , R373M (●), and N386D (♦). A double mutant, H364s.R373M (o) was also generated.

References:

1. **Li, Y., S. A. Migueles, B. Welcher, K. Svehla, A. Phogat, M. K. Louder, X. Wu, G. M. Shaw, M. Connors, R. T. Wyatt, and J. R. Mascola. 2007. Broad HIV-1 neutralization mediated by CD4-binding site antibodies. Nat Med 13:1032-4.**
2. **Liu, J., A. Bartesaghi, M. J. Borgnia, G. Sapiro, and S. Subramaniam. 2008. Molecular architecture of native HIV-1 gp120 trimers. Nature 455:109-13.**
3. **Sather, D. N., J. Armann, L. K. Ching, A. Mavrantoni, G. Sellhorn, Z. Caldwell, X. Yu, B. Wood, S. Self, S. Kalams, and L. Stamatatos. 2009. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. J Virol 83:757-69.**
4. **Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731-9.**