

## Supplementary Materials for

### **Coexistence of potent HIV-1 broadly neutralizing antibodies and antibody-sensitive viruses in a viremic controller**

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Table S3 (Microsoft Excel format). IC<sub>50</sub> and IC<sub>80</sub> values in TZM-bl assay of the 1:1:1 combination of NC37, BG1, and BG18 against a 118-virus panel (provided in Excel).

Table S4 (Microsoft Excel format). IC<sub>50</sub> and IC<sub>80</sub> values in TZM-bl assay of antibodies BG8, BG18, PGT121, and 12A12 against selected HIV-1 V3 envelope mutants (provided in Excel).

Table S5 (Microsoft Excel format). Crystallization statistics (provided in Excel).

## **Supplementary Materials**

### **Supplementary Methods**

#### **HIV-1-infected subjects**

Purified IgG from donor EB354 ranked in the top 1% for neutralization breadth and potency in a cohort of 394 HIV-1 infected long-term non-progressors. This donor was diagnosed with HIV-1 Clade B in 1986. He received treatment with didanosine and stavudine between 1995 and 1998, but has had no antiretroviral therapy since that time. In 2002 his viral load was <400 copies/mL and CD4 and CD8 counts were 954 and 1046 cells/mm<sup>3</sup>, respectively. He had three documented peaks in viremia between 2002 and 2006. HLA typing revealed HLA A\*01:01, 24:02, B\*27:05, 57:01, C\*01:02, 06:02.

#### **ELISAs**

High-binding 96-well ELISA plates (Costar) were coated overnight with 5 µg/mL of purified 2CC core, gp120.YU2 (wild type or mutants) or gp140.YU2 foldon trimer (24) in PBS. After washing 6 times with PBS + 0.05 % Tween 20, the plates were blocked for 2 h with 2% BSA, 1 µM EDTA and 0.05% Tween-PBS (“blocking buffer”), and then incubated for 1 h with IgGs that were added as seven consecutive 1:4 dilutions in PBS from an initial concentration of 4 µg/mL. After additional washing, the plates were developed by incubation with goat HRP-conjugated anti-human IgG antibodies (Jackson ImmunoResearch) (at 0.8 µg/mL in blocking buffer) for 1 h followed by HRP chromogenic substrate (ABTS solution; Invitrogen). All experiments were performed at least 3 times.

For ELISAs using BG505 SOSIP.664 trimers with a D7324 epitope tag (BG505 SOSIP.664-D7324 (26)) the plates were coated overnight with 5 µg /mL of D7324 antibody

as previously described(26), and then washed and incubated with 500 ng/mL of the trimer (22, 26). After a further wash, IgGs were added for 1 h as seven consecutive 1:4 dilutions in PBS from initial concentrations of 4 µg/mL . The endpoint was generated by incubation with goat HRP-conjugated anti-human IgG antibodies, as described above. All experiments were performed at least 3 times.

For competitive ELISAs, the plates were coated with 0.5 µg/mL BG505.SOSIP.664 (26), washed, blocked for 2 h with blocking buffer and then incubated for 1 h with competing antibodies added as seven consecutive 1:4 dilutions in PBS from an initial concentration of 32 µg/mL, and in the presence of biotinylated BG1 or BG18 at a constant concentration of 4 µg/mL. The plates were then incubated for 1 h with HRP-conjugated streptavidin (Jackson ImmunoResearch) at 1 µg/mL in blocking buffer, followed by HRP chromogenic substrate (ABTS solution; Invitrogen). All experiments were performed at least 3 times.

### **HIV-1<sub>YU2</sub> envelope mutants**

Single, double and triple mutations were introduced into wild-type HIV-1<sub>YU2</sub> envelope using the QuikChange (multi-) site-directed mutagenesis kit, according to the manufacturer's specifications (Agilent Technologies).

### **Protein Production and Purification for structural studies.**

6x-His-tagged BG18, BG18<sub>N26Q</sub>, NC102, and NC37 Fabs were expressed by transient transfection in HEK293-6E cells and purified from transfected cell supernatants using Ni<sup>2+</sup>-NTA affinity chromatography (GE Healthcare) and Superdex 200 16/60 size exclusion chromatography (SEC) (GE Healthcare). Truncated, His-tagged 93TH057 gp120 core protein was produced in baculovirus-infected insect cells and purified as described using Ni<sup>2+</sup>

affinity chromatography (GE Healthcare) (44, 92). Prior to crystallization trials, purified NC37 and 93TH057 proteins were coincubated (2:1 molar ratio of Fab to gp120 core) and treated with 5 kU of Endoglycosidase H per mg of gp120 protein for 3 h at 25°C. Endoglycosidase H-treated complex was purified by size exclusion chromatography using a Superdex 200 10/300 GL column (GE Healthcare).

Soluble BG505 SOSIP.664 trimers for EM studies were constructed as described (26). HEK293-6E cells treated with 5 μM kifunensine (Sigma) were co-transfected with plasmids encoding BG505 SOSIP.664 and soluble furin at a ratio of 4:1. BG505 SOSIP.664 protein was harvested from cell supernatants using a 2G12 immunoaffinity column made by covalently coupling 2G12 IgG monomer to an NHS-activated Sepharose column (GE Healthcare). Protein was eluted with 3 M MgCl<sub>2</sub> followed by immediate buffer exchange into Tris-buffered saline (TBS) pH 7.4 (50 mM NaCl). Trimers were further purified using Mono Q 5/50 GL (GE Healthcare) followed by Superose 6 10/300 SEC (GE Healthcare).

### Negative-stain single particle EM

Purified BG505 SOSIP.664–BG18–179NC75 complexes were diluted to 10 μg/mL in TBS immediately before adding 3 μL to a glow-discharged ultrathin C film on holey carbon support film, 400 mesh, Cu grids (Ted Pella, Inc.). Samples on the grids were cross-linked using glutaraldehyde vapor and then stained with 3% uranyl acetate. Data were collected using a FEI Tecnai T12 transmission electron microscope operating at 120 keV equipped with a Gatan Ultrascan 2k X 2k CCD. Images were acquired using a 0.5 s exposure time at a nominal magnification of 42,000x at 1 μm defocus, resulting in 2.5 Å per pixel. A total of 25639 particles were picked using swarm picking in EMAN2.1 (90), and the CTF

correction was done using EMAN2.1 (90). Initial reference-free 2D class averaging was performed using RELION and all particles were sorted into 250 classes. 9827 particles corresponding to good class averages were selected, and the particles were further sorted using 3D classification in RELION, after which 7925 particles are selected for refinement. To obtain a reference structure for 3D classification and refinement, we collected data from an independent BG505 SOSIP.664–BG18–179NC75 complex sample by cryoelectron tomography and obtained a 40 Å sub-tomogram averaged structure using previously-described methods (93). The sub-tomogram averaged structure was low-pass filtered to 80 Å for use as the reference structure for single particle reconstruction. The resolution of the final single particle reconstruction was ~25 Å calculated using RELION (91) and a gold-standard FSC with a 0.143 cutoff as recommended for resolution estimations for single particle EM reconstructions (94).

### Fitting of EM density maps

EM structures were visualized using UCSF Chimera (95, 96). Coordinates from crystal structures were fit into the sub-tomogram averaged or negative stain single particle EM structures using the fit in map utility within UCSF Chimera with the following options: real-time correlation/average update, use map simulated from atoms, resolution 25 Å. We first fit a BG505 SOSIP.664 structure (PDB 4TVP) into the density, and then fit coordinates for BG18 Fab (this study) and CH103 Fab (PDB 4JAM; as a model for 179NC75 Fab) into corresponding densities individually. The C<sub>H</sub>1-C<sub>L</sub> domain regions of the Fabs exhibited poor densities, as expected given variability in Fab elbow angles (97).

## **Crystallization, X-ray data collection and structure determinations**

Crystals of BG18 Fab in which a potential *N*-linked glycosylation site at heavy chain position 26 was removed by mutating Asn<sub>26<sub>HC</sub></sub> to Gln showed superior size and morphology compared with crystals of wild-type BG18 Fab, thus the structure determination was done using BG18<sub>N26Q</sub> Fab. Crystals of BG18<sub>N26Q</sub> Fab (space group P2<sub>1</sub>;  $a = 46.12 \text{ \AA}$ ,  $b = 71.04 \text{ \AA}$ ,  $c = 69.54 \text{ \AA}$ ;  $\beta = 98.48^\circ$ ; 1 molecule per asymmetric unit); were obtained by combining 0.2  $\mu\text{L}$  of an 18 mg/mL protein solution with 0.2  $\mu\text{L}$  of 0.1 M sodium acetate pH 4.5, 26.8% (v/v) polyethylene glycol (PEG) 400 and 13.4% (w/v) PEG 8,000 at 20°C, cryoprotected in mother liquor supplemented with 20% (v/v) ethylene glycol, and flash cooled in liquid nitrogen. Crystals of NC102 Fab (space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>;  $a = 60.2 \text{ \AA}$ ,  $b = 82.5 \text{ \AA}$ ,  $c = 117.2 \text{ \AA}$ ; 1 molecule per asymmetric unit) were produced by vapor diffusion in sitting drops with a reservoir of 0.1 M sodium acetate trihydrate pH 4.6 and 2.0 M ammonium sulfate using 250 nL drops with a 1.5:1 protein:reservoir ratio. Crystals were cryoprotected in mother liquor supplemented with 25% (v/v) ethylene glycol and flash cooled in liquid nitrogen. Crystals of a NC37–93TH057 complex (space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>;  $a = 63.6 \text{ \AA}$ ,  $b = 67.4 \text{ \AA}$ ,  $c = 210.4 \text{ \AA}$ ; 1 complex per asymmetric unit) were produced by vapor diffusion in 250 nL sitting drops by incubating protein and reservoir (comprising 0.1 M HEPES pH 7.5 and 20% (w/v) PEG 10,000) at a 1.5:1 protein:reservoir ratio. Crystals were cryoprotected in mother liquor supplemented with 20% (w/v) PEG 400 and flash cooled in liquid nitrogen.

X-ray diffraction data were collected at the Stanford Synchrotron Radiation Lightsource beamline 12-2 outfitted with a Pilatus 6M pixel detector (Dectris). XDS was used to index, integrate and scale the data (88). Crystals of BG18<sub>N26Q</sub> Fab diffracted to 1.5  $\text{\AA}$ , and the structure was solved by molecular replacement using 10-1074 Fab (PDB code 4FQ2)

$V_H V_L$  with CDR loops removed and  $C_H 1 C_L$  as search models. The structure was refined using an iterative approach of refinement with Phenix (43) and manual model building in Coot (89). The final model of BG18<sub>N26Q</sub> Fab ( $R_{work} = 18.0\%$ ,  $R_{free} = 18.9\%$ ) had 98.3%, 1.7% and 0% of residues in the favored, allowed, and disallowed regions, respectively, of the Ramachandran plot. NC102 Fab diffracted to 1.6 Å, and the structure was solved by molecular replacement using a model of NIH45-46 (PDB code 3U7W) with  $V_H V_L$  with CDR loops removed and  $C_H 1 C_L$  as search models. The final model of NC102 Fab ( $R_{work} = 18.0\%$ ,  $R_{free} = 20.0\%$ ) had 98%, 2% and 0% of residues in the favored, allowed, and disallowed regions, respectively, of the Ramachandran plot. The NC37–93TH057 gp120 complex structure was solved by molecular replacement using search models of NC102  $V_H V_L$ ,  $C_H 1 C_L$ , and a truncated gp120 core (from PDB 3U7Y) as search models. The final model of the NC37–93TH057 complex ( $R_{work} = 21.0\%$ ,  $R_{free} = 26.0\%$ ) had 96%, 4% and 0% of residues in the favored, allowed, and disallowed regions, respectively, of the Ramachandran plot. Data collection and refinement statistics are presented in Table S5.

### TZM-bl assays of Env glycol-mutant viruses

Pseudoviruses were generated by transfection of 293T cells (ATCC) with an HIV-1 Env expressing plasmid and the Env-deficient genomic backbone plasmid called pSG3ΔEnv ([AIDSreagent.org](http://AIDSreagent.org)), as described previously (86). Pseudoviruses were harvested 72 h post-transfection for use in neutralization assays. Neutralizing activity was assessed using a single round of replication pseudovirus assay and TZM-Bl target cells ([AIDSreagent.org](http://AIDSreagent.org)), as described previously (86). Briefly, TZM-bl cells were seeded in a 96-well flat bottom plate. To this plate was added pseudovirus, which was preincubated with serial dilutions of

antibody for 1 h at 37°C. Luciferase reporter gene expression was quantified 72 h after infection upon lysis and addition of Bright-Glo™ Luciferase substrate (Promega). To determine IC<sub>50</sub> values, dose-response curves were fit by nonlinear regression. All assays were performed in duplicate.

### **Synthesis of the HIV-1 V3 glycopeptides**

The synthesis of the V3 glycopeptides derived from several HIV-1 strains was achieved by using a chemoenzymatic method consisting of automated solid-phase peptide synthesis of the GlcNAc-peptide precursor and subsequent enzymatic transglycosylation of the GlcNAc-peptide to provide the target glycopeptides, following the previously published procedures (98). The interactions between the biotinylated synthetic glycopeptides and the BG18 and BG8 IgG antibodies were evaluated by SPR using a BIACore T200 system (GE Healthcare) at 25 °C. Biotinylated glycopeptides were immobilized on neutravidin-coated CM5 sensor chips in HBS-P buffer (10 mM HEPES, 150 mM NaCl, P20 surfactant 0.05% v/v, pH 7.4) until 200 response unit (RU) was achieved. BG18, BG8, PGT121 or 10-1074 were injected at two-fold series dilutions of concentration starting at 4 mM in BS-P buffer with a flow rate of 40 µL /min for 180 s. BS-P buffer with a flow rate of 40 µL/min was then injected for 120s to allow for dissociation. Regeneration was performed by injection of 3M MgCl<sub>2</sub> with a flow rate of 50 µL/min for 3 min followed by injection of HBS-P buffer with a flow rate of 50 µL/min for 5 min.

### **Autologous plasma virus single genome sequencing primers sequences**

envB5out: 5’– TAGAGCCCTGGAAGCATCCAGGAAG

envB3out: 5'– TTGCTACTTGTGATTGCTCCATGT

envB5in: 5'– TTAGGCATCTCCTATGGCAGGAAGAAG

envB3in: 5'– GTCTCGAGATACTGCTCCCACCC

### Bioinformatic processing of MiSeq env-sequences

Sequence adapters were removed using Cutadapt v1.8.3. Read assembly for each virus was performed in three steps. First, de novo assembly was performed using Spades v3.6.1 to yield long contig files. Contigs longer than 255 bp were subsequently aligned to an HIV envelope reference sequence and a consensus sequence was generated using Geneious 8. Finally, reads were re-aligned to the consensus sequence to close gaps and a final consensus was generated. Sequences with double peaks (cutoff consensus identity for any residue < 75%) were omitted from downstream analysis.

### Analysis of viral evolution

Alignments of env nucleotide sequences were generated using ClustalW (version 2.11) (99) or via manual alignment using Geneious (version 8.1.6) sequence analysis software (100). Regions that could not be unambiguously aligned were removed from phylogenetic analysis and diversity calculations. Evolutionary model classes for maximum likelihood phylogenetic analyses were selected using jModelTest (101). Maximum likelihood phylogenetic trees were generated using PhyML (version 3) (102) with joint estimation of model parameter values and phylogenies. Pairwise genetic diversity was compared among samples using a two-sample U statistic test (103) in the DIVEIN webtool (104).

### Generation of CMV-Env viruses

CMV-env expression cassettes were generated according to an established protocol (36). Briefly, the CMV promoter was amplified from pcDNA 3.1D/V5-His-TOPO Expression vector using the primers:

CMVenv 5'AGTAATCAATTACGGGGTCATTAGTTCAT 3'and

CMVenv1A 5'CATA GGAGATGCCTAACGCCGGTGGAGCTCTGCTTATATAGACCTC

3'. The PCR product was purified using the Macherey-Nagel PCR and Gel Purification Kit. 1 μL of first round PCR product was amplified using primers:

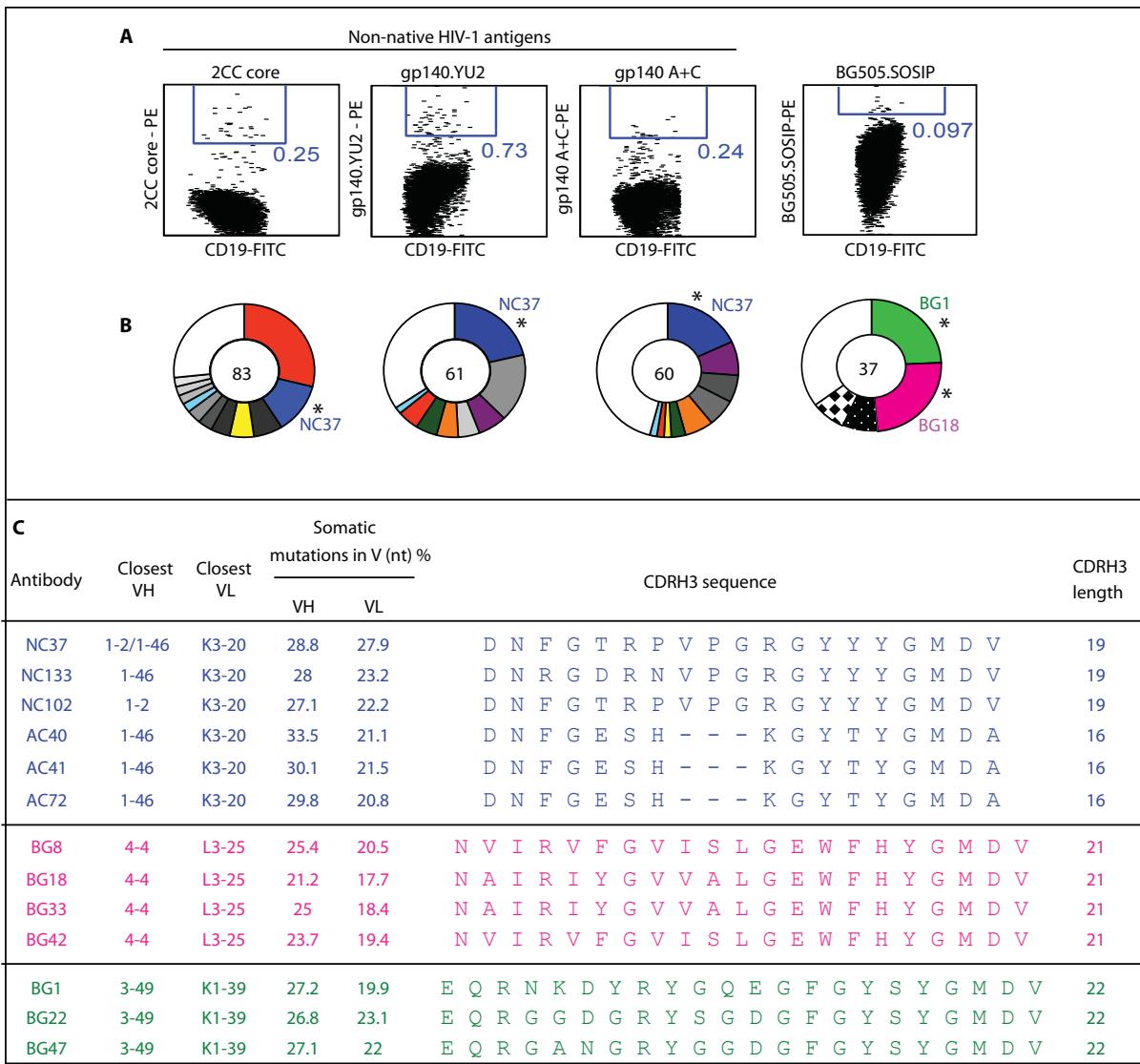
env1ATOPO 5' CACC GGCTTAGGCATCTCCTATGGCAGGAAGAA 3' and

Rev19 5' ACTTTTGACCACTTGCCACCCAT 3' in a 20 μL volume containing 1x High Fidelity Buffer, 2 mM MgSO<sub>4</sub>, 0.2 mM dNTPs, 0.5 units of High Fidelity Platinum and 0.2 μM of each primer. Cycling conditions were 94° C, 2 min; (94° C, 15 s; 55° C 30 s; 68° C, 4 min) x 35; 68° C, 10 min. The presence of env was validated by analysis on a 0.7% Agarose gel and the product was purified using the Macherey-Nagel Gel and PCR Purification Kit. 10 ng of envelope and 0.5 ng of CMV were then subjected to overlapping PCR with primers CMVenv and Rev19 in triplicates. Total reaction volume 6 was 50 μL containing 1x High Fidelity Buffer, 0.2 μM MgS04, 0.2 mM dNTPs, 1 U of Platinum Taq High Fidelity and 0.4 μM of each primer. PCR was carried out at 94°C, 2min; (94° C, 30 s; 60° C 30 s; 68° C, 4min) x 25; 68°C, 10 min. 500 ng of CMV-env were co-transfected with pSG3Δenv in 6-well plates into 293T cells and supernatant was harvested after 48 h. Supernatants were subjected to neutralization testing by TZM-bl as described above.

## Viral Outgrowth Cultures

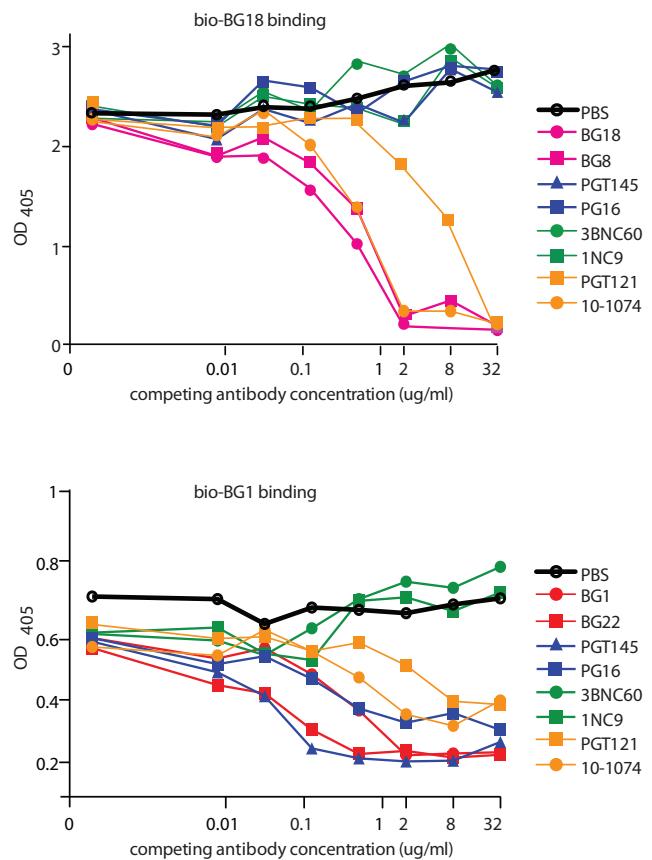
Virus from donor EB354 was obtained by co-culture of patient peripheral blood mononuclear cells (PBMCs) with healthy donor PBMCs as previously described (37). Healthy donor PBMCs were obtained from patients by leukapheresis under study protocol MNU-0628 at Rockefeller University. All donors provided written informed consent before participation. Healthy donor PBMCs were pre-stimulated at a density of  $5 \times 10^6$  cells/mL in IMDM containing 10% FBS, 1% Penicillin-Streptomycin and PHA at 1  $\mu\text{g}/\text{mL}$  for 2-3 days at 37°C and 5% CO<sub>2</sub>.  $6 \times 10^6$  of the stimulated donor PBMCs were then transferred to IMDM containing 10% FBS, 1% Penicillin-Streptomycin, 10 IU/mL IL-2 and 5  $\mu\text{g}/\text{mL}$  polybrene and co-cultured with  $5-10 \times 10^6$  EB354 PBMCs at 37°C and 5% CO<sub>2</sub>. Media was replaced weekly and the presence of p24 in culture supernatant was quantified by Lenti-X p24 Rapid Titer Kit (Clontech). Cultures exceeding 1 ng of p24 per mL of supernatant were frozen and stored at -80°C. Determination of tissue culture infectious dose 50 (TCID<sub>50</sub>) and subsequent testing for sensitivity of autologous viruses to different broadly neutralizing antibodies and autologous serum IgG was carried out using a TZM-bl neutralization assay according to established protocols (86). All neutralization assays were run in duplicate.

## Supplementary Figures:

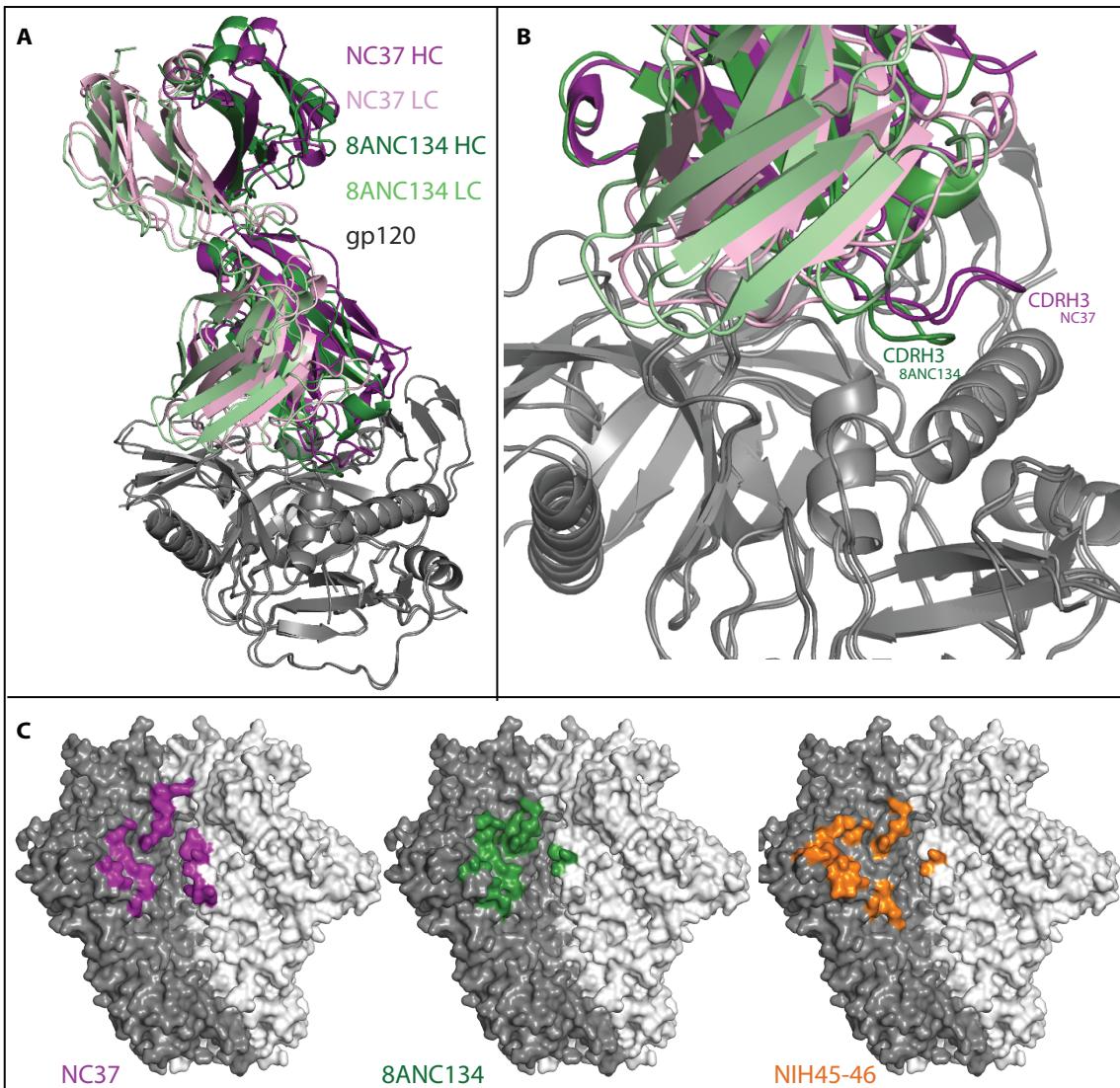


**Figure S1: Isolation of neutralizing antibody clones from donor EB354.** (A) Upper panels: CD19 pre-enriched IgG+ memory B cells were stained with CD19 and with three non-native HIV-1 baits: 2CC core, gp140<sub>YU2</sub> fold-on trimer and gp140<sub>92UG37.8</sub> + gp140<sub>CZA79012</sub> (gp140 A+C) fold-on trimmers, and one native bait BG505.SOSIP-AviB. The

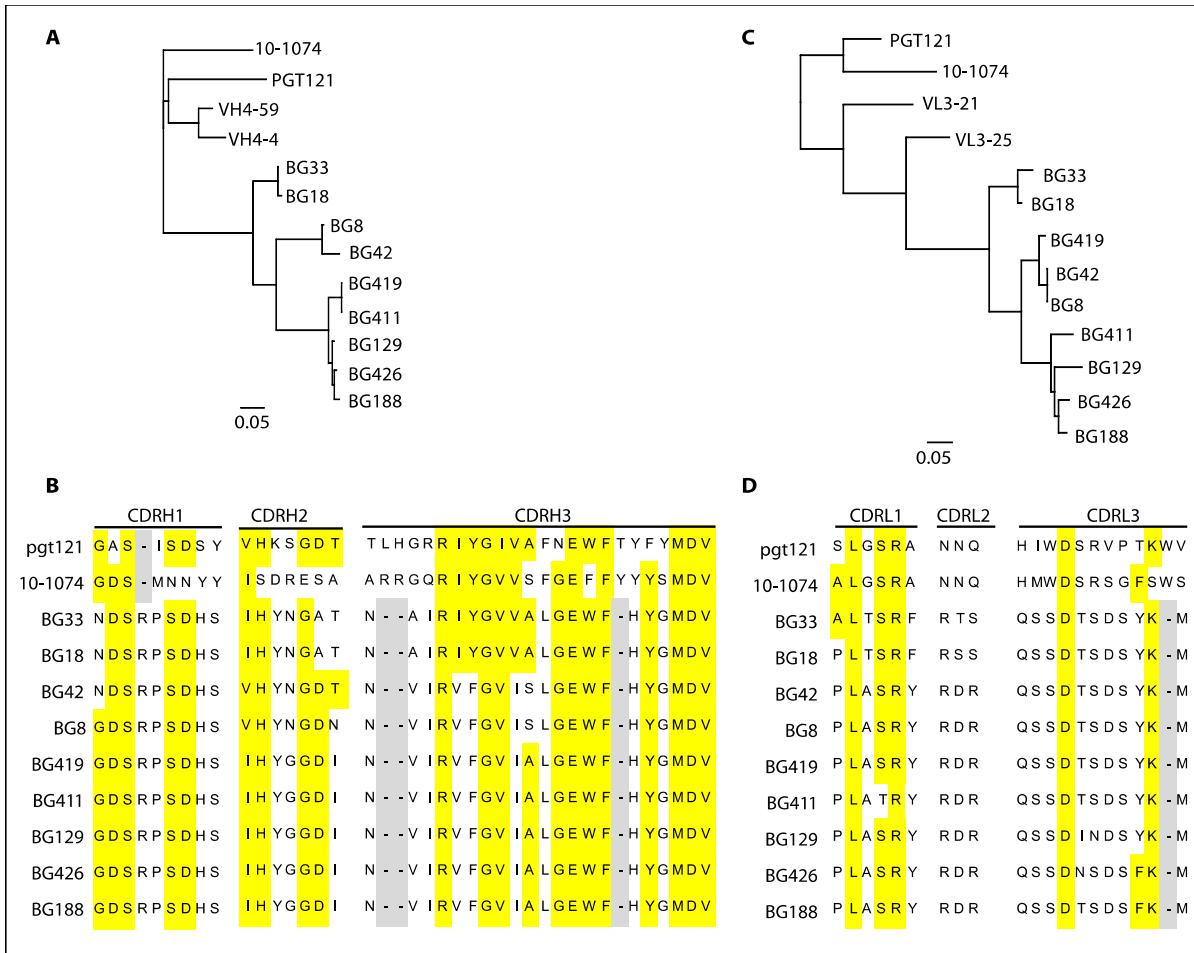
populations in the blue insert represents cells that were single cell sorted and the numbers indicate the percent of cells that were bait positive out of the total IgG+ B cells. (B) Pie charts represent the total Ig sequences that were amplified from the single sorted cells from the corresponding FACS plot shown in (A). The number in the middle of the pies represents the total number of antibodies sequenced. White slices represent sequences that appeared only once and did not have any clonal relatives. The slices represent antibody clones and are proportional to the number of sequences in every clone. Gray shaded slices represent clones that were obtained in only one sort. Colored slices that represent antibody clones that were sorted with at least two different non-native baits. The clones marked with an asterisk are clones representative antibodies of which exhibited tier 2 neutralizing activity. These are represented by the variants NC37 (blue clone), BG18 (magenta clone) and BG1 (green clone). (C) Genetic characteristic of the 13 antibodies, representative variants from each of the three clones (blue, magenta and green) that showed neutralizing activity. The V<sub>H</sub>, V<sub>L</sub> and level of mutations, as well as the sequence of the CDRH3 (based on IMGT definition) are indicated.



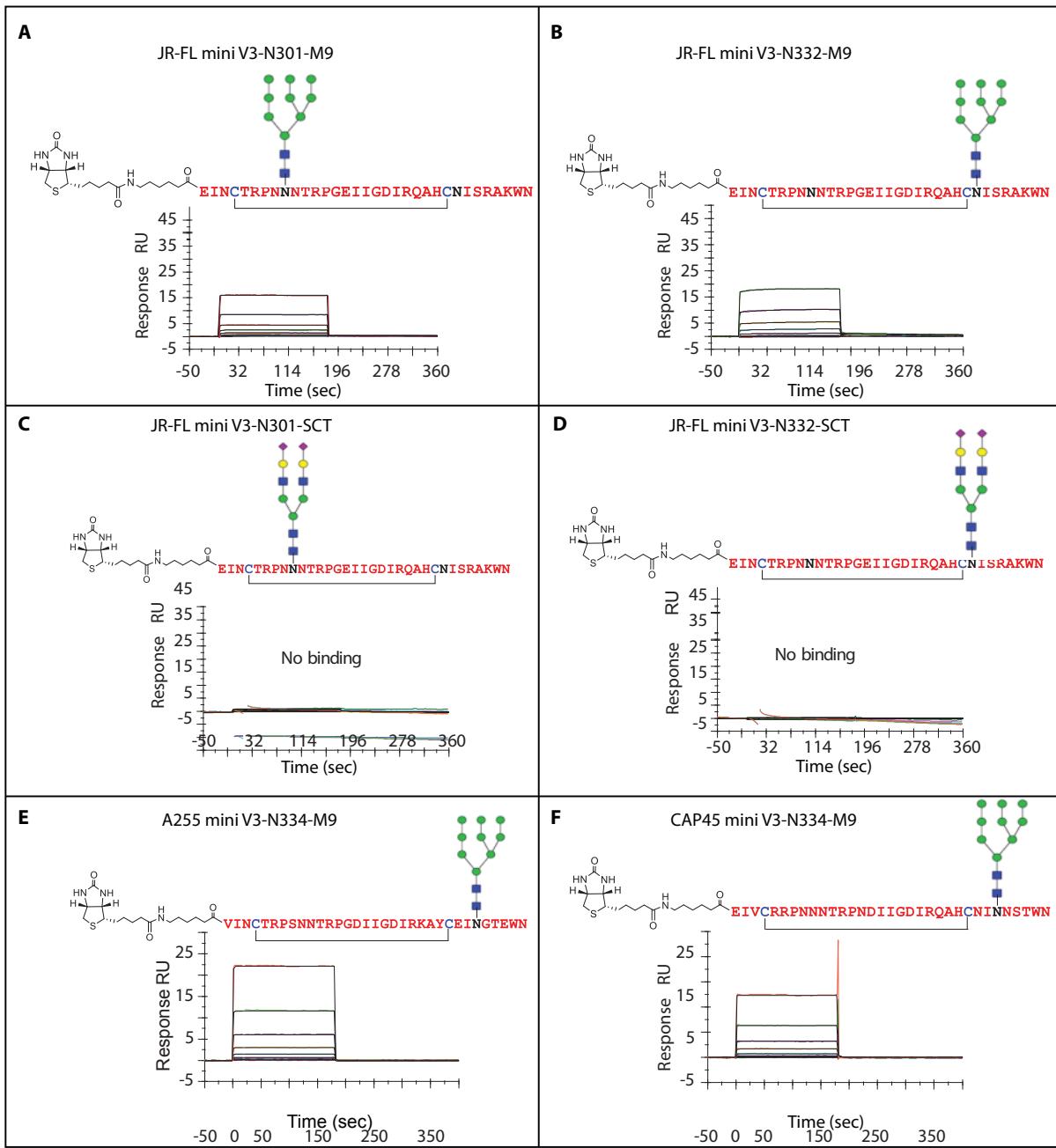
**Figure S2: Competitive ELISA.** An equal amount of (4  $\mu$ g/mL) of biotinylated BG18 (top) and BG1 (bottom) were assayed for binding in ELISA to BG505 SOSIP.664 in the presence of increasing amounts of various competing antibodies. Detection was performed using Streptavidin HRP. The black line indicates biotinylated antibody binding in the absence of competition.



**Figure S3: NC37 Fab-gp120 complex structure.** (A) 2.7 Å crystal structure of a NC37 Fab-gp120 complex superimposed on an 8ANC134 Fab-gp120 complex structure (PDB 4RX4). (B) Close-up of the NC37 Fab-gp120 interface compared to the 8ANC134 Fab-gp120 interface. (C) Side view of a BG505 trimer structure in surface representation (PDB 4TVP) with predicted epitopes for NC37 (purple), 8ANC134 (green) and NIH45-46 (orange) shown on one protomer (dark gray) of the trimer.

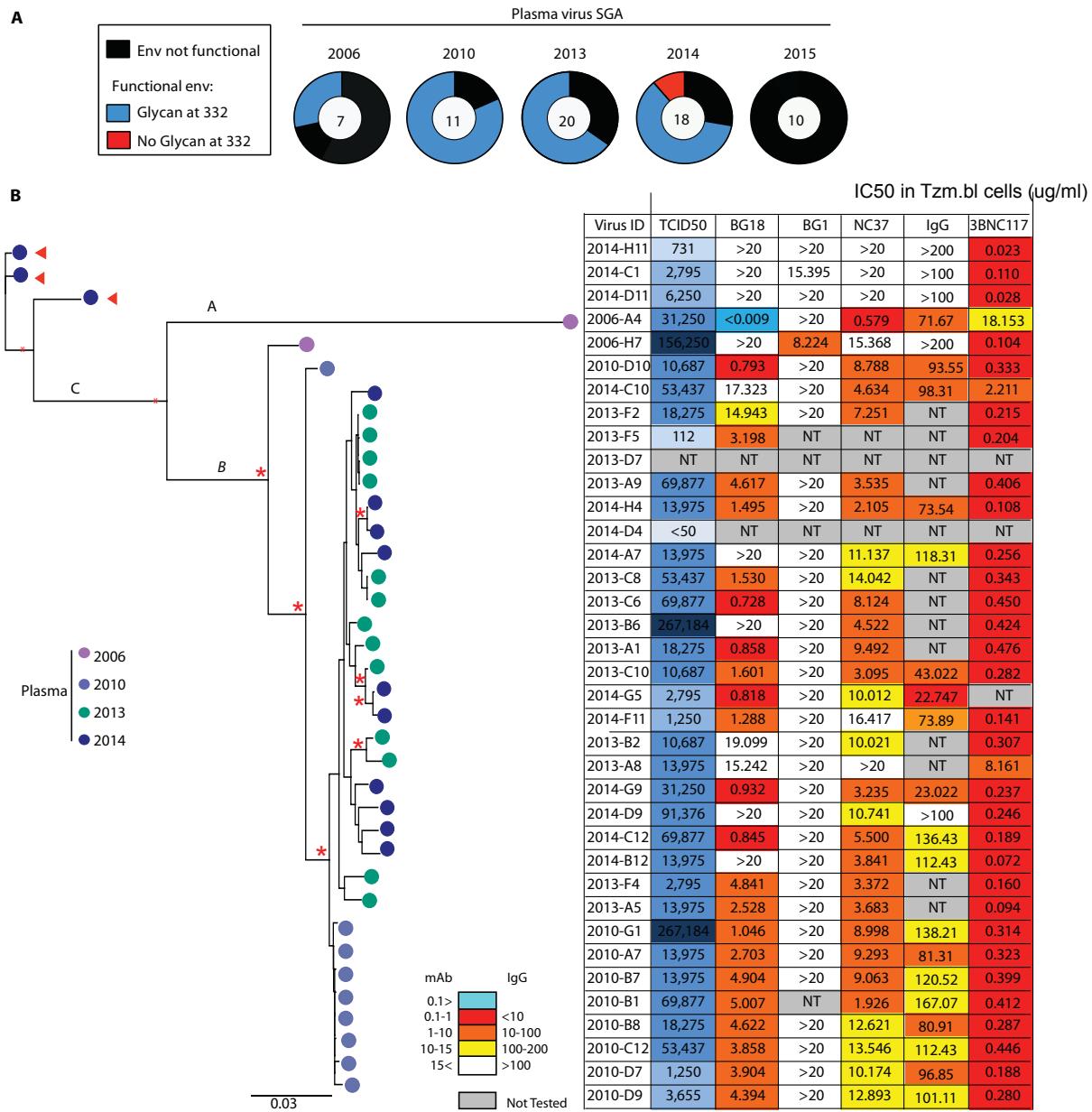


**Figure S4: Sequence comparison between BG18, PGT121, and 10-1074.** (A) and (C): Dendrogram of the sequences of the heavy chains and light chains, respectively, of PGT121, 10-1074, the nine BG18 clonal variants, and their predicted germlines. (B) and (D): Amino acid sequence alignment of the heavy chains and the light chains CDRs, respectively. Positions common to PGT121/10-1074 and BG18 variants are highlighted. Gaps in alignment are shaded in gray.



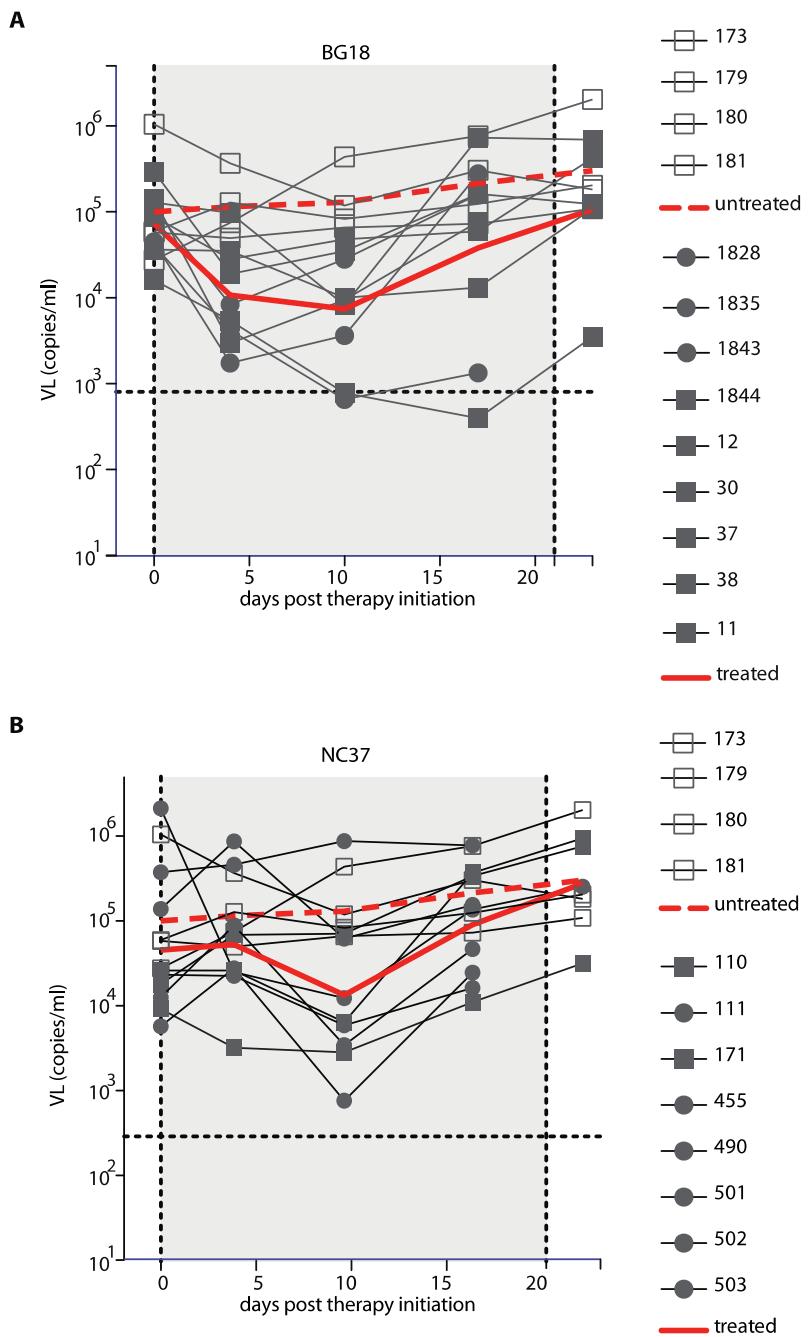
**Figure S5: BG18 antibody binding to synthetic HIV-1 V3 glycopeptides.** Biotin-tagged V3 glycopeptides were immobilized on a neotrvavidin chip and BG18 IgG was used as the analyte. (A) Interaction between BG18 and a JR-FL mini-V3 glycopeptide carrying a Man<sub>9</sub>GlcNAc<sub>2</sub> glycan at N301. (B) Interaction between BG18 and a JR-FL mini-V3

glycopeptide carrying a Man<sub>9</sub>GlcNAc<sub>2</sub> glycan at N332. (C) Interaction between BG18 IgG and a JR-FL mini-V3 glycopeptide carrying a biantennary complex type *N*-glycan at N301. (D) Interaction between BG18 IgG and a JR-FL mini-V3 glycopeptide carrying a biantennary complex type *N*-glycan at N332. (E) Interaction between BG18 IgG and an A244 mini-V3 glycopeptide carrying a Man<sub>9</sub>GlcNAc<sub>2</sub> glycan at N334. (F) Interaction between BG18 IgG and a CAP45 mini-V3 glycopeptide carrying a Man<sub>9</sub>GlcNAc<sub>2</sub> glycan at N334.



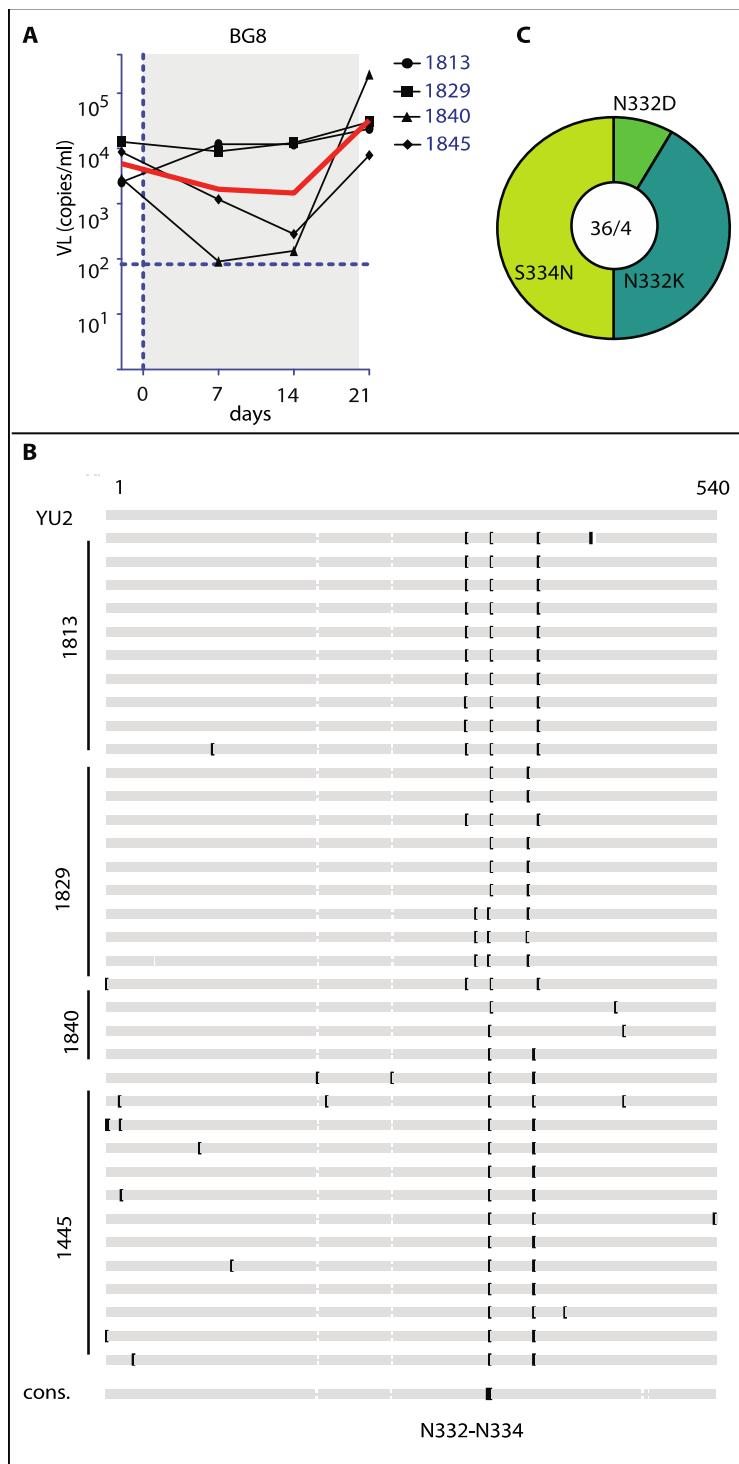
**Figure S6: env SGS of plasma virus from donor EB354.** (A) Pie charts representing the total *env* sequences amplified by single genome PCR for every collection time point. The total number of sequences obtained from every time point is denoted in the middle of the pie, and the different slices are proportional to the number of sequences. Black slices represent non-functional *env* sequences carrying either frame shifts or stop codons. Blue slice indicate functional full-length *env* carrying intact N332 PNGS. Red slice indicate functional full-

length *env* where the PNGS in position N332 was mutated. (B) Maximum-likelihood phylogenetic tree of single genome-derived *env* gene sequences from donor EB354 sampled on 2006, 2010, 2013 and 2014. A red asterisk indicates clades with bootstrap support  $\geq 90\%$ . The table on the right side shows TCID<sub>50</sub> values colored from dark blue (for high TCID<sub>50</sub>) to light blue (for low TCID<sub>50</sub>). IC<sub>50</sub> values in TZM-bl assay of BG18, BG1, NC37, IgG from the contemporaneous serum and the control antibody 3BNC117 against autologous pseudoviruses using EB354 *env* sequences. A color key for IC<sub>50</sub> is shown.



**Figure S7: Viral loads in HIV-1<sub>YU2</sub>-infected, BG18- and NC37-treated humanized mice.** (A) Viral load values in HIV1<sub>YU2</sub>-infected humanized mice before and after monotherapy with BG18 (top) and NC37 (bottom). The gray area on the graph indicates the period of time during which antibody was given. Each graph shows two independent

experiments indicated by circles (one experiment) and by squares (second experiment). Filled and open symbols indicate treated and control mice, respectively. The dashed and solid red lines indicate the average values for control and treated mice, respectively.



**Figure S8: BG8 therapy in HIV1YU2-infected humanized mice.** (A) Viral load values in four hu-mice before and after administration of BG8. The gray shaded area on the graph indicates the period of time during which antibody was administered. The bold red line

indicate the geometric mean value (B) Amino acid sequence alignment of gp120 sequences from viruses cloned on day 21 after therapy. Each horizontal gray bar represents the sequence of a single gp120 clone aligned to HIV-1<sub>YU2</sub>. Amino acid substitutions are indicated as black ticks. The mouse ID from which the sequence was obtained is indicated on the vertical black bars. An expanded view of the boxed areas is shown on the right in each panel. (C) Pie chart showing the recurrent mutations in gp120 compared to the wild-type HIV-1<sub>YU2</sub> sequence following BG8 therapy. The number in the center of the pie denotes the total number of sequences cloned; the slices represent the most consistently mutated areas in gp120 and are proportional to the number of sequences that carried mutations.

#### **Supplementary Tables:**

**Table S1. Clinical information, donor EB354**

**Table S2. IC<sub>50</sub> and IC<sub>80</sub> values in TZM-bl assay for antibodies NC37, BG1, BG18, BG8, and 10-1074 and PGT121 against a 118-virus panel**

**Table S3. IC<sub>50</sub> and IC<sub>80</sub> values in TZM-bl assay of the 1:1:1 combination of NC37, BG1, and BG18 against a 118-virus panel**

**Table S4. IC<sub>50</sub> and IC<sub>80</sub> values in TZM-bl assay of antibodies BG8, BG18, PGT121, and 12A12 against selected HIV-1 V3 envelop mutants.**

**Table S5. Crystallization statistics**