SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Molecular dynamics simulations on glycan-removed HIV-1 gp140 proteins

We constructed an all-atom fully glycosylated trimer model using the coordinates of the BG505 X-ray structure (PDB ID 4TVP) as a template. Any missing segments in the BG505 X-ray structure were modeled using LoopyMod (Soto et al., 2008) based on the native BG505 sequence. Missing side chains were modeled using the program SCAP (Xiang and Honig, 2001). Using in-house software we modeled a mannose$_5$ sugar moiety at each *N*-linked glycosylation sequon along the BG505 protein sequence. The resulting fully glycosylated trimer model, BG505-Man-5, served as the starting template for two additional glycosylated trimer models where glycans surrounding the CD4bs were removed. The first glycosylated trimer model where glycans surrounding the CD4bs were removed, BG505-Δgly-VRC01-Man-5, had glycans N197, N276, N362 and N461 deleted from the BG505-Man-5 model. The second glycosylated trimer model where glycans surrounding the CD4bs were removed, BG505- Δgly-VRC13-Man-5, had glycans N133, N197, N301 and N362 deleted from the BG505-Man-5 model.

All three models (BG505-Man-5, BG505-Δgly-VRC01-Man-5 and BG505- Δgly-VRC13-Man-5) were fully solvated in a water box of dimensions 175 × 175 × 175 Å^3 with a 17 Å padding. The system was neutralized by the addition of NaCl at a concentration of 150 mM. The CHARMM36 force field was used for the parameterization of the protein (including CMAP corrections) (Best et al., 2012) and the mannose-9 (Guvench et al., 2011). TIP3P parameterization (Jorgensen, 1983) was used to describe the water molecules.

All molecular dynamics (MD) simulations involving the three fully glycosylated trimer models were performed with ACEMD software (Harvey et al., 2009) in explicit solvent on a METROCUBO workstation (https://www.acellera.com/products/GPU-hardware-molecular-dynamics-metrocubo/). The system was minimized for 2000 steps, followed by equilibration in the NPT ensemble for 50 ns at 1 atm and 300 K using a time-step of 2 fs, rigid bonds, cutoff of 9 Å and PME for long range electrostatics. During the equilibration, heavy protein atoms were constrained by a 1 kcal/mol·Å^2 spring constant and slowly relaxed over the first 5 ns, and the protein was allowed to move freely thereafter. The system was then simulated for 500 ns under the NVT ensemble using ACEMD in the NVT ensemble using a Langevin thermostat with damping of 0.1 ps^{-1} and hydrogen mass repartitioning scheme to achieve time-steps of 4 fs.

Snapshots from each of the three the MD simulation were taken at 0.1 ns time intervals for structural analysis. To assess the internal geometry of each glycan moiety, we analyzed the distribution of GlcNAc-GlcNAc-β-mannose angles (kink angle) for each simulation. The kink angle is defined as the angle formed between the geometric centers of the first three saccharide rings originating from the Nδ2 atom of a glycosylated asparagine residue. We also assessed the distribution of glycan-glycan contacts by a defining a contact as any glycan that has 10 or more atoms within a distance of 5.0 Å to another glycan.

Glycan-antibody overlap analysis

For the glycan-antibody overlap analysis we determined the number of atoms from each glycan that occupied the same volume that would be potentially occupied by an antibody. We used 8 structures that were co-crystallized with gp120 (PDB IDs: 4JAN, 4JM2, 4YDJ, 4YE4, 3U2S, 5FYJ, 4P9H and 2NY7), two antibody structures that were co-crystallized an HIV-1 prefusion trimer (PGT122 and 35O22; PDB ID: 4TVP), one antibody structure docked onto a HIV-1 prefusion trimer using a cryoEM density map (PGT151; PDB ID: 4NUG) and one antibody structure co-crystallized with CD4 (PDB ID: 1GC1). Each co-crystallized structure was aligned to each of the three MD trajectories at 0.1 ns time points using TM-align (Zhang and Skolnick, 2005). After superimposition, we determined all glycan atoms from the MD trajectory within 3.0 Å of the antibody or CD4 structures.

Fab expression and purification

109L+3H (Garces et al., 2015) and 35O22 IgG heavy chain plasmids containing the HRV3C cleavage site after Lys 218 in the hinge region were co-transfected with counterpart light chain plasmids in EXPI (35O22) or HEK 293S GnTI$^-$ (PGT122) cells using TrueFect-Max transfection reagent (United Biosystems) according to manufacturer's protocol. Cultures were fed with fresh 293FreeStyle media (Life Technologies) 4 h post-transfection and then with HyClone SFM4HEK293 enriched medium (HyClone) containing valproic acid (4 mM

final concentration) 24 h after transfection. Cultures were incubated at 33º C for 6 days, and supernatants harvested, clarified by centrifugation and filtration and passed over a protein A affinity column. After PBS washing and low pH elution, the eluate was pH neutralized with 1M Tris pH 8.0 and protein solutions were extensively dialyzed against PBS. Fabs were obtained using HRV3C digestion overnight at room temperature and collected from flow-through from a protein A column to remove Fc fragments. Fabs were then further purified through a Superdex 200 column in PBS.

Crystallization of glycan-removed BG505 SOSIP

The BG505 SOSIP.664 N137A, S198A, T278A, S365A, T464A protein was expressed in HEK 293 GnTI$^-$ cells, captured with a VRC01 and eluted with 3 M $MgCl_2$ then purified through a Superdex 200 column in PBS in a similar protocol as described previously (Pancera et al., 2014; Stewart-Jones et al., 2016). 2-fold molar excess 109L+3H and 35O22 Fabs were mixed with the Env trimer and incubated overnight at room temperature. Complexes were purified through a Superdex 200 column with 5 mM HEPES, pH 7.5, 150 mM NaCl and 0.02% azide.

HIV-1 trimer complexes were screened for crystallization using 572 conditions from Hampton, Wizard and Precipitant Synergy (Majeed et al., 2003) screens using a Cartesian Honeybee crystallization robot as described previously (McLellan et al., 2011) and a mosquito robot using 100 nl of reservoir solution and 100 nl of protein solution.

Crystals of glycosylated BG505 SOSIP.664 N137A, S198A, T278A, S365A, T464A complexed with 109L+3H and 35O22 Fabs were obtained from 0.1M $CaCl_2$, 9.9% 2-methyl-2,4-pentanediol, 4.95% PEG8000, 0.1M NaAc pH5.5 and grew to dimensions of 600 µm x 100 µm x 100 µm. Crystals were cryoprotected in solutions of 20% ethylene glycol, maintaining the mother liquor components at the original concentrations, and flash-frozen in liquid nitrogen. Data were collected at a wavelength of 1.00 Å at the SER-CAT beamline ID-22 (Advanced Photon Source, Argonne National Laboratory).

X-ray data collection, structure solution and model building

Crystals for glycan-deleted BG505 SOSIP.664 N137A, S198A, T278A, S365A, T464A-109L+3H-35O22 were in space group $P6_3$, and diffraction extended to 2.9 Å resolution along the *c* axis (c-axis $I/\sigma I > 3.0$) and to lower resolutions along *a* and *b* axes, resulting in an overall dataset resolution with $I/\sigma I$ of ≥ 2.0 of 3.65 Å with 81.4 % completeness in this range (**Table S1B**). Diffraction data were processed with the HKL2000 suite (Otwinowski and Minor, 1997). The data were corrected for anisotropy by http://services.mbi.ucla.edu/anisoscale/ with truncations to 3.65 Å, 3.65 Å, 2.9 Å along a, b, and c axes, respectively. Structure solution was obtained with Phaser (McCoy et al., 2007) using BG505 SOSIP.664-N137A -109L+3H-35O22 (PDB ID: 5CEZ) (Garces et al., 2015), as search model. Refinement was carried out with Phenix (Adams et al., 2004) and Buster (Bricogne, 2011). Model building was carried out with Coot (Emsley and Cowtan, 2004). Carbohydrates were modelled into *Fo*-*Fc* maps and further refined using *Fo*-*Fc*, *2Fo*-*Fc* and feature-enhanced maps (Afonine et al., 2015). Glycan geometries and real-space correlation coefficients were determined using the program Privateer (Agirre et al., 2015). Structure parameters such as the Ramachandran plot as well as rotamers were validated by MOLPROBITY (Davis et al., 2004) during refinement. Data collection and refinement statistics are shown in Table S1.

Design of glycan-removed HIV-1 gp140 proteins from diverse HIV-1 strains

The 4-glycan-deleted BG505.DS-SOSIP were synthesized with the third amino acid in sequons of 4 potential glycosylation sites at the vicinity of HIV-1 CD4-binding site, N197, N276, N362 and N462, mutated to Alanine. Glycan-deleted DS-SOSIP trimers from clade B and C were constructed as chimeric proteins with the BG505.DS-SOSIP as backbone. Regions of the Env sequences (residues 31-664) from diverse HIV-1 strains were replaced with corresponding gp120 residues (31-45 and 478-511) and the full gp41 region of the BG505 molecule to keep the core of trimer the same as that of BG505.DS-SOSIP, in which disulfide bridges between residues 201 and 433 (named DS) and residues 501 and 605 (named SOS), Ile to Pro mutation at gp41 residue 559 (named IP) and an improved cleavage site (RRRRRR) were introduced. Sequon Glycosylation sites N197, N276, N362 and N462, if present in the native sequences of respective HIV-1 strains, were all mutated to NxA.

Production, negatively selection and storage of glycan-removed HIV-1 gp140 proteins from diverse HIV-1 strains

Glycan-removed DS-SOSIP trimer mutants from clades A, B and C were produced in 293 FreeStyle cells, as described previously (Pancera et al., 2014). Briefly, 600 µg of DS-SOSIP trimer construct was co-transfected with 150 µg of furin plasmid DNA into 1 liter of cells. The transfected supernatants were harvest and filtered with 0.22 µm filters after 6 days. The supernatants were then loaded over a VRC01-affinity column. After washing with phosphate-buffered saline (PBS), the trimer proteins were eluted with 3 M MgCl2 and 30 mM Tris at a pH of 7.0, and concentrated to 2-3 ml using Amicon Ultracel-50K (Millipore) before loading onto a Superdex200 16/600 gel filtration column (GE Healthcare) equilibrated in PBS. The peak corresponding to trimeric HIV-1 Env was identified and pooled. To remove any aberrant trimers in non-prefusion states, the purified trimer proteins were negatively selected first with a protein A immobilized V3-antibody 447-52D (PDB 4M1D) column, and subsequently with a V3 antibody cocktail column containing 6 immobilized V3-directed antibodies: 1006-15D, 2219, 2557, 3074, and 50.1 (PDB 3MLW, 2B0S, 3MLS, 3UJI, 3MLX, and IGGI). Endotoxin levels of the purified trimer proteins were assessed to ensure that they were all below 20 EU/100 µg of protein for use in non-human primates. 10% Glycerol was added to purified proteins as cryo-protectant before freezing with liquid nitrogen. All frozen proteins were stored at -80 ºC until use.

Antigenic analysis of glycan-removed DS-SOSIP trimers

Standard 96-well bare MULTI-ARRAY Meso Scale Discovery (MSD) Plates (MSD, Cat# L15XA-3) were coated with a panel of HIV neutralizing antibodies targeting different antigenic sites and different conformational states, specifically, for CD4-binding site: VRC01, b12 and VRC13; for glycan-V3 site: PGT121, PGT128, 2G12; for quaternary conformation: PGT145 and CAP256-VRC26.25; for gp41gp120 interface and cleavage: 35O22, 8ANC195 and PGT151. In addition, non-neutralizing CD4-binding site antibodies F105, 17b and 48D and V3-loop antibodies 447-52D, 3074 and 2557 were used to coat the MSD plate. Non-cognate antibodies (anti-influenza antibodies CR9114 and anti-RSV antibodies D25 were also included to identify non-specific interaction). Antibodies were coated in duplicate (30 µL/well) at a concentration of 4 µg/ml diluted in 1X PBS by incubating overnight at 4º C. The plates were washed (wash buffer: 0.05% Tween-20 + 1 X PBS) and blocked with 150 µL of blocking buffer (5% [W/V] MSD Blocker A (MSD, Cat# R93BA-4)) by incubating for 1 hr on a vibrational shaker (Heidolph TITRAMAX 100; Cat# P/N: 544-11200-00) at 650 rpm before a washing step. While the incubation in progress, HIV-1 DS-SOSIP trimers were titrated in serial 2X dilutions starting at a concentration of 5 µg/ml of the trimer in the assay diluent (1% [W/V] MSD blocker A + 0.05% Tween-20). For soluble CD4 (sCD4) induction, the trimer was combined with sCD4 at a constant molar concentration of 1 µM before being added to the MSD plate. Diluted trimer was transferred (25 µl/well) to the MSD plates and incubated for 2 hr on the vibrational shaker at 650 rpm. After the 2 hr incubation with trimer, the plates were washed again. 2G12 antibody, labeled with MSD SULFOTAG (MSD; Cat #R91AO-1) at a conjugation ratio of 1:15 (2G12:SULFOTAG), was diluted in assay diluent at 2 ug/ml and added to the plates (25 µL/well), and incubated for 1 hr on the vibrational shaker at 650 rpm. The plates were washed and read using 1X read buffer (MSD Read Buffer T (4x); Cat# R92TC-1) on the MSD Sector Imager 2400.

Negative stain electron microscopy

Samples were adsorbed to freshly glow-discharged carbon-film grids, rinsed twice with buffer and stained with freshly made 0.75% uranyl formate. Images were recorded on an FEI T20 microscope with a 2,000 × 2,000 Eagle CCD camera at a pixel size of 1.5 Å. Image analysis and 2D averaging was performed with Bsoft54 and EMAN55.

Glycan analysis

An aliquot of each sample was denatured by incubating with 10 mM of dithiothreitol at 56 °C for an hour and alkylated by 55 mM of iodoacetamide for 45 minutes in dark prior to digestion with proteases optimized based on amino acid sequence of each target protein. Specifically, each aliquot was treated with a combination of proteases including trypsin (Promega), Lys-C (Promega), Arg-C (Promega), and Glu-C (Promega). Following digestion, the samples were deglycosylated by Endo-H (Promega) followed by PNGaseF (Glyko®, Prozyme) treatment in the presence of O18-water. The resulting peptides were separated on an Acclaim PepMap RSLC C18

column (75 µm x 15 cm) and eluted into the nano-electrospray ion source of an Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer (Thermo Fisher Scientific) with a 180-min linear gradient consisting of 0.5-100% solvent B over 150 min at a flow rate of 200 nL/min. The spray voltage was set to 2.2 kV and the temperature of the heated capillary was set to 280 °C. Full MS scans were acquired from m/z 300 to 2000 at 120k resolution, and MS2 scans following collision-induced fragmentation were collected in the ion trap for the most intense ions in the Top-Speed mode within a 3-sec cycle using Fusion instrument software (v2.0, Thermo Fisher Scientific). The resulting spectra were analyzed using SEQUEST (Proteome Discoverer 1.4, Thermo Fisher Scientific) with full MS peptide tolerance of 20 ppm and MS2 peptide fragment tolerance of 0.5 Da, and filtered using ProteoIQ (v2.7, Premier Biosoft) at the protein level to generate a 1% false discovery rate for protein assignments. Site occupancy was calculated using spectral counts assigned to the O18-Asp-containing (PNGaseF-cleaved) and/or HexNAc-modified (EndoH-cleaved) peptides and their unmodified counterparts. Note that quantification by spectral counts, as opposed to area-under-the-curve, may overestimate the presence of minor glycoforms (Cao et al., 2017).

Animal protocols and immunization

For immunization studies, animals were housed and cared for in accordance with local, state, federal, and institute policies in an American Association for Accreditation of Laboratory Animal Care-accredited facility at VRC, NIAID, NIH or at a contract facility (Bioqual Inc, MD). All animal experiments were reviewed and approved by the Animal Care and Use Committee of the Vaccine Research Center, NIAID, NIH. The animal work was covered under protocol VRC 13-431 for guinea pigs, and VRC 13-450 for monkeys.

Female Hartley guinea pigs with body weights of 300 grams were purchased from Charles River Laboratories (MA). For each immunization, 400 µl of immunogen mix, containing 25 µg of specified, filter-sterilized protein immunogen and 80 µl of Adjuplex (Sigma-Aldrich Inc, MO) in PBS, was injected to muscles of the two hind legs. Blood was collected through retro-orbital bleeding under anesthesia for serological analyses.

Female or male Indian Rhesus Macaca with body weights of 2-9 kg were used for immunization studies. For each immunization, 1 ml of immunogen mix, containing 100 µg of specified, filter-sterilized protein immunogen and 200 µl of Adjuplex (Sigma-Aldrich Inc, MO) in PBS, were injected via a needle syringe to the caudle thigh of the two hind legs. Blood was collected for serological analyses.

Determination of optimal adjuvant

Besides Adjuplex, Alhydrogel (aluminum hydroxide) and ISCOMatrix adjuvants were used for the optimization of adjuvants study in NHP. Alhydrogel was purchased from Sergeant Adjuvants (Clifton, NJ) and mixed with immunogens at 10:1 weight ratio. ISCOMatrix was obtained from CSL (Australia) and 75 Isocounits was used for each immunization.

Enzyme-linked immunosorbent assay (ELISA) for sera competition with VRC01

Biotinylation of VRC01

Monoclonal VRC01 antibody (500 µl of 1 mg/ml) was concentrated to 200-250 µl in PBS using a 30,000 NMWL filter (Amicon Ultra®, Millipore) spun at 14,000 xg. 15 µl of 50 µg/ml Blue Dextran were added to the concentrated VRC01. The VRC01 solution was added to an Ilustra Nap-5 column (Sephadex™ G25 DNA Grade, GE Healthcare) pre-washed 5 times with Reaction Buffer (100mM Carbonate, pH 8.4) and eluted with 750 µl Reaction Buffer to collect the Blue Dextran-stained fraction. 80 µg *N*-hydroxysulfosuccinimidobiotin (10 mg/ml in DMSO, Pierce, Rockford, IL) were added per 1 mg VRC01 and incubated on a rotator for 4 hours at room temperature. The reaction solution was added to a PD-10 (GE Healthcare) desalting column pre-washed 5 times with Storage Buffer (10mM Tris, 150mM NaCl, 0.1% NaN_3, pH 8.2 100 mM Carbonate, pH 8.4) and eluted with consecutive additions of 750 µl Storage Buffer to collect the Blue Dextran-stained fraction. The final concentration of biotinylated VRC01 was estimated by the ratio of the pre- to post-biotinylation solution volume.

Procedure for Enzyme-linked immunosorbent assay (ELISA) for sera competition with VRC01

Non-human primate sera were assayed using 96-well plates (Costar® High Binding Half-Area, Corning, Kennebunk, ME) coated with 50 µl/well of 2 µg/ml BG505 DS SOSIP Δgly4 or CH505 DS SOSIP Δgly4 antigen overnight at 4°C. Between subsequent steps, except for the addition of biotinylated VRC01 (see Biotinylation of

VRC01), plates were washed 5 times with PBS-T (PBS + 0.05% Tween) and incubated at 37°C for 1 hour. After coating, plates were blocked with 100 µl/well of blocking buffer (B3T: 150 mM NaCl, 50 mM Tris-HCl, 1 mM EDTA, 3.3% fetal bovine serum, 2% bovine albumin, 0.07% Tween 20, 0.02% Thimerosal). Next, 50 µl/well of 1:500 B3T-diluted rhesus macaque sera were added column-wise. This was followed, without washing, by serially-diluted (5-fold, Starting Dilution: 2µg/ml) biotinylated VRC01 at 50 µl/well, which incubated for 30min at 37°C. Afterward, streptavidin-conjugated horseradish peroxidase (KPL, Gaithersburg, MD) diluted 1:200 in B3T was added at 50 µl/well. Plates were developed with tetramethylbenzidine (TMB) substrate (SureBlueTM, KPL, Gaithersburg, MD) for 10 minutes before adding 1N sulfuric acid (Fisher Chemical) to stop the reaction. Plates were read at 450nm (Molecular Devices, SpectraMax using SoftMax Pro 5 software) and the optical densities (OD) analyzed following subtraction of the non-specific horseradish peroxidase background activity.

Guinea pig sera were analyzed using the above protocol with the following modifications: the samples were diluted 1:5,000 in B3T, unlabeled VRC01 was substituted for biotinylated VRC01 as the guinea pig sera do not cross-react with the secondary antibody, and goat anti-human IgG antibody (Jackson ImmunoResearch, West Grove, PA) diluted 1:5000 in B3T was used as the secondary antibody.

The area under the curve (AUC) was calculated for each sample using PRISM (PRISM 6 for Windows, GraphPad Software) and normalized to the AUC for biotinylated VRC01 in the absence of a competitor. The percent reduction in VRC01 binding was defined as the value of one hundred multiplied by the quantity of one minus the Week 18/Prebleed AUC ratio. Within each immunogen group, the calculated percent reduction values were normalized to the greatest individual value. An alpha level of 0.05 was used for all statistical tests using PRISM. The intragroup VRC01 binding reduction between the Prebleed and Week 18 timepoints was assessed by t-test while the correlation of the serum neutralization titers with the degree of VRC01 binding reduction was determined by a two-tailed Pearson's correlation.

Construction and characterization of HIV-1 virus panels with glycans removed around the CD4-binding site

To quantify and map the effects of glycan removal on immune responses of the glycan-deleted HIV-1 immunogens, HIV-1 gp160 with 4-glycan-deleted at glycosylation sites 197, 276, 362 and 462 were synthesized for preparing pseudoviruses (ΔGly4). A panel of viruses were also constructed in which each of the 4 glycans in the 4-glycan-deleted pseudoviruses was restored to create s series of "3-glycan-removed" pseudoviruses, named as ΔGly4 + the site restored. The panel of 3-glycan-removed pseudoviruses (ΔGly4+197, ΔGly4+276, ΔGly4+362, ΔGly4+462) allow to assess the effect of added glycan to the neutralization activity of sera from 4-glycan-deleted immunogens.

Neutralization assays

Single-round-of-replication Env pseudoviruses were prepared, titers were determined, and the pseudoviruses were used to infect TZM-bl target cells as described previously (Montefiori, 2009; Shu et al., 2007). Neutralization curves were fit by nonlinear regression using a 5-parameter hill slope equation as previously described (Seaman et al., 2010). The 50% and 80% inhibitory dilutions (ID_{50} and ID_{80}) were reported as the serum concentrations required to inhibit infection by 50% and 80% respectively; inhibitory concentrations (IC_{50} or IC_{80}) for mAbs were calculated the same way.

Serum adsorption with HIV-1 gp140 probes and neutralization

Competition of serum or mAb neutralization was assessed as described previously (Wu et al., 2010) by adding a fixed concentration (25 µg/ml) of the gp140 glycoprotein to serial dilutions of antibody for 15 min prior to the addition of virus. The resulting IC_{50} values were compared to the control with mock protein added (PBS only). The neutralization blocking effect of the proteins was calculated as the percent reduction in the IC_{50} or ID_{50} value of the antibody in the presence of protein compared to PBS.

Quantification of difference in antibody sensitivity for wild type and ΔGly4 virus

The fold difference in sensitivity between wild type and ΔGly4 viruses for each antibody was calculated as the geometric mean of the ratios of the IC_{50} for wild type virus to the IC_{50} for the ΔGly4 virus over all strains. Strains with IC_{50} values above upper limit or below lower limit were excluded from the calculations. The fold difference for CD4-supersite antibody sensitivity was defined as the geometric mean of the fold differences for VRC01, VRC-CH31, N6, b12, VRC13, CH103, and CH235.12. The fold difference for V1V2-apex antibody sensitivity was defined as the geometric mean of the fold differences for PG9 and PGDM1400. The fold difference of PGT128 sensitivity was used to represent the fold difference of V3-glycan antibody sensitivity.

Homology and glycan modeling

Homology models were built for CH505 DS SOSIP chim, 426c, CH505 DS-SOSIP, and ZM106.9 strains of HIV-1 Envelope glycoproteins. Using YASARA (Krieger et al., 2002) , gp120 and gp41 monomer and trimer crystals were chosen automatically as templates based on sequence alignment and Z-scores before a hybrid model was built for each strain. Man5 glycans were modeled on each of the homology models using the approach described in (He and Zhu, 2015).

Antibody accessible - protein surface area calculation

The mean antibody accessible-protein surface area for BG505 WT, ΔGly4, ΔGly4+197, ΔGly4+276, ΔGly4+363, and ΔGly4+462 were extracted from 500ns (5000 snap shots) of production run from molecular dynamics simulation of BG505 SOSIP with modeled Man5 glycans (Stewart-Jones et al., 2016) calculated with Naccess (http://www.bioinf.manchester.ac.uk/naccess/) using a 10 Å probe radius. The antibody accessible-protein surface area for various glycosylated states of CH505 DS SOSIP, 426c, CH505 DS-SOSIP, and ZM106.9 strains were also calculated based on the homology models. The residues that are at the bottom of the Env trimer, defined by residues that were within 15 Å from residues 31, 505, or 664, were excluded from the calculation.

Supplementary figures

Figure S1

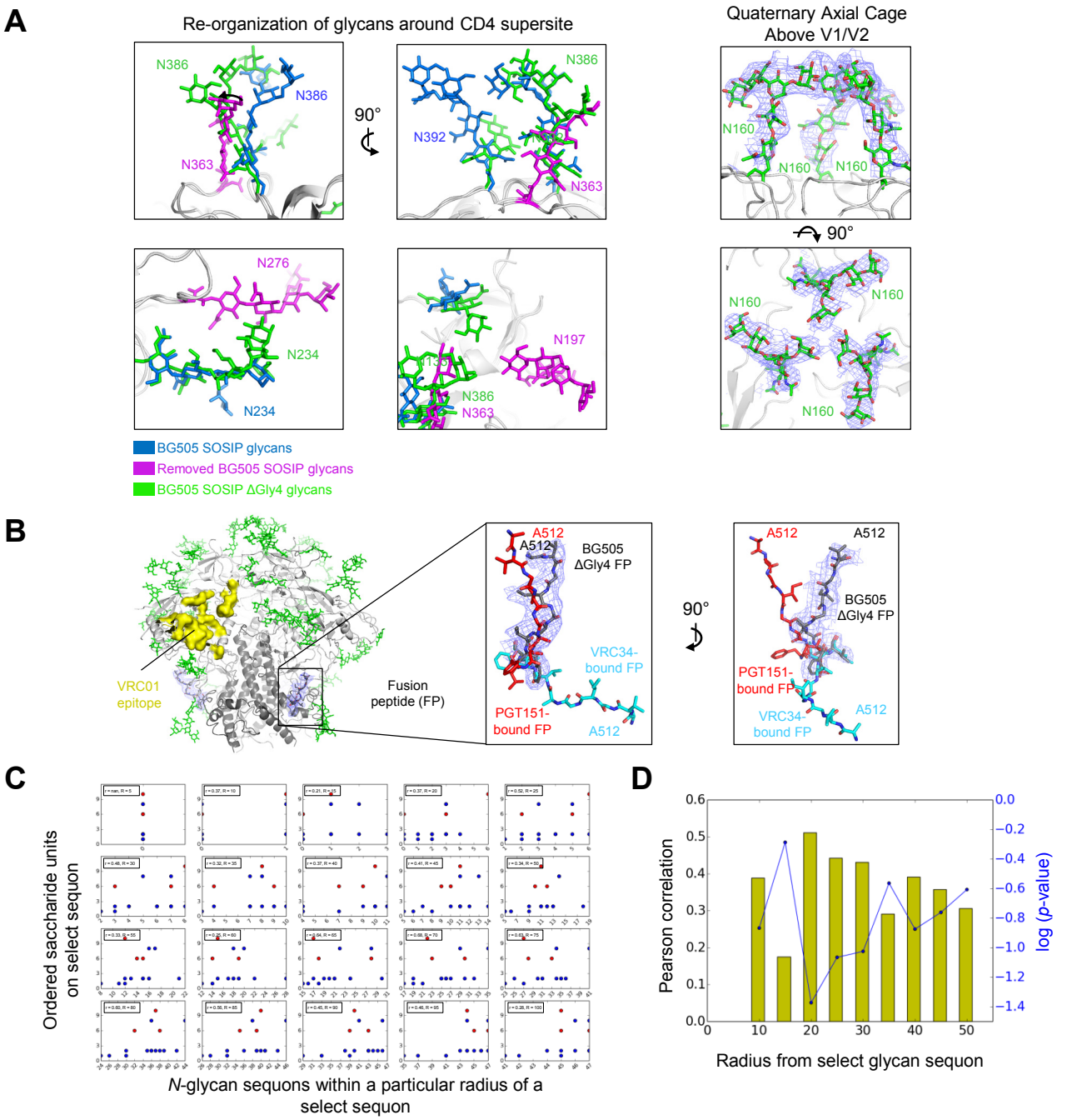


Figure S1. Structural features of 4-glycan-deleted BG505.DS-SOSIP. Related to Figure 1.

(A) Structural effects of removing select glycans around the CD4 supersite on neighboring glycans where adjacent glycans adjust and can occupy space previously occupied by select glycans, for example glycan N386 re-orientates into the glycan N363 (top left, middle panels), and it appears the disorder of glycan N392 residues increases in the absence of glycan N363 (top middle panels). Similar adjustments occur with glycan N234 when glycan N276 is removed (lower left panel). Some longer-range effects are apparent at glycan N133 (lower middle panel) and the presence of an ordered assembly of glycan N160 at the apex (top and lower right panels). Representative *2Fo-Fc* glycan electron density at 0.8 σ is shown in right panels.

(B) The BG505 SOSIP ΔGly4 structure revealed an ordered fusion peptide, shown with *2Fo-Fc* electron density at 1 σ, which adopted a unique conformation compared to the bound fusion peptide conformations in PGT151 and VRC34 structures.

(C) Number of crystallographically ordered saccharide units on a particular sequon relative to the density of neighboring N-linked glycans. Radii (R) range from 5 to 100 Å and are centered on Nδ2 of the Asn residue in the N-linked glycan sequon. There were a total of 16 visibly occupied sequons in the 4-glycan-deleted BG505 N137A SOSIP.664 Env trimer structure. Red circles show the glycans (N88, N301, N332) contacting antibodies 109L+3H or 35O22.

(D) Pearson correlation values (mustard) and associated log(*p*-value) values (blue) plotted for radii ranging from 10 to 50 Å.

Figure S2. Design of 4-glycan-deleted HIV-1 DS-SOSIP trimeric immunogens. Related to Figure 2.
Sequence alignment. Sequences of 4-glycan-deleted HIV-1 DS-SOSIP trimers were aligned with potential glycosylation sites highlighted in green and the removed-glycosylation sites highlighted in gray.

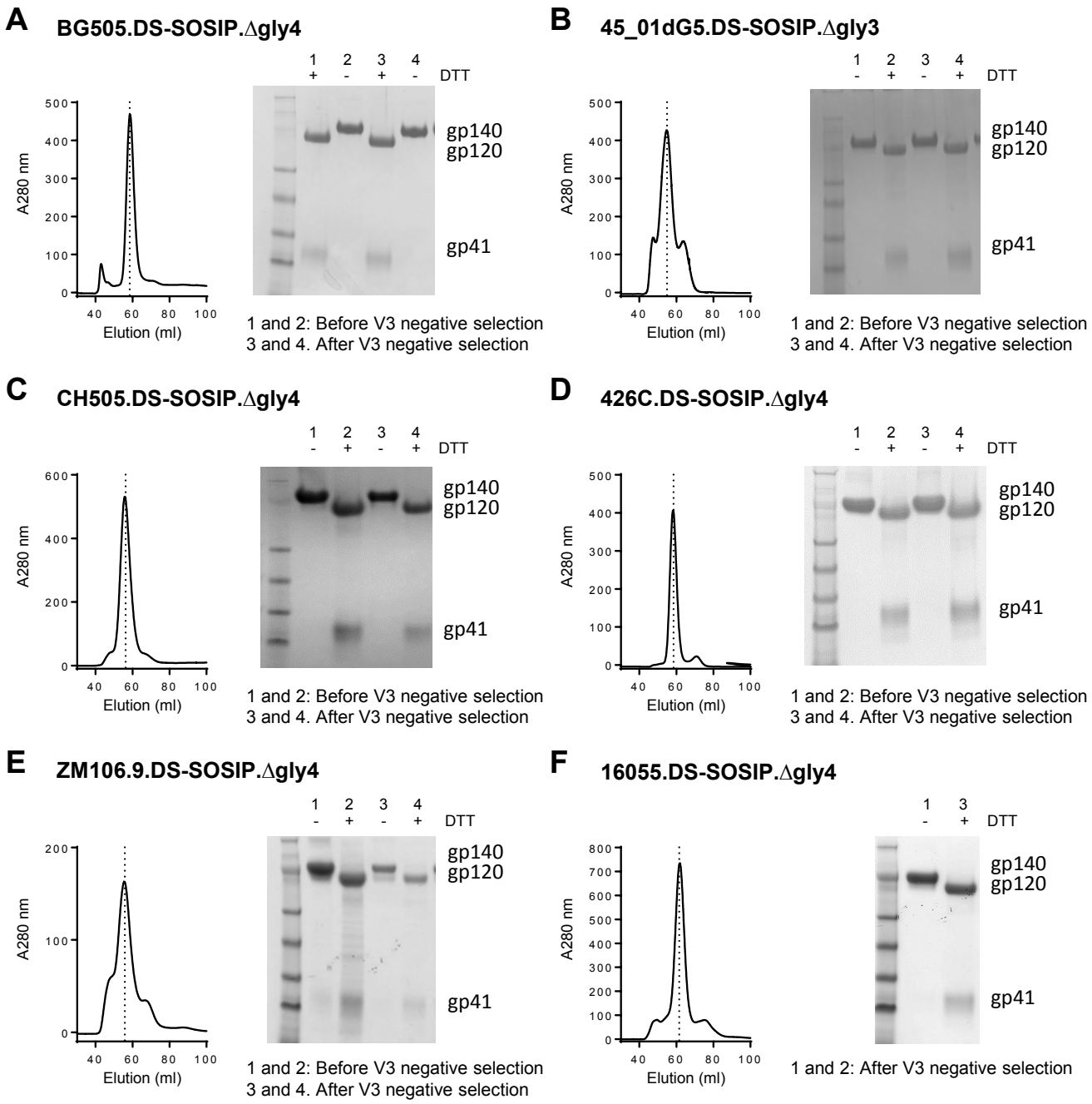
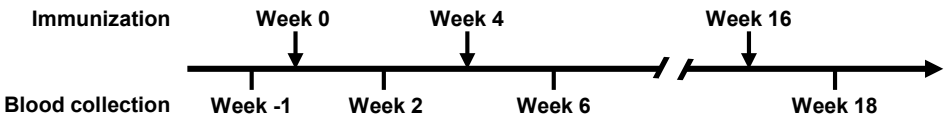


Figure S3. Purification and characterization of 4-glycan-deleted HIV-1 Env trimers. Related to Figure 2.
A-F: Gel filtration profile of diverse HIV-1 Env DS-SOSIP trimer proteins with 4 glycans deleted around the CD4-binding site are shown to the left and SDS-PAGE in non-reducing and reducing conditions are shown to the right. Purity before and after negative selection by V3-antibody affinity columns are compared on SDS-PAGE gels.

Figure S4

A



B

ID50	Virus	BG505.W6M.C2.T332N		BG505.W6M.C2.ΔGly4		Q23.17		Q168.a2		DJ263.8		JRCSF.JB		SF162.LS		TRO.11.		CAP256.206.C9		DU156.12		MW965.26		ZM233.6		SIVmac251.30	
	Clade	A		A		A		AD		AG		B		B		B		C		C		C		C		non HIV	
Animal group	Serum week	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18
BG505 DS-SOSIP Alum	A1V183	<10	15		291	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	11	50	<10	<10	<10	<10
	13N001	<10	129		209	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	51	<10	<10	<10	<10
	A1V185	11	<10		<10	25	<10	11	<10	74	<10	11	<10	<10	<10	11	<10	12	<10	11	<10	85	<10	38	<10	53	<10
	A1V030	53	<10		21	56	<10	28	<10	32	<10	62	<10	57	<10	14	<10	24	<10	24	<10	50	35	15	<10	53	<10
	A1V093	40	30		35	11	<10	13	<10	14	<10	<10	<10	<10	<10	<10	<10	14	<10	<10	<10	50	48	10	<10	12	<10
BG505 DS-SOSIP Adjuplex	A1V163	<10	<10		37	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	137	<10	<10	<10	<10
	A1V193	<10	36		320	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	89	<10	<10	<10	<10
	A1V168	<10	104		2,949	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	12	122	<10	<10	<10	<10
	A1V049	11	466		2,994	10	<10	10	<10	22	<10	10	<10	<10	<10	10	<10	13	<10	<10	<10	98	176	14	<10	10	<10
	A1V145	86	<10		165	83	<10	46	<10	95	<10	78	<10	80	<10	61	<10	56	<10	84	<10	93	137	87	<10	25	<10
BG505 DS-SOSIP ISCO Matrix	A1V139	11				53		10		29		11		<10		10		13		<10		11		10		10	
	A1V165	<10	428		300	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	20	162	<10	<10	<10	<10
	A1V010	<10	<10		991	<10	<10	<10	<10	<10	<10	<10	<10	<10	30	<10	<10	<10	<10	<10	<10	10	590	<10	<10	<10	<10
	A1V039	58	344		257	15	<10	14	<10	47	<10	15	<10	13	<10	14	<10	13	<10	14	<10	71	117	15	<10	13	<10
	A1V038	<10	<10		116	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	10	42	<10	<10	<10	<10
Control		0.109	0.025		0.0003	0.163	0.071	0.191	0.099	0.189	0.240	0.494	0.065	0.315	0.038	1.05	0.402	2.05	1.12	0.216	0.091	0.102	0.021	3.49	2.58	>10	>50

ID80	Virus	BG505.W6M.C2.T332N		BG505.W6M.C2.ΔGly4		Q23.17		Q168.a2		DJ263.8		JRCSF.JB		SF162.LS		TRO.11		CAP256.206.C9		DU156.12		MW965.26		ZM233.6		SIVmac251.30	
	Clade	A		A		A		AD		AG		B		B		B		C		C		C		C		non HIV	
Animal group	Serum week	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18	6	18
BG505 DS-SOSIP Alum	A1V183	<10	<10		107	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	49	<10	<10	<10	<10
	13N001	<10	40		45	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	45	<10	<10	<10	<10
	A1V185	<10	<10		<10	11	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	20	<10
	A1V030	21	<10		<10	24	<10	12	<10	<10	<10	27	<10	<10	<10	<10	<10	<10	<10	10	<10	<10	<10	<10	<10	24	<10
	A1V093	<10	<10		<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
BG505 DS-SOSIP Adjuplex	A1V163	<10	<10		<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	51	<10	<10	<10	<10
	A1V193	<10	<10		83	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	42	<10	<10	<10	<10
	A1V168	<10	49		883	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	52	<10	<10	<10	<10
	A1V049	<10	143		847	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	30	53	<10	<10	<10	<10
	A1V145	51	<10		64	50	<10	15	<10	63	<10	21	<10	<10	<10	28	<10	28	<10	41	<10	57	39	50	<10	<10	<10
BG505 DS-SOSIP ISCO Matrix	A1V139	<10				<10		<10		<10		<10		<10		<10		<10		<10		<10		<10		<10	
	A1V165	<10	25		<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	53	<10	<10	<10	<10
	A1V010	<10	<10		235	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	10	214	<10	<10	<10	<10
	A1V039	33	74		20	10	<10	<10	<10	15	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	41	46	<10	<10	<10	<10
	A1V038	<10	<10		32	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Control		0.345	0.140		0.0006	0.486	0.258	0.701	0.263	1.50	2.00	2.21	0.483	1.68	0.313	4.80	1.56	7.35	3.30	0.877	0.308	0.255	0.104	>10	8.37	>10	>50

Color coding for serum neutralization:

- >100,000
- 10,000-100,000
- 1000-10,000
- 100-1000
- 10-100

Color coding for antibody neutralization:

- <0.100
- 0.100 - 1.00
- 1.00 - 10.0
- >10.0

Figure S4. Effects of adjuvants on immune responses to trimeric HIV-1 Env in non-human primates. Related to Figure 3 and 5.

(A) Immunization scheme for non-human primates extending over 18 weeks.

(B) Effects of adjuvants on immunogenicity of BG505 DS-SOSIP. Neutralization titers of week 6 and week 18 serum against a panel of viruses were shown.

Figure S5

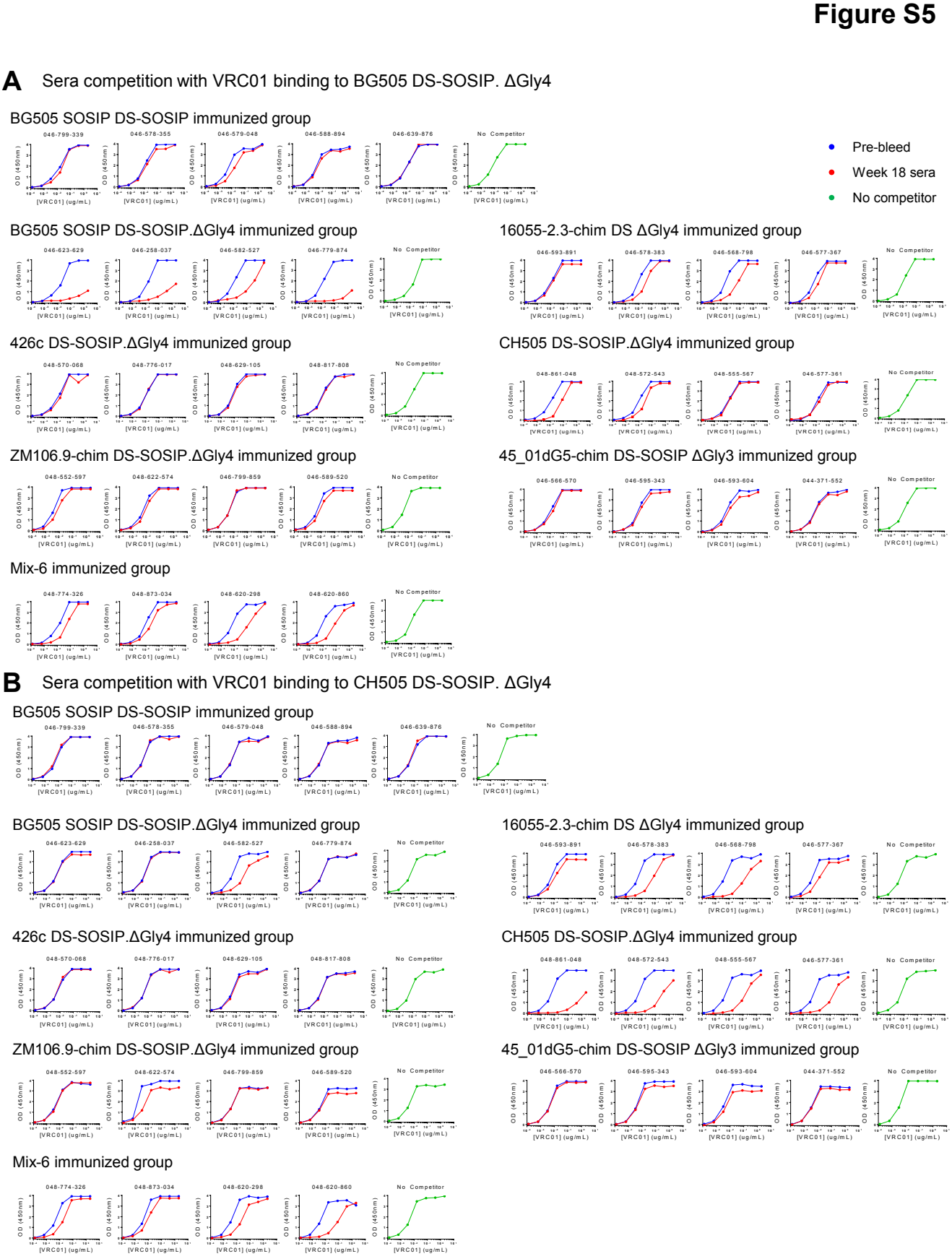
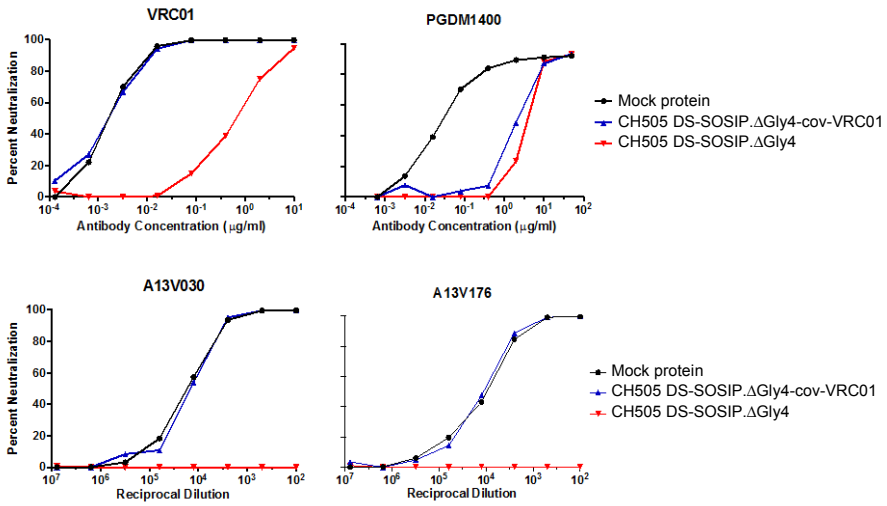


Figure S5. Week 18 guinea pig sera competition of VRC01 binding to HIV-1 trimer Env. Related to Figure 4.

(A) Sera competition on VRC01 binding to BG505 DS-SOSIP.ΔGly4.

(B) Sera competition on VRC01 binding to CH505 DS-SOSIP.ΔGly4. The right most panel in each group is the control experiment in which no sera (green) was added to standardize readings of each plate. Other panels represent pre-bleed (blue) and week 18 sera (red) of each animal in respective groups.

A



B

Epitope	mAb	IC_{50} μg/ml in the presence of: Mock Protein	CH505 DS-SOSIP.ΔGly4	CH505 DS-SOSIP.ΔGly4-cov-VRC01	Fold change in IC_{50}: CH505 DS-SOSIP.ΔGly4	CH505 DS-SOSIP.ΔGly4-cov-VRC01
CD4bs	VRC01	0.002	0.631	0.002	316	1
	VRC01	0.002	1.01	0.003	505	2
	CH235.12	0.002	5.70	0.006	2850	3
	b12	0.008	3.19	0.014	399	2
	VRC13	0.009	0.442	0.010	49	1
V1V2	PGDM1400	0.026	3.10	2.08	119	80
	PG9	0.332	2.23	1.41	7	4
gp120-gp41 Interface	PGT151	0.009	8.64	0.044	960	5
	N123-VRC34.01	1.22	17.8	16.9	15	14
MPER	10E8	2.77	2.86	2.58	1	1

Immunogen	NHP sera, wk18	ID_{50} in the presence of: Mock Protein	CH505 DS-SOSIP.ΔGly4	CH505 DS-SOSIP.ΔGly4-cov-VRC01	Fold change in IC_{50}: CH505 DS-SOSIP.ΔGly4	CH505 DS-SOSIP.ΔGly4-cov-VRC01
CH505 DS-SOSIP.ΔGly4	A13V030	15,909	<100	13,595	>159	1
	A13V124	55,310	<100	3,603	>553	15
	A13V099	6,579	<100	4,174	>66	2
	A13V103	6,482	<100	4,976	>65	1
	A11V093	6,101	<100	14,957	>61	0
	A11V051	99,364	<100	21,614	>994	5
Mix-4	A13V176	9,711	<100	11,392	>97	1
	A13V067	20,246	<100	5,027	>202	4
	A13V200	7,654	<100	5,124	>77	1
	A13V148	23,016	<100	23,048	>230	1
	A11E102	6,755	<100	4,791	>68	1
	A9V006	10,231	<100	2,314	>102	4

Immunogen	GP Sera, wk18	ID_{50} in the presence of: Mock Protein	CH505 DS-SOSIP.ΔGly4	CH505 DS-SOSIP.ΔGly4-cov-VRC01	Fold change in IC_{50}: CH505 DS-SOSIP.ΔGly4	CH505 DS-SOSIP.ΔGly4-cov-VRC01
CH505 DS-SOSIP.ΔGly4	516-1	532,680	417	93,633	1277	6
	516-2	310,803	400	80,610	777	4
	516-3	208,513	287	81,079	727	3
	516-4	130,395	497	41,006	262	3
Mix-6	522-1	37,488	<100	8,224	>375	5
	522-2	23,193	<100	4,426	>232	5
	522-3	34,527	<100	3,640	>345	9
	522-4	153,922	129	24,176	>1193	6

Figure S6. Competition by Env proteins of neutralization by week 18 Guinea pig and NHP sera. Related to Figures 4 and 6.

(A) Examples of neutralization curves in the presence of competitors. Antibodies or sera were incubated first with competitor protein, then with virus. CH505 DS-SOSIP.ΔGly4 protein competed most of the activity of the CD4bs-directed antibody VRC01, all of the activity of the two sera shown, and most of the activity of the V1V2-directed antibody PGDM1400. In contrast, CH505 DS-SOSIP.ΔGly4-cov-VRC01, which has single-chain VRC01 covalently bound and blocking the CD4bs, does not compete VRC01 or serum activity, but competes PGDM1400 as well as the parental protein does.

(B) Raw data and fold change for antibodies and sera competed by CH505.DS-SOSIP.ΔGly4 or VRC01-bound CH505.DS-SOSIP.ΔGly4 . Fold change is the ratio of IC_{50}s for samples with protein or mock protein.

Figure S7

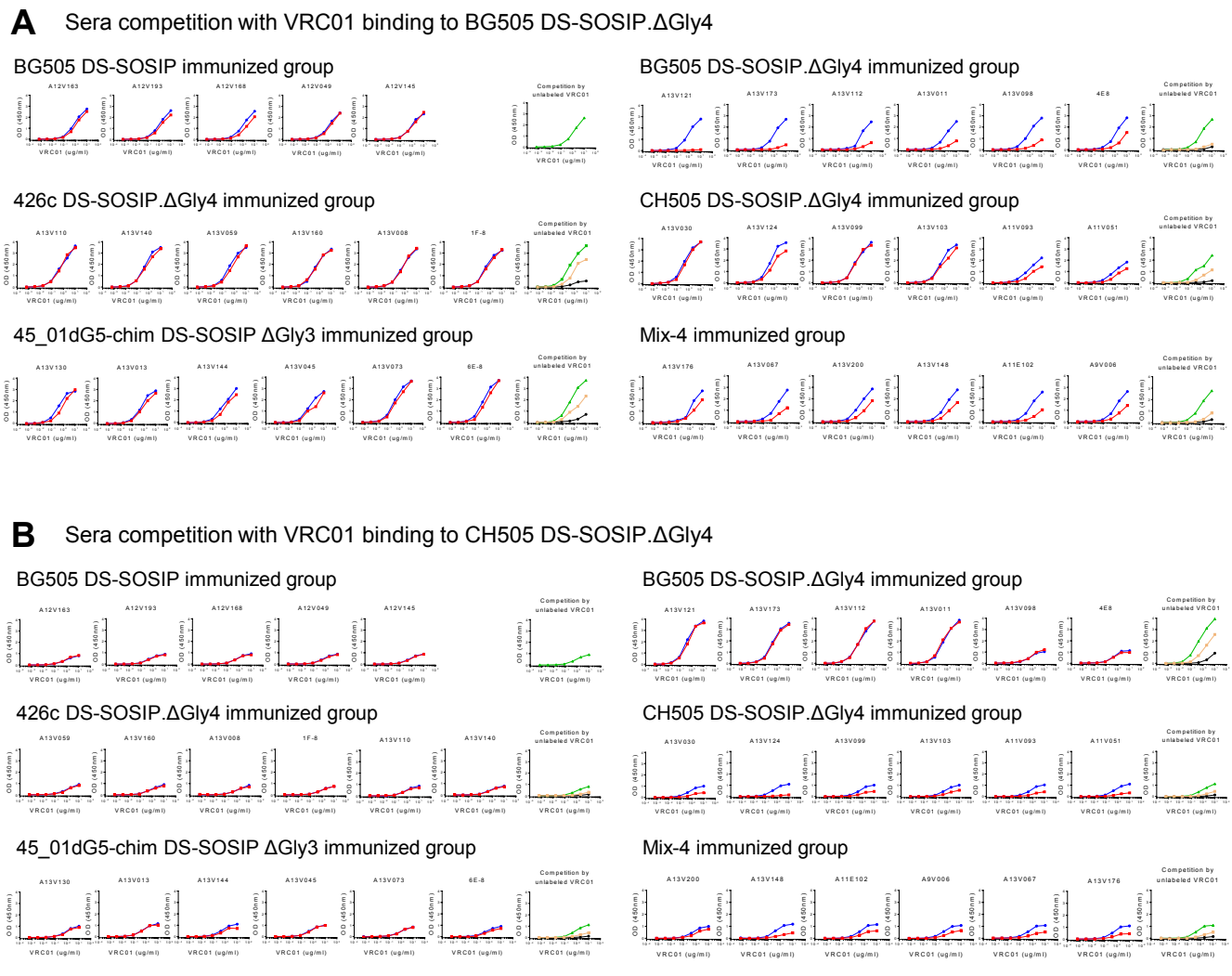


Figure S7. Week 18 NHP sera competition of VRC01 binding to HIV-1 trimer Env and correlation with antibody accessible-protein surface area. Related to Figure 6 and 7.

(A) ELISA titration curves of sera competition on VRC01 binding to BG505 DS-SOSIP.ΔGly4.

(B) ELISA titration curves of sera competition on VRC01 binding to CH505 DS-SOSIP.ΔGly4. The right most panel in each group is the control experiment in which no sera (green), or 0.128 μg/ml (orange square) and 0.64 μg/ml (black dot) unlabeled VRC01 were used to standardize readings in each plate. Other panels represent pre-bleed (blue) and week 18 sera (red) of each animal in respective groups.

(C) Pearson correlation of mean antibody accessible-protein surface area extracted from Man5 glycosylated BG505 SOSIP molecular dynamics simulation (calculated using a 10 Å probe by Naccess) with BG505-DS SOSIP ΔGly4 immunogenicity (log(ID_{50})) (see Table S7), for WT, ΔGly4, and ΔGly3 BG505 viruses.

(D) Pearson correlations between anitbody accessible-protein surface area and immunogenicity (log(ID_{50}) (see Table S7) for WT, ΔGly4, and ΔGly3 viruses, for 426c.DS-SOSIP ΔGly4 (left) and CH505 DS-SOSIP ΔGly4 (right). Accessible surface areas were calculated based on Man5 glycosylated homology models with a 10 Å probe using Naccess.

Supplemental Tables

Table S1A. Antibody-glycan overlap analysis for BG505.DS-SOSIP.ΔGly4. Related to Figure 1.

mAb \ Glycan		N88	N133	N137	N156	N160	N187 C	N187 F	N197	N234	N262	N276	N295	N301	N332	N339	N355	N363	N386	N392	N398	N406	N411	N448	N462	N611	N618	N625	N637
Glycan-dependent	PG9	0.00	0.26	11.02	41.24	49.89	25.76	42.38	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	PGT122	0.00	0.30	26.17	3.35	0.00	2.26	0.01	0.00	0.00	0.00	0.00	1.81	7.62	34.52	0.00	0.00	0.00	0.06	0.01	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	PGT135	0.00	8.99	5.51	0.00	0.00	0.74	0.01	0.00	0.00	0.00	0.00	0.10	0.00	23.20	0.08	0.00	0.00	7.15	16.14	2.46	23.64	0.66	0.00	0.00	0.00	0.00	0.00	0.00
CD4-super site	B12	0.00	22.40	10.45	0.90	0.47	36.94	8.63	0.00	0.04	17.10	0.00	20.92	58.71	4.93	0.00	0.00	0.00	71.22	0.46	0.00	0.13	0.28	0.03	0.00	0.00	0.00	0.00	0.00
	CH103	0.00	13.91	0.16	0.17	0.00	26.28	6.68	0.00	0.14	0.81	0.00	0.00	12.42	0.00	0.00	0.00	0.00	48.23	8.12	3.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	HJ16	0.00	0.63	0.01	0.00	0.00	1.71	0.03	0.00	0.94	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	4.04	15.09	10.57	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	VRC01	0.00	0.01	0.00	0.00	0.00	0.61	0.00	0.00	7.44	2.84	0.00	0.00	3.80	0.00	0.00	0.01	0.00	4.55	0.81	0.15	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.13
	VRC13	0.00	24.69	4.83	0.03	0.01	32.03	10.45	0.00	0.10	3.80	0.00	6.38	29.08	0.70	0.00	0.00	0.00	75.84	5.90	0.06	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
CD4	CD4	0.00	0.01	0.04	0.00	0.00	0.49	0.01	0.00	0.33	0.15	0.00	0.00	2.14	0.00	0.00	0.00	0.00	4.91	0.15	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
gp120 and gp41 interface	PGT151	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.36	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.70	16.40	0.00	0.60	0.03	0.00	4.70
	8ANC 195	1.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	45.55	0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.99	7.52	1.34	70.42
	35O22	42.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.83	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.16	44.01	67.50	3.53

Table S1B. X-ray crystallography of BG505 SOSIP.ΔGly4. Related to Figure 1.

HIV-1 gp140 trimer	Glycosylated BG505 SOSIP.664 N137A, S198A, T278A, S365A, T464A, Fabs 109L+3H & 35O22
PDB ID	5V7J
Data collection	
Space group	$P6_3$
Cell dimensions	
a, *b*, *c* (Å)	131.16, 131.16, 315.42
α, β, γ (°)	90.0, 90.0, 120.0
Resolution (Å)	50.00 – 2.90* (3.94-3.65, 3.65-3.44, 3.44-3.27, 3.27-3.12, 3.12-3.00, 3.00-2.90)
Unique reflections	67,932
R_{sym} or R_{merge}	0.242 (0.391, 0.384, 0.505, 0.546, 0.742, 0.814)
R_{pim}	0.131 (0.202, 0.186, 0.243, 0.262, 0.360, 0.432)
$I/\sigma I$	3.82 (2.00, 1.84, 1.55, 1.46, 1.08, 0.84)
Completeness (%)	51.3* (53.5, 38.2, 25.3, 17.3, 12.9, 10.6)
Redundancy	3.1 (2.9, 3.3, 3.9, 4.2, 4.2, 3.7)
Refinement	
Resolution (Å)	45.0-2.91 (3.70-3.56, 3.56-3.40, 3.40-3.28, 3.28-3.18, 3.18-3.09, 3.09-3.01, 3.01-2.93)
Reflections	33,547
R_{work}/ R_{free}	30.2/33.6 (0.33/0.35, 0.35/0.41, 0.37/0.44, 0.40/0.42, 0.47/0.42, 0.60/0.57, 0.93/0.83)
No. atoms	
Protein	11480
Carbohydrate	945
B-factors	
Protein	131
Carbohydrate	169
R.m.s deviations	
Bond lengths (Å)	0.017
Bond angles (°)	1.749
Ramachandran statistics	
Allowed (%)	99.2
Favored (%)	85.8
$^4C_1$ carbohydrate geometry (%)	100
MolProbity all-atoms clashscore	5.8
MolProbity score	2.99

* Completeness in resolution range 50.00-3.65 Å is 81.4 %.

Table S2. Glycosylation site occupancy of CH505.DS-SOSIP, BG505.DS-SOSIP and 4-glycan-deleted variants. Related to Figure 2.

Protein	Position	Wild type DS.SOSIP				4-glycan-deleted DS.SOSIP			
		Motif	Site Occupancy (%)	EndoH sensitive (%)	PNGaseF sensitive (%)	Motif	Site Occupancy (%)	EndoH sensitive (%)	PNGaseF sensitive (%)
BG505.DS. SOSIP	N88	NVT	97.69	29	71	NVT	96.36	4	96
	N133	NVT	91.64	90	10	NVT	58.78	84	16
	N137	NIT	93.23	19	81	NIT	81.76	18	82
	N156	NCS	98.64	98	2	NCS	100.00	61	39
	N160	NMT	98.84	98	2	NMT	100.00	61	39
	N187C	NRS	40.00	19	81	NRS	83.71	3	97
	N187F	NNS	82.39	0	100	NNS	97.37	37	63
	N197	NTS	63.46	79	21	NTA	0.00	N/A	N/A
	N234	NGT	99.30	91	9	NGT	96.63	90	10
	N262	NGS	100.00	98	2	NGS	100.00	99	1
	N276	NIT	22.03	46	54	NIA	0.00	N/A	N/A
	N295	NCT	84.26	73	27	NCT	68.61	91	9
	N301	NNT	91.07	94	6	NNT	99.02	95	5
	N332	NVS	100.00	0	100	NVS	100.00	0	100
	N339	NET	89.36	86	14	NET	75.00	92	8
	N355	NNT	88.14	25	75	NNT	92.80	17	83
	N363	NSS	99.24	94	6	NSA	0.00	N/A	N/A
	N386	NTS	59.93	66	34	NST	63.64	88	12
	N392	NST	92.39	42	58	NTS	94.81	63	37
	N398	NTS	96.15	12	88	NTS	93.46	4	96
	N405	NST	100.00	2	98	NST	100.00	2	98
	N411	NDS	95.54	39	61	NDS	97.52	34	66
	N448	NIT	98.72	96	4	NIT	99.39	100	0
	N462	NST	100.00	4	96	NSA	0.00	N/A	N/A
	N611	NSS	95.27	9	91	NSS	92.78	6	94
	N618	NLS	100.00	2	98	NLS	100.00	0	100
	N625	NMT	79.65	89	11	NMT	63.39	72	28
	N637	NYT	98.81	71	29	NYT	97.33	49	51
CH505.DS. SOSIP	N88	NVT	57.14	42	58	NVT	58.00	86	14
	N130	NCT	81.05	81	19	NCT	84.44	92	8
	N133	NAT	77.08	77	23	NAT	80.00	81	19
	N138	NSS	77.55	25	75	NSS	86.67	31	69
	N156	NCS	64.41	96	4	NCS	89.97	98	2
	N160	NIT	95.99	88	12	NIT	99.16	95	5
	N187	NSS	96.45	57	43	NSS	97.78	30	70
	N197	NTS	96.90	72	28	NTA	0.00	N/A	N/A
	N230	NKT	93.75	47	53	NKT	77.05	100	0
	N241	NVS	88.47	92	8	NVS	97.29	92	8
	N262	NGS	87.15	96	4	NGS	93.79	99	1
	N276	NIT	82.02	61	39	NIA	0.00	N/A	N/A
	N289	NES	96.33	91	9	NES	98.73	97	3
	N301	NKT	100.00	0	100	NKT	100.00	0	100
	N334	NES	96.77	86	14	NES	92.56	91	9
	N339	NET	90.91	98	2	NET	100.00	100	0
	N358	NIT	87.41	94	6	NIT	85.71	99	1
	N386	NTS	96.43	86	14	NTS	98.47	95	5
	N392	NRT	97.30	96	4	NRT	100.00	98	2
	N398	NST	65.48	69	31	NST	31.03	28	72
	N404	NST	86.99	8	92	NST	98.89	0	100
	N411	NST	100.00	1	99	NST	100.00	2	98
	N442	NIT	80.56	62	38	NIT	88.89	63	37
	N448	NIT	97.92	64	36	NIT	97.30	69	31
	N461	NNT	68.86	56	44	NNA	0.00	N/A	N/A
	N611	NSS	95.18	28	72	NSS	95.62	20	80
	N618	NLS	100.00	0	100	NLS	100.00	0	100
	N625	NMT	55.00	100	0	NMT	88.24	100	0
	N637	NYT	89.47	75	25	NYT	93.13	39	61

Table S3. Characterization of 3- and 4-glycan-deleted HIV-1 viruses. Related to Figure 3, Figure 4, Figure 5, Figure 6 and Figure 7.

This table is presented as a separate Excel file

Table S4. ID_{80} values for neutralization by week 18 guinea pig sera. Related to Figure 3 and Figure 4.

Table S4A. ID_{80} values for neutralization of HIV-1 strains homologous to immunogens by week 18 guinea pig sera

Immunogen	Virus	Clade A				Clade C						Clade B	
		BG505.T332N		16055		426c		CH505s.T/F		ZM106.9		45_01dG5	
	Animal ID	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4
BG505 DS-SOSIP	606-1	766	12,500	<10	<10	<10	<10	<10	<20	<10	<10	<10	<10
	606-2	287	232	<10	<10	<10	<10	<10	<20	<10	<10	<10	<10
	606-3	5,743	10,426	<10	<10	<10	<10	<10	<20	<10	<10	<10	<10
	606-4	161	307	<10	<10	<10	<10	<10	66	<10	<10	<10	<10
	606-5	142	429	<10	<10	<10	<10	<10	<20	<10	<10	<10	<10
BG505 DS-SOSIP.ΔGly4	517-1	219	461,270	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	517-2	144	289,544	<10	<10	<10	67	<10	<10	<10	<10	<10	<10
	517-3	<10	357,559	<10	16,277	<10	12	<10	<10	<10	<10	<10	<10
	517-4	551	175,486	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
16055-2.3-chim.DS-SOSIP.ΔGly4	519-1	<10	<10	10	31,402	<10	<10	<10	670	<10	<10	<10	<10
	519-2	<10	56,328	<10	88,842	<10	<10	<10	277,596	<10	<10	<10	<10
	519-3	<10	36,004	<10	74,873	<10	<10	<10	197,449	<10	<10	<10	<10
	519-4	<10	6,166	139	31,704	<10	<10	<10	48,992	<10	<10	<10	<10
426c.DS-SOSIP.ΔGly4	521-1	<10	<10	<10	<10	<10	189,732	<10	<10	<10	<10	<10	<10
	521-2	<10	<10	<10	<10	<10	142,746	<10	<10	<10	<10	<10	<10
	521-3	<10	<10	<10	<10	<10	316,274	<10	<10	<10	<10	<10	<10
	521-4	<10	<10	<10	<10	<10	215,691	<10	<10	<10	<10	<10	<10
CH505 DS-SOSIP.ΔGly4	516-1	<10	29,272	<10	54,041	<10	<10	<10	413,806	<10	<10	<10	<10
	516-2	<10	2,012	<10	8,280	<10	<10	<10	210,163	<10	<10	<10	<10
	516-3	<10	<10	<10	1,370	<10	<10	<10	199,369	<10	<10	<10	<10
	516-4	<10	<10	<10	1,668	<10	<10	<10	169,615	<10	<10	<10	<10
ZM106.9-chim.DS-SOSIP.ΔGly4	518-1	<10	<10	<10	<10	<10	<10	<10	<10	<10	953	<10	<10
	518-2	<10	<10	<10	<10	<10	<10	<10	<10	<10	405	<10	<10
	518-3	<10	<10	<10	<10	<10	<10	<10	<10	<10	13,888	<10	<10
	518-4	<10	<10	<10	<10	<10	<10	<10	<10	<10	1,116	<10	<10
45_01dG5.DS-SOSIP.ΔGly3	520-1	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	520-2	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	520-3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	520-4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Mix-6	522-1	<10	90,477	<10	18,193	<10	60,641	56	85,248	<10	2,189	<10	16,841
	522-2	<10	22,679	<10	11,371	<10	13,815	<10	26,307	<10	22,279	<10	51,598
	522-3	<10	45,924	<10	17,248	<10	143,881	<10	54,805	<10	50,118	<10	24,186
	522-4	<10	75,658	<10	84,914	<10	32,336	<10	158,932	<10	59,176	<10	9,694

Table S4B. ID_{80} values for neutralization of HIV-1 strains heterologous to immunogens by week 18 guinea pig sera

Immunogen	Virus	Clade AE				Clade C							
		CNE5		CNE55		26191-2.48		3168.V4.C10		0013095-2.11		001428.2.42	
	Animal ID	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4
CH505 DS-SOSIP.ΔGly4	516-1	<20	<20	<20	21	<20	613,148	<20	<20	<20	<20	<20	<20
	516-2	<20	<20	<20	<20	<20	70,600	<20	<20	<20	<20	<20	<20
	516-3	<20	<20	<20	<20	<20	14,294	<20	<20	<20	<20	<20	<20
	516-4	<20	<20	<20	<20	<20	57,719	<20	<20	<20	<20	<20	<20
16055-2.3-chim.DS-SOSIP.ΔGly4	519-1	<20	<20	<20	<20	<20	3,500	<20	<20	<20	<20	<20	570
	519-2	<20	<20	<20	<20	<20	1,292,629	<20	<20	<20	<20	<20	304
	519-3	<20	<20	<20	<20	<20	477,164	<20	<20	<20	<20	<20	77
	519-4	<20	<20	<20	<20	<20	65,114	<20	<20	<20	<20	<20	103
Mix-6	522-1	<20	<20	<20	66	<20	55,982	<20	80	<20	102	<20	3,340
	522-2	<20	<20	<20	4,274	<20	23,959	<20	4,612	<20	<20	<20	17,028
	522-3	<20	<20	<20	8,970	<20	38,365	<20	6,857	<20	<20	<20	37,500
	522-4	<20	745	<20	17,815	<20	94,570	<20	6,180	<20	<20	<20	66,508

Color coding for neutralization titer: 1000-10,000; 100-1000; 10-100; >100,000; 10,000-100,000

Table S5. ID_{80} values for neutralization by week 18 NHP sera. Related to Figure 5 and Figure 6.

Table S5A. ID_{80} values for neutralization of HIV-1 strains homologous to immunogens by week 18 non-human primate sera

Immunogen	Virus	Clade A		Clade C				Clade B	
		BG505.T332N		426c		CH505s.T/F		45_01dG5	
	Animal ID	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4
BG505 DS-SOSIP	A12V163	<10	<20	<20	<20	<20	<20	<20	<20
	A12V193	<10	53	<20	<20	<20	<20	<20	<20
	A12V168	49	371	<20	<20	<20	<20	<20	<20
	A12V049	143	466	<20	<20	<20	<20	<20	<20
	A12V145	<10	22	<20	<20	<20	<20	<20	<20
BG505 DS-SOSIP.ΔGly4	A13V121	<10	312	<10	<10	<10	<10	<10	<10
	A13V173	<10	4,735	<10	<10	<10	<10	<10	<10
	A13V112	<10	106	<10	89	<10	<10	<10	<10
	A13V011	<10	5,572	<10	<10	<10	<10	<10	<10
	A13V098	<10	5,115	<10	<10	<10	<10	<10	<10
	4E-8	<10	227	<10	<10	<10	22	<10	<10
426c.DS-SOSIP.ΔGly4	A13V110	<10	<10	57	298,813	<10	<10	<10	<10
	A13V140	<10	<10	<10	63,234	<10	<10	<10	<10
	A13V059	<10	<10	101	204,673	<10	<10	<10	<10
	A13V160	<10	<10	156	282,482	<10	<10	<10	<10
	A13V008	<10	<10	186	77,283	<10	<10	<10	<10
	1F-8	<10	<10	51	8,039	<10	<10	<10	<10
CH505 DS-SOSIP.ΔGly4	A13V030	<10	<10	<10	<10	11	5,084	<10	<10
	A13V124	<10	277	<10	<10	55	9,795	<10	<10
	A13V099	<10	91	<10	<10	11	2,776	<10	<10
	A13V103	<10	137	<10	<10	<10	2,121	<10	<10
	A11V093	<10	1,175	<10	<10	<10	8,321	<10	<10
	A11V051	<10	2,146	<10	<10	15	11,916	<10	<10
45_01dG5.DS-SOSIP.ΔGly3	A13V130	<10	<10	<10	<10	<10	<10	<10	692
	A13V013	<10	<10	<10	<10	<10	<10	<10	427
	A13V144	<10	<10	<10	<10	<10	<10	126	1,461
	A13V045	<10	<10	<10	47	<10	<10	<10	96
	A13V073	<10	<10	<10	<10	<10	<10	18	734
	6E-8	<10	<10	<10	280	<10	<10	190	4,042
Mix-4	A13V176	<10	379	<10	20,402	<10	5,341	<10	34
	A13V067	<10	28	<10	6,969	<10	7,403	<10	82
	A13V200	<10	92	87	7,787	<10	2,403	<10	143
	A13V148	<10	12	<10	6,190	13	6,111	<10	81
	A11E102	<10	589	175	3,465	<10	2,119	10	885
	A9V006	<10	<10	<10	1,603	<10	4,139	<10	81

Table S5B. ID_{80} values for neutralization of HIV-1 strains heterologous to immunogens by week 18 non-human primate sera

Immunogen	Virus	Clade AE				Clade C											
		CNE5		CNE55		26191-2.48		3168.V4.C10		0013095-2.11		001428.2.42		16055		ZM106.9	
	Animal ID	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4
CH505 DS.SOSIP.ΔGly4	A13V030	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	13	<20	62
	A13V124	<20	<20	<20	<20	<20	386	<20	<20	<20	<20	<20	<20	<20	926	<20	<10
	A13V099	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<10	<20	<10
	A13V103	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	10	<20	<10
	A11V093	<20	<20	<20	85	<20	<20	<20	<20	<20	194	<20	780	<20	871	<20	401
	A11V051	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	4107	<20	39	<20	<10
Mix-4	A13V176	<20	<20	<20	<20	<20	92	<20	<20	<20	<20	<20	73	<20	102	<20	<10
	A13V067	<20	50	<20	<20	<20	<20	<20	70	<20	<20	<20	88	<20	36	<20	<10
	A13V200	<20	<20	<20	<20	<20	<20	<20	29	<20	<20	<20	104	<20	48	<20	<10
	A13V148	<20	<20	<20	102	<20	<20	<20	21	<20	<20	<20	<20	<20	<10	<20	<10
	A11E102	<20	<20	<20	<20	<20	<20	<20	82	<20	<20	<20	704	<20	114	<20	239
	A9V006	<20	<20	<20	<20	<20	143	<20	<20	<20	68	<20	<20	<20	179	<20	<10

Neutralization titers are colored with the same scheme in Table S3

Table S6. ID_{80} values for neutralization of 3-glycan-deleted viruses by week 18 guinea pig sera. Related to Figure 7.

		BG505.W6M.C2.T332N.SG3						16055-2.3.SG3					426c.SG3					CH505s.T/F.SG3					ZM106.9.SG3					45_01dG5.SG3				
Immunogen	Animal ID	WT	ΔG4	197	276	363	462	WT	ΔG4	197	276	463	WT	ΔG4	197	276	463	WT	ΔG4	197	276	461	WT	ΔG4	197	276	464	WT	ΔG4	197	362	463
BG505 DS-SOSIP.ΔGly4	517-1	219	461,270	496,874	116,154	229,618	310	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	517-2	144	289,544	136,518	40,836	54,118	550	<10	<10	<10	<10	<10	<10	67	82	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	517-3	<10	357,559	33,825	5,495	81,858	41,970	<10	16,277	1,743	<10	12,111	<10	12	<10	<10	<10	<10	<10	15,095	<10	56,145	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	517-4	551	175,486	318,483	100,650	130,023	510	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
16055-2.3-chim.DS-SOSIP.ΔGly4	519-1	<10	<10	<10	<10	<10	<10	10	31,402	9,563	1,390	25,549	<10	<10	<10	<10	<10	<10	670	<10	<10	2,690	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	519-2	<10	56,328	6,179	<10	24,145	13,032	<10	88,842	18,618	715	95,843	<10	<10	<10	<10	<10	<10	277,596	38,845	<10	216,921	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	519-3	<10	36,004	3,393	<10	13,494	9,268	<10	74,873	7,841	<10	42,317	<10	<10	<10	<10	<10	<10	197,449	27,350	<10	85,718	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	519-4	<10	6,166	661	<10	2,470	1,411	139	31,704	6,951	4,808	22,514	<10	<10	<10	<10	<10	<10	48,992	8,747	<10	36,525	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
426c.DS-SOSIP.ΔGly4	521-1	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	189,732	99,874	<10	23,417	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	521-2	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	142,746	67,302	90	16,174	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	521-3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	316,274	142,959	273	28,354	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	521-4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	215,691	95,338	792	19,470	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
CH505 DS-SOSIP.ΔGly4	516-1	<10	29,272	2,357	<10	14,373	13,992	<10	94,041	4,415	<10	21,302	<10	<10	<10	<10	<10	<10	413,806	84,870	<10	215,416	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	516-2	<10	2,012	115	<10	1,385	823	<10	8,280	705	<10	3,424	<10	<10	<10	<10	<10	<10	210,163	34,515	<10	102,806	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	516-3	<10	<10	<10	<10	<10	<10	<10	1,370	78	<10	491	<10	<10	<10	<10	<10	<10	199,369	25,089	<10	83,205	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	516-4	<10	<10	<10	<10	<10	<10	<10	1,668	169	<10	633	<10	<10	<10	<10	<10	<10	169,615	29,308	<10	111,689	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
ZM106.9-chim.DS-SOSIP.ΔGly4	518-1	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	953	<10	342	365	<10	<10	<10	<10	<10
	518-2	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	405	<10	156	231	<10	<10	<10	<10	<10
	518-3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	13,889	737	95	1,677	<10	<10	<10	<10	<10
	518-4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	1,116	<10	380	188	<10	<10	<10	<10	<10
45_01dG5.DS-SOSIP.ΔGly3	520-1	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	520-2	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	520-3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	520-4	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Mix-6	522-1	<10	90,477	19,712	5,933	29,453	15,643	<10	18,193	1,697	13	10,229	<10	60,641	20,280	303	4,836	56	85,248	5,978	185	25,062	<10	2,189	<10	<10	690	<10	16,841	160	950	<10
	522-2	<10	22,679	3,748	935	9,283	5,632	<10	11,371	1,021	191	9,671	<10	13,815	5,404	<10	1,314	<10	26,307	3,015	534	10,094	<10	22,279	966	<10	4,835	<10	51,598	9,815	11,771	8,075
	522-3	<10	45,924	14,043	5,020	19,989	10,914	<10	17,248	1,787	<10	9,567	<10	143,881	61,621	<10	13,035	<10	54,805	5,636	<10	19,108	<10	50,118	2,827	<10	19,295	<10	24,186	1,034	6,471	6,938
	522-4	<10	75,658	5,728	87	32,521	27,689	<10	84,914	5,122	<10	30,431	<10	32,336	12,603	<10	1,509	<10	158,932	16,201	339	59,044	<10	59,176	3,956	<10	16,124	<10	9,694	554	1,770	1,574

Sera neutralization titers are colored with the same scheme in Table S3

Table S7. Mapping the effects of individual glycan on neutralization by week 18 NHP sera. Related to Figure 7.

ID_{50}		BG505.W6M.C2.T332N						426C					CH505s.T/F					45-01dG5					16055-2.3					ZM106.9				
Immunogen	Animal ID	WT	ΔGly 4	197	276	363	462	WT	ΔGly4	197	276	463	WT	ΔGly4	197	276	463	WT	ΔGly 4	197	362	463	WT	ΔGly 4	197	276	463	WT	ΔGly 4	197	362	464
BG505 DS-SOSIP.ΔGly4	A13V121	106	16,261	79,004	9,396	4,024	466	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V173	<10	27,073	55,984	12,325	21,384	119	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V112	<10	3,934	23,643	19,134	32,477	88	<10	225	200	42	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	134	455
	A13V011	<10	28,118	166,989	50,869	47,545	131	<10	11	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V098	<10	23,364	67,572	10,285	37,923	<10	<10	15	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	4E-8	<10	2,297	2,054	828	489	361	<10	<10	<10	<10	<10	<10	47	<10	99	47	<10	<10	<10	<10	<10	<10	120	22	22	<10	<10	<10	<10	<10	<10
426c.DS-SOSIP.ΔGly4	A13V110	<10	<10	<10	<10	<10	<10	<10	1,054,096	233,478	1,141	23,657	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V140	<10	<10	<10	<10	<10	<10	<10	245,983	174,075	1,384	17,017	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	16	<10	<10	<10	<10	<10	<10	<10	<10
	A13V059	<10	<10	<10	<10	<10	<10	<10	755,489	240,255	745	25,282	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V160	<10	<10	<10	<10	<10	<10	<10	2,634,856	224,137	7,684	16,318	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	397	<10	32,100	<10	<10	<10	<10	<10	<10
	A13V008	<10	<10	<10	<10	<10	<10	<10	287,038	68,596	2,157	10,137	<10	10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	1F-8	<10	<10	<10	<10	<10	<10	<10	26,520	16,854	<10	2,927	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
CH505 DS-SOSIP.ΔGly4	A13V030	<10	<10	142	150	<10	<10	<10	<10	<10	<10	<10	28	19,895	12,229	5,999	792	<10	10	<10	<10	<10	<10	47	<10	<10	<10	92	<10	<10	<10	<10
	A13V124	<10	2,697	1,672	790	1,625	993	<10	<10	<10	<10	<10	97	35,699	69,634	1,616	10,330	<10	<10	<10	<10	<10	<10	1,222	<10	<10	68	<10	<10	<10	<10	<10
	A13V099	<10	741	383	187	513	149	<10	<10	<10	<10	<10	17	17,994	3,898	3,075	2,051	<10	<10	<10	<10	<10	<10	15	<10	<10	<10	16	115	54,777	<10	
	A13V103	94	3,388	1,020	187	1,043	217	<10	<10	<10	<10	<10	81	12,029	5,138	1,516	412	<10	<10	<10	<10	<10	<10	11	<10	<10	<10	<10	<10	<10	<10	<10
	A11V093	<10	5,859	3,143	12,049	4,009	1,390	<10	35	<10	<10	<10	66	34,645	10,015	12,788	5,549	<10	<10	<10	<10	<10	<10	3,232	17	70	<10	<10	1,242	<10	<10	67
	A11V051	<10	8,826	9,470	7,655	8,718	19	<10	<10	<10	<10	<10	67	40,700	25,651	5,631	4,227	<10	<10	<10	<10	<10	<10	78	11,335	<10	<10	<10	15	<10	<10	<10
45_01dG5.DS-SOSIP.ΔGly3	A13V130	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	3,186	<10	1,600	1,418	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V013	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	25	<10	<10	<10	<10	1,566	<10	916	1,530	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V144	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	660	5,179	2,552	4,445	1,907	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V045	<10	<10	<10	<10	<10	<10	<10	67	<10	<10	<10	<10	<10	<10	<10	<10	<10	598	<10	90	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V073	<10	<10	<10	<10	<10	<10	<10	11	<10	<10	<10	<10	<10	<10	<10	<10	63	3,052	230	1,986	581	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6E-8	<10	<10	<10	<10	<10	<10	<10	931	105	<10	22	<10	22	<10	<10	<10	457	11,276	2,078	7,891	3,174	<10	85	27	94	<10	<10	133	24	<10	555
Mix-4	A13V176	<10	3,653	4,000	1,552	2,445	280	<10	244,151	17,986	314	2,573	<10	37,397	6,026	737	2,909	<10	62	<10	<10	<10	<10	470	<10	216	<10	<10	<10	<10	<10	<10
	A13V067	<10	2,284	9,512	1,539	266	100	<10	23,223	14,261	<10	1,689	<10	42,332	40,122	397	1,974	<10	358	<10	110	<10	<10	74	<10	<10	<10	<10	<10	<10	<10	<10
	A13V200	<10	2,227	5,137	3,533	1,121	151	<10	25,324	14,007	263	1,996	<10	10,456	4,532	200	1,520	<10	529	<10	176	<10	<10	77	<10	<10	<10	<10	12	<10	<10	<10
	A13V148	<10	61	4,784	5,483	72	100	<10	19,037	21,218	<10	2,591	24	24,561	21,027	6,272	2,914	<10	226	<10	143	<10	<10	<10	<10	<10	<10	<10	31	<10	<10	78
	A11E102	<10	6,519	9,452	1,308	2,842	917	<10	10,301	14,766	138	834	<10	9,078	3,062	510	1,167	29	1,918	<10	690	<10	<10	428	<10	14	<10	<10	849	1,157	23	70
	A9V006	<10	1,840	7,151	<10	118	100	<10	4,636	4,587	45	<10	<10	16,976	2,876	1,427	2,933	<10	973	<10	138	<10	<10	788	<10	217	25	<10	<10	<10	<10	<10
Control antibody*	VRC01	0.0350	0.0006	0.0040	<0.0001	<0.0001	0.0004	0.7870	0.0030	0.0100	0.0240	0.0720	0.1090	0.0090	0.024	0.0010	0.3060	0.0030	0.0007	0.0160	0.0030	0.0030		>10	0.0050	0.0220		0.0009	>10	1.7100	0.0040

ID_{80}		BG505.W6M.C2.T332N						426C					CH505s.T/F					45-01dG5					16055-2.3					ZM106.9				
Immunogen	Animal ID	WT	ΔGly 4	197	276	363	462	WT	ΔGly4	197	276	463	WT	ΔGly4	197	276	463	WT	ΔGly 4	197	362	463	WT	ΔGly 4	197	276	463	WT	ΔGly 4	197	362	464
BG505 DS-SOSIP.ΔGly4	A13V121	<10	312	3,865	11	36	114	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V173	<10	4,735	14,284	<10	6,081	78	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V112	<10	106	2,318	64	99	18	<10	89	93	18	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	37	117
	A13V011	<10	5,572	25,382	1,040	9,114	66	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V098	<10	5,115	15,293	<10	7,202	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	4E-8	<10	227	<10	<10	60	118	<10	<10	<10	<10	<10	<10	22	<10	43	19	<10	<10	<10	<10	<10	<10	50	<10	16	<10	<10	<10	<10	<10	<10
426c.DS-SOSIP.ΔGly4	A13V110	<10	<10	<10	<10	<10	<10	57	298,813	73,098	427	8,025	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V140	<10	<10	<10	<10	<10	<10	<10	63,234	62,242	557	6,601	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V059	<10	<10	<10	<10	<10	<10	101	204,673	74,416	120	9,788	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V160	<10	<10	<10	<10	<10	<10	156	282,482	68,980	3,105	4,963	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	119	<10	10,007	<10	<10	<10	<10	<10	<10
	A13V008	<10	<10	<10	<10	<10	<10	186	77,283	25,925	901	3,332	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	1F-8	<10	<10	<10	<10	<10	<10	51	8,039	7,489	<10	956	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
CH505 DS-SOSIP.ΔGly4	A13V030	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	11	5,084	2,687	1,654	263	<10	<10	<10	<10	<10	<10	13	<10	<10	<10	62	<10	<10	<10	<10
	A13V124	<10	277	191	<10	204	284	<10	<10	<10	<10	<10	55	9,795	10,166	438	2,362	<10	<10	<10	<10	<10	<10	926	<10	<10	10	<10	<10	<10	<10	<10
	A13V099	<10	91	144	15	201	41	<10	<10	<10	<10	<10	11	2,776	739	838	511	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	49	14,585	<10	
	A13V103	<10	137	301	<10	271	83	<10	<10	<10	<10	<10	<10	2,121	1,359	399	123	<10	<10	<10	<10	<10	<10	10	<10	<10	<10	<10	<10	<10	<10	<10
	A11V093	<10	1,175	1,175	1,711	944	381	<10	<10	<10	<10	<10	<10	8,321	2,869	3,194	1,550	<10	<10	<10	<10	<10	<10	871	<10	<10	<10	401	<10	<10	<10	<10
	A11V051	<10	2,146	3,572	766	2,720	<10	<10	<10	<10	<10	<10	15	11,916	9,171	2,126	1,124	<10	<10	<10	<10	<10	<10	39	3,359	<10	<10	<10	<10	<10	<10	<10
45_01dG5.DS-SOSIP.ΔGly3	A13V130	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	692	<10	465	325	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V013	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	427	<10	258	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V144	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	126	1,461	977	1,222	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V045	<10	<10	<10	<10	<10	<10	<10	47	<10	<10	<10	<10	<10	<10	<10	<10	<10	96	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	A13V073	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	18	734	<10	619	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6E-8	<10	<10	<10	<10	<10	<10	<10	280	62	<10	<10	<10	<10	<10	<10	<10	190	4,042	1,055	2,961	<10	<10	51	<10	61	<10	<10	14	<10	174	
Mix-4	A13V176	<10	379	814	291	334	122	<10	20,402	7,137	56	838	<10	5,341	1,437	300	754	<10	34	<10	<10	<10	<10	102	<10	68	<10	<10	<10	<10	<10	<10
	A13V067	<10	28	<10	67	<10	98	<10	6,969	6,116	<10	558	<10	7,403	7,808	152	464	<10	82	<10	78	<10	<10	36	<10	<10	<10	<10	<10	<10	<10	<10
	A13V200	<10	92	221	45	67	84	87	7,787	5,431	111	645	<10	2,403	985	100	402	<10	143	<10	105	<10	<10	48	<10	<10	<10	<10	<10	<10	<10	<10
	A13V148	<10	12	85	67	21	99	<10	6,190	6,042	<10	680	13	6,111	3,638	1,612	737	<10	81	<10	82	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	26
	A11E102	<10	589	464	107	284	275	175	3,465	4,135	45	254	<10	2,119	576	176	326	10	885	<10	246	<10	<10	114	<10	<10	<10	<10	239	370	<10	<10
	A9V006	<10	<10	<10	<10	<10	51	<10	1,603	1,285	21	<10	<10	4,139	773	404	748	<10	81	<10	86	<10	<10	179	<10	74	<10	<10	<10	<10	<10	<10
Control Antibody*	VRC01	0.2200	0.0010	0.0170	0.0050	0.0010	0.0030	0.0330	0.0010	0.0370	0.0570	0.1870	0.4170	0.0020	0.0820	0.0050	1.9700	0.0440	0.0020	0.0180	0.0120	0.0140	0.3430	0.0020	>10	0.0170	0.0580			>10	4.1500	0.0170

Sera neutralization titers are colored with the same scheme in Table S3.

*Color shading of VRC01 potency values (μg/ml).

<0.001	0.001-0.01	0.01-0.100	0.100-1.00	1.00-10.0	10.1 - 50

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