Science Translational Medicine
AAAS

Supplementary Materials for

Stabilized HIV-1 envelope immunization induces neutralizing antibodies to the CD4bs and protects macaques against mucosal infection

Kevin O. Saunders *et al.*

Corresponding author: Priyamvada Acharya, priyamvada.acharya@duke.edu; Kevin O. Saunders, kevin.saunders@duke.edu; Barton F. Haynes, barton.haynes@dm.duke.edu

Sci. Transl. Med. **14**, eabo5598 (2022)
DOI: 10.1126/scitranslmed.abo5598

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MDAR Reproducibility Checklist

Materials and Methods

Intrarectal low-dose mucosal SHIV challenge. The SHIV.CH505.375H.dCT(s1) challenge stock was expanded in activated, primary rhesus macaque $CD4^+$ T cells, as previously described (*69*, *70*). Intrarectal inoculations were performed by inserting a flexible lubricated pediatric feeding tube atraumatically 3 to 5 cm into the rectum of animals lying in a Trendelenburg position followed by the administration by slow push of 1 ml of a 1:150 diluted virus stock that was grown in rhesus macaque $CD4^+$ T cells. This inoculum corresponds to a 25% animal infectious dose (AID_{25}) and 1,241 IU or 45,313 IU when titered on primary rhesus $CD4^+$ T cells or TZM-bl cells, respectively (*69*, *70*). Animals were maintained in the Trendelenburg position for 30 minutes before being repositioned and returned to their cages to recover from anesthesia.

The SHIV.CH505.375H.dCT(s1) challenge stock was expanded in activated, primary rhesus $CD4^+$ T cells, as previously described (*69*, *70*). Intrarectal inoculations were performed by inserting a flexible lubricated pediatric feeding tube atraumatically 3 to 5 cm into the rectum of animals lying in a Trendelenburg position followed by the administration of 1 ml of a 1:150 diluted virus stock by slow push. Animals were maintained in the Trendelenburg position for 30 minutes before being repositioned and returned to their cages to recover from anesthesia.

Plasma vRNA quantification. Plasma viral load measurements were performed by the National Institutes of Health/National Institute of Allergy and Infectious Disease-sponsored Nonhuman Primate Virology Core Laboratory at the Duke Human Vaccine Institute as previously described (*33*). The Nonhuman Primate Virology Core Laboratory is Clinical Laboratory Improvement Amendments (CLIA) certified. Viral load was determined with a highly standardized, quality-controlled Applied Biosystems Real-Time SIV viral RNA PCR assay. PCR reactions were setup using QIAsymphony SP and QIAgility automated platforms (QIAGEN). Viral RNA was extracted and purified from plasma, annealed to a target specific primer and reverse-transcribed into cDNA. The cDNA was treated with RNase and added to a custom real-time PCR master mix containing target specific primers and a fluorescently-labeled hydrolysis probe (*71*). Thermal cycling was performed on a QuantStudio3 (Thermo Fisher Scientific) real-time quantitative PCR (qPCR) instrument. Viral RNA copies were interpolated using quantification cycle and a standard curve. To account for starting sample volume, the mean viral RNA copies/mL was calculated. The limit of quantification using 0.5 ml of NHP plasma was set at 62 RNA copies/mL with a 94% detection and a Clopper-Pearson binomial confidence interval of 84.8 and 98.3.

Envelope-specific single B cell sorting. Macaque B cells. Cryopreserved peripheral blood mononuclear cells (PBMC) were washed, counted, and stained with natural killer (NK), T, and B cell surface markers and fluorophore-labeled envelope protein for 1 hour at 4 °C(***72***). Antibodies used for staining were CD20 fluorescein isothiocyanate (FITC) clone L27 (BD Biosciences Cat No. 347673), CD3 peridinin chlorophyll protein (PerCP) Cy5.5 clone SP34-2 (BD Biosciences Cat No. 552852), IgD phycoerythrin (PE) polyclonal (Southern Biotech Cat No. 2030-09), CD8 PE Texas Red clone 3B5 (Invitrogen Cat No. MHCD0817), IgM PE Cy5 clone G20-127 (BD Biosciences Cat No. 551079), CD16 PE Cy7 clone 3G8 (BD Biosciences Cat No. 557744), Live / Dead Aqua (Invitrogen Cat No. L34957), CD14 brilliant violet (BV) 570 clone M5E2 (BioLegend Cat No. 301832), and CD27 allophycocyanin (APC) Cy7 clone O323 (BioLegend Cat No. 302816). Each lot of antibody is titrated on one million rhesus macaque PBMCs and the minimum concentration of antibody that reaches saturation of mean fluorescence intensity was used. Envelope reactive, live, IgD^- single B cells were sorted into individual wells of a 96-well PCR plate that contained cell lysis buffer and 5X first-strand synthesis buffer. Plates were frozen on dry ice and ethanol and stored at -80 °C until reverse transcription of RNA.

Human single B cell sorting. Human B cell sorting was performed as described previously (*73*). Fluorescence activated cell sorting (FACS) staining consisted of LIVE/DEAD Fixable Aqua Stain (Thermo Fisher Scientific), anti-human IgM (FITC), anti-CD235a (PE Cy5), anti-CD3 (PE Cy5), anti-CD19 (APC Cy7), anti-CD27 (PE Cy7) (BD Biosciences); anti-IgD (PE); anti-CD10 (R phycoerythrin-Texas Red-X; ECD), anti-CD38 (APC-Alexa fluor (AF) 700), anti-CD19 (APC-Cy7), anti-CD16 (BV570), anti-CD14 (BV605) (BioLegend). Samples were also stained with CH505 TF chTrimer in both AF647 and BV421 (*74*). Memory B cells were gated as PBMCs that were Aqua $Stain^-$, $CD14^-$, $CD16^-$, $CD3^-$, $CD235a^-$, $CD19^+$, and surface IgD^-. B cells that were double-positive for AF647 and BV421 were sorted into lysis buffer for RT-PCR.

Antibody gene RT-PCR. Superscript III (Thermo Fisher Scientific Cat No. 18080044) and immunoglobulin constant region-specific reverse primers were used to reverse transcribe B cell RNA (*72, 75, 76*). Five microliters of complementary DNA were subjected to two rounds of nested PCR for heavy and light chain variable region amplification. PCR reactions yielding immunoglobulin variable region amplicons were identified by 96 well E-gel agarose electrophoresis (Thermo Fisher Scientific). PCR amplicons were purified using the PCR clean-up kit (Qiagen). Purified PCR amplicons were sequenced with 4 μM of forward and reverse primers. Contigs of the amplified immunoglobulin sequences were made by the Duke automated sequence analysis pipeline (Duke ASAP).

Immunogenetics of rhesus macaque and human immunoglobulin genes were determined with the macaque or human reference library using Cloanalyst (*72*, *77*) respectively. Similarly, recombination summaries of the immunogenetics of identified antibodies were partitioned into clones and an unmutated common ancestor (UCA) was inferred from select clones with the infer UCA function in Cloanalyst. A phylogenetic tree for the DH950 lineage was generated using the heavy chain sequences since we only performed next generation sequencing (NGS) of VH regions. A second aliquot of the purified PCR amplicon was used for overlapping PCR to generate a linear expression cassette in order to express small amounts of antibody for binding screens. The expression cassette was transfected into Expi293F cells (Thermo Fisher Scientific, Cat No. A14527) with Expifectamine (Thermo Fisher Scientific, Cat No. A14526). Three days after transfection, cell culture media was cleared of cells and secreted recombinant antibodies in the cell culture media were tested for binding to HIV-1 envelope. From the binding screen, antibodies were selected for gene synthesis. The synthesized heavy and light chains were cloned into gamma, kappa, or lambda expression vectors (GenScript). Plasmids were prepared for transient transfection of Expi293F cells using the MidiPrep or MegaPrep plasmid plus kit (Qiagen). Antibodies were produced as described below.

Recombinant antibody expression. Expi293F cells (Thermo Fisher Scientific) were diluted to 2.5 million cells/mL in Expi293 media on the day of transfection. One liter of Expi293F cells were co-transfected with 400 μg of heavy chain plasmid and 400 μg of light chain plasmid using Expifectamine per the manufacturer's protocol. Five days after transfection, the cells were centrifuged and the cell culture supernatant was collected and filtered with a 0.8 µm filter. The cell-free supernatant was concentrated to approximately 50 mL total volume and incubated with protein A beads (Thermo Fisher Scientific) overnight at 4 °C. The protein A beads were centrifuged for 5 minutes at 1200 rpm in a Sorval table top centrifuge. The protein A resin was washed by resuspending it in 25 mL of Tris-buffered saline (TBS; 10 mM Tris pH7, 340 mM NaCl) and pipetted into an empty gravity flow column (Bio-Rad). The antibody was eluted off of the beads with two elutions of 15 mL each of 2% glacial acetic acid. The pH was neutralized by adding 1M Tris pH8.0 to a final pH of 6. The eluate was concentrated in a Vivaspin 15 and buffer exchanged into citrate buffered saline (25 mM sodium citrate pH6, 150 mM NaCl pH6) with successive rounds of centrifugation.

Next generation sequencing of antibody genes. Illumina MiSeq sequencing of antibody heavy chain VDJ sequences was performed on peripheral B cells as previously described (*78*). For each timepoint, sequencing was performed twice on independent cDNA samples to confirm the absence or presence of antibody sequences of interest. From single template NGS experiments, we have observed that the number of errors introduced in PCR amplification in our NGS sample preparation rarely exceeds four base pairs (<1%). Thus, two sequences that each differ by 4 base pairs (8 total base pair differences) cannot be reliably determined to derive from two unique B cells. To conservatively account for this, we clustered together sequences with 8 or fewer base pair differences. Counts of these clusters were treated as a lower bound estimate of the number of unique DH950 clonal member sequences. V, D, and J gene segment inference, clonal relatedness testing and reconstruction of clonal lineage trees were performed using the Cloanalyst software package (*77*).

Competition enzyme-linked immunosorbent assay (ELISA) binding assays. Nuncsorp 384-well plates were coated with 15 µL of streptavidin at 2 µg/mL in 0.1 M sodium bicarbonate overnight at 4 °C. The unbound streptavidin was aspirated and each well was washed once with SuperWash. Each well was blocked with 40 µL of superblock for 1 hour at room temperature. Biotinylated CH505 TF chTrimer envelope at 1 µg/mL was captured on the streptavidin-coated plate for 1 hour at room temperature. The plate was washed and 100 µg/mL of blocking antibody in SuperBlock was added to the CH505 TF chTrimer for 90 minutes. Antibodies to epitopes not of interest were used as negative controls. As a positive control, antibodies were used to block themselves. To determine total binding no blocking antibody was added to the wells. After 90 minutes, the plate was washed twice with SuperWash and serial dilutions of the binding antibody of interest were incubated in the wells for 1 hour. When the binding and blocking antibody were from the same species, then the binding antibody was biotinylated and the Env was captured with a mouse antibody against the Avi tag on the envelope. When the binding antibody was from rhesus macaques and the blocking antibody was from humans, a rhesus-specific anti-IgG conjugated to horseradish peroxidase (HRP, Southern Biotech Cat No: Cat. No.: 4700-05) was used to detect the presence of the binding antibody in the wells. Each well was washed and binding of biotinylated mAbs was determined with a 1:30000 dilution of HRP-conjugated streptavidin. HRP was detected with Tetramethylbenzidine and stopped with 1% HCl. The absorbance at 450 nm of each well was read with a Spectramax plate reader (Molecular Devices). Binding titers determined as area under the log-transformed curve (log AUC) were assessed in the presence and absence of blocking antibodies were used to determine the percent blocking. Assays were considered valid if the positive control antibodies blocked greater than 20% of the binding antibody binding.

NSEM and modeling. Fab-trimer complexes were formed by mixing 10 µg CH505 TF chTrimer with 36 µg of Fab and diluting with HEPES-buffered saline (HBS), containing 150 mM NaCl, 20 mM HEPES, pH 7.4, to a final trimer concentration of 100 to 200 µg/ml. After incubation overnight at 4 °C. Fab-trimer complexes were brought to room temperature and diluted with four volumes of HBS augmented with 0.1% glutaraldehyde and fixed for 5 minutes, then excess glutaraldehyde was quenched by adding sufficient 1 M Tris buffer, pH 7.4, for a final Tris concentration of 75 mM. Quenched samples were transferred to a 100-kDa molecular weight cutoff spin concentrator (Millipore Sigma UCF5100), centrifuged for three minutes at 14,000 g to concentrate, re-diluted with the addition of 500 µl of HBS, centrifuged again, and the concentrate then collected. After measuring the nominal trimer concentration, the Fab-trimer complex was diluted to 100 µg/ml with HBS augmented with 5% glycerol, if needed, or used without dilution for negative stain. For negative stain, 300-mesh carbon-coated EM grids were glow-discharged for 30 seconds, and 5 µl of sample was applied and incubated for 5 to 20 seconds. Grids were then blotted, and stained with 2% uranyl formate for 1 minute, blotted and allowed to air dry. Images were obtained with a Philips 420 electron microscope operated at 120 kV, and a nominal magnification 82,000×, and images captured with a 2k x 2k CCD camera with a nominal pixel size of 4.02 Å/pixel. Images were analyzed by 2D class averages and 3D reconstructions calculated using standard protocols with Relion 3.0 (*79*).

Cryo-EM. Purified HIV-1 envelope CH505 TF.M5 chTrimer preparations were diluted to a final concentration of about 1 mg/mL in 2 mM Tris pH 8.0, 200 mM NaCl and 0.02% sodium azide, were mixed with 6-fold molar excess of DH1025.2 Fab and incubated for 2 hours at room temperature. 2.5 µL of protein was deposited on a Quantifoil 1.2/1.3 holey carbon grid that had been glow discharged for 30 seconds in a PELCO easiGlow Glow Discharge Cleaning System. After a 30 second incubation in greater than 95% humidity, excess protein was blotted away for 2.5 seconds before being plunge frozen into liquid ethane using a Leica EM GP2 plunge freezer (Leica Microsystems). Cryo-EM imaging was performed on a FEI Titan Krios microscope (Thermo Fisher Scientific) operated at 300 kV. Data were acquired with a Gatan K3 detector operated in counting mode. Data processing was performed within cryoSPARC (*80*) including particle picking, multiple rounds of 2D classification, ab initio reconstruction, heterogeneous and homogeneous map refinements, and non-uniform map refinements. ChimeraX (*81*), Coot (*82*), Isolde (*83*) and Phenix (*84*) were used for model-building and refinement.

Serum cytokine analyses. Serum was collected from individual macaques 24 hours post vaccination. Cytokine concentrations in non-heat-inactivated serum were determined by Luminex using the Cytokine & Chemokine 30-Plex NHP ProcartaPlex Kit (Thermo Fisher Scientific). This kit was selected due to the inclusion of interferon alpha.

Serum samples were processed according to the manufacturer's protocol. Cytokine concentrations were read on a Luminex Bioplex200 instrument.

HIV-1 neutralization assays. Neutralizing antibody activity was measured in 96-well culture plates by using Tat-regulated luciferase (Luc) reporter gene expression to quantify reductions in virus infection in TZM-bl cells. TZM-bl cells were obtained from the NIH AIDS Research and Reference Reagent Program, as contributed by John Kappes and Xiaoyun Wu. Assays were performed with HIV-1 Env-pseudotyped viruses as described previously (*85*). Test serum samples were diluted over a range of 1:20 to 1:43740 in cell culture medium and pre-incubated with virus (about 150,000 relative light unit equivalents) for 1 hour at 37 °C before addition of cells. Similarly, mAbs were serially diluted three-fold in cell culture medium starting at 50 or 25 µg/mL and pre-incubated with virus under the above conditions. Following a 48 hour incubation, cells were lysed and Luc activity determined using a microtiter plate luminometer and BriteLite Plus Reagent (Perkin Elmer). Neutralization titers are the sample dilution (for serum) or antibody concentration (for mAbs) at which relative luminescence units (RLU) were reduced by 80% or 50% (ID_{80} and ID_{50} respectively) compared to RLU in virus control wells after subtraction of background RLU in cell control wells. Serum samples were heat-inactivated at 56 °C for 30 minutes prior to assay.

Recombinant HIV-1 gp140 SOSIP production. Soluble HIV-1 Env trimers were expressed, purified and characterized in vitro as previously described (*7*, *86*). The soluble CH505 TF chTrimer was designed was expressed in Freestyle293F cells (Thermo Fisher Scientific Cat No. K900001) by transient transfection with plasmid DNA complexed with 293Fectin (Thermo Fisher Scientific Cat No. 12347019). 650 µg of SOSIP expressing plasmid and 150 µg of furin expressing plasmid were used for each 1L of cells. Cell-free culture supernatant was 0.8 µm filtered and concentrated to approximately 150 mL. The SOSIP protein was purified from the cell culture supernatant with PGT145 affinity chromatography. Cell-free supernatant was applied to the column at 2 mL/min using an AKTA Pure (GE Healthcare Cat No. 29018224), washed, and protein was eluted off the column with 3M $MgCl_2$. The eluate was immediately diluted in 10 mM Tris pH 8, 0.2 µm filtered, and concentrated down to 2 mL for size exclusion chromatography. Trimeric protein was purified with a Superose6 16/600 column (GE Healthcare Cat No. 29323952) in 10 mM Tris pH 8, 500 mM NaCl. Trimeric HIV-1 Env protein was sterile-filtered, snap frozen, and stored at -80°C. The formation of trimers was determined by negative stain electron microscopy, blue native-polyacrylamide gel electrophoresis (PAGE), and analytical size exclusion chromatography.

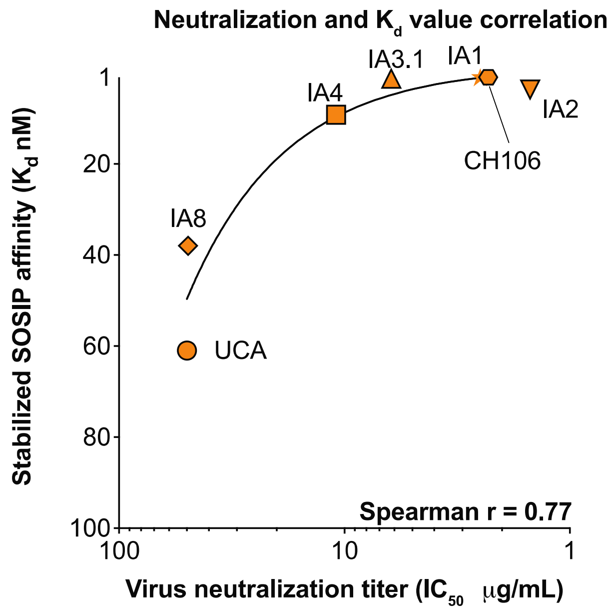
Recombinant gp120, gp140CFI, and gp120 core production. Each envelope was expressed by transient transfection using Freestyle 293F (Thermo Fisher Scientific) cells and polyethylenimine (PEI) transfection as described previously (*87*). HIV-1 envelope was purified by overnight incubation with lectin agarose resin (Vector Labs). Monomeric gp120 or gp120 core proteins were purified by Superdex200 size exclusion chromatography (GE Healthcare), and trimeric gp140CFI proteins were purified withSuperose6 (GE Healthcare) size exclusion chromatography. Size exclusion chromatography was performed with on an AKTA Pure (GE Healthcare).

HIV-1 envelope site-directed mutagenesis. Single mutations were introduced into HIV-1 envelope genes using the QuickChange Lightning Multi Site-Directed Mutagenesis Kit (Agilent). Oligonucleotides were synthesized and purified by standard desalting (Integrated DNA Technologies). Fifty nanograms of purified DNA was used as the template for mutagenesis. Mutagenesis reactions were conducted per the manufacturer's instructions except the volume of DpnI was doubled and the DpnI digestion was conducted for 1 hour. Mutated DNA was transformed into XL10Gold (Agilent). Mutated plasmid DNA was confirmed to encode the mutations by direct colony sequencing (GeneWiz) and alignment of sequencing contigs to reference genes using AlignX (Invitrogen).

HIV-1 envelope indirect ELISA. ELISAs were performed as described previously (*88*). Nuncsorp 384-well plates were coated the antibody of interest at 2 μg/mL in 0.1 M sodium bicarbonate overnight at 4 °C. Plates were washed once with SuperWash and blocked with SuperBlock for 1 hour at room temperature. The plate was washed twice with SuperWash and an envelope dilution series was incubated with the antibody on the plate for 90 minutes. The plate was washed twice and a 1:500 dilution of rabbit anti-envelope serum was added to the plate for 60 minutes. The plate was washed twice and a 1:16000 dilution of anti-rabbit IgG conjugated to HRP was incubated in the plate for 1 hour. For a subset of assays using gp140 oligomers or gp120s, the envelope was coated on the plate. Antibody was serially diluted and added to the plate for 90 minutes. Subsequently, anti-human or rhesus IgG conjugated to HRP was added to the plate. The plate was washed four times with SuperWash. HRP was detected by TMB (KPL) followed by the addition of 1% HCl. The absorbance at 450 nm of each well was read with a SpectraMax plate reader (Molecular Devices) and SoftMax Pro v5.3 (Molecular Devices).

Biolayer interferometry binding assays. Anti-Human IgG Fc capture (AHC; FortéBio) sensor tips were hydrated in phosphate-buffered saline (PBS) for at least 10 minutes at room temperature. Antibodies were immobilized on anti-Human IgG Fc capture sensor tips by immersion in 20 μg/ml mAb in PBS for 300 seconds followed by washing in PBS for 60 seconds at 1000 rpm. The mAb captured sensor tips were then immersed in 50 μg/ml of SOSIP protein for 400 seconds at 1000 rpm to measure association of the ligand and analyte. The complex was dissociated by immersing the sensor tips in PBS for 600 seconds. The sensor tips were regenerated once using glycine pH 2.0 for an immersion time of 20 seconds between measurements. Values obtained for the anti-influenza hemagglutinin (HA) CH65 control mAb binding to envelope was subtracted from each value as non-specific binding. Reported binding corresponds to values and the end of the association phase. Data was evaluated using the Octet Data Analysis HT 12.0 software (FortéBio). For CH505 TF chTrimer affinity measurements, serial dilutions of CH505 TF chTrimer (concentrations ranging from 4.75nM to 475nM) were assessed for binding to CH103 lineage antibodies captured on an anti-human IgG Fc biosensor tips (AHC, Molecular Devices) as described earlier (*89*). Non-specific binding was subtracted using the control anti-respiratory syncytial virus mAb, Palivizumab (Synagis). Rate constants and apparent dissociation constant (K_d) were calculated by global curve fitting analyses to the Langmuir 1:1 model using the BIAevaluation software (GE Healthcare).

A



B

CH103 clonal linage

CH505 TF	UCA	IA_8	IA_4	IA_3.1	IA_2	IA_1	CH106
K_d (nM)	61	38	9.2	1.3	3.7	1	1
IC_{50} (µg/mL)	>50	49.4	10.9	6.2	1.5	2.5	2.3

Fig. S1. Binding of CH103 lineage antibodies to CH505 transmitted/founder (TF) chTrimer correlates with neutralization of CH505 TF pseudovirus. (**A**) Spearman rank correlation of apparent binding affinity (K_d) and IC_{50} neutralization titers (µg/mL) are shown for individual antibodies throughout the CD4 binding site (CD4bs) broadly neutralizing antibody (bnAb) CH103 lineage. Neutralization titers are shown as antibody concentration in µg/mL required to neutralize 50% of virus replication (IC_{50}). (**B**) A table of apparent binding affinities and IC_{50} neutralization titers are shown. Antibodies are in order from left to right of increase divergence from the unmutated common ancestor (UCA) inferred to be the precursor of the CH103 lineage.

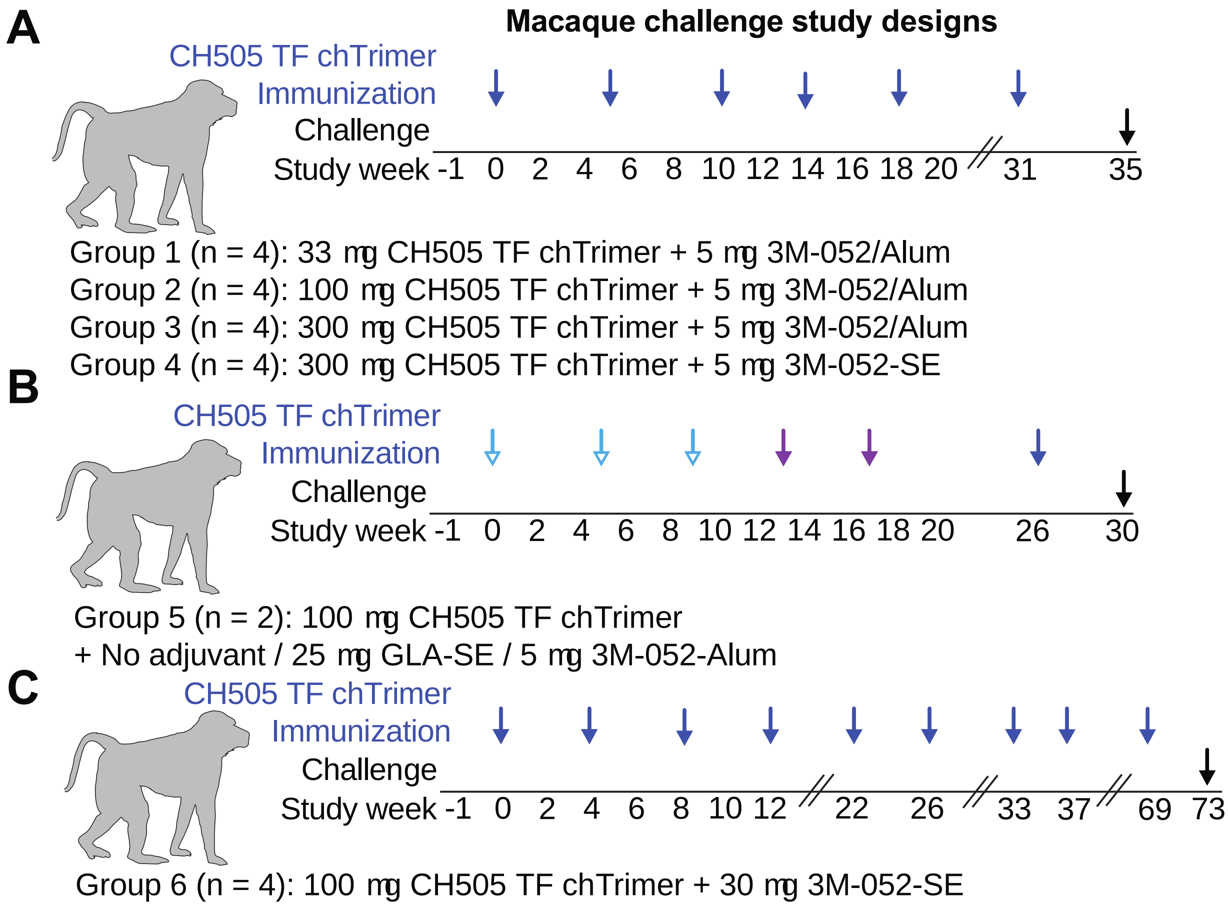


Fig. S2. CH505 TF chTrimer immunization regimens administered to macaques challenged intrarectally with Simian-human immunodeficiency virus (SHIV) CH505TF.375H.dCT. (**A to C**) Macaques were immunized with various concentrations of CH505 TF chTrimer and either 3M-052 plus alum, 3M-052 stable emulsion (3M-052-SE), or glucopyranosyl lipid A stable emulsion (GLA-SE). (**A**) Animals received different doses of CH505 TF chTrimer with the indicated adjuvants. (**B**) Animals were immunized with CH505 TF chTrimer without adjuvant (light blue arrows) followed by immunization with CH505 TF chTrimer and GLA-SE adjuvant (purple arrows). Animals were then immunized with 3M-052-Alum (dark blue arrows). (**C**) Animals received CH505 TF chTrimer immunization with 3M-052-SE. All macaques were challenged on the same day along with negative control macaques despite beginning studies at different times.

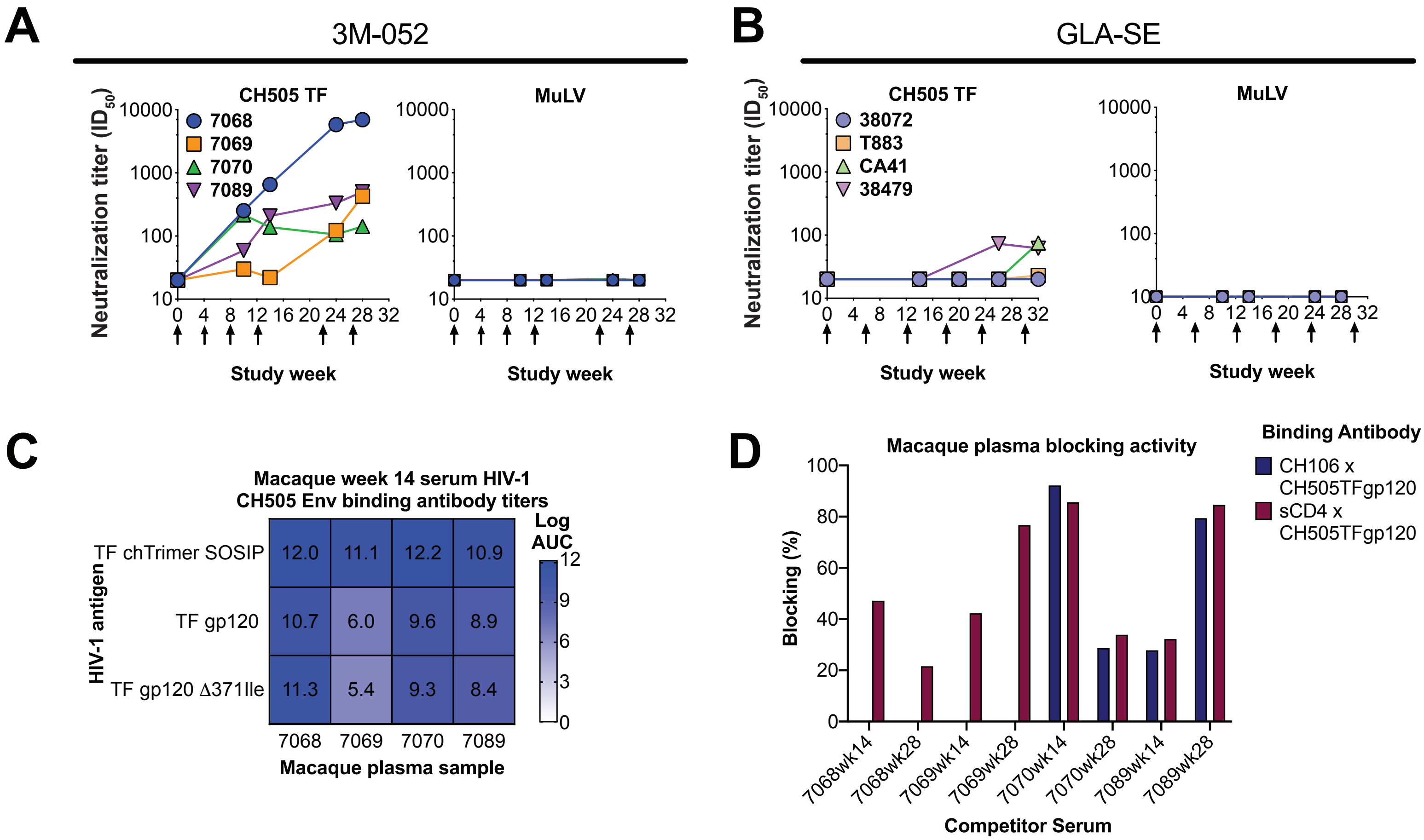


Fig. S3. TLR7/8 adjuvant increases vaccine induction of CH505 TF trimer-reactive antibodies. (**A and B**) Macaque serum neutralization of HIV-1 infection of TZM-bl cells was measured. Pseudotyped murine leukemia virus (MLV) was used as a negative control virus. Each symbol represents an individual macaque. Macaques were immunized with either (**A**) TLR7/8 agonist 3M-052 or (**B**) TLR4 agonist GLA-SE. Arrows below each plot indicate an immunization. (**C**) Serum antibody binding was measured against CH505 TF SOSIP gp140 stabilized trimer, recombinant gp120 subunit, and recombinant gp120 subunit with the CD4bs knocked-out by a deletion of Isoleucine at position 371 (Δ371Ile). Binding is presented as log area under the curve (LogAUC) as measured by enzyme-linked immunosorbent assay (ELISA). (**D**) Macaque plasma blocking of CD4 and CD4 bnAb CH106 binding to CH505 TF gp120 was measured. Plasma was used from macaques immunized four times (week 14) or six times (week 28) with CH505 TF chTrimer adjuvanted 3M-052.

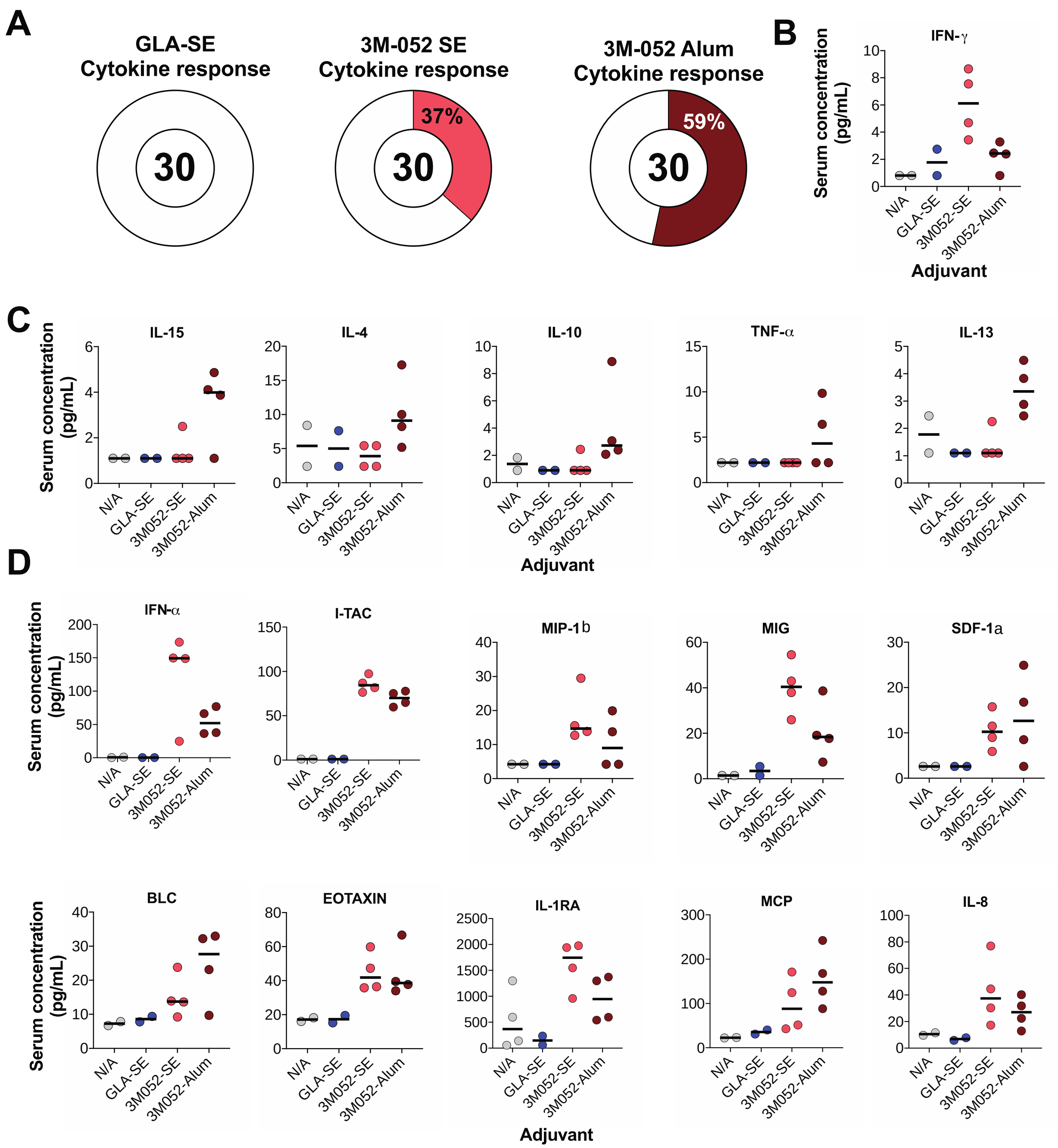


Fig. S4. TLR7/8 agonists activate cytokine production in macaques after vaccination compared to a TLR4 agonist. (**A**) The numbers and percentages of cytokines that were detectable in serum 24 hours after vaccination are shown for each adjuvant. (**B**) Interferon-γ (IFN-γ) secretion into the serum was induced by 3M-052-SE but not GLA-SE or 3M-052-Alum. (**C**) Cytokines preferentially induced by 3M-052-Alum formulation compared to 3M-052-SE are shown. IL, interleukin; TNF-α, tumor necrosis factor-α. (**D**) Cytokines induced by vaccination with 3M-052 formulations but not GLA-SE are shown. I-TAC, Interferon–inducible T Cell Alpha Chemoattractant; MIP-1β, Macrophage inflammatory protein-1 beta; MIG, monokine induced by gamma interferon; SDF-1 α, Stromal cell-derived factor 1 alpha; BLC, B lymphocyte chemoattractant; MCP, Monocyte Chemoattractant Protein-1. Horizontal bars indicate group mean. N/A indicates no adjuvant control immunization.

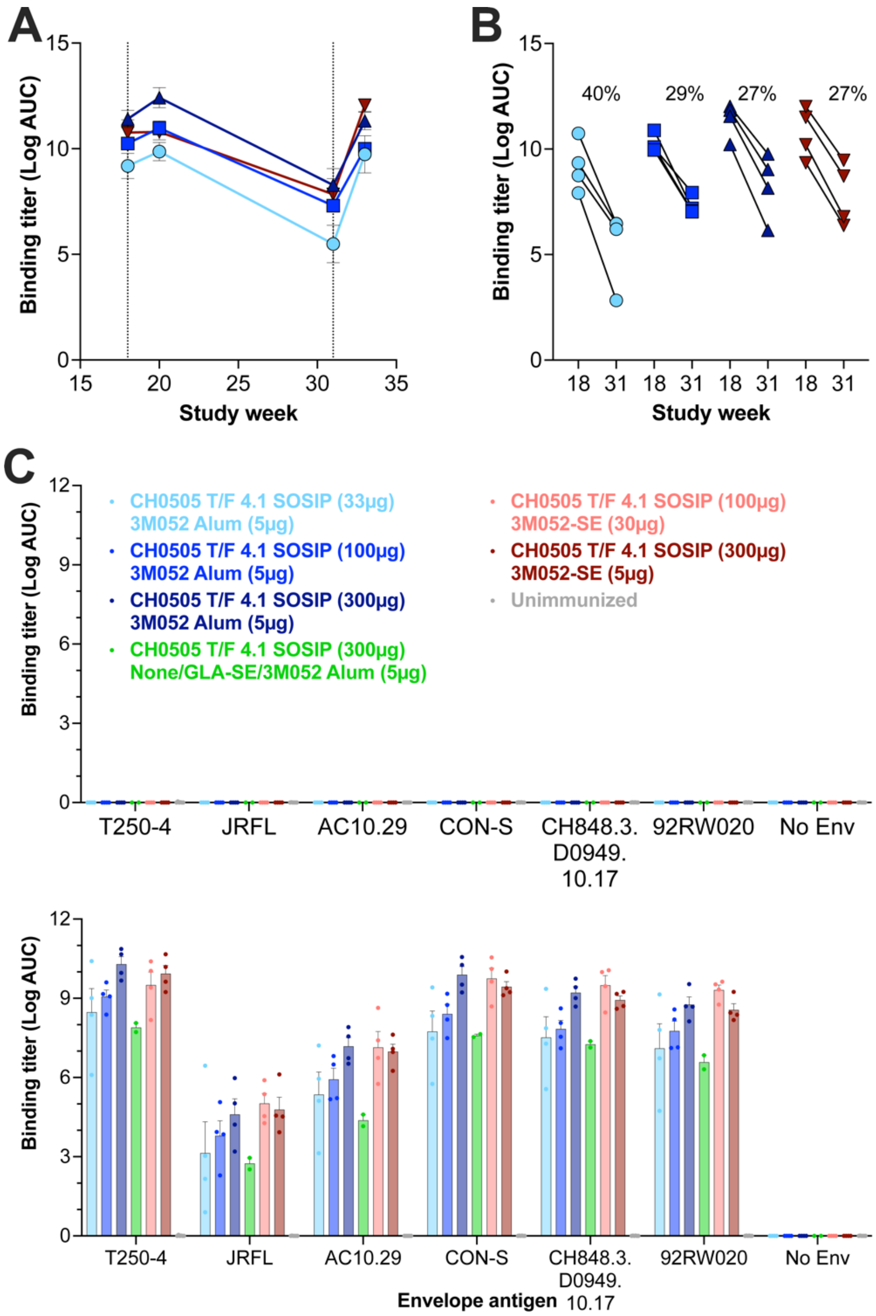


Fig. S5. Plasma IgG binding breadth and durability after vaccination of macaques with different immunogen formulations. (**A**) A time-course of plasma IgG binding to biotinylated CH505 TF chTrimer is shown. Vertical dashed lines show timepoints for immunization. Symbols represent the mean and standard error for each group of macaques (n = 4 macaques). Lines are color-coded as in (C). (**B**) The Percent decrease in plasma IgG titers is shown at thirteen weeks after immunization. Connected symbols represent the same macaque at each timepoint. The mean decrease in binding titer for the group is indicated above the symbols. Symbols are color-coded as in (C). (**C**) Plasma IgG binding titers to a panel of biotinylated HIV-1 envelope SOSIP gp140 proteins are shown as LogAUC values. Binding IgG titer (Top) before any immunization and (Bottom) two weeks prior to challenge ae shown.

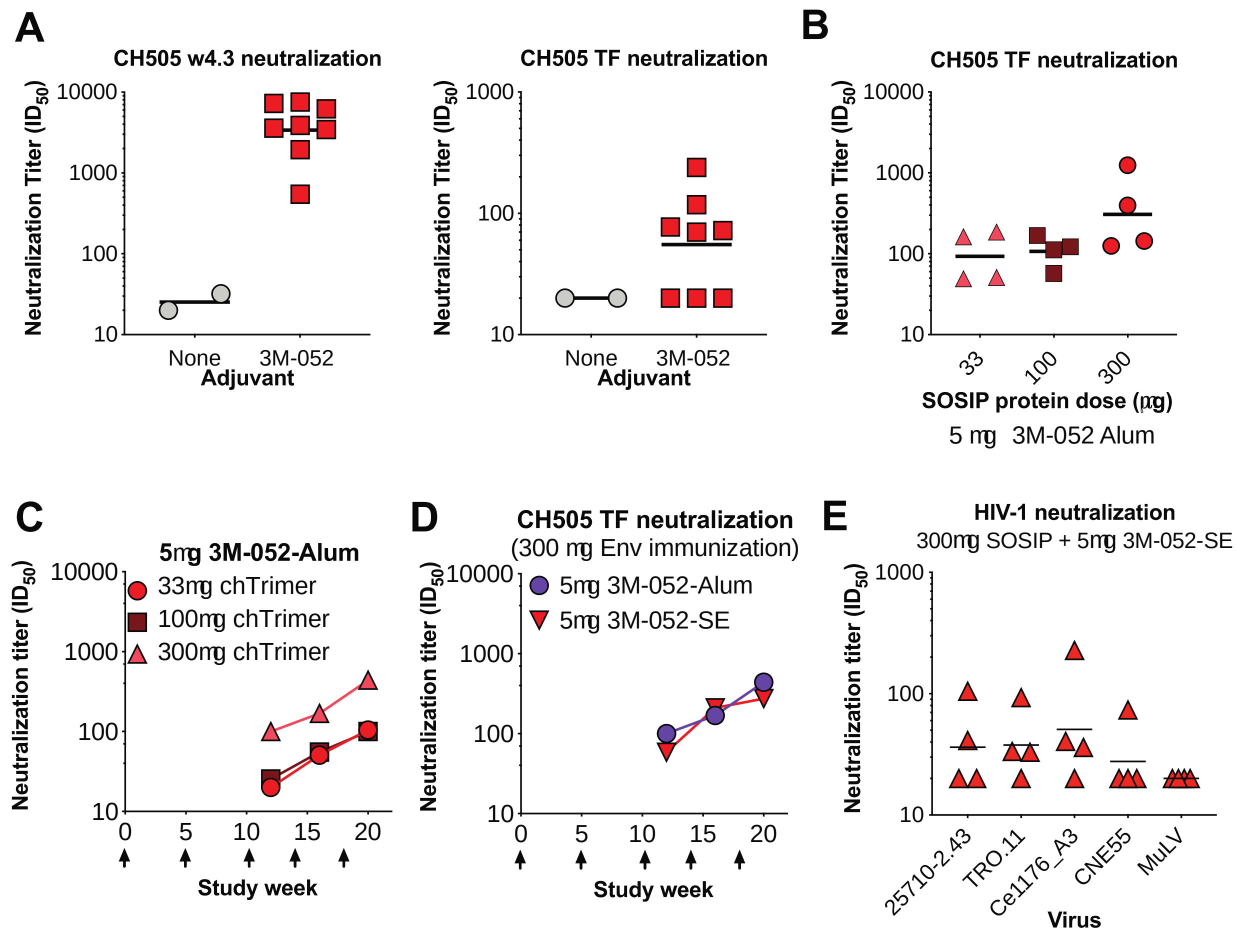


Fig. S6. Adjuvant and immunogen dose are critical determinants for vaccine induction of CH505 TF neutralizing antibodies. (**A**) Vaccinated macaque serum neutralization of tier 1 A virus CH505 w4.3 (left) or tier 2 virus CH505 TF (right) infection of TZM-bl cells was measured. Neutralizing antibody titers were measured two weeks after two immunizations with 300 µg of CH505 TF chTrimer without any adjuvant or formulated with 5µg of 3M-052-alum (n = 2 or 8 macaques). (**B**) Shown are neutralization titers against CH505 TF infection of TZM-bl cells by serum collected two weeks after immunization with different doses of CH505 TF chTrimer formulated with 5 µg of 3M-052-Alum (n = 4 macaques). (**C**) Shown are group geometric mean ID_{50} neutralization titers over time for macaques (n = 4) immunized with different doses of CH505 TF chTrimer formulated with 5µg of 3M-052-Alum. (**D**) Shown are group geometric mean ID_{50} neutralization titers over time for macaques (n = 4) immunized with CH505 TF chTrimer formulated with 5µg of 3M-052-Alum or 3M-052-SE. (**E**) Neutralization of TZM-bl cell infection with heterologous HIV-1 isolates by vaccinated macaque serum is shown. Serum was collected three weeks after five immunizations with 300µg CH505 TF chTrimer plus 5µg of 3M-052-SE (n = 4 macaques). ID_{50} neutralization titers are shown as reciprocal serum dilution. Individual macaques are shown as symbols. Horizontal bars indicate group geometric mean titers.

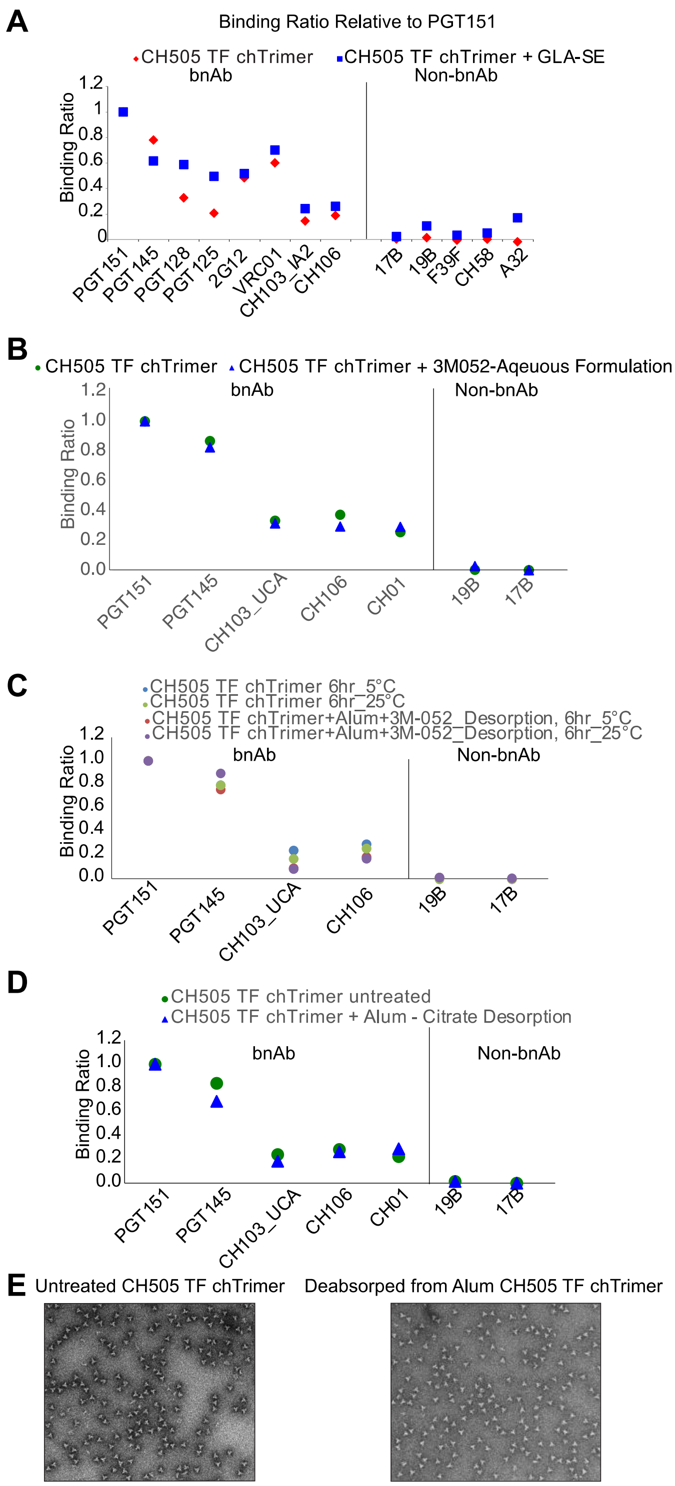
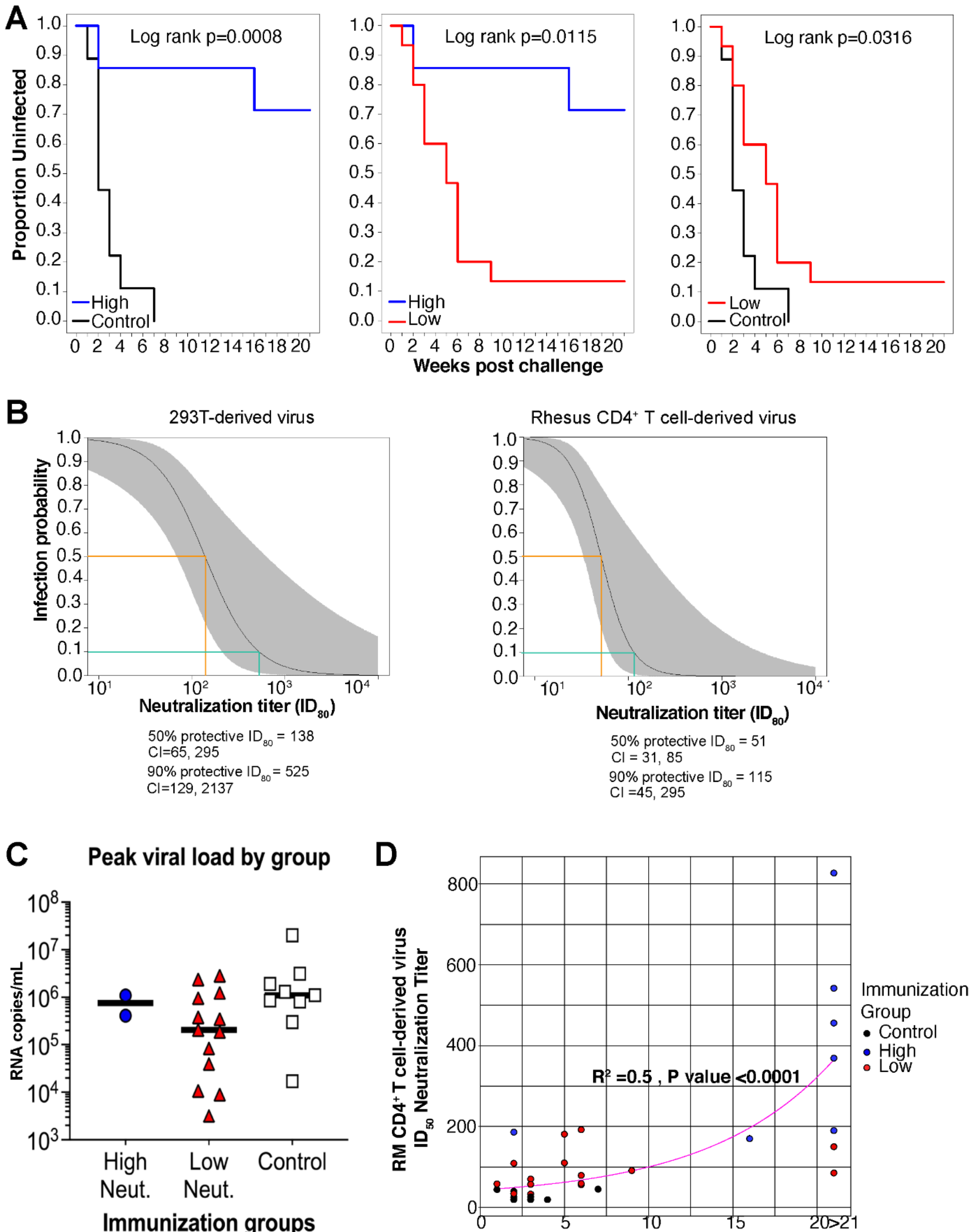


Fig. S7. Antigenicity and structure of CH505 TF chTrimer in different adjuvant formulations. (**A to D**) CH505 TF chTrimer was mixed with (**A**) GLA-SE, (**B**) 3M-052 aqueous formulation (AF), (**C**) 3M-052 plus alum, or (**D**) alum alone to compare to the change in antigenicity relative to CH505 TF chTrimer in Tris-NaCl buffer. Binding is normalized to PGT151 binding in each assay to account for assay variation in antibody loading. (**E**) Negative stain electron microscopy (NSEM) is shown for CH505 TF chTrimers that was untreated or absorbed to Alum and deabsorbed by a sodium citrate incubation.



A
Log rank p=0.0008
Log rank p=0.0115
Log rank p=0.0316
Proportion Uninfected
High
Control
High
Low
Low
Control
Weeks post challenge
B
293T-derived virus
Rhesus CD4+ T cell-derived virus
Infection probability
Neutralization titer (ID80)
50% protective ID80 = 138
CI=65, 295
90% protective ID80 = 525
CI=129, 2137
50% protective ID80 = 51
CI = 31, 85
90% protective ID80 = 115
CI =45, 295
C
Peak viral load by group
RNA copies/mL
High Neut.
Low Neut.
Control
Immunization groups
D
RM CD4+ T cell-derived virus ID50 Neutralization Titer
R² =0.5 , P value <0.0001
Immunization Group
Control
High
Low
Weeks to infection

Fig. S8. Kaplan-Meier analysis of infection probability at different challenge timepoints. (**A**) Kaplan-Meier curves depict the proportion of macaques with undetectable viremia in each group over time. Arrows indicate the timepoints for challenge. Pairwise comparisons of infection control macaques (n=9 macaques), vaccinated macaques with low neutralization titers (n=15 macaques), or vaccinated macaques with high neutralization titers (n=7 macaques) are shown. (**B**) Logistic regression modeling was used to predict the probability of a macaque becoming infected given a certain ID_{80} plasma or serum neutralization titer. The ID_{80} neutralization titer required in macaque plasma to confer protection of 50% (orange line) and 90% (teal line) of macaques are shown. Neutralization titers were determined for (left) 293T-produced SHIV CH505.TF.375H virus and (right) rhesus $CD4^+$ T cell-derived SHIV.CH505.375H dCT challenge virus. ID_{80} neutralization titers and conference intervals (CI) are provided below the graphs. (**C**) Comparison of peak viral loads in infected macaques. Each symbol represents a single macaque and horizontal bars indicate group median. The median viral loads for the infection control, low neutralization (Neut.) titer, and high neutralization titer groups were $1.1x10^6$, $2.03x10^5$, and $7.47x10^5$ RNA copies respectively. The peak viral loads for infection control and immunized macaques with low neutralization titers were not significantly different (Exact Wilcoxon test, p = 0.071 , n = 9 or 13 macaques). Horizontal bars indicate group mean. (**D**) Linear regression correlation of ID_{50} neutralization titer at the time of first challenge and the number of weeks it took for the rhesus macaque (RM) to become infected. A significant correlation was found between the two variables. The data points are colored based on neutralization titer.

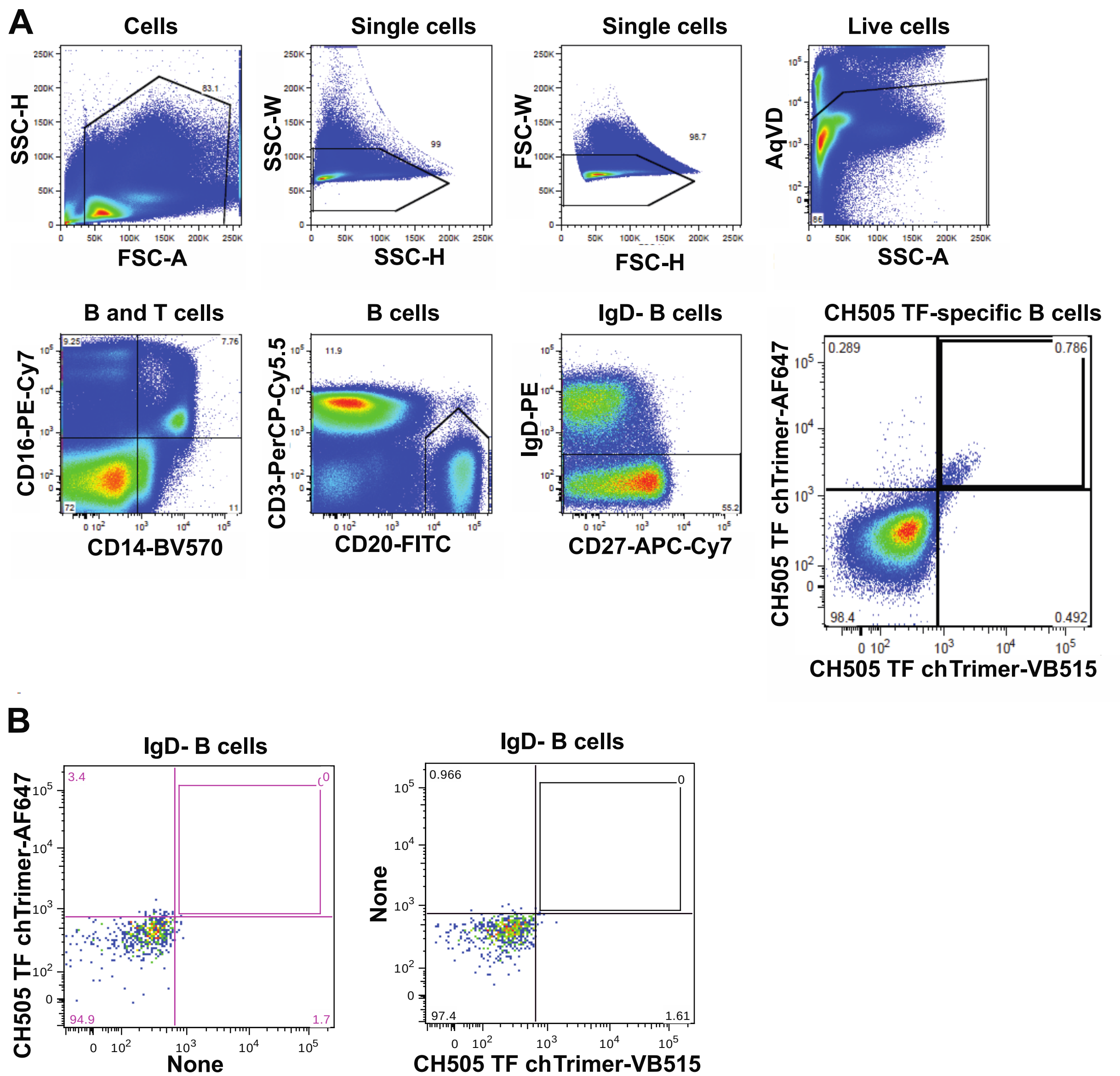


Fig. S9. Single B cell sorting gating strategy for HIV-1 Env-specific B cells from rhesus macaque 7068. (**A**) Peripheral blood memory B cells that bound to CH505 TF chTrimer labeled with two different fluorochromes were isolated two weeks after the sixth immunization. SSC-H, side scatter height; FSC-A, forward scatter area; SSC-W, side scatter width; FSC-W, forward scatter width; AqVD, aqua viability dye; PE, phycoerythrin; VB, VioBright; PerCP, peridinin chlorophyll protein; APC, allophycocyanin; AF, Alexa fluor; FITC, Fluorescein isothiocyanate. (**B**) FACS dot plots of negative control staining experiments, where only one of two CH505 TF chTrimer antigens were used to stain cells, are shown. B cells shown were gated as in (A).

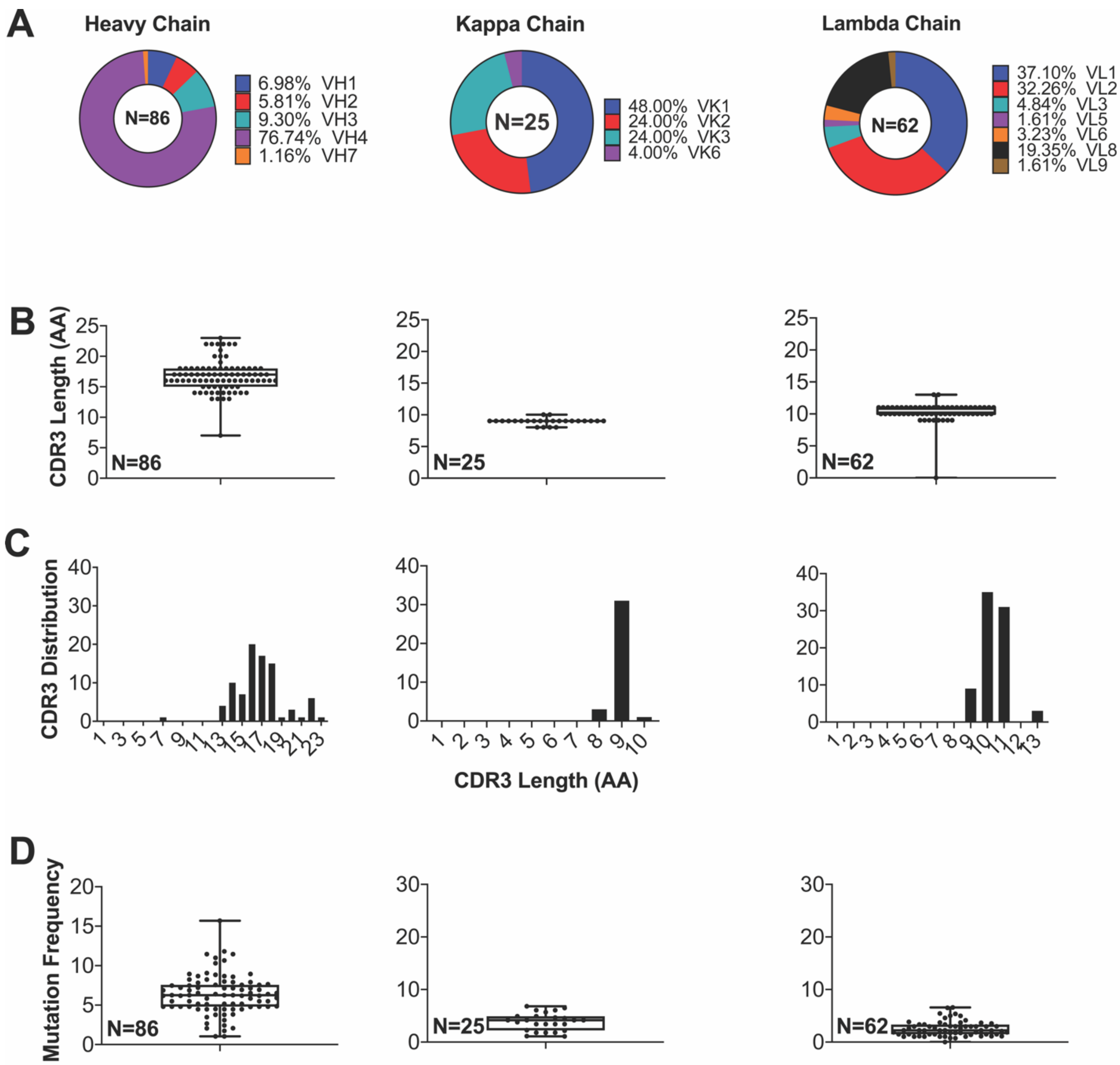


Fig. S10. Immunogenetics of CH505 TF chTrimer specific antibodies isolated from 7068. The first, second, and third columns show the immunogenetics of heavy, kappa, or lambda chain variable regions, respectively. (**A**) The percentage of immunoglobulin chains derived from a particular V gene segment are shown. Each antibody lineage was counted once. (**B**) Amino acid length of the third complementarity determining region (CDR3) is shown for each observed antibody lineage. (**C**) Histograms show the distribution of CDR3 lengths in amino acids among antibody lineages. (**D**) V gene segment somatic mutation percentages are shown. Each symbol indicates a unique antibody lineage. The most mutated member of the lineage is shown in (D). Data in (B) and (D) are presented as box-and-whisker plot showing minimum, maximum, interquartile range, and median.

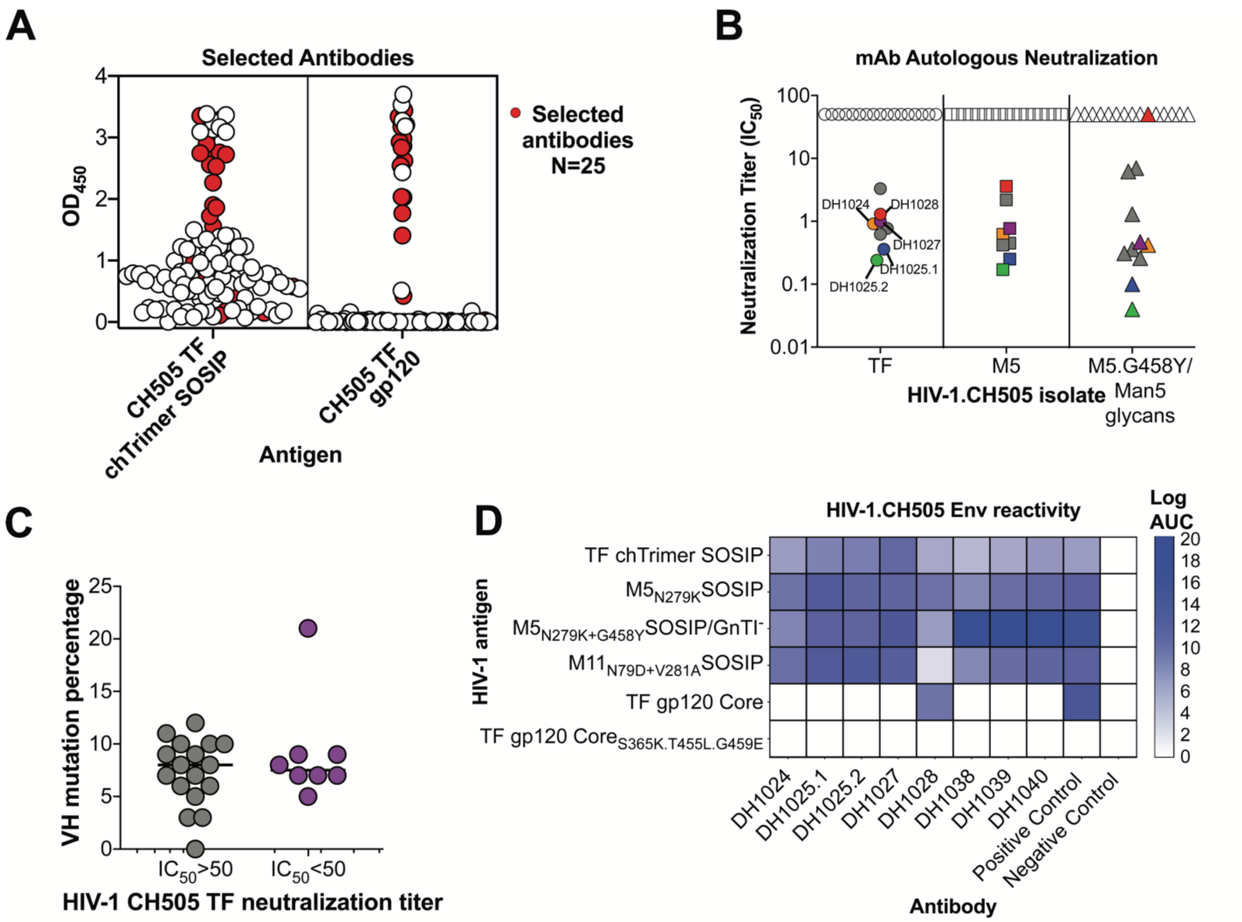


Fig. S11. Functional characterization of CH505 TF chTrimer-specific antibodies isolated from 7068. (**A**) Cell culture supernatants containing recombinant antibodies cloned from sorted single B cells were screened by ELISA for binding to CH505 TF chTrimer or gp120. Antibodies selected for medium-scale expression and further analysis based on V gene usage, CDR3 length, and CH505 TF envelop reactivity are colored in red (n = 25). (**B**) Shown is monoclonal antibody (mAb) neutralization of CH505 TF, N279K mutated TF (M5), N279K and G458Y mutated TF enriched for Man5Glc NAc2 glycans (M5.G458Y) virus infection of TZM-bl cells. IC_{50} titers are shown in µg/mL. Symbols represent individual antibodies and are color-coded to correspond to Fig. 3 and 6. (**C**) Mutation percentages of neutralizing and non-neutralizing antibodies are similar. (**D**) Neutralizing mAb binding to SOSIP gp140 and gp120 core versions of CH505 envelope was quantified. S365K.T455L.G459E mutations knockout CD4bs bnAb binding, but not non-binding of the neutralizing antibody F105. ELISA binding titers are shown as LogAUC values in a heatmap. CH103 was used as a positive control and CH65 (anti-influenza HA) was used as a negative control IgG.

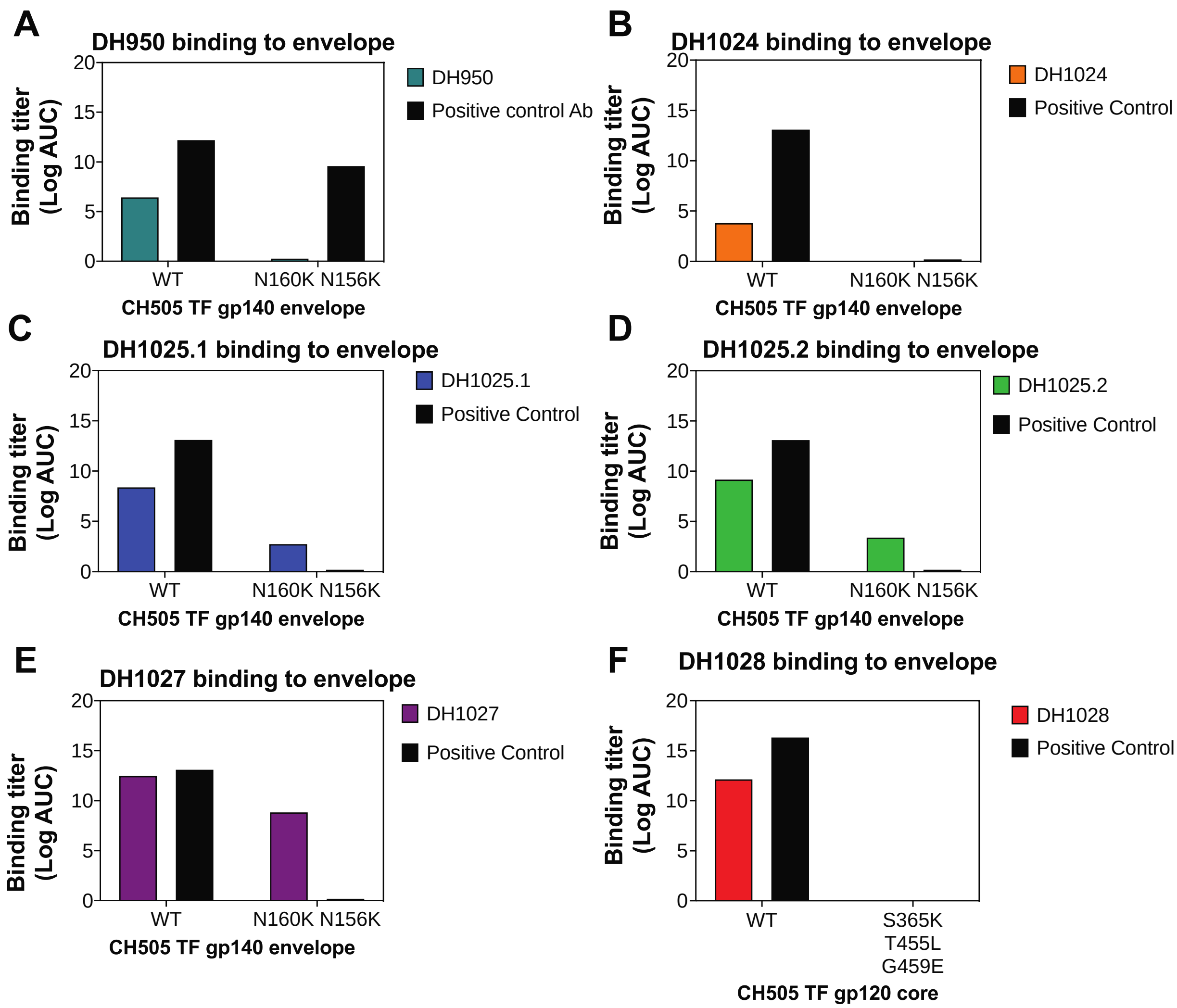


Fig. S12. Neutralizing antibody binding to wild-type (WT) and mutant CH505 envelops shows antibodies are sensitive to CD4bs or V1V2 glycan mutations. (**A to F**) Binding was assessed for uncleaved gp140 oligomers and gp120 core proteins for (**A**) DH950, (**B**) DH1204, (**C**) DH1025.1, (**D**) DH1025.2, (**E**) DH1027, and (**F**) DH1028. Binding titers are shown as LogAUC values. PG9 was used the positive control in (B to E). CH103 was used as the positive control in (A) and (F).

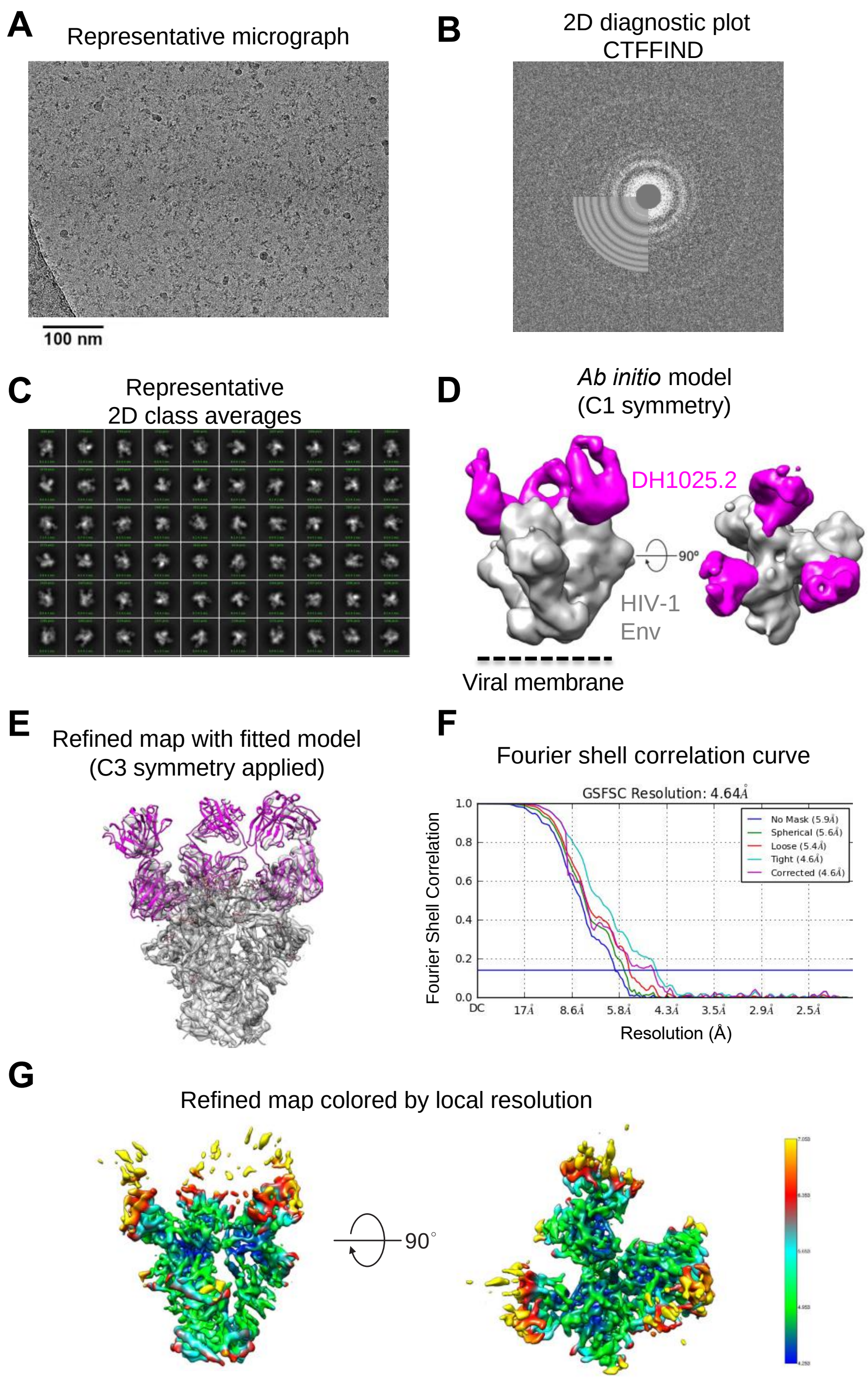


Fig. S13. Cryo-electron microscopy data and structure validation for DH1025.2 Fab in complex with CH505 M5 SOSIP G458Y Env trimer. (**A**) Shown is a representative micrograph of DH1025.2 Fab in complex with CH505.M5.SOSIP G458Y Env trimer. (**B**) A 2D diagnostic plot of the micrographs using CTFFIND4 is shown. (**C**) Representative 2D class averages were calculated from selected particles. (**D**) An Ab initio model showing CH505.M5.SOSIP G458Y Env trimer (gray) and DH1025.2 Fab (magenta) is shown. (**E**) A C3 symmetry applied refined map of ab initio generated model refined against cleaned-up particles is shown. (**F**) Fourier shell correlation (FSC) curves of the 3D reconstructions are shown, with the horizontal blue line indicating $FSC_{0.143}$. (**G**) A refined 3D density map colored by local resolution is shown in side and top views.

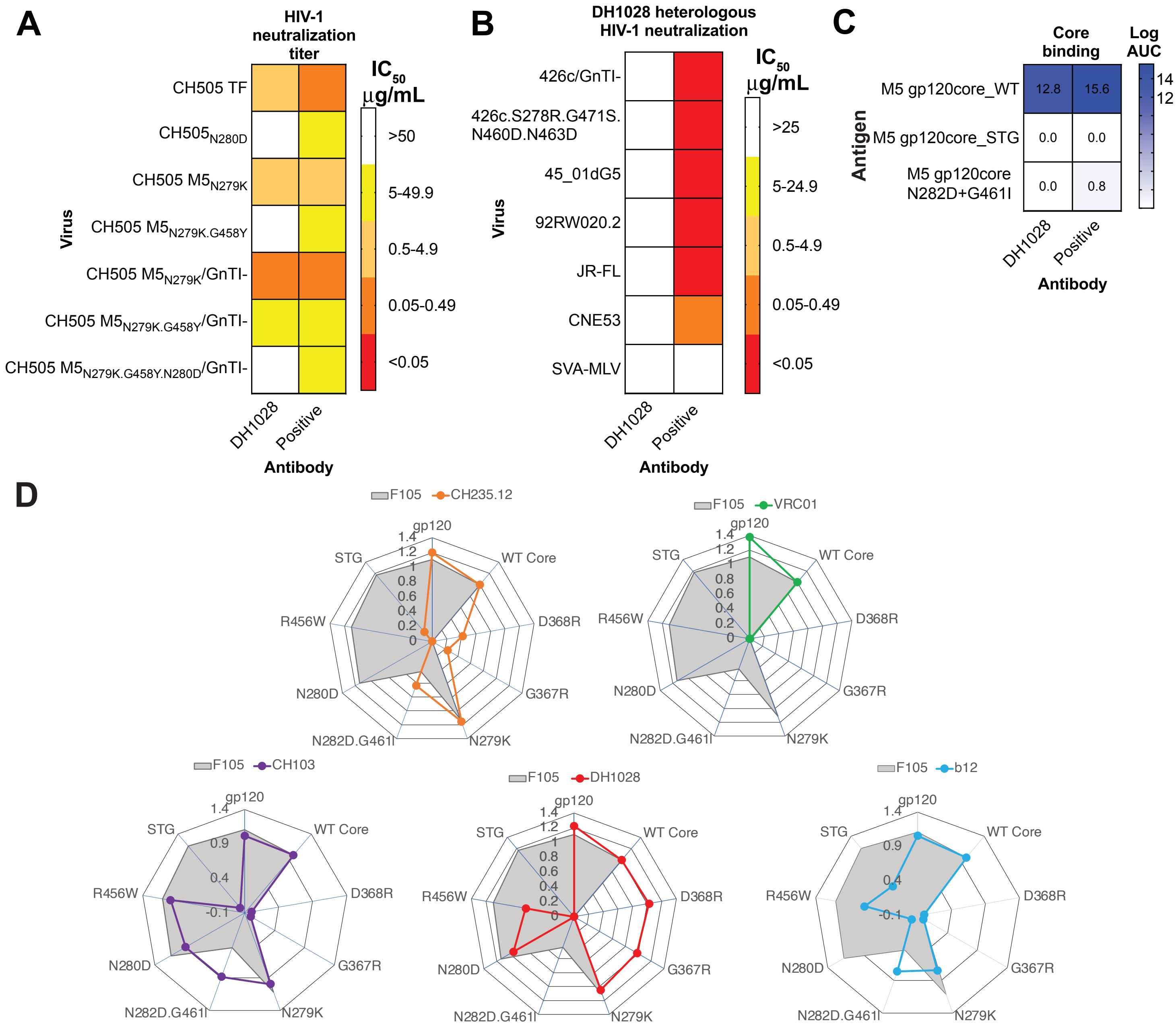


Fig. S14. DH1028 neutralization specificity and breadth. (**A**) DH1029 neutralization sensitivity to CD4bs mutations was measured. A mixture of CH01 and CH31 was used as a positive control broadly neutralizing IgG sample. (**B**) The ability of DH1028 to neutralize heterologous HIV-1 isolate infection of TZM-bl cells is shown. A mixture of CH01 and CH31 was used as a positive control broadly neutralizing IgG sample. (**C**) DH1029 sensitivity was measured to CD4bs mutations that preferentially knockout bnAb binding to gp120 core. CH103 was used as a positive control CD4bs bnAb. (**D**) Radar plots show binding of indicated antibodies to HIV-1 envelope gp120 and gp120 cores with CD4bs mutations. Binding is shown normalized to WT CH505 TF core protein.

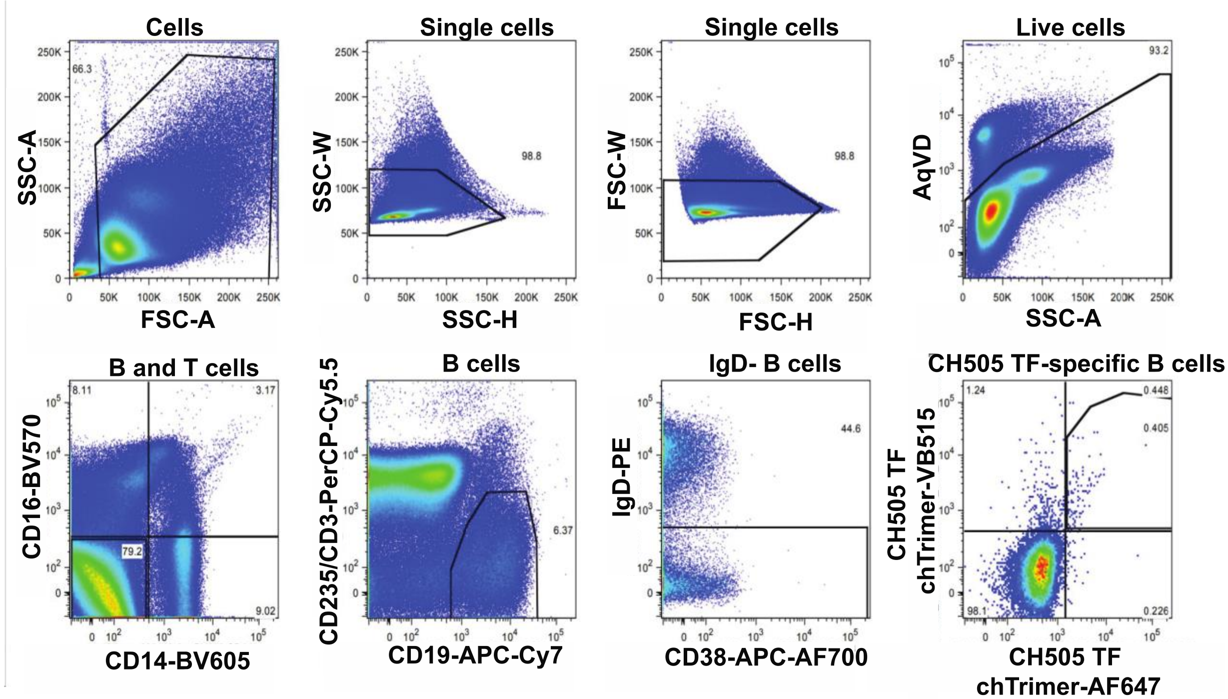


Fig. S15. Single B cell sorting gating strategy for HIV-1 Env-specific B cells from CH505, an individual living with HIV. B cells from week 78 post infection that bound to CH505 TF chTrimer were labeled with two different fluorochromes and were sorted as shown.

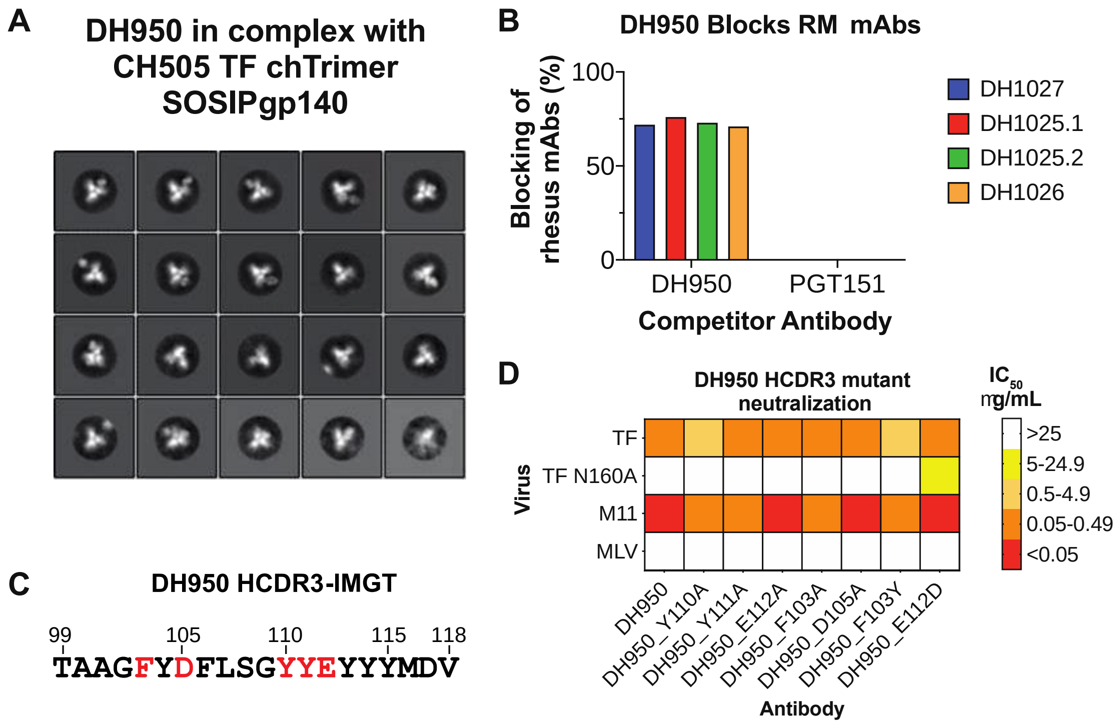


Fig. S16. Characterization of the DH950 epitope and paratope. (**A**) 2D class averages of NSEM images of DH950 Fab bound to CH505 TF chTrimer are shown. (**B**) Competition ELISA results are shown; envelope bound to DH950 showed reduced binding to rhesus macaque neutralizing mAbs. Binding PGT151 to envelope showed no blocking of rhesus macaque antibodies. (**C**) Alanine scanning mutagenesis of Tyrosine and introduction of YYD motifs in the DH950 HCDR3 was done to determine the amino acids required for neutralization of CH505 TF or CH505 M11 viruses. F104Y or E112D mutations introduced YYD motifs into the HCDR3 of DH950. The HCDR3 was determined using the International ImMunoGeneTics (IMGT) system definition for start and stop sites of HCDR3 (HCDR3-IMGT). (**D**) Neutralizing IC_{50} titers are shown as a heatmap. MLV was used as the negative control virus.

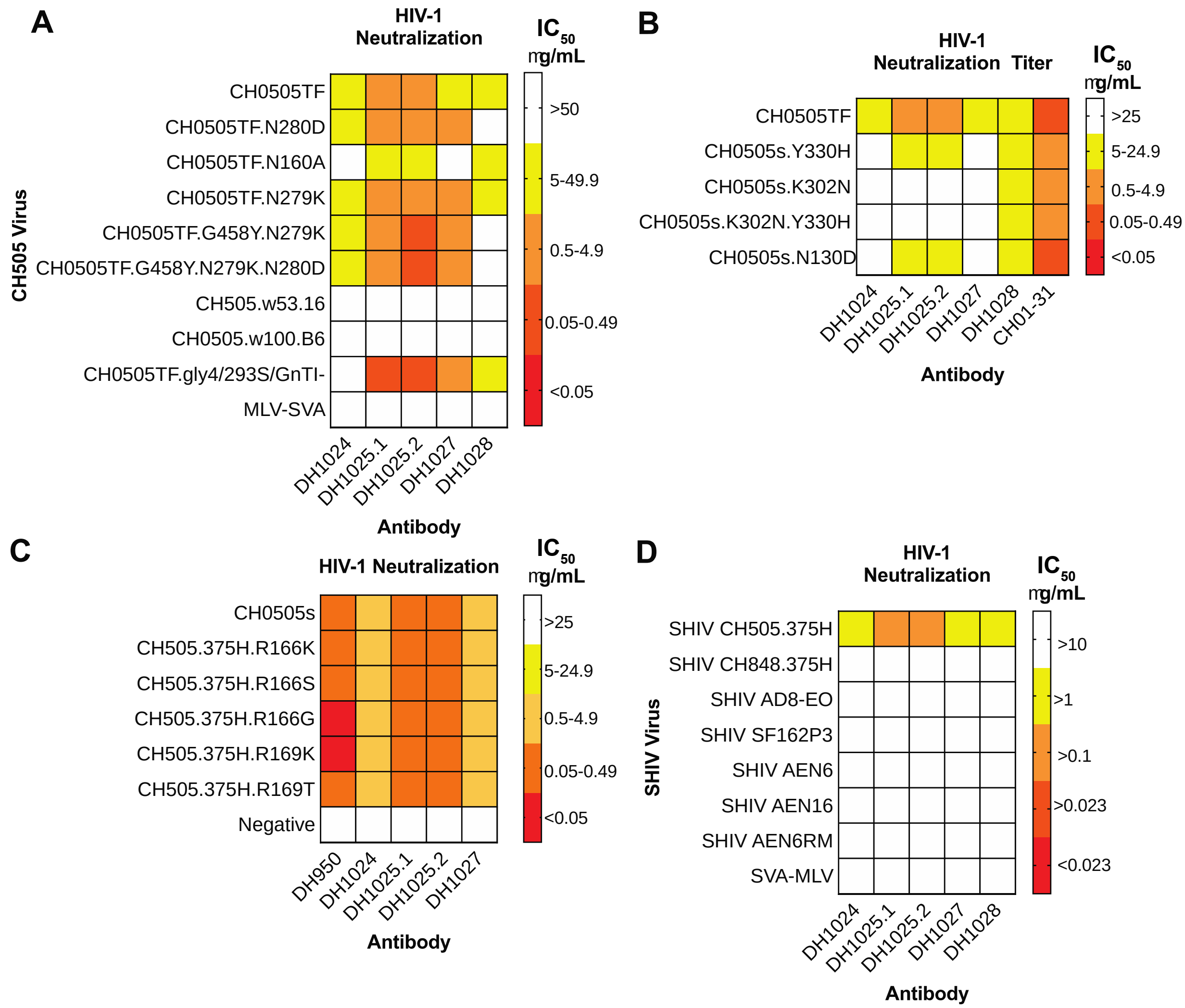


Fig. S17. Characterization of rhesus macaque autologous neutralization specificity. (**A**) mAb neutralization of CH505 TF, CH505 TF CD4bs mutants, CH505 TF V2 glycan mutant, and CH505 chronic infection isolates (w53.16 and w100.B6) infection of TZM-bl cells is shown. (**B**) Neutralizing mAb sensitivity was tested against mutations that arise in the V1 region (N130D), V3 region proximal to the N301 glycan (K302N), or V3 region proximal to the GDIR motif (Y330H) during CH505 SHIV infection. (**C**) mAb neutralization capacity was tested against CH505 TF virus with a 375H mutation and V2 region mutations. Neutralizing mAbs were unaffected by V2 region mutation, but were affected by the V1 and V3 region mutations presented in (**B**). (**D**) mAb neutralization of autologous and heterologous SHIV infection of TZM-bl cells is shown. SVA-MLV is a murine leukemia virus that was used as a negative control. IC_{50} titers are shown in µg/mL and are color-coded in the heatmaps.

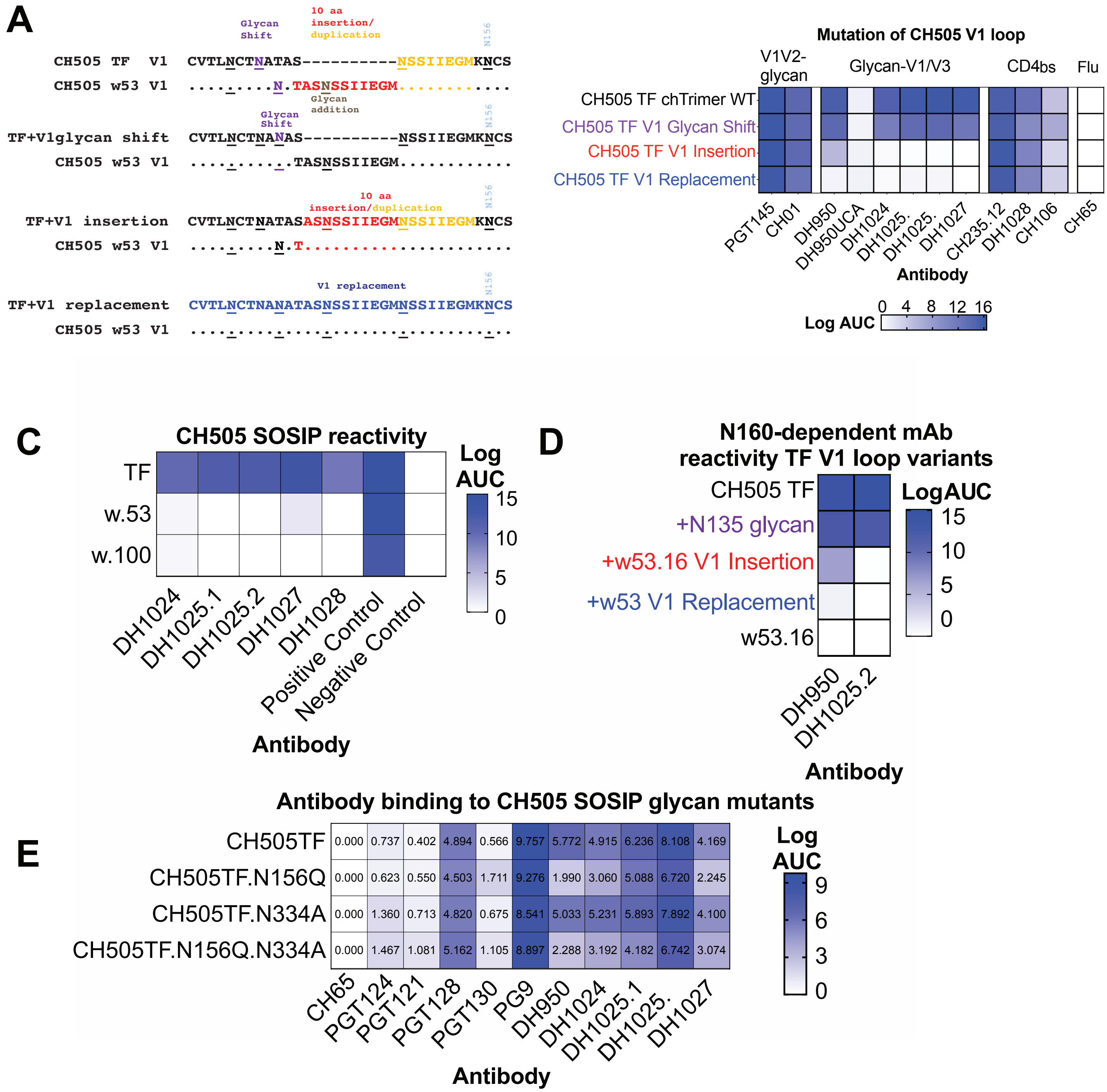


Fig. S18. N156 mutation reduces binding reactivity of glycan-V1/V3-reactive autologous neutralizing. **(A)** Amino acid alignments showing changes in the V1 region of CH505 TF and w53.16 envelopes. Alignments for chimeric envelopes are shown below the WT TF and w53.16 alignment. **(B)** Heatmap of ELISA binding titers for antibodies with different specificities shown as log AUC. Binding was assessed for CH505 TF with modifications to the V1 region to include w53.16 sequence elements. PGT145, CH01, and CH106 were used as positive controls and CH65 (anti-influenza HA) was used as a negative control. **(C)** Heatmap of ELISA binding titers as log AUC for CH505 TF, w53.16, and w100.B6 closed state-stabilized SOSIPv4.1 gp140 trimers. CH103 was used as a positive control and CH65 (anti-influenza HA) was used as a negative control. **(D)** A heatmap comparing ELISA binding titers for DH950 and DH1025.2 as Log AUC for CH505 TF with modifications to the V1 region to include w53.16 sequence elements. **(E)** Heatmap of ELISA binding titers as Log AUC for CH505 TF wildtype and N334 and N156 mutants. Autologous neutralizing antibody binding decreased in the presence of N156 mutation but not N334 mutation. CH65 (anti-influenza HA) was used as a negative control.

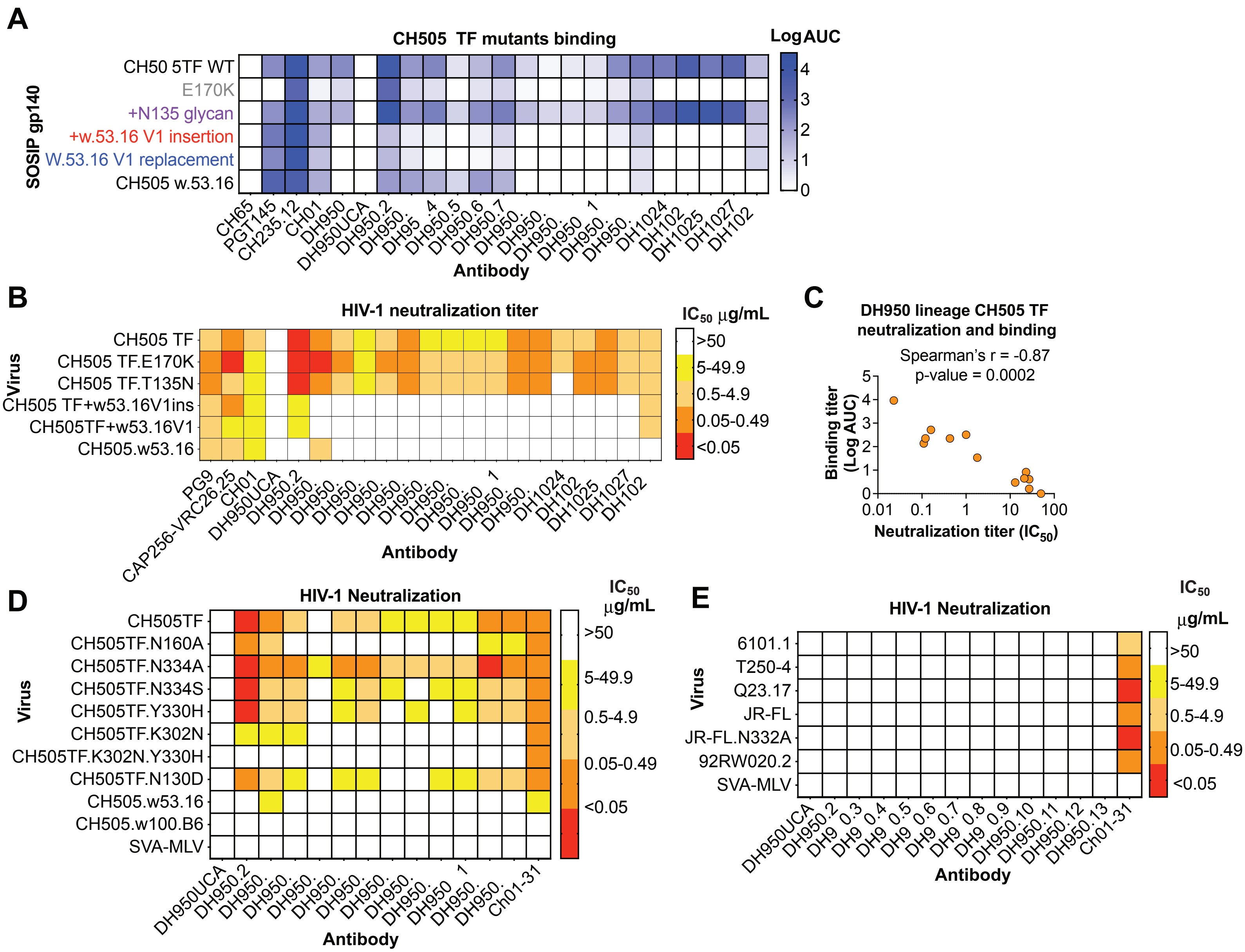


Fig. S19. Neutralization and binding activity of select members of the DH950 antibody lineage. (**A**) The heatmap shows ELISA titers as LogAUC of human and rhesus macaque glycan-V1/V3-directed antibodies binding to pre-fusion stabilized SOSIPv4.1 gp140 proteins. Antibody binding was assessed for CH505 TF, CH505 w53.16, and CH505 variants with V1 modified to include sequence elements from CH505 w53.16. (**B**) DH950 lineage mAb neutralizing activity was measured for CH505 TF and w53.16 V1 chimeric virus infection of TZM-bl cells. An E170K mutation was also introduced to assess whether a more positively charged V2 loop would increase binding to neutralizing antibodies. (**C**) The Spearman correlation of DH950 lineage antibody neutralization IC_{50} titer and binding log AUC titer is shown. Each symbol represents a single antibody in the DH950 lineage (n = 13). (**D**) DH950 lineage mAb neutralizing activity was measured for CH505 TF, late isolates (w53.16 and w100.B6), and TF mutant virus infection of TZM-bl cells. Mutations Y330H, K302N, and N130D in CH505 TF were identified during SHIV infection. N160 and N334 mutations were made to examine V2-glycan and V3-glycan dependence. (**E**) DH950 lineage mAb neutralizing activity was measured for heterologous HIV-1 infection of TZM-bl cells. Neutralization IC_{50} titers are shown as a heatmap in (A), (B), (D), and (E). MLV was used as a negative control virus. A mixture of CH01 and CH31 was used as a positive control.

Table S1. 50% and 90% protective ID_{50} and ID_{80} serum neutralization titers (shown as reciprocal dilution) against SHIV.CH505 TF challenge.

Serum neutralization titer	Virus production cell type	50% Infection Titer (95% CI)	10% Infection Titer (95% CI)	C statistics	Hosmer and Lemeshow Goodness-of-Fit Test
		50% Protection Titer (95% CI)	90% Protection Titer (95% CI)		
ID_{50}	293T/17	409 (182, 871)	1954 (417, 8913)	0.894	0.257
ID_{50}	Rh $CD4^{+}$	155 (98, 245)	364 (148, 891)	0.914	0.58
ID_{80}	293T/17	138 (65, 295)	525 (129, 2137)	0.914	0.007
ID_{80}	Rh $CD4^{+}$	51 (31, 85)	115 (45, 295)	0.896	0.483

Table S2. Macaque neutralizing antibody immunogenetics.

Macaque	Antibody	VH gene segment	HCDR3 Length (Amino Acids)	VH mutation (%)	VL gene segment	LCDR3 Length (Amino Acids)	VL mutation (%)
7068	DH1024	*IGHV4-f*02*	22	6.5	*IGVL2~k*	8	1
7068	DH1025.1	*IGHV4-f*02*	23	6.5	*IGVK1~h*	9	4.2
7068	DH1025.2	*IGHV4-f*02*	23	6.5	*IGVK1~h*	9	6.5
7068	DH1027	*IGHV4-j*02*	18	6.25	*IGVK1~r*	9	6.8
7068	DH1028	*IGHV2-d*01*	13	5.9	*IGVK1~r*	9	2.7

Table S3. Cryo-EM data collection, refinement, and validation

Data Collection	CH505 M5 SOSIP G458Y+ DH1025.2 Fab
Microscope	FEI Titan Krios
Voltage (kV)	300
Electron dose (e-/Å2)	57.6
Detector	Gatan K3
Pixel Size (Å)	1.08
Defocus Range (μm)	~1.5-3
Magnification	92000
Reconstruction Software	cryoSparc
Particles	86587
Symmetry	C3
Box size (pix)	320
Resolution (Å) (FSC 0.143)*	4.64

*Resolutions are reported according to the FSC 0.143 gold-standard criterion

Table S4. DH950 antibody immunogenetics.

Participant	Antibody	VH gene segment	JH gene segment	H CDR3 length (Amino Acids)	VH mutation (%)	VL gene segment	KCDR3 length (Amino Acids)	VK/L Mutation (%)
CH505	DH950	IGHV3-15	6*03	21	9	IGKV3-15	10	9

Immunogenetics were inferred using the human reference library in Cloanalyst

Data file S1. Raw, individaul-level data for experiments where n<20.