# Structure and immunogenicity of a stabilized HIV-1 envelope trimer based on a group-M consensus sequence

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                                                                     49
                TPA leader                                         47 49            64
HxB2 gp160      MRVKEKYQHLWRWGWRWGTMLLGMLMICS------ATEKLWVTVYYGVPVWKEATTTLFCASDAKAYDTE
ConM gp160      MRVMGIQRNCQHL-WRWGILIFGMLIICS------AAENLWVTVYYGVPVWKEANTTLFCASDAKAYDTE
ConS gp160      MRVRGIQRNCQHL-WRWGTLILGMLMICS------AAENLWVTVYYGVPVWKEANTTLFCASDAKAYDTE
ConM SOSIP.v7   MDAMKRGLCCVLL-LCGAVFVSPSQEIHARFRRGARAENLWVTVYYGVPVWKDAETTLFCASDAKAYDTE
BG505 SOSIP.664 MDAMKRGLCCVLL-LCGAVFVSPSQEIHARFRRGARAENLWVTVYYGVPVWKDAETTLFCASDAKAYETK
BG505 SOSIP.v6  MDAMKRGLCCVLL-LCGAVFVSPSQEIHARFRRGARAENLWVTVYYGVPVWKDACTTLFCASDAKAYETK

               6566    73                               106
HxB2 gp160      VHNVWATHACVPTDPNPQEVVLVNVTENFNMWKNDMVEQMHEDIISLWDQSLKPCVKLTPLCVSLKCTDL
ConM gp160      VHNVWATHACVPTDPNPQEIVLENVTENFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTDV
ConS gp160      VHNVWATHACVPTDPNPQEIVLENVTENFNMWKNNMVEQMHEDIISLWDQSLKPCVKLTPLCVTLNCTNV
ConM SOSIP.v7   KRNVWATHCCVPTDPNPQEIVLENVTENFNMWKNNMVEQMHTDIISLWDQSLKPCVKLTPLCVTLNCTDV
BG505 SOSIP.664 KHNVWATHACVPTDPNPQEIHLENVTEEFNMWKNNMVEQMHTDIISLWDQSLKPCVKLTPLCVTLQCTNV
BG505 SOSIP.v6  KHNVWATHCCVPTDPNPQEIHLENVTEEFNMWKNNMVEQMHTDIISLWDQSLKPCVKLTPLCVTLQCTNV

                                                  165
HxB2 gp160      KNDTNTNSSSGR-----MIMEKGEIKNCSFNISTSIRGKVQKEYAFFYKLDIIPIDNDTTS--------Y
ConM gp160      NATNNTTNNEE-------------IKNCSFNITTEIRDKKKKVYALFYKLDVVPIDDNNS---------Y
ConS gp160      NVTNTTNNTEEK----------GEIKNCSFNITTEIRDKKQKVYALFYRLDVVPIDDNNNNSSN-----Y
ConM SOSIP.v7   NATNNTTNNEE-------------IKNCSFNITTELRDKKKKVYALFYKLDVVPIDDNNS---------Y
BG505 SOSIP.664 T-NNITDDMR------------GELKNCSFNMTTELRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEY
BG505 SOSIP.v6  T-NNITDDMR------------GELKNCSFNMTTELRDKKQKVYSLFYRLDVVQINENQGNRSNNSNKEY

HxB2 gp160      KLTSCNTSVITQACPKVSFEPIPIHYCAPAGFAILKCNNKTFNGTGPCTNVSTVQCTHGIRPVVSTQLLL
ConM gp160      RLINCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCNDKKFNGTGPCKNVSTVQCTHGIKPVVSTQLLL
ConS gp160      RLINCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCNDKKFNGTGPCKNVSTVQCTHGIKPVVSTQLLL
ConM SOSIP.v7   RLINCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCNDKKFNGTGPCKNVSTVQCTHGIKPVVSTQLLL
BG505 SOSIP.664 RLINCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQLLL
BG505 SOSIP.v6  RLINCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCKDKKFNGTGPCPSVSTVQCTHGIKPVVSTQLLL

                                                                     316
HxB2 gp160      NGSLAEEEVVIRSVNFTDNAKTIIVQLNTSVEINCTRPNNNTRKRIRIQRGPGRAFVTIG-KIGNMRQAH
ConM gp160      NGSLAEEEIIIRSENITNNAKTIIVQLNESVEINCTRPNNNTRKSIRI--GPGQAFYATGDIIGDIRQAH
ConS gp160      NGSLAEEEIIIRSENITNNAKTIIVQLNESVEINCTRPNNNTRKSIRI--GPGQAFYATGDIIGDIRQAH
ConM SOSIP.v7   NGSLAEEEIIIRSENITNNAKTIIVQLNESVEINCTRPNNNTRKSIRI--GPGQWFYATGDIIGDIRQAH
BG505 SOSIP.664 NGSLAEEEVMIRSENITNNAKNILVQFNTPVQINCTRPNNNTRKSIRI--GPGQAFYATGDIIGDIRQAH
BG505 SOSIP.v6  NGSLAEEEVMIRSENITNNAKNILVQFNTPVQINCTRPNNNTRKSIRI--GPGQWFYATGDIIGDIRQAH

HxB2 gp160      CNISRAKWNNTLKQIASKLREQFGNNKTIIFKQSSGGDPEIVTHSFNCGGEFFYCNSTQLFNSTWFNSTW
ConM gp160      CNISRTKWNKTLQQVAKKLREHFN-KTII-FNPSSGGDLEITTHSFNCGGEFFYCNTSELFNSTWNG---
ConS gp160      CNISGTKWNKTLQQVAKKLREHFNNKTII-FKPSSGGDLEITTHSFNCRGEFFYCNTSGLFNSTWIGN--
ConM SOSIP.v7   CNISRTKWNKTLQQVAKKLREHFN-KTII-FNPSSGGDLEITTHSFNCGGEFFYCNTSELFNSTWNG---
BG505 SOSIP.664 CNVSKATWNETLGKVVKQLRKHFGNNTIIRFANSSGGDLEVTTHSFNCGGEFFYCNTSGLFNSTWISN-T
BG505 SOSIP.v6  CNVSKATWNETLGKVVKQLRKHFGNNTIIRFANSSGGDLEVTTHSFNCGGEFFYCNTSGLFNSTWISN-T

                                           429 432
HxB2 gp160      STEGSNNTEGSDTITLPCRIKQIINMWQKVGKAMYAPPISGQIRCSSNITGLLLTRDGGNSNNE-SEIFR
ConM gp160      ---------TNNTITLPCRIKQIINMWQGVGQAMYAPPIEGKIRCTSNITGLLLTRDGGNNNT---ETFR
ConS gp160      --GTKNNNNTNDTITLPCRIKQIINMWQGVGQAMYAPPIEGKITCKSNITGLLLTRDGGNNNTNETEIFR
ConM SOSIP.v7   ---------TNNTITLPCRIKQIINMWQRVGQAMYAPPIEGKIRCTSNITGLLLTRDGGNNNT---ETFR
BG505 SOSIP.664 SVQGSNSTGSNDSITLPCRIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGLILTRDGGSTNST-TETFR
BG505 SOSIP.v6  SVQGSNSTGSNDSITLPCRIKQIINMWQRIGQAMYAPPIQGVIRCVSNITGLILTRDGGSTNST-TETFR

                                            500501                                535
HxB2 gp160      PGGGDMRDNWRSELYKYKVVKIEPLGVAPTKAKRRVVQREKR--AVGIGALFLGFLGAAGSTMGAASMTL
ConM gp160      PGGGDMRDNWRSELYKYKVVKIEPLGVAPTKAKRRVVEREKR--AVGIGAVFLGFLGAAGSTMGAASITL
ConS gp160      PGGGDMRDNWRSELYKYKVVKIEPLGVAPTKAKRRVVEREKR--AVGIGAVFLGFLGAAGSTMGAASITL
ConM SOSIP.v7   PGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVERRRRRRAVGIGAVFLGFLGAAGSTMGAASMTL
BG505 SOSIP.v6  PGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASMTL
BG505 SOSIP.v6  PGGGDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVGRRRRRRAVGIGAVFLGFLGAAGSTMGAASMTL

                    543        555 559 561                                        605
HxB2 gp160      TVQARQLLSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAVERYLKDQQLLGIWGCSGKLICTTA
ConM gp160      TVQARQLLSGIVQQQSNLLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLKDQQLLGIWGCSGKLICTTN
ConS gp160      TVQARQLLSGIVQQQSNLLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLKDQQLLGIWGCSGKLICTTT
ConM SOSIP.v7   TVQARNLLSGIVQQQSNLLRAPECQQHLLQLTVWGIKQLQARVLAVERYLKDQQLLGIWGCSGKLICCTN
BG505 SOSIP.664 TVQARNLLSGIVQQQSNLLRAPEAQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCTN
BG505 SOSIP.v6  TVQARNLLSGIVQQQSNCLRAPECQQHLLKLTVWGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCTN

                                                                       664
HxB2 gp160      VPWNASWSNKSLEQIWNHTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWFNITN
ConM gp160      VPWNSSWSNKSQDEIWDNMTWMEWDKEINNYTDIIYSLIEESQNQQEKNEQELLALDKWASLWNWFDITN
ConS gp160      VPWNSSWSNKSQDEIWDNMTWMEWEREINNYTDIIYSLIEESQNQQEKNEQELLALDKWASLWNWFDITN
ConM SOSIP.v7   VPWNSSWSNKSQDEIWDNMTWMEWDKEINNYTDIIYSLIEESQNQQEKNEQELLALD-------------
BG505 SOSIP.v6  VPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD-------------
BG505 SOSIP.v6  VPWNSSWSNRNLSEIWDNMTWLQWDKEISNYTQIIYGLLEESQNQQEKNEQDLLALD-------------
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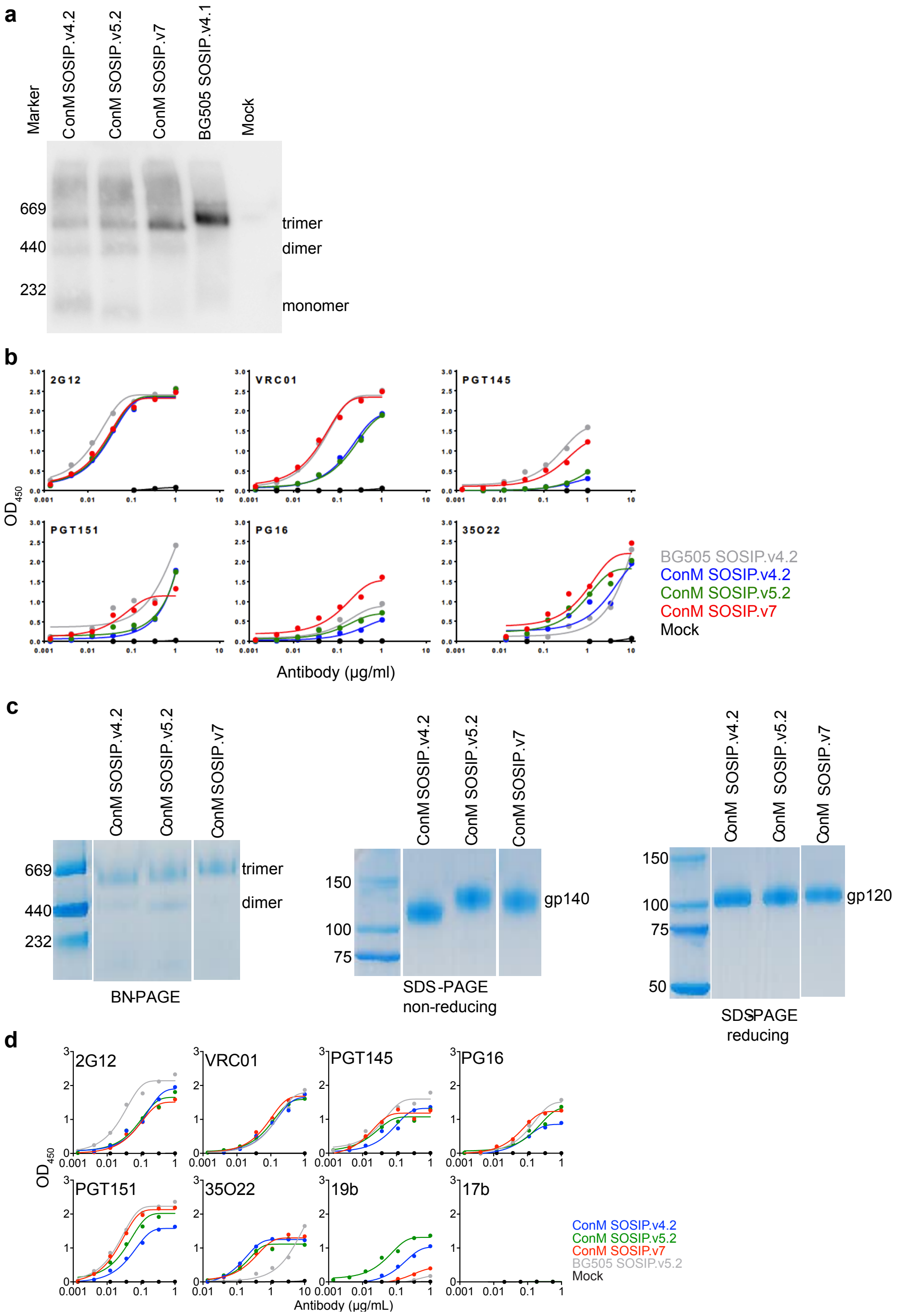
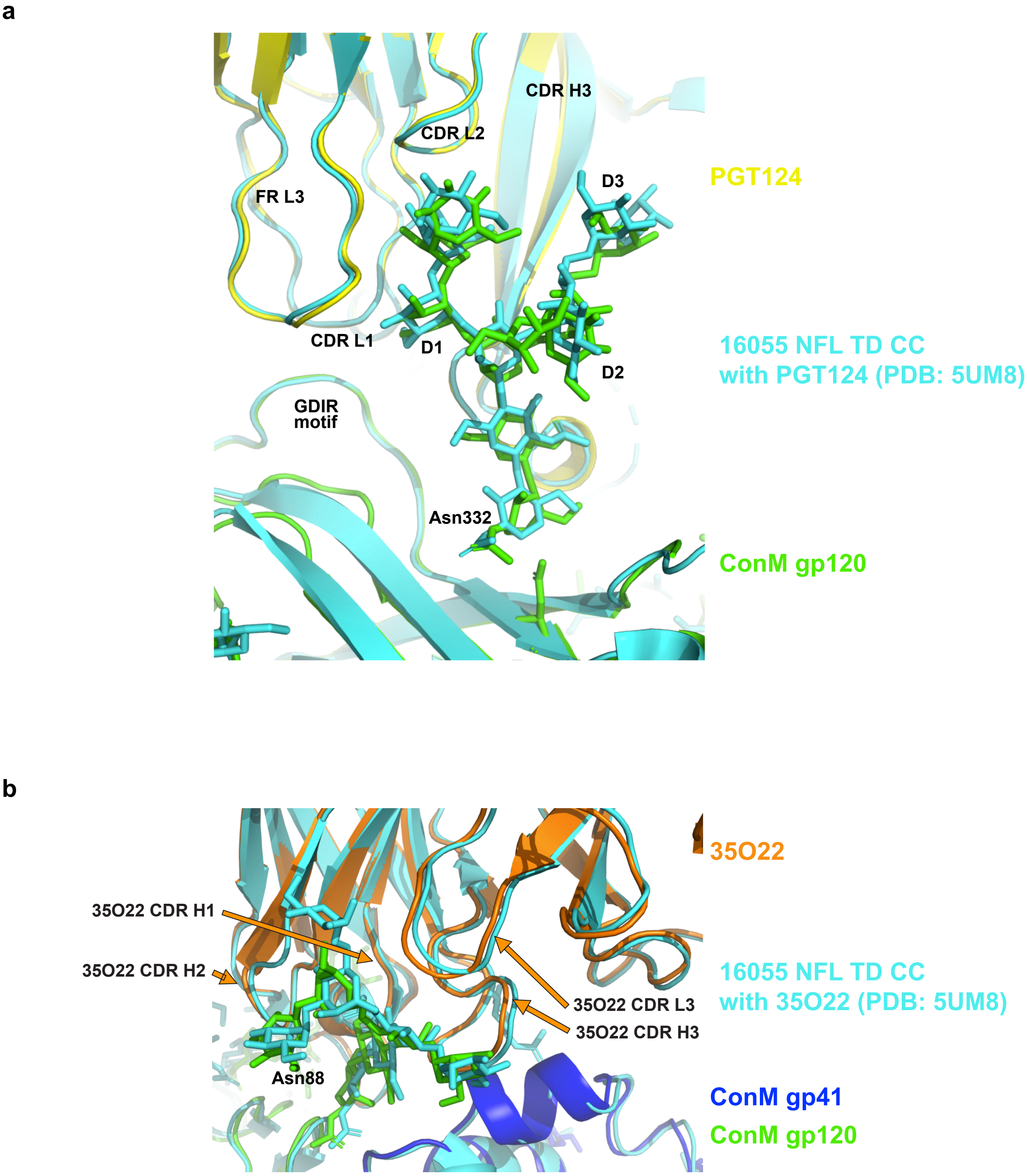
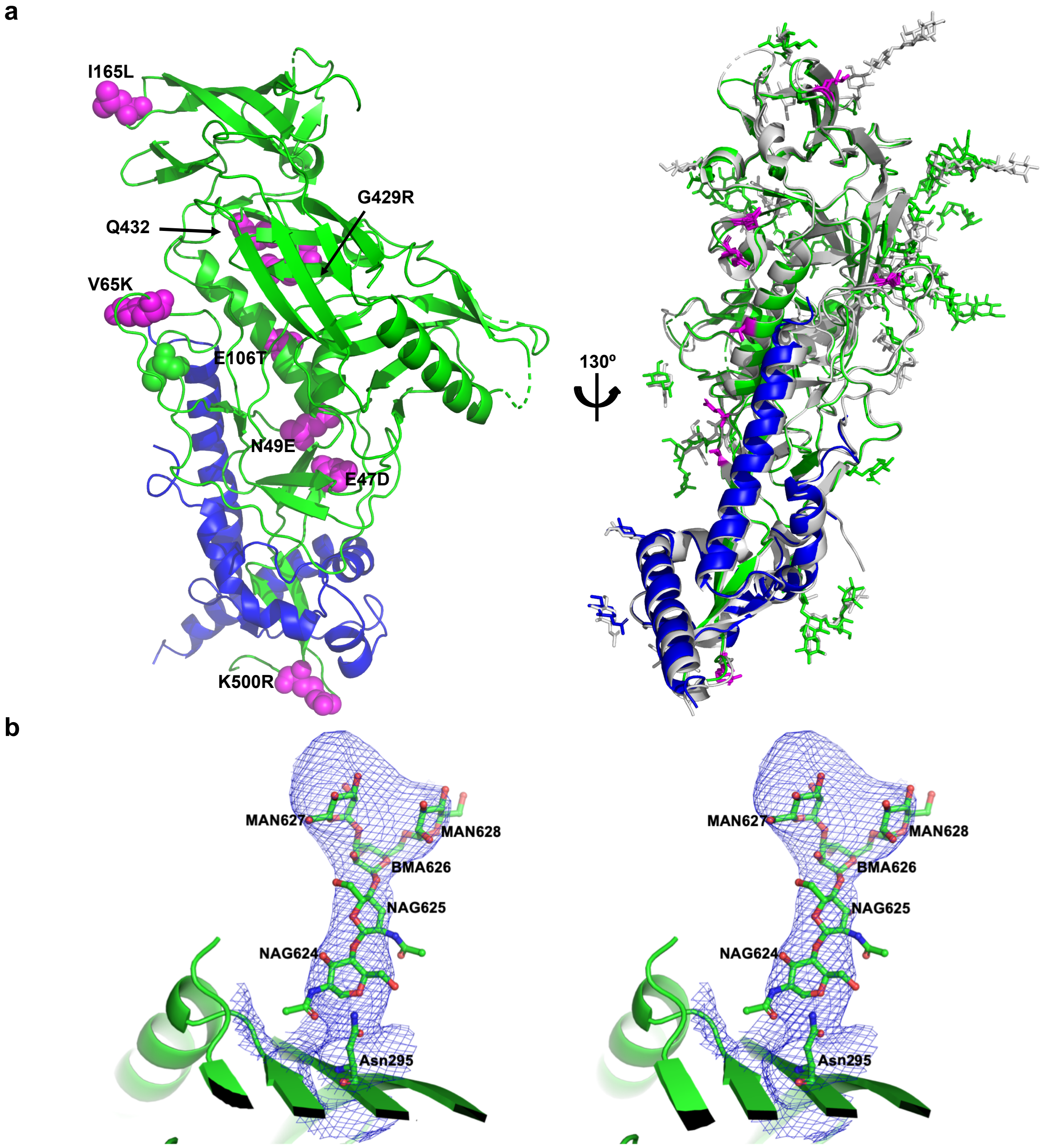
**Supplementary Figure 1.** Amino acid sequence alignment of ConM SOSIP.v7 with HxB2 gp160, ConM gp160 (alignment ID: 104CP2), ConS gp160, BG505 SOSIP.664 and BG505 SOSIP.v6. The SOSIP design, trimer-stabilizing and improved trimerization mutations are indicated in bold[1]; the extra intermolecular disulfide bond in red[2]; and the TD8 mutations in blue[3] (HxB2 numbering).



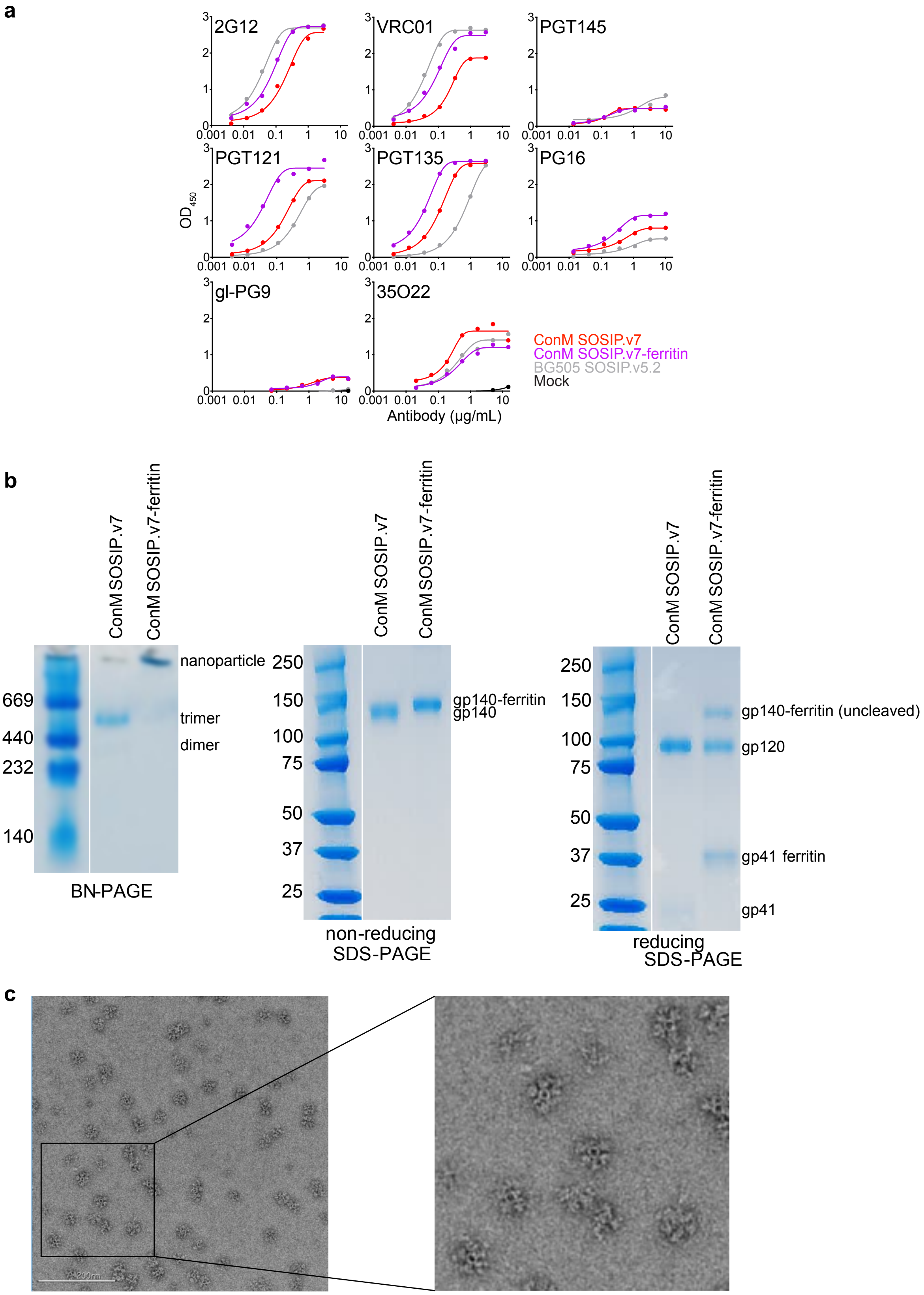
**Supplementary Figure 2.** Screening and characterization of ConM SOSIP.v4.2, SOSIP.v5.2 and SOSIP.v7 trimers. **a** BN-PAGE analysis of unpurified D7324-tagged SOSIP trimers expressed in 293T cells followed by Western blotting with 2G12/VRC01. **b** D7324-capture ELISA with unpurified D7324-tagged SOSIP trimers. **c** PGT145-purified SOSIP trimers were analyzed by BN-PAGE analysis, reducing (+DTT) and non-reducing (-DTT) SDS-PAGE followed by Coomassie Blue staining. We note the difference in migration pattern of SOSIP.v4 and SOSIP.v5 trimers in non-reducing SDS-PAGE gels, which are consistent with previous results showing that SOSIP.v5 proteins, which contain an extra intraprotomer disulfide bond between the gp120 and gp41, migrate more slowly than their SOSIP.664 and SOSIP.v4 counterparts. The likely explanation is a decrease in SDS uptake when a protein becomes more compact [2]. **d** D7324-capture ELISA with PGT145-purified ConM SOSIP trimer (D7324-tagged) expressed in 293F cells against a panel of bNAbs.



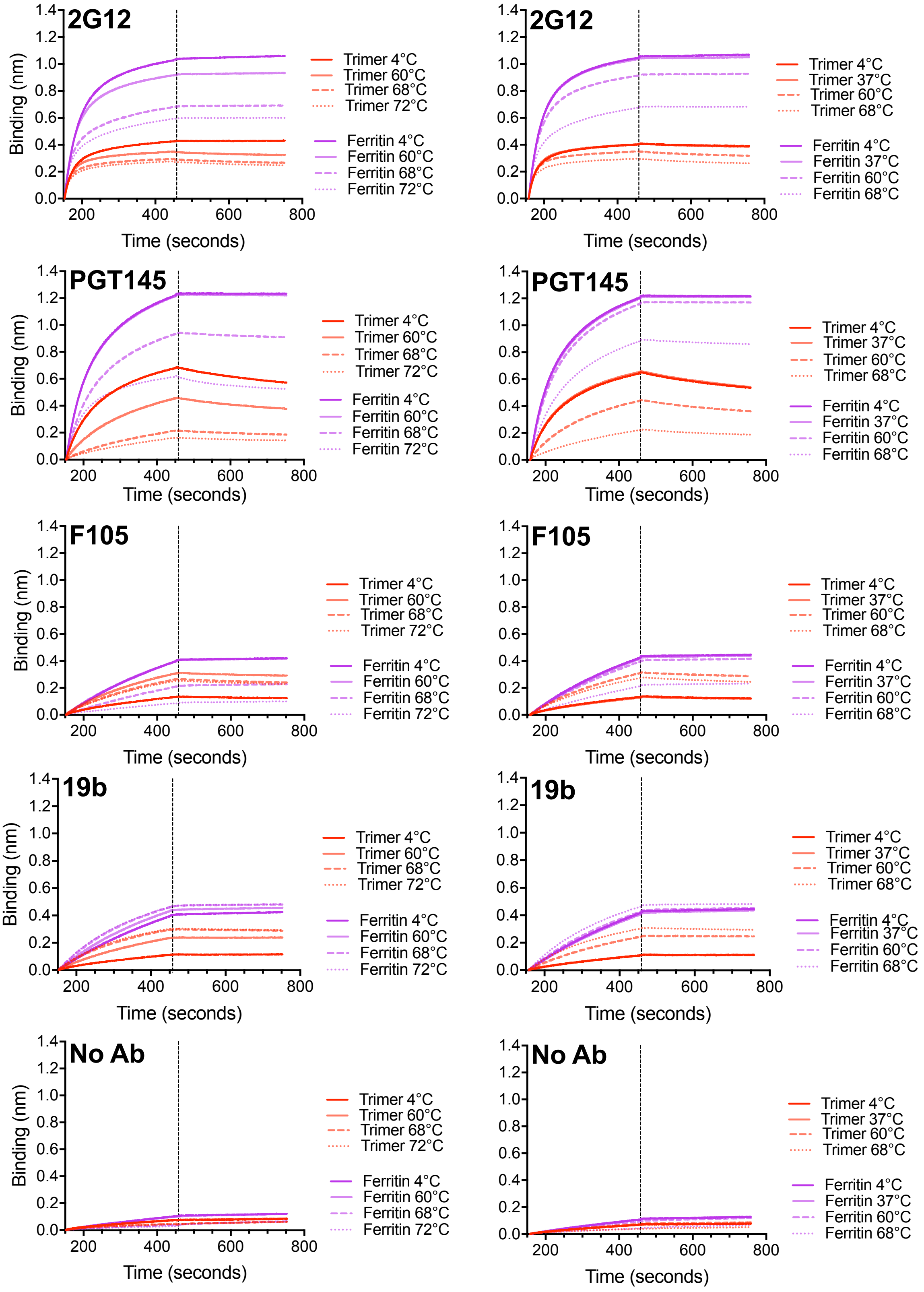
**Supplementary Figure 3.** Binding modes of PGT124 and 35O22 to ConM SOSIP.v7. **a.** Interaction of the CDR loops of PGT124 in yellow with the oligomannose glycan at the Asn332 supersite in green stick representation and $^{324}$GDIR$^{327}$ motif at the V3 loop base. The $^{324}$GDIR$^{327}$ motif and D1/D2/D3 arms in the oligomannose glycan at Asn332 are labeled. The structure of 16055 NFL TD CC in complex with PGT124 and 35O22 is represented in cyan cartoon for comparison. **b.** Interaction of the CDR loops of 35O22 in orange with the gp120/gp41 interface. gp120 and gp41 are represented as green and blue cartoon, respectively.



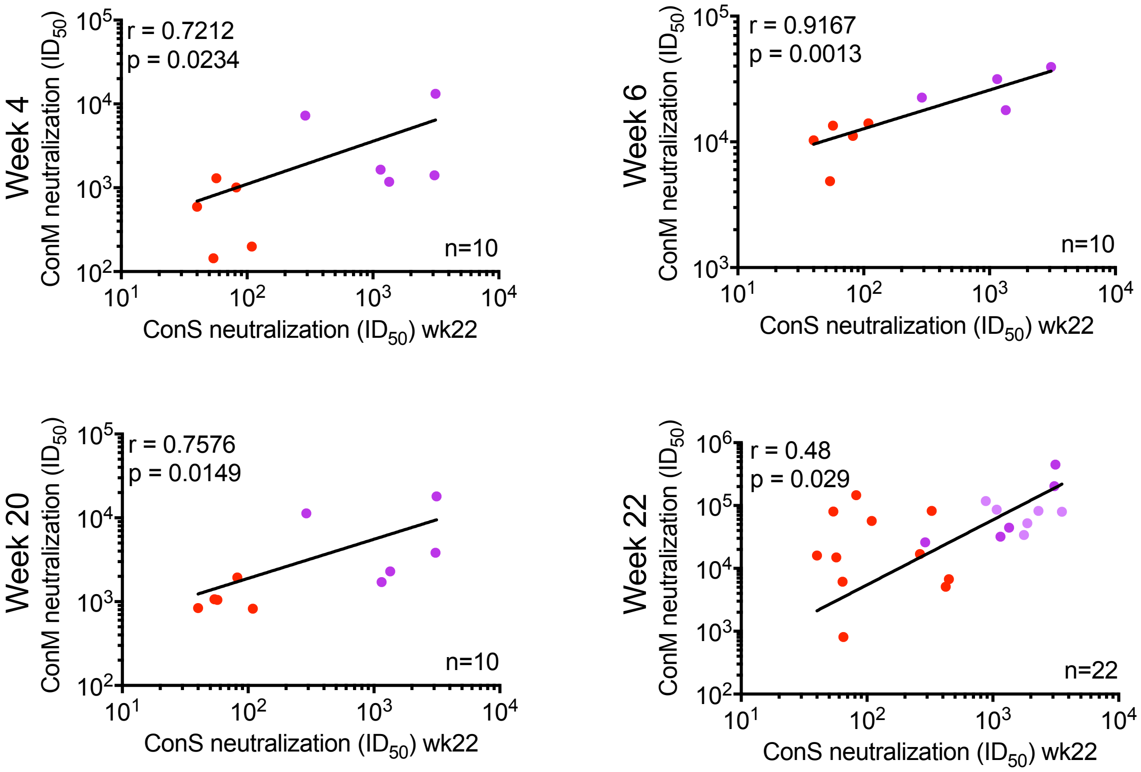
**Supplementary Figure 4.** Structural features of ConM SOSIP.v7. **a.** Characteristics of TD8 residues of ConM SOSIP.v7. All TD8 residues (E47D, N49E, V65K, E106T, I165L, G429R, Q432, and K500R) could be resolved in the crystal structure of ConM SOSIP.v7 in complex with bNAbs PGT124 and 35O22. Gp120 and gp41 are represented by green and blue cartoons, respectively, and TD8 residues are shown as magenta spheres (left panel). Superposition of ConM SOSIP.v7 with BG505 SOSIP.664 (PDB ID: 5CEZ) shows that the TD8 residues in ConM SOSIP.v7 are structurally similar to those in BG505 SOSIP.664 in stabilizing the Env trimers (right panel). The BG505 SOSIP.664 structure is represented in gray cartoon model and the TD8 residues in ConM SOSIP.v7 and BG505 SOSIP.664 are shown in magenta stick models. **b.** Stereo image of Asn295 and linked glycans (NAG624-MAN628). 2m*Fo*-D*Fc* electron density maps are shown at 1.0σ. Asn295 and linked glycans are represented by stick models, and carbon, nitrogen, and oxygen atoms are shown in green, blue, and red, respectively.



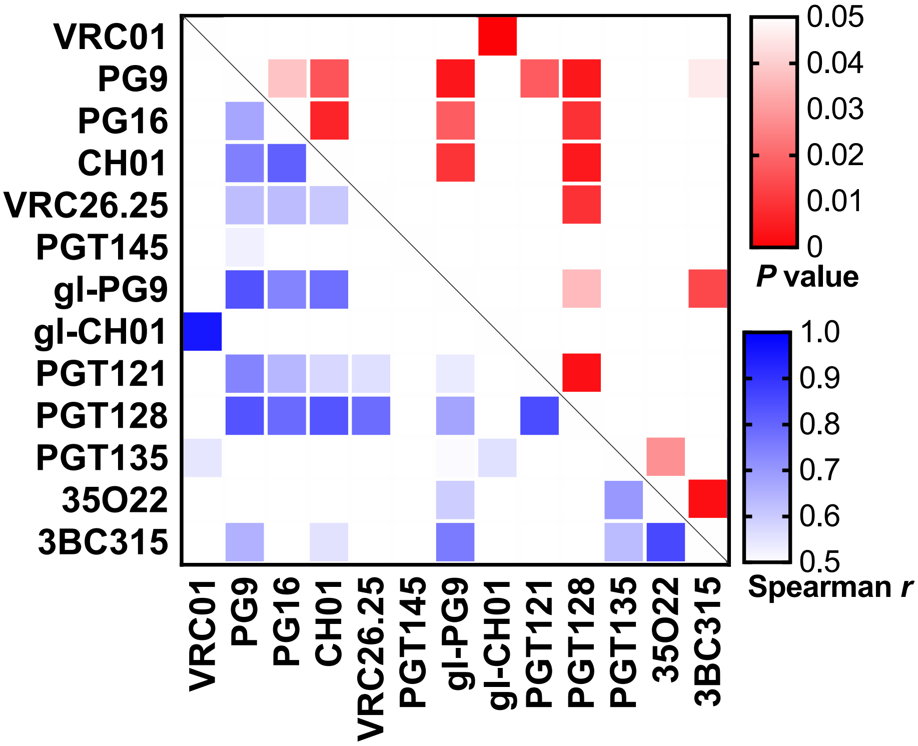
**Supplementary Figure 5** Characterization of ConM SOSIP.v7-ferritin nanoparticles. **a.** Lectin-capture ELISA with PGT145-purified ConM SOSIP.v7 trimers and ConM SOSIP.v7-ferritin nanoparticles expressed in 293F cells. **b.** PGT145-purified SOSIP-ferritin was analyzed by BN-PAGE analysis, reducing (+DTT) and non-reducing (-DTT) SDS-PAGE followed by Coomassie Blue staining. **c.** NS-EM images of PGT145-purified ConM SOSIP.v7-ferritin.



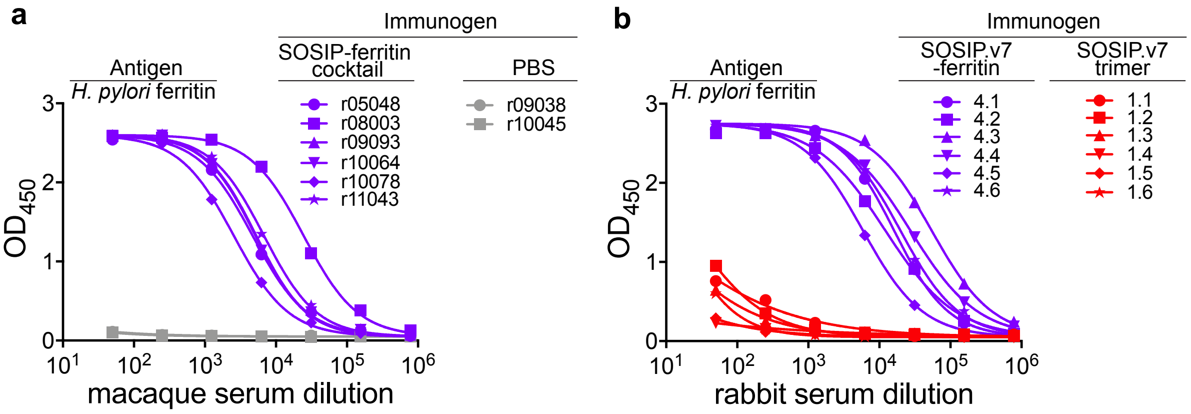
**Supplementary Figure 6.** Thermostability of antibody epitopes on ConM SOSIP.v7 trimers and nanoparticles. BLI binding curves of 2G12, PGT145, F105 and 19b to ConM SOSIP.v7 trimers and ConM SOSIP.v7-ferritin nanoparticles incubated at different temperatures. The results of two independent experiments are shown (left and right panels) and the average AUC values are plotted in Fig. 5f.



**Supplementary Figure 7.** Correlation between ConM and ConS neutralization. Plots show the correlation between the neutralization titers ($ID_{50}$) of the autologous Tier 1A virus at different timepoints with the related Tier 1B ConS virus at week 22. Titers against ConM for weeks 4, 6 and 20 are from Fig. 4C (n=10) and for week 22 from Supplementary Table 4 (n=22). ConM trimer immunized animals are in red and the nanoparticle immunized animals in magenta. Spearman r and p values are given.



**Supplementary Figure 8.** Correlation matrix of bNAb binding interference by sera of ConM immunized rabbits. The competition ELISA values are shown in Fig. 5a. Spearman r values are represented in blue of increasing intensity and the corresponding P values are in red of increasing intensity.



**Supplementary Figure 9.** Immunogenicity of the ferritin component of ConM SOSIP.v7 ferritin nanoparticles. ELISA binding curves against His-tagged ferritin (devoid of Env) using week 22 sera from immunized macaques (**a**) and rabbits (**b**).

|  |  |  | 501C-605C | I559P | R6 | ΔMPER | 64K | 66R | 316W | 535M | 543Q | 543N | 72C-564C | 73C-561C | 49C-555C | 47D[c] | 49E[c] | 65K[c] | 106T[c] | 165L[c] | 429R[c] | 432Q[c] | 500R[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SOSIP.681 | SOSIP.v1 | SOSIP.v1 | 🟩 | 🟩 | 🟩 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 |
| SOSIP.664 | SOSIP.v2 | SOSIP.v2 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 |
|  | SOSIP.v3 | SOSIP.v3.1 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟥 | 🟥 | 🟩 | 🟩 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 |
|  |  | SOSIP.v3.2 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟥 | 🟥 | 🟩 | 🟥 | 🟩 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 |
|  | SOSIP.v4[a] | SOSIP.v4.1 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 |
|  |  | SOSIP.v4.2 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 |
|  | SOSIP.v5[b] | SOSIP.v5.1 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 |
|  |  | SOSIP.v5.2 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟩 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 |
|  | SOSIP.v6[b] | SOSIP.v6 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟩 | 🟩 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 | 🟥 |
|  | SOSIP.v7 | SOSIP.v7 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟥 | 🟩 | 🟥 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |

**Supplementary Table 1.** Nomenclature for the stabilized ConM SOSIP trimers, related to Fig. 1b. The modifications included in each SOSIP version are indicated in green (present) and red (not present).

[a] described in de Taeye et al. 2015[1]

[b] described in Torrents de la Peña et al. 2017[2]

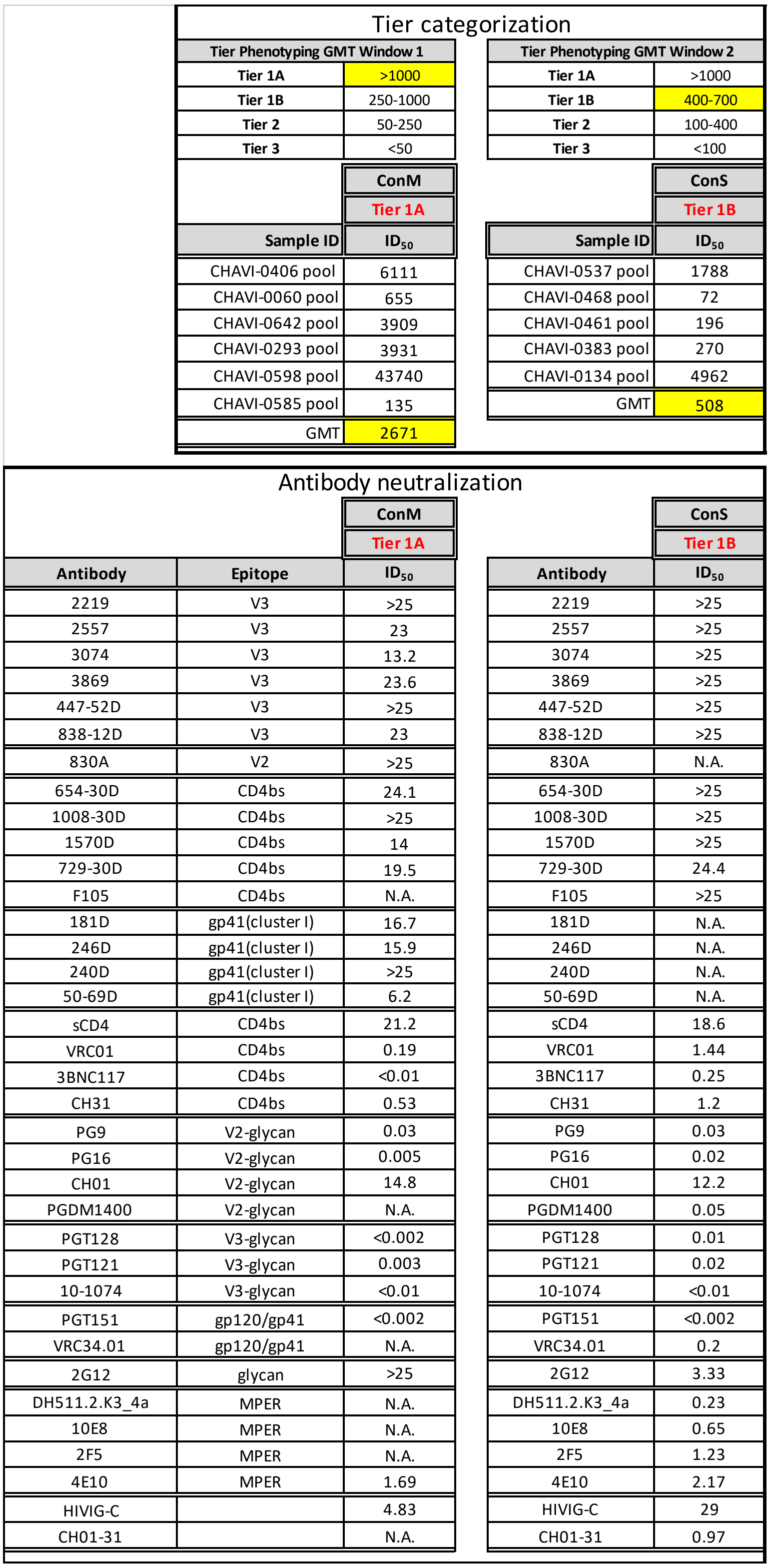
[c] described in Guenaga et al. 2015[3]

|  | ConM SOSIP.v7 |  | Clade A (BG505, PDB 5FYL) |  | Clade B (JR-FL, PDB 5FYK) |  | Clade G (X1193.c1, PDB 5FYJ) |  |
|---|---|---|---|---|---|---|---|---|
| N glycan | Residue | Glycan moieties (residue number in PDB file) | N glycan | Glycan moieties | N glycan | Glycan moieties | N glycan | Glycan moieties |
| N88 | N88 | 6 (601-606) | N88 | 7 | N88 | 7 | N88 | 8 |
| N130 | N130 | 1 (607) |  |  |  |  |  |  |
| N133 | D133 |  | N133 | 2 |  |  | N133 | 1 |
| N135 | N135 |  |  |  | N135 | 1 |  |  |
| N137 | T137 |  | N137 | 7 |  |  |  |  |
| N142 | N142 |  |  |  |  |  | N142 | 1 |
| N156 | N156 |  | N156 | 9 | N156 | 9 | N156 | 11 |
| N160 | N160 | 1 (608) | N160 | 8 | N160 | 7 | N160 | 9 |
| N187 | N187 |  |  |  | N187 | 7 |  |  |
| N188 | N188 |  |  |  |  |  | N188 | 10 |
| N190 |  |  |  |  |  |  |  |  |
| N190C |  |  |  |  |  |  |  |  |
| N197 | N197 | 1 (609) | N197 | 6 |  |  | N197 | 3 |
| N234 | N234 | 4 (610-613) | N234 | 5 |  |  | N234 | 6 |
| N241 | N241 | 2 (614-615) |  |  | N241 | 4 | N241 | 7 |
| N262 | N262 | 7 (616-622) | N262 | 8 | N262 | 9 | N262 | 11 |
| N276 | N276 | 1 (623) | N276 | 5 | N276 | 8 | N276 | 9 |
| N293 | E293 |  |  |  |  |  | N293 | 9 |
| N295 | N295 | 5 (624-628) | N295 | 7 | N295 | 5 |  |  |
| N301 | N301 | 2 (629-630) | N301 | 8 | N301 | 8 | N301 | 6 |
| N332 | N332 | 10 (631-639, 635A) | N332 | 10 | N332 | 10 | N332 | 10 |
| N339 | N339 |  | N339 | 1 | N339 | 8 |  |  |
| N344 | Q344 |  |  |  |  |  | N344 | 7 |
| N355 | N355 |  | N355 | 1 | N355 | 7 | N355 | 3 |
| N362 | N362 |  |  |  | N362 | 4 |  |  |
| N363 | P363 |  | N363 | 3 |  |  |  |  |
| N386 | N386 | 5 (640-644) | N386 | 6 | N386 | 8 | N386 | 8 |
| N392 | N392 | 2 (645-646) | N392 | 6 | N392 | 6 | N392 | 1 |
| N397 | N396 |  |  |  | N397 | 2 |  |  |
| N413 | N411 or N412 |  |  |  |  |  | N413 | 10 |
| N442 | K442 |  |  |  |  |  | N442 | 6 |
| N448 | N448 | 1 (647) | N448 | 6 | N448 | 6 |  |  |
| N462 | N462 |  |  |  |  |  |  |  |
| N463 | N463 |  |  |  | N463 | 2 |  |  |
| N464 | N464 |  |  |  |  |  | N464A | 2 |
| N611 | N611 | 1 (701) | N611 | 1 | N611 | 3 | N611 | 2 |
| N616 | N616 |  |  |  | N616 | 1 | N616 | 2 |
| N618 | S618 |  | N618 | 1 |  |  |  |  |
| N625 | N625 |  |  |  | N625 | 1 | N625 | 3 |
| N637 | N637 | 1 (702) | N637 | 3 | N637 | 1 | N637 | 2 |

**Supplementary Table 2.** Glycans observed in the ConM SOSIP.v7 structure in complex with PGT124 and 35O22. Possible Asn sequons were summarized from BG505 SOSIP.664 (clade A, [4]), BG505 (clade A, PDB: 5FYL), JR-FL (clade B, PDB: 5FYK), and X11.93.c1 (clade G, PDB: 5FYJ) [5].

| Residue No. | ConM gp140 wt | Amino acid conservation rate | BG505 gp140 T332N wt | Amino acid conservation rate | Conservation rate gap between ConM and BG505 | Consensus |
|---|---|---|---|---|---|---|
| 630 | E | 35.23% | Q | 62.40% | 27.17% | Q 62.40%; E 35.23%; D 1.37%; K 0.54%; A 0.15%; X 0.10%; R 0.07%; G 0.05%; L 0.02%; M 0.02%; P 0.02%; S 0.02% |
| 636 | N | 17.49% | S | 40.61% | 23.12% | S 40.61%; D 32.72%; N 17.49%; E 3.88%; G 2.63%; I 0.83%; H 0.51%; A 0.41%; R 0.28%; T 0.23%; X 0.11%; Q 0.10%; F 0.05%; Y 0.05%; K 0.03%; V 0.03%; C 0.02% |
| 152 | E | 22.15% | G | 41.51% | 19.36% | G 41.51%; E 22.15%; D 10.47%; K 3.93%; T 3.70%; N 3.58%; R 2.39%; S 2.23%; Q 2.08%; I 1.96%; A 1.60%; V 1.16%; M 0.79%; P 0.61%; X 0.56%; L 0.52%; Y 0.11%; W 0.08%; H 0.07%; C 0.05%; F 0.02% |
| 646 | I | 40.72% | L | 58.97% | 18.25% | L 58.97%; I 40.72%; X 0.08%; F 0.05%; T 0.03%; A 0.02%; C 0.02%; H 0.02%; P 0.02%; R 0.02%; S 0.02%; V 0.02%; Y 0.02% |
| 412 | N | 4.97% | D | 23.09% | 18.12% | D 23.09%; G 17.24%; S 14.69%; E 13.60%; T 10.47%; N 4.97%; K 4.27%; A 3.58%; R 1.52%; I 1.36%; L 0.92%; V 0.90%; P 0.80%; Q 0.61%; X 0.57%; Y 0.21%; H 0.18%; F 0.11%; M 0.10%; C 0.05%; W 0.02% |
| 170 | K | 30.76% | Q | 46.88% | 16.12% | Q 46.88%; K 30.76%; R 9.55%; H 2.13%; E 2.11%; T 1.44%; L 1.01%; N 0.69%; S 0.46%; M 0.26%; X 0.20%; V 0.16%; I 0.13%; Y 0.13%; A 0.07%; D 0.07%; F 0.03%; P 0.03%; W 0.03%; # 0.02%; G 0.02% |
| 389 | E | 4.52% | G | 18.91% | 14.39% | Q 24.97%; K 24.53%; G 18.91%; N 6.09%; R 5.97%; E 4.52%; P 3.81%; S 3.37%; D 2.63%; A 1.88%; I 0.80%; L 0.70%; T 0.67%; H 0.38%; X 0.29%; V 0.16%; Y 0.15%; C 0.03%; # 0.02%; F 0.02%; M 0.02%; W 0.02% |
| 619 | Q | 21.32% | L | 34.96% | 13.64% | L 34.96%; Y 21.91%; Q 21.32%; F 3.81%; E 3.66%; H 3.19%; K 2.77%; V 2.34%; M 2.21%; I 1.28%; R 1.05%; W 0.33%; X 0.26%; T 0.21%; S 0.18%; D 0.13%; N 0.13%; A 0.08%; G 0.03%; * 0.02%; P 0.02% |
| 133 | D | 36.45% | N | 43.98% | 7.53% | N 43.98%; D 36.45%; E 5.50%; K 3.62%; T 2.59%; S 2.05%; H 1.52%; A 1.16%; Y 0.70%; Q 0.46%; X 0.39%; P 0.34%; G 0.33%; I 0.29%; R 0.16%; M 0.10%; V 0.10%; C 0.05%; L 0.05%; F 0.03%; * 0.02% |
| 410 | T | 16.03% | S | 21.19% | 5.16% | S 21.19%; T 16.03%; N 15.13%; G 11.52%; E 6.77%; D 6.66%; L 3.03%; A 2.86%; C 2.32%; K 2.18%; I 2.01%; P 1.95%; R 1.47%; V 1.37%; Q 0.75%; Y 0.70%; H 0.46%; X 0.38%; F 0.34%; M 0.29%; W 0.10%; # 0.03% |
| 446 | T | 8.88% | V | 11.53% | 2.65% | S 31.81%; K 12.30%; V 11.53%; N 10.52%; T 8.88%; E 7.00%; R 6.22%; I 4.61%; L 3.30%; A 1.19%; D 1.16%; Q 0.28%; H 0.25%; X 0.20%; M 0.18%; P 0.15%; G 0.13%; F 0.08%; Y 0.07%; C 0.05%; # 0.02%; * 0.02% |
| 151 | N | 4.89% | R | 7.53% | 2.64% | K 19.78%; T 15.85%; E 10.44%; G 7.80%; R 7.53%; N 4.89%; D 4.22%; S 3.71%; I 3.57%; Q 3.45%; P 3.34%; V 2.83%; A 2.65%; M 2.23%; Y 1.98%; L 1.01%; X 0.93%; C 0.49%; W 0.49%; F 0.34%; H 0.29%; * 0.05% |
| 442 | K | 5.53% | V | 7.84% | 2.31% | N 29.14%; Q 21.34%; I 7.95%; V 7.84%; L 6.59%; K 5.53%; E 3.93%; S 3.88%; P 3.78%; R 3.53%; T 1.98%; Y 1.00%; A 0.98%; H 0.82%; D 0.67%; M 0.54%; X 0.13%; F 0.11%; W 0.11%; G 0.07%; C 0.03%; # 0.02% |
| 186 | D | 16.34% | E | 16.43% | 0.09% | N 35.00%; E 16.43%; D 16.34%; G 12.42%; K 7.82%; S 6.23%; T 1.29%; R 1.05%; A 0.80%; Q 0.62%; P 0.61%; X 0.31%; H 0.25%; V 0.25%; I 0.23%; L 0.08%; M 0.07%; Y 0.07%; C 0.02%; F 0.02% |
| 397 | G | 11.78% | S | 11.75% | -0.03% | N 12.91%; G 11.78%; S 11.75%; E 4.65%; P 4.63%; D 4.45%; T 4.32%; A 3.71%; F 3.53%; V 3.24%; K 3.22%; W 2.65%; I 2.55%; R 2.42%; L 1.75%; Y 1.18%; H 1.08%; Q 0.75%; X 0.74%; M 0.43%; C 0.08%; * 0.02% |
| 137 | T | 33.56% | N | 31.51% | -2.05% | T 33.56%; N 31.51%; S 7.98%; A 4.22%; K 3.22%; I 3.17%; D 3.08%; V 2.95%; G 2.01%; E 1.16%; R 1.15%; P 0.88%; X 0.75%; L 0.72%; H 0.64%; Y 0.51%; C 0.47%; Q 0.29%; F 0.23%; M 0.11%; W 0.08%; # 0.05% |
| 189 | S | 4.27% | G | 0.95% | -3.32% | N 6.99%; S 4.27%; K 1.47%; T 1.37%; D 1.15%; G 0.95%; E 0.69%; I 0.51%; A 0.34%; P 0.34%; R 0.34%; V 0.28%; Q 0.23%; H 0.18%; Y 0.10%; M 0.05%; L 0.03%; X 0.03% |
| 440 | E | 12.70% | Q | 7.72% | -4.98% | A 27.80%; R 20.21%; S 14.76%; E 12.70%; K 10.00%; Q 7.72%; P 4.40%; N 0.52%; G 0.47%; T 0.44%; D 0.29%; X 0.20%; I 0.13%; M 0.13%; V 0.10%; L 0.07%; H 0.03%; C 0.02% |
| 346 | A | 32.98% | V | 24.71% | -8.27% | A 32.98%; V 24.71%; S 11.01%; K 9.44%; G 7.64%; T 6.00%; R 4.66%; E 1.18%; I 0.80%; Q 0.34%; X 0.25%; M 0.21%; D 0.20%; N 0.16%; Y 0.13%; F 0.11%; L 0.05%; H 0.03%; # 0.02%; W 0.02% |
| 340 | K | 27.67% | E | 18.36% | -9.31% | K 27.67%; N 27.49%; E 18.36%; D 8.20%; T 4.81%; R 4.73%; S 2.85%; Q 1.62%; A 1.44%; G 0.97%; I 0.75%; X 0.34%; H 0.29%; V 0.18%; Y 0.16%; M 0.10%; L 0.05% |
| 644 | S | 13.35% | G | 3.17% | -10.18% | T 22.10%; R 19.52%; N 15.72%; S 13.35%; E 7.04%; K 6.25%; D 6.22%; G 3.17%; Q 3.13%; A 0.92%; W 0.70%; M 0.51%; H 0.44%; X 0.25%; Y 0.25%; I 0.16%; L 0.16%; F 0.03%; C 0.02%; P 0.02%; V 0.02% |
| 185 | D | 37.50% | N | 26.90% | -10.60% | D 37.50%; N 26.90%; E 11.08%; G 9.42%; K 5.48%; S 4.34%; T 1.34%; R 0.70%; A 0.67%; H 0.54%; Q 0.41%; X 0.34%; I 0.29%; V 0.26%; P 0.23%; Y 0.13%; L 0.10%; F 0.08%; M 0.05% |
| 188 | N | 12.32% | Q | 0.98% | -11.34% | N 12.32%; S 4.38%; K 3.35%; D 2.39%; E 1.54%; Q 0.98%; T 0.90%; G 0.88%; R 0.54%; A 0.33%; V 0.28%; H 0.18%; I 0.16%; P 0.16%; X 0.10%; Y 0.10%; L 0.08%; M 0.05%; F 0.02% |
| 135 | N | 32.35% | T | 20.48% | -11.87% | N 32.35%; T 20.48%; K 12.27%; S 5.42%; V 5.04%; I 4.53%; R 4.34%; L 2.96%; G 2.21%; E 2.11%; D 1.65%; Q 1.10%; A 0.98%; P 0.83%; W 0.77%; X 0.69%; Y 0.61%; H 0.41%; C 0.29%; F 0.28%; M 0.23% |
| 640 | D | 19.73% | Q | 7.82% | -11.91% | N 22.84%; G 20.44%; D 19.73%; S 11.39%; Q 7.82%; E 6.92%; K 3.01%; R 1.69%; H 1.59%; Y 1.31%; T 1.13%; A 0.79%; X 0.64%; V 0.21%; L 0.20%; I 0.13%; F 0.10%; M 0.02%; P 0.02% |
| 161 | I | 35.85% | M | 19.22% | -16.63% | I 35.85%; V 22.91%; M 19.22%; T 11.04%; A 9.20%; S 0.67%; X 0.21%; F 0.20%; L 0.15%; Y 0.13%; N 0.07%; R 0.07%; K 0.03%; E 0.02%; G 0.02%; P 0.02% |
| 336 | T | 35.00% | A | 17.78% | -17.22% | T 35.00%; A 17.78%; E 10.57%; K 8.16%; S 7.89%; G 4.84%; D 3.14%; Q 2.39%; R 2.11%; V 1.95%; N 1.64%; I 1.18%; X 0.56%; L 0.46%; P 0.33%; H 0.23%; M 0.18%; Y 0.11%; W 0.05%; # 0.02%; F 0.02% |
| 620 | D | 27.26% | S | 9.83% | -17.43% | D 27.26%; E 23.54%; N 16.48%; S 9.83%; T 8.30%; K 4.52%; G 3.16%; A 2.26%; Q 1.85%; H 1.10%; R 0.49%; X 0.47%; P 0.21%; I 0.20%; V 0.10%; L 0.07%; W 0.05%; Y 0.05%; # 0.02%; C 0.02% |
| 142 | N | 20.75% | D | 2.45% | -18.30% | N 20.75%; T 19.00%; S 11.60%; A 3.62%; G 3.47%; D 2.45%; I 2.24%; V 1.90%; K 1.73%; E 0.98%; Y 0.97%; R 0.87%; X 0.77%; H 0.67%; Q 0.67%; L 0.59%; P 0.51%; C 0.46%; M 0.33%; F 0.29%; W 0.08%; # 0.02%; * 0.02% |
| 141 | T | 21.35% | D | 2.50% | -18.85% | N 22.87%; T 21.35%; S 11.47%; A 4.63%; V 3.52%; G 3.13%; D 2.50%; K 2.32%; I 2.23%; L 1.28%; R 1.18%; Y 1.03%; E 0.93%; Q 0.85%; X 0.64%; H 0.62%; C 0.57%; F 0.51%; P 0.46%; M 0.41%; W 0.23% |
| 139 | N | 23.85% | I | 2.78% | -21.07% | T 29.53%; N 23.85%; S 11.50%; A 5.78%; V 4.34%; G 3.42%; I 2.78%; D 2.57%; K 1.98%; L 1.03%; R 1.03%; C 0.92%; E 0.87%; Y 0.79%; H 0.65%; X 0.62%; F 0.52%; Q 0.38%; P 0.36%; M 0.21%; W 0.07%; # 0.02% |
| 335 | R | 30.76% | K | 9.16% | -21.60% | R 30.76%; G 22.73%; K 19.16%; E 13.71%; A 3.70%; S 3.37%; T 1.49%; I 1.16%; Q 0.88%; N 0.72%; L 0.43%; X 0.43%; V 0.41%; M 0.26%; F 0.16%; Y 0.15%; W 0.10%; D 0.08%; H 0.05% |
| 343 | Q | 29.19% | G | 4.74% | -24.45% | Q 29.19%; K 24.10%; E 13.01%; R 10.88%; G 4.74%; H 3.99%; N 3.57%; T 2.57%; Y 2.19%; S 1.70%; D 0.92%; I 0.75%; A 0.64%; L 0.61%; X 0.34%; V 0.33%; W 0.15%; F 0.13%; M 0.07%; P 0.05%; # 0.02%; * 0.02% |
| 270 | I | 61.14% | V | 36.50% | -24.64% | I 61.14%; V 36.50%; T 1.54%; M 0.20%; A 0.13%; L 0.13%; E 0.10%; X 0.10%; D 0.05%; P 0.05%; F 0.02%; K 0.02%; N 0.02% |
| 363 | P | 34.38% | N | 8.92% | -25.46% | P 34.38%; Q 21.99%; S 17.41%; N 8.92%; H 5.56%; K 4.84%; R 2.75%; A 1.13%; E 0.97%; T 0.92%; G 0.41%; X 0.25%; D 0.15%; L 0.13%; I 0.08%; M 0.02%; V 0.02%; Y 0.02% |
| 344 | Q | 45.21% | K | 17.93% | -27.28% | Q 45.21%; K 17.93%; R 15.89%; E 6.66%; N 3.71%; G 2.63%; L 2.59%; M 1.57%; W 1.24%; H 0.82%; D 0.51%; A 0.28%; S 0.26%; Y 0.23%; X 0.20%; T 0.07%; V 0.07%; I 0.05%; F 0.02%; P 0.02% |
| 396 | N | 34.51% | I | 4.71% | -29.80% | N 34.51%; T 9.28%; L 6.63%; S 6.20%; I 4.71%; Y 4.60%; D 4.52%; M 3.75%; K 3.32%; E 3.06%; P 2.91%; F 2.85%; G 2.80%; H 2.13%; R 1.88%; V 1.64%; W 1.60%; Q 0.93%; A 0.82%; X 0.46%; C 0.07%; * 0.03% |
| 460 | N | 36.50% | S | 6.32% | -30.18% | N 36.50%; T 7.98%; K 7.71%; S 6.32%; G 6.28%; I 5.89%; D 5.69%; E 4.30%; V 3.27%; A 2.47%; Q 2.27%; R 1.96%; H 1.67%; L 1.36%; P 1.24%; Y 0.83%; X 0.49%; M 0.39%; F 0.16%; C 0.08%; * 0.02%; W 0.02% |
| 461 | N | 39.79% | T | 9.51% | -30.28% | N 39.79%; G 10.19%; T 9.51%; D 8.38%; S 8.13%; E 6.18%; K 3.55%; I 1.83%; R 1.37%; H 1.26%; V 1.24%; Q 1.11%; A 1.08%; P 0.87%; X 0.47%; L 0.46%; M 0.33%; Y 0.28%; C 0.05%; F 0.05%; W 0.02% |
| 337 | K | 34.73% | T | 1.46% | -33.27% | K 34.73%; E 16.80%; Q 12.19%; D 9.75%; N 7.95%; A 7.67%; R 2.55%; G 1.49%; T 1.46%; S 1.42%; L 0.98%; H 0.67%; I 0.51%; X 0.43%; V 0.39%; M 0.08%; Y 0.02% |
| 271 | I | 44.39% | M | 8.84% | -35.55% | I 44.39%; V 41.20%; M 8.84%; K 1.80%; R 1.39%; T 0.98%; A 0.67%; Q 0.25%; E 0.16%; L 0.13%; X 0.08%; S 0.05%; D 0.02%; G 0.02% |
| 240 | K | 36.91% | P | 0.15% | -36.76% | K 36.91%; T 22.92%; N 18.26%; R 6.18%; H 5.51%; Q 3.17%; S 2.93%; E 0.95%; Y 0.69%; G 0.56%; L 0.47%; A 0.39%; X 0.34%; D 0.16%; I 0.16%; P 0.15%; M 0.10%; C 0.05%; V 0.03%; F 0.02% |
| 154 | I | 42.21% | L | 4.38% | -37.83% | I 42.21%; M 37.22%; V 14.01%; L 4.38%; R 0.65%; T 0.34%; X 0.31%; A 0.23%; Q 0.15%; E 0.11%; F 0.10%; K 0.10%; G 0.03%; N 0.03%; S 0.03%; W 0.03%; Y 0.03% |
| 507 | E | 45.17% | G | 3.89% | -41.28% | E 45.17%; Q 44.96%; G 3.89%; K 1.93%; D 1.69%; R 0.69%; A 0.52%; T 0.51%; N 0.20%; S 0.11%; H 0.10%; P 0.05%; L 0.03%; V 0.03%; X 0.03%; M 0.02%; W 0.02% |
| 588 | K | 63.11% | R | 21.53% | -41.58% | K 63.11%; R 21.53%; Q 11.08%; G 1.19%; S 0.56%; E 0.49%; H 0.44%; T 0.36%; A 0.26%; N 0.23%; M 0.20%; X 0.20%; V 0.10%; W 0.10%; Y 0.10%; I 0.03%; C 0.02%; L 0.02% |
| 183 | P | 63.45% | Q | 20.81% | -42.64% | P 63.45%; Q 20.81%; S 6.58%; K 3.14%; E 2.08%; A 1.00%; L 0.92%; R 0.54%; H 0.38%; N 0.29%; T 0.21%; G 0.20%; X 0.10%; D 0.07%; V 0.03%; # 0.02%; I 0.02%; Y 0.02% |
| 333 | I | 65.12% | V | 20.98% | -44.14% | I 65.12%; V 20.98%; L 13.53%; M 0.11%; X 0.08%; F 0.05%; T 0.05%; N 0.02%; P 0.02%; S 0.02% |
| 351 | E | 63.60% | K | 16.56% | -47.04% | E 63.60%; K 16.56%; G 3.83%; T 2.81%; D 2.47%; S 2.19%; Q 1.82%; N 1.70%; A 1.51%; R 1.21%; V 0.72%; I 0.36%; X 0.29%; P 0.28%; H 0.25%; L 0.16%; Y 0.07%; F 0.03%; M 0.03%; # 0.02%; C 0.02% |
| 413 | T | 53.08% | S | 2.05% | -51.03% | T 53.08%; N 25.88%; I 7.15%; V 2.55%; P 2.36%; S 2.05%; D 1.67%; K 0.79%; E 0.74%; L 0.59%; H 0.56%; Y 0.54%; X 0.43%; A 0.29%; F 0.26%; M 0.15%; R 0.15%; Q 0.08%; C 0.02%; G 0.02%; W 0.02% |
| 283 | T | 67.67% | N | 12.68% | -54.99% | T 67.67%; I 13.74%; N 12.68%; V 4.25%; S 1.03%; A 0.23%; P 0.15%; X 0.08%; D 0.03%; M 0.03%; # 0.02%; G 0.02%; K 0.02%; L 0.02%; Y 0.02% |
| 629 | M | 71.14% | L | 10.98% | -60.16% | M 71.14%; I 11.16%; L 10.98%; T 2.73%; V 1.51%; R 1.03%; Q 0.57%; K 0.34%; N 0.26%; A 0.15%; X 0.07%; # 0.02%; C 0.02%; W 0.02% |
| 356 | K | 70.40% | N | 2.34% | -68.06% | K 70.40%; T 14.25%; R 5.69%; N 2.34%; S 2.26%; A 1.69%; E 0.85%; G 0.82%; I 0.39%; Q 0.38%; X 0.25%; D 0.20%; P 0.10%; V 0.08%; M 0.07%; H 0.05%; L 0.03%; # 0.02%; F 0.02%; Y 0.02% |
| 130 | N | 69.00% | Q | 0.88% | -68.12% | N 69.00%; E 6.05%; D 5.64%; H 5.58%; K 4.74%; T 2.96%; S 1.85%; R 1.18%; I 0.90%; Q 0.88%; Y 0.38%; X 0.29%; L 0.21%; M 0.11%; # 0.07%; A 0.03%; F 0.03%; V 0.03%; C 0.02%; W 0.02% |
| 92 | N | 75.18% | E | 6.90% | -68.28% | N 75.18%; E 6.90%; D 6.64%; K 4.14%; Y 2.55%; S 1.26%; T 0.88%; P 0.79%; H 0.57%; Q 0.39%; R 0.21%; G 0.13%; X 0.13%; A 0.05%; M 0.05%; V 0.05%; I 0.03%; L 0.02%; W 0.02% |
| 453 | L | 84.00% | I | 14.92% | -69.08% | L 84.00%; I 14.92%; M 0.52%; V 0.23%; F 0.08%; X 0.07%; G 0.05%; P 0.03%; R 0.02%; S 0.02%; T 0.02%; Y 0.02% |
| 348 | K | 81.14% | Q | 11.71% | -69.43% | K 81.14%; Q 11.71%; E 3.98%; R 1.23%; T 0.52%; A 0.36%; L 0.26%; I 0.16%; X 0.15%; N 0.11%; S 0.11%; H 0.08%; V 0.07%; # 0.03%; F 0.02%; G 0.02%; M 0.02% |
| 617 | K | 84.88% | R | 13.40% | -71.48% | K 84.88%; R 13.40%; T 0.52%; S 0.21%; N 0.11%; X 0.11%; I 0.10%; E 0.08%; L 0.08%; G 0.07%; D 0.03%; V 0.03%; Y 0.03%; * 0.02%; H 0.02%; M 0.02%; Q 0.02% |
| 618 | S | 77.08% | N | 4.71% | -72.37% | S 77.08%; T 16.87%; N 4.71%; P 0.34%; D 0.16%; A 0.15%; X 0.11%; Y 0.11%; K 0.10%; E 0.07%; G 0.05%; L 0.05%; # 0.02%; F 0.02%; Q 0.02%; R 0.02%; V 0.02% |
| 371 | I | 86.34% | V | 11.68% | -74.66% | I 86.34%; V 11.68%; L 0.93%; T 0.64%; M 0.11%; X 0.10%; S 0.05%; N 0.03%; # 0.02%; D 0.02%; F 0.02%; Q 0.02%; R 0.02% |
| 567 | Q | 85.24% | K | 10.50% | -74.74% | Q 85.24%; K 10.50%; R 4.06%; X 0.07%; E 0.05%; L 0.05%; N 0.02%; P 0.02% |
| 174 | A | 88.45% | S | 10.75% | -77.70% | A 88.45%; S 10.75%; V 0.28%; T 0.20%; G 0.18%; D 0.03%; E 0.03%; K 0.03%; X 0.03%; P 0.02% |
| 288 | L | 92.52% | F | 7.28% | -85.24% | L 92.52%; F 7.28%; M 0.07%; X 0.03%; # 0.02%; P 0.02%; S 0.02%; V 0.02%; W 0.02% |
| 430 | V | 90.92% | I | 1.11% | -89.81% | V 90.92%; A 4.55%; T 2.83%; I 1.11%; L 0.20%; G 0.11%; P 0.11%; E 0.08%; * 0.03%; S 0.02% |
| 285 | I | 97.32% | L | 2.09% | -95.23% | I 97.32%; L 2.09%; M 0.33%; T 0.15%; V 0.07%; A 0.02%; X 0.02% |
| 241 | N | 97.19% | S | 0.39% | -96.80% | N 97.19%; D 0.98%; K 0.92%; S 0.39%; E 0.15%; H 0.07%; T 0.07%; X 0.05%; Y 0.05%; I 0.03%; Q 0.03%; # 0.02%; C 0.02%; G 0.02%; M 0.02%; R 0.02% |

**Supplementary Table 3.** Amino acid conservation analysis of ConM and BG505 sequences used for generating Fig. 2c



Tier categorization

| Tier Phenotyping GMT Window 1 | | Tier Phenotyping GMT Window 2 | |
|---|---|---|---|
| **Tier 1A** | >1000 | **Tier 1A** | >1000 |
| **Tier 1B** | 250-1000 | **Tier 1B** | 400-700 |
| **Tier 2** | 50-250 | **Tier 2** | 100-400 |
| **Tier 3** | <50 | **Tier 3** | <100 |

| | **ConM** | | **ConS** |
|---|---|---|---|
| | **Tier 1A** | | **Tier 1B** |
| **Sample ID** | **ID$_{50}$** | **Sample ID** | **ID$_{50}$** |
| CHAVI-0406 pool | 6111 | CHAVI-0537 pool | 1788 |
| CHAVI-0060 pool | 655 | CHAVI-0468 pool | 72 |
| CHAVI-0642 pool | 3909 | CHAVI-0461 pool | 196 |
| CHAVI-0293 pool | 3931 | CHAVI-0383 pool | 270 |
| CHAVI-0598 pool | 43740 | CHAVI-0134 pool | 4962 |
| CHAVI-0585 pool | 135 | GMT | 508 |
| GMT | 2671 | | |

Antibody neutralization

| | | **ConM** | | **ConS** |
|---|---|---|---|---|
| | | **Tier 1A** | | **Tier 1B** |
| **Antibody** | **Epitope** | **ID$_{50}$** | **Antibody** | **ID$_{50}$** |
| 2219 | V3 | >25 | 2219 | >25 |
| 2557 | V3 | 23 | 2557 | >25 |
| 3074 | V3 | 13.2 | 3074 | >25 |
| 3869 | V3 | 23.6 | 3869 | >25 |
| 447-52D | V3 | >25 | 447-52D | >25 |
| 838-12D | V3 | 23 | 838-12D | >25 |
| 830A | V2 | >25 | 830A | N.A. |
| 654-30D | CD4bs | 24.1 | 654-30D | >25 |
| 1008-30D | CD4bs | >25 | 1008-30D | >25 |
| 1570D | CD4bs | 14 | 1570D | >25 |
| 729-30D | CD4bs | 19.5 | 729-30D | 24.4 |
| F105 | CD4bs | N.A. | F105 | >25 |
| 181D | gp41(cluster I) | 16.7 | 181D | N.A. |
| 246D | gp41(cluster I) | 15.9 | 246D | N.A. |
| 240D | gp41(cluster I) | >25 | 240D | N.A. |
| 50-69D | gp41(cluster I) | 6.2 | 50-69D | N.A. |
| sCD4 | CD4bs | 21.2 | sCD4 | 18.6 |
| VRC01 | CD4bs | 0.19 | VRC01 | 1.44 |
| 3BNC117 | CD4bs | <0.01 | 3BNC117 | 0.25 |
| CH31 | CD4bs | 0.53 | CH31 | 1.2 |
| PG9 | V2-glycan | 0.03 | PG9 | 0.03 |
| PG16 | V2-glycan | 0.005 | PG16 | 0.02 |
| CH01 | V2-glycan | 14.8 | CH01 | 12.2 |
| PGDM1400 | V2-glycan | N.A. | PGDM1400 | 0.05 |
| PGT128 | V3-glycan | <0.002 | PGT128 | 0.01 |
| PGT121 | V3-glycan | 0.003 | PGT121 | 0.02 |
| 10-1074 | V3-glycan | <0.01 | 10-1074 | <0.01 |
| PGT151 | gp120/gp41 | <0.002 | PGT151 | <0.002 |
| VRC34.01 | gp120/gp41 | N.A. | VRC34.01 | 0.2 |
| 2G12 | glycan | >25 | 2G12 | 3.33 |
| DH511.2.K3_4a | MPER | N.A. | DH511.2.K3_4a | 0.23 |
| 10E8 | MPER | N.A. | 10E8 | 0.65 |
| 2F5 | MPER | N.A. | 2F5 | 1.23 |
| 4E10 | MPER | 1.69 | 4E10 | 2.17 |
| HIVIG-C | | 4.83 | HIVIG-C | 29 |
| CH01-31 | | N.A. | CH01-31 | 0.97 |

**Supplementary Table 4.** Characterization of the ConM and ConS viruses. Top: tier categorization of the ConM and ConS viruses using a panel of reference sera pool. Bottom: analysis of the sensitivity of ConM and ConS to a panel of bNAbs and non-NAbs. The TZM-bl cell assays were performed at DUMC. GMT: geometric mean titer. N.A: not tested.

| | | Control | | Autologous | | | | Heterologous Tier 1 | | | | | | | | Heterologous Tier 2 | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Week 22 | Virus | MLV | MLV | ConM | ConM | ConM | ConM | SF162 | SF162 | MW965.26 | AMC008 | ZM197M | ConS | ConS | ConS | BG505 | 246-F3_C10_2 [a] | CNE55 [a] | REJO | SHIVp3 | SHIVp3 | TRO.11 | WITO | BJOX002000.03.2 [a] | CH119.10 [a] | 25710-2.43 | Ce1176_A3 | Ce703010217_B6 [a] | TV1.21 | TV1.21 | TV1.29 | X1632-S2-B10 [a] |
| | Tier | | | 1A | 1A | 1A | 1A | 1A | 1A | 1A | 1B | 1B | 1B | 1B | 1B | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | Clade | | | | | | | B | B | C | B | C | | | | A | AC | CRF01_AE | B | B | B | B | B | CRF07_BC | CRF07_BC | C | C | C | C | C | C | G |
| Immunogen | Lab | AMC | DUMC | AMC | DUMC | OSR | ISCIII | AMC | ISCIII | DUMC | AMC | AMC | AMC | DUMC | OSR | AMC | DUMC | DUMC | AMC | AMC | OSR | DUMC | AMC | DUMC | DUMC | DUMC | DUMC | DUMC | AMC | DUMC | OSR | DUMC |
| ConM SOSIP.v7 (ISCOMATRIX™) | 1839 | <20 | <20 | 56944 | 46649 | 58493 | 49939 | 29 | <20 | 449 | <20 | <20 | 231 | 109 | 175 | <20 | <20 | <20 | <20 | <20 | | <20 | <20 | <20 | <20 | <20 | <20 | <20 | | <20 | | <20 |
| | 1840 | <20 | <20 | 146656 | 143538 | 72497 | 88150 | 26 | <20 | 218 | <20 | <20 | 308 | 82 | 127 | <20 | <20 | <20 | 34 | 37 | | <20 | <20 | <20 | <20 | <20 | <20 | <20 | | <20 | | <20 |
| | 1841 | <20 | <20 | 80585 | 64681 | 48667 | 91919 | <20 | <20 | 116 | <20 | <20 | 123 | 54 | 44 | <20 | <20 | <20 | 20 | <20 | | <20 | <20 | <20 | <20 | <20 | <20 | <20 | | <20 | | <20 |
| | 1842 | <20 | <20 | 16010 | 16106 | 10482 | 24314 | 24 | 62 | 114 | <20 | <20 | 71 | 40 | 216 | <20 | <20 | <20 | 40 | <20 | | <20 | <20 | <20 | <20 | <20 | <20 | <20 | | <20 | | <20 |
| | 1843 | <20 | <20 | 15008 | 26484 | 13017 | 43449 | 27 | 26 | 76 | <20 | <20 | 126 | 57 | 43 | <20 | <20 | <20 | <20 | <20 | | <20 | <20 | <20 | <20 | <20 | <20 | <20 | | <20 | | <20 |
| ConM SOSIP.v7-ferritin (ISCOMATRIX™) | 1809 | <20 | <20 | 31880 | 36017 | 28178 | 21227 | 52 | 27 | 21599 | <20 | <20 | 2163 | 1149 | 797 | <20 | <20 | <20 | <20 | <20 | | <20 | <20 | <20 | <20 | <20 | <20 | <20 | | 207 | | <20 |
| | 1810 | <20 | <20 | 25953 | 56137 | 16014 | 8007 | 61 | 28 | 3732 | <20 | <20 | 391 | 290 | 696 | <20 | 34 | <20 | <20 | <20 | | <20 | <20 | 26 | 23 | <20 | <20 | <20 | | 107 | | <20 |
| | 1811 | <20 | <20 | 44432 | 70273 | 26597 | 10609 | 583 | 72 | 9982 | <20 | <20 | 3321 | 1344 | 1485 | <20 | <20 | <20 | 26 | 62 | | <20 | <20 | <20 | <20 | <20 | <20 | <20 | | 158 | | <20 |
| | 1812 | <20 | <20 | 203036 | 164177 | 152493 | 43901 | 1437 | 405 | 3486 | <20 | <20 | 6199 | 3090 | 2639 | <20 | <20 | <20 | <20 | <20 | | <20 | <20 | <20 | <20 | <20 | <20 | <20 | | 65 | | <20 |
| | 1813 | <20 | <20 | 449906 | 354473 | 245476 | 125261 | 274 | 58 | 29493 | <20 | <20 | 3993 | 3141 | 1399 | <20 | 78 | <20 | <20 | <20 | | <20 | <20 | 73 | <20 | <20 | <20 | <20 | | 410 | | <20 |
| ConM SOSIP.v7 (SE) | 1.1 | <20 | | 5110 | | 2107 | | <20 | | | | | 423 | | 116 | | | | | | <20 | | | | | | | | 23 | | <20 | |
| | 1.2 | <20 | | 6757 | | 3012 | | <20 | | | | | 448 | | 83 | | | | | | <20 | | | | | | | | 33 | | <20 | |
| | 1.3 | <20 | | 16917 | | 3893 | | <20 | | | | | 264 | | 33 | | | | | | <20 | | | | | | | | <20 | | <20 | |
| | 1.4 | <20 | | 82485 | | 34782 | | <20 | | | | | 327 | | 64 | | | | | | <20 | | | | | | | | 24 | | <20 | |
| | 1.5 | <20 | | 809 | | 204 | | <20 | | | | | 65 | | 20 | | | | | | <20 | | | | | | | | 24 | | <20 | |
| | 1.6 | <20 | | 6162 | | 6944 | | <20 | | | | | 64 | | 166 | | | | | | <20 | | | | | | | | 27 | | <20 | |
| ConM SOSIP.v7-ferritin (SE) | 4.1 | 23 | | 79605 | | 52368 | | 488 | | | | | 3548 | | 757 | | | | | | <20 | | | | | | | | 127 | | <20 | |
| | 4.2 | <20 | | 52461 | | 16086 | | 192 | | | | | 1877 | | 496 | | | | | | <20 | | | | | | | | <20 | | <20 | |
| | 4.3 | <20 | | 34131 | | 8033 | | 691 | | | | | 1771 | | 1211 | | | | | | <20 | | | | | | | | 422 | | <20 | |
| | 4.4 | <20 | | 86348 | | 16255 | | 8650 | | | | | 1072 | | 126 | | | | | | <20 | | | | | | | | 169 | | <20 | |
| | 4.5 | <20 | | 117823 | | 23534 | | 178 | | | | | 880 | | 382 | | | | | | <20 | | | | | | | | 60 | | <20 | |
| | 4.6 | <20 | | 82326 | | 25108 | | 1231 | | | | | 2306 | | 782 | | | | | | <20 | | | | | | | | 123 | | <20 | |
| | Rabbit ID | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**Supplementary Table 5.** Midpoint neutralization titers for sera from rabbits immunized with ConM SOSIP.v7 or ConM SOSIP.v7-ferritin. Sera (from week 22, unless otherwise noted) were tested against a panel of Env-pseudotyped viruses in a standard TZM-bl assay. Upper part: neutralization titers for sera from rabbits immunized with ConM SOSIP.v7 or ConM SOSIP.v7-ferritin receiving ISCOMATRIX™ adjuvant. Immunizations were carried out by Covance (Denver, USA). The neutralization assays were performed at AMC, DUMC, OSR and ISCIII.
Lower part: neutralization titers for week 22 sera from rabbits immunized with ConM SOSIP.v7 or ConM SOSIP.v7-ferritin formulated with Squalene Emulsion (SE) adjuvant. Immunizations were carried out by NFCSO-DVMP (Gödölló, Hungary). The TZM-bl cell assays were performed at AMC and OSR.
The boxes are colored according to their magnitude. ID50 <20 in white; ID50 20-40 in grey; ID50 40-100 in yellow; ID50 100-1000 in orange; ID50 1000-10000 in red; ID50 10000-100000 in pink; ID50 >100000 in purple; [a]week 38 sera dilutions.

| | | | Autologous | | |
|---|---|---|---|---|---|
| Week 22 | Virus | MLV | ConM | ConM | ConM |
| | Tier | | 1A | 1A | 1A |
| Immunogen | Dump-in Protein | | | V3 Peptide | ConM D368R |
| ConM SOSIP.v7 | 1839 | <20 | >4860 | >4860 | 46 |
| | 1840 | 31 | >4860 | >4860 | 53 |
| | 1841 | <20 | >4860 | >4860 | 55 |
| | 1842 | <20 | >4860 | >4860 | 26 |
| | 1843 | <20 | >4860 | >4860 | <20 |
| ConM SOSIP.v7-ferritin | 1809 | <20 | >4860 | >4860 | 49 |
| | 1810 | <20 | >4860 | >4860 | 45 |
| | 1811 | <20 | >4860 | >4860 | 45 |
| | 1812 | <20 | >4860 | >4860 | 38 |
| | 1813 | <20 | >4860 | >4860 | 135 |
| | Rabbit ID | | | | |

**Supplementary Table 6.** Neutralization depletion experiment with ConM virus. Midpoint neutralization titers for sera from rabbits against ConM virus with or without cyclic ConM V3 peptide (which is identical to that of BG505) and ConM SOSIP.v7 D368R trimer as competitors.

| Week 22 | Virus | ConS$^{a}$ | | | | | | | | | | | TV1.21 | | | | | TV1.21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mutant | | Y173A | T236K | N295A | N301A | G324A | N332A | V518L | T607S | T607K | W680R K683Q | | N160A | N332A | K169E | N276A | |
| | | | V1V2 glycan | gp120-gp41 | 2G12 sens | V3 glycan | V3 glycan | V3 glycan | fusion peptide | gp120-gp41 | gp120-gp41 | MPER | | V1V2 glycan | V3 glycan | V1V2 glycan | CD4BS | |
| Immunogen | Clade | | | | | | | | | | | | C | C | C | C | C | C |
| ConM SOSIP.v7 | 1839 | 100 | 25 | 120 | 110 | <20 | <20 | 118 | 161 | 234 | 385 | 81 | | | | | | <20 |
| | 1840 | 79 | <20 | 85 | 81 | <20 | <20 | 109 | 88 | 133 | 146 | 67 | | | | | | <20 |
| | 1841 | 52 | <20 | 61 | 43 | <20 | <20 | 72 | 51 | 62 | 52 | 38 | | | | | | <20 |
| | 1842 | 57 | <20 | 69 | 56 | <20 | <20 | 55 | 73 | 79 | 109 | 42 | | | | | | <20 |
| | 1843 | 57 | 28 | 65 | 62 | <20 | <20 | 82 | 64 | 79 | 83 | 46 | | | | | | <20 |
| ConM SOSIP.v7-ferritin | 1809 | 519 | 87 | 489 | 422 | 266 | 26 | 697 | 461 | 347 | 553 | 403 | 121 | 312 | 782 | <20 | 146 | 207 |
| | 1810 | 209 | 108 | 209 | 189 | 97 | 42 | 238 | 201 | 172 | 250 | 176 | 81 | 148 | 317 | <20 | 83 | 107 |
| | 1811 | 624 | 306 | 566 | 390 | 244 | 54 | 878 | 519 | 501 | 678 | 466 | 142 | 332 | 973 | <20 | 179 | 158 |
| | 1812 | 1333 | 169 | 1284 | 1012 | 268 | 44 | 1990 | 989 | 1265 | 1675 | 1065 | 48 | 102 | 222 | <20 | 80 | 65 |
| | 1813 | 1689 | 922 | 1989 | 2102 | 1336 | 106 | 3148 | 1230 | 1566 | 1652 | 1616 | 290 | 588 | 1210 | <20 | 333 | 410 |
| ConM SOSIP.v7 (fold change) | 1839 | | 4.0 | 0.8 | 0.9 | 5.0 | 5.0 | 0.8 | 0.6 | 0.4 | 0.3 | 1.2 | | | | | | |
| | 1840 | | 4.0 | 0.9 | 1.0 | 4.0 | 4.0 | 0.7 | 0.9 | 0.6 | 0.5 | 1.2 | | | | | | |
| | 1841 | | 2.6 | 0.9 | 1.2 | 2.6 | 2.6 | 0.7 | 1.0 | 0.8 | 1.0 | 1.4 | | | | | | |
| | 1842 | | 2.9 | 0.8 | 1.0 | 2.9 | 2.9 | 1.0 | 0.8 | 0.7 | 0.5 | 1.4 | | | | | | |
| | 1843 | | 2.0 | 0.9 | 0.9 | 2.9 | 2.9 | 0.7 | 0.9 | 0.7 | 0.7 | 1.2 | | | | | | |
| ConM SOSIP.v7-ferritin (fold change) | 1809 | | 6.0 | 1.1 | 1.2 | 2.0 | 20.0 | 0.7 | 1.1 | 1.5 | 0.9 | 1.3 | | 0.4 | 0.2 | 6.1 | 0.8 | |
| | 1810 | | 1.9 | 1.0 | 1.1 | 2.2 | 5.0 | 0.9 | 1.0 | 1.2 | 0.8 | 1.2 | | 0.5 | 0.3 | 4.1 | 1.0 | |
| | 1811 | | 2.0 | 1.1 | 1.6 | 2.6 | 11.6 | 0.7 | 1.2 | 1.2 | 0.9 | 1.3 | | 0.4 | 0.1 | 7.1 | 0.8 | |
| | 1812 | | 7.9 | 1.0 | 1.3 | 5.0 | 30.3 | 0.7 | 1.3 | 1.1 | 0.8 | 1.3 | | 0.5 | 0.2 | 2.4 | 0.6 | |
| | 1813 | | 1.8 | 0.8 | 0.8 | 1.3 | 15.9 | 0.5 | 1.4 | 1.1 | 1.0 | 1.0 | | 0.5 | 0.2 | 14.5 | 0.9 | |
| | Rabbit ID | | | | | | | | | | | | | | | | | |

| Week 22 | Virus | MW965.26 | | | SF162.LS | | | TV1.21 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mutant | | | | | | | N160A | | | N332A | | | |
| | | - | scramble | ConM V3 | - | scramble | ConM V3 | - | scramble | ConM V3 | - | scramble | ConM V3 | depletion peptide |
| Immunogen | Clade | C | C | C | B | B | B | C | C | C | C | C | C | |
| ConM SOSIP.v7 | 1839 | 861 | 849 | 359 | 194 | 210 | 138 | <20 | <20 | <20 | <20 | <20 | <20 | |
| | 1840 | 883 | 1051 | 441 | 450 | 428 | 327 | 162 | 153 | 115 | 287 | 171 | 170 | |
| | 1841 | 372 | 472 | 170 | 50 | 62 | 42 | 102 | 107 | 79 | 151 | 143 | 112 | |
| | 1842 | 330 | 304 | 212 | 180 | 172 | 129 | 87 | 75 | 93 | 114 | 123 | 103 | |
| | 1843 | 276 | 326 | 200 | 236 | 206 | 114 | 96 | 79 | 91 | 123 | 121 | 122 | |
| ConM SOSIP.v7-ferritin | 1809 | 18574 | 13341 | 3326 | 801 | 601 | 181 | 458 | 422 | 187 | 1246 | 715 | 281 | |
| | 1810 | 3973 | 2671 | 813 | 256 | 209 | 201 | 214 | 175 | 138 | 412 | 341 | 186 | |
| | 1811 | 11760 | 6534 | 1647 | 1889 | 1062 | 509 | 394 | 304 | 194 | 891 | 902 | 286 | |
| | 1812 | 3850 | 2921 | 1452 | 2367 | 3312 | 730 | 211 | 154 | 129 | 326 | 325 | 193 | |
| | 1813 | 23015 | 22572 | 3181 | 625 | 462 | 266 | 1109 | 719 | 338 | 2931 | 1866 | 635 | |
| ConM SOSIP.v7 (fold change) | 1839 | | 1.0 | 2.4 | | 0.9 | 1.4 | | | | | | | |
| | 1840 | | 0.8 | 2.0 | | 1.1 | 1.4 | | 1.1 | 1.4 | | 1.7 | 1.7 | |
| | 1841 | | 0.8 | 2.2 | | 0.8 | 1.2 | | 1.0 | 1.3 | | 1.1 | 1.3 | |
| | 1842 | | 1.1 | 1.6 | | 1.0 | 1.4 | | 1.2 | 0.9 | | 0.9 | 1.1 | |
| | 1843 | | 0.8 | 1.4 | | 1.1 | 2.1 | | 1.2 | 1.1 | | 1.0 | 1.0 | |
| ConM SOSIP.v7-ferritin (fold change) | 1809 | | 1.4 | 5.6 | | 1.3 | 4.4 | | 1.1 | 2.4 | | 1.7 | 4.4 | |
| | 1810 | | 1.5 | 4.9 | | 1.2 | 1.3 | | 1.2 | 1.6 | | 1.2 | 2.2 | |
| | 1811 | | 1.8 | 7.1 | | 1.8 | 3.7 | | 1.3 | 2.0 | | 1.0 | 3.1 | |
| | 1812 | | 1.3 | 2.7 | | 0.7 | 3.2 | | 1.4 | 1.6 | | 1.0 | 1.7 | |
| | 1813 | | 1.0 | 7.2 | | 1.4 | 2.3 | | 1.5 | 3.3 | | 1.6 | 4.6 | |
| | Rabbit ID | | | | | | | | | | | | | |

**Supplementary Table 7.** Epitope mapping of sera from ConM SOSIP.v7 immunized rabbits for ConS, TV1.21, MW965.26 and SF162.LS viruses. Left: midpoint neutralization titers for the sera against a panel of ConS and TV1.21 mutant viruses. Right: midpoint titers of MW965.26, SF162.LS and two TV1.21 mutants (N160A and N332A) after depleting the sera using scrambled or ConM V3 peptide. The TZM-bl cell assay was performed at DUMC. The lower part depicts the fold changes in ID50 titers compared to WT virus or non-depleted serum (ID50 virus/ID50 mutant virus). Fold change <0.25 in grey; 0.25-2.5 in white; 2.5-10 in light blue; >10 in dark blue. $^{a}$week 38 sera dilutions.

|  |  | Control |  | Autologous |  |  |  | Heterologous Tier 1 |  |  |  | Heterologous Tier 2 |  |  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Week 22 | Virus | MLV | MLV | ConM | ConM | AMC008 | AMC011 | SF162 | MW965.26 | ConS | ConS | 398_F1_F6_20 | 246-F3_C10_2 | CNE55 | REJO | SHIVp3 | TRO.11 | X2278-C2-B6 | BJOX002000.03.2 | CH119.10 | 25710-2.43 | Ce1176_A3 | Ce703010217_B6 | TV1.21 | X1632-S2-B10 |
|  | Tier |  |  | 1A | 1A | 1B | 2 | 1A | 1A | 1B | 1B | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
|  | Clade |  |  |  |  | B | B | B | C |  |  | A | AC | CRF01_AE | B | B | B | B | CRF07_BC | CRF07_BC | C | C | C | C | G |
| Immunogen | Lab | AMC | DUMC | AMC | DUMC | AMC | AMC | AMC | DUMC | AMC | DUMC | DUMC | DUMC | DUMC | AMC | AMC | DUMC | DUMC | DUMC | DUMC | DUMC | DUMC | DUMC | DUMC | DUMC |
| SOSIP-ferritin cocktail | r05048 | <20 | 23 | 1416 | 890 | <20 | <20 | 152 | 403 | <20 | 117 | 26 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 |
|  | r10078 | <20 | <20 | 1750 | 801 | <20 | <20 | 286 | 490 | <20 | 100 | 22 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 |
|  | r11043 | <20 | <20 | >4860 | 2417 | <20 | <20 | 720 | 955 | 58 | 129 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 |
|  | r08003 | <20 | <20 | >4860 | 5227 | <20 | <20 | 3124 | 3369 | 140 | 210 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 |
|  | r09093 | <20 | <20 | >4860 | 1377 | <20 | <20 | 1248 | 801 | 103 | 148 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 |
|  | r10064 | <20 | <20 | 1923 | 2383 | <20 | <20 | 955 | 677 | 23 | 74 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 |
| negative controls | r09038 | <20 | 23 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 |  |  |  | <20 | <20 |  |  |  |  |  |  |  |  |  |
|  | r10045 | <20 | 27 | <20 | <20 | <20 | <20 | <20 | <20 | <20 | <20 |  |  |  | <20 | <20 |  |  |  |  |  |  |  |  |  |
|  | Macaque ID |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

**Supplementary Table 8.** Neutralization by sera from macaques immunized with a trivalent cocktail of SOSIP-ferritin nanoparticles. Midpoint neutralization titers were determined for a panel of Env-pseudotyped viruses in the TZM-bl cell assay (performed at AMC and DUMC).

| Week 22 plasma | Virus | MLV | ConS | | | | | | | | | | TV1.21 | | | | | MW965.26 | | | SF162.LS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mutant | | | Y173A | T236K | N295A | N301A | G324A | N332A | V518L | T607S | W680R K683Q | | N160A | N332A | K169E | N276A | | | | | | | |
| | | | | V1V2 glycan | gp120-gp41 | 2G12 sens | V3 glycan | V3 glycan | V3 glycan | fusion peptide | gp120-gp41 | MPER | | V1V2 glycan | V3 glycan | V1V2 glycan | CD4BS | - | scramble | ConM V3 | - | scramble | ConM V3 | depletion peptide |
| Immunogen | Clade | - | | | | | | | | | | | C | C | C | C | C | C | C | C | B | B | B | |
| SOSIP-ferritin cocktail | r05048 | 70 | 458 | 297 | 294 | 203 | 172 | 468 | 127 | 158 | 143 | 343 | 48 | 56 | 79 | <30 | 73 | 629 | 476 | 345 | 543 | 518 | 223 | |
| | r10078 | 57 | 459 | 227 | 249 | 478 | 363 | 417 | 285 | 442 | 140 | 529 | 60 | 47 | 78 | <30 | 68 | 851 | 585 | 500 | 868 | 1059 | 367 | |
| | r11043 | 43 | 324 | 193 | 281 | 233 | 195 | 241 | 241 | 275 | 131 | 301 | 30 | 55 | 125 | <30 | 74 | 1763 | 1074 | 397 | 1159 | 1116 | 574 | |
| | r08003 | 65 | 503 | 307 | 421 | 489 | 460 | 568 | 277 | 490 | 275 | 675 | 148 | 148 | 748 | 34 | 208 | 3915 | 4219 | 1541 | 5018 | 4548 | 2601 | |
| | r09093 | 45 | 287 | 240 | 204 | 234 | 168 | 325 | 132 | 206 | 150 | 295 | 61 | 58 | 110 | <30 | 74 | 968 | 1064 | 569 | 1317 | 1184 | 657 | |
| | r10064 | 39 | 266 | 175 | 179 | 193 | 195 | 295 | 96 | 202 | 96 | 385 | 67 | 56 | 88 | <30 | 75 | 849 | 752 | 461 | 1045 | 1116 | 430 | |
| negative controls | r09038 | 38 | 94 | 78 | 112 | 45 | 45 | 140 | 49 | 72 | 32 | 87 | <20 | <30 | 36 | <30 | 40 | 53 | 58 | 48 | 52 | 40 | 34 | |
| | r10045 | 46 | 146 | 78 | 157 | 35 | 78 | 177 | 30 | 106 | 34 | 137 | <20 | <30 | 36 | <30 | 40 | 64 | 68 | 54 | 69 | 59 | 110 | |
| SOSIP-ferritin cocktail (fold change) | r05048 | | | 1.5 | 1.6 | 2.3 | 2.7 | 1.0 | 3.6 | 2.9 | 3.2 | 1.3 | | | | | | | 1.3 | 1.8 | | 1.0 | 2.4 | |
| | r10078 | | | 2.0 | 1.8 | 1.0 | 1.3 | 1.1 | 1.6 | 1.0 | 3.3 | 0.9 | | | | | | | 1.5 | 1.7 | | 0.8 | 2.4 | |
| | r11043 | | | 1.7 | 1.2 | 1.4 | 1.7 | 1.3 | 1.3 | 1.2 | 2.5 | 1.1 | | | | | | | 1.6 | 4.4 | | 1.0 | 2.0 | |
| | r08003 | | | 1.6 | 1.2 | 1.0 | 1.1 | 0.9 | 1.8 | 1.0 | 1.8 | 0.7 | | | | | | | 0.9 | 2.5 | | 1.1 | 1.9 | |
| | r09093 | | | 1.2 | 1.4 | 1.2 | 1.7 | 0.9 | 2.2 | 1.4 | 1.9 | 1.0 | | | | | | | 0.9 | 1.7 | | 1.0 | 2.0 | |
| | r10064 | | | 1.5 | 1.5 | 1.4 | 1.4 | 0.9 | 2.8 | 1.3 | 2.8 | 0.7 | | | | | | | 1.5 | 1.8 | | 0.8 | 2.4 | |
| negative controls (fold change) | r09038 | | | 1.2 | 0.8 | 2.1 | 2.1 | 0.7 | 1.9 | 1.3 | 2.9 | 1.1 | | | | | | | 1.6 | 1.1 | | 1.0 | 1.5 | |
| | r10045 | | | 1.9 | 0.9 | 4.2 | 1.9 | 0.8 | 4.9 | 1.4 | 4.3 | 1.1 | | | | | | | 0.9 | 1.2 | | 1.1 | 0.6 | |
| | Macaque ID | | | | | | | | | | | | | | | | | | | | | | | |

**Supplementary Table 9.** Mapping of the neutralizing epitopes on ConS, TV1.21, MW965.26 and SF162.LS for immunized macaques. Midpoint neutralization titers of plasma from macaques immunized with a trivalent cocktail of SOSIP-ferritin against a panel of heterologous ConS and TV1.21 mutant viruses (left) and MW965.26 and SF162.LS depleting the sera using scrambled or ConM V3 peptide (right). The TZM-bl cell assays were performed at DUMC. Note the relatively high MLV neutralization titers.
Lower part: fold change in ID50 titers (ID50 virus/ID50 mutant virus). Fold change <0.25 in grey; 0.25-2.5 in white; 2.5-10 in light blue; >10 in dark blue.

## Supplementary References

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