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Supplemental Information

Rational Design of DNA-Expressed Stabilized Native-Like HIV-1 Envelope Trimers

Yoann Aldon, Paul F. McKay, Joel Allen, Gabriel Ozorowski, Réka Felfödiné Lévai, Monica Tolazzi, Paul Rogers, Linling He, Natalia de Val, Katalin Fábián, Gabriella Scarlatti, Jiang Zhu, Andrew B. Ward, Max Crispin, and Robin J. Shattock

SUPPLEMENTAL FIGURES

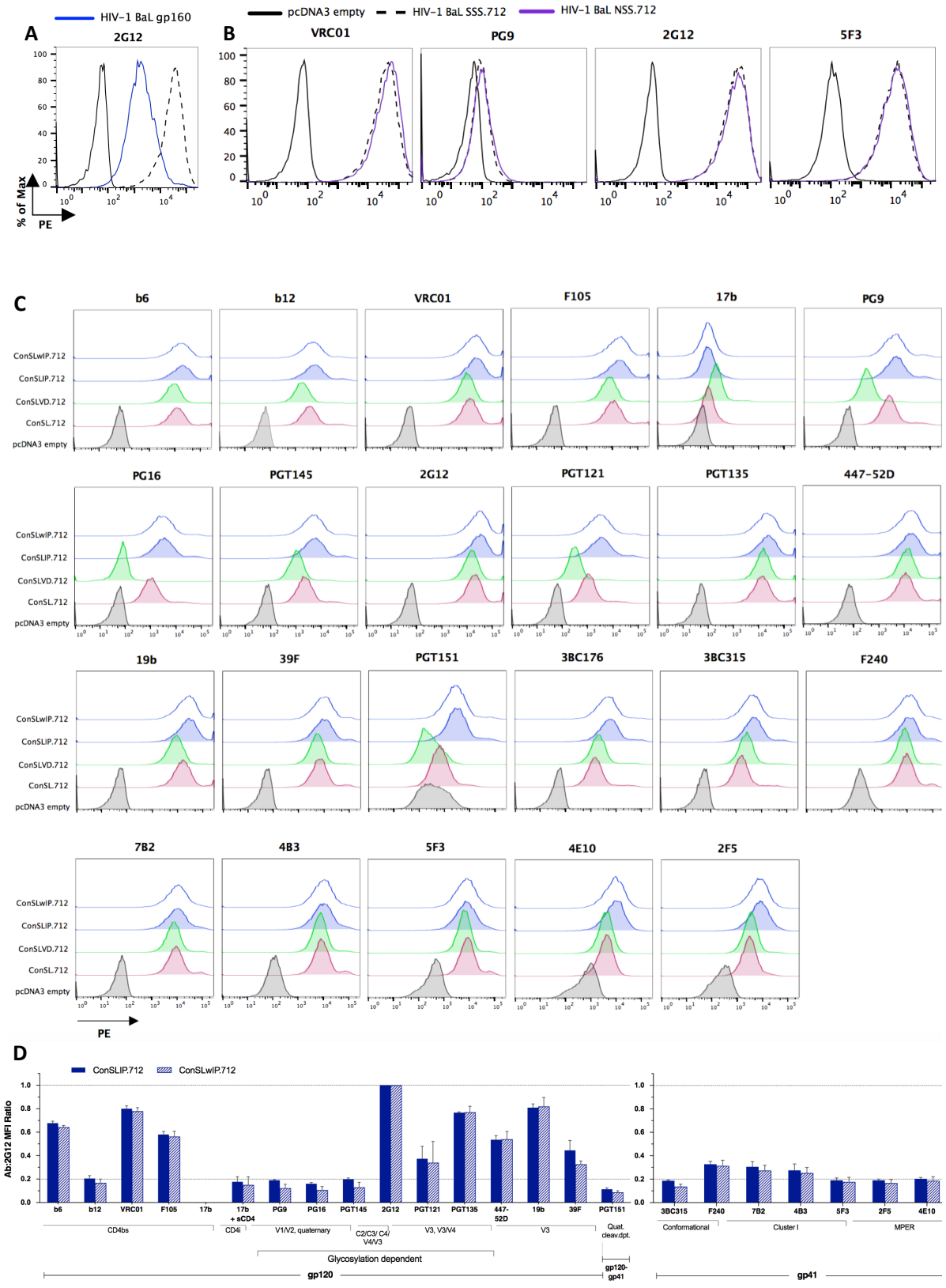


Figure S1. Effects of truncation at position 712 and signal sequence on surface expression of BaL and impact of IP, VD and linker orientation on the cleavage independent ConSL.712 design. Related to Figures 2 and 3. (**A**) FC analysis of HIV-1 BaL Env comparing the expression levels of gp160 and truncated version BaL SSS.712 transiently expressed in 293T.17 cells. Representative of n ≥ 2 independent experiments.

(**B**) Comparison of native signal sequence (NSS) versus secretion signal sequence (SSS) and their impact on Env surface antigenicity in 293T.17 cells. 293T.17 cells transfected with a pcDNA3 empty vector were included as a negative control to determine each mAb background. Representative of $n \geq 2$ independent experiments. (**C**) 293T.17 cells were transiently transfected with the indicated constructs and traces were plotted in FlowJo. Representative of $n \geq 2$ independent experiments. (**D**) Ab binding profile of ConSLIP.712 and ConSLwIP.712 were analysed as in Figure 2. Error bars represent mean ± SEM with $n \geq 2$ independent experiments.

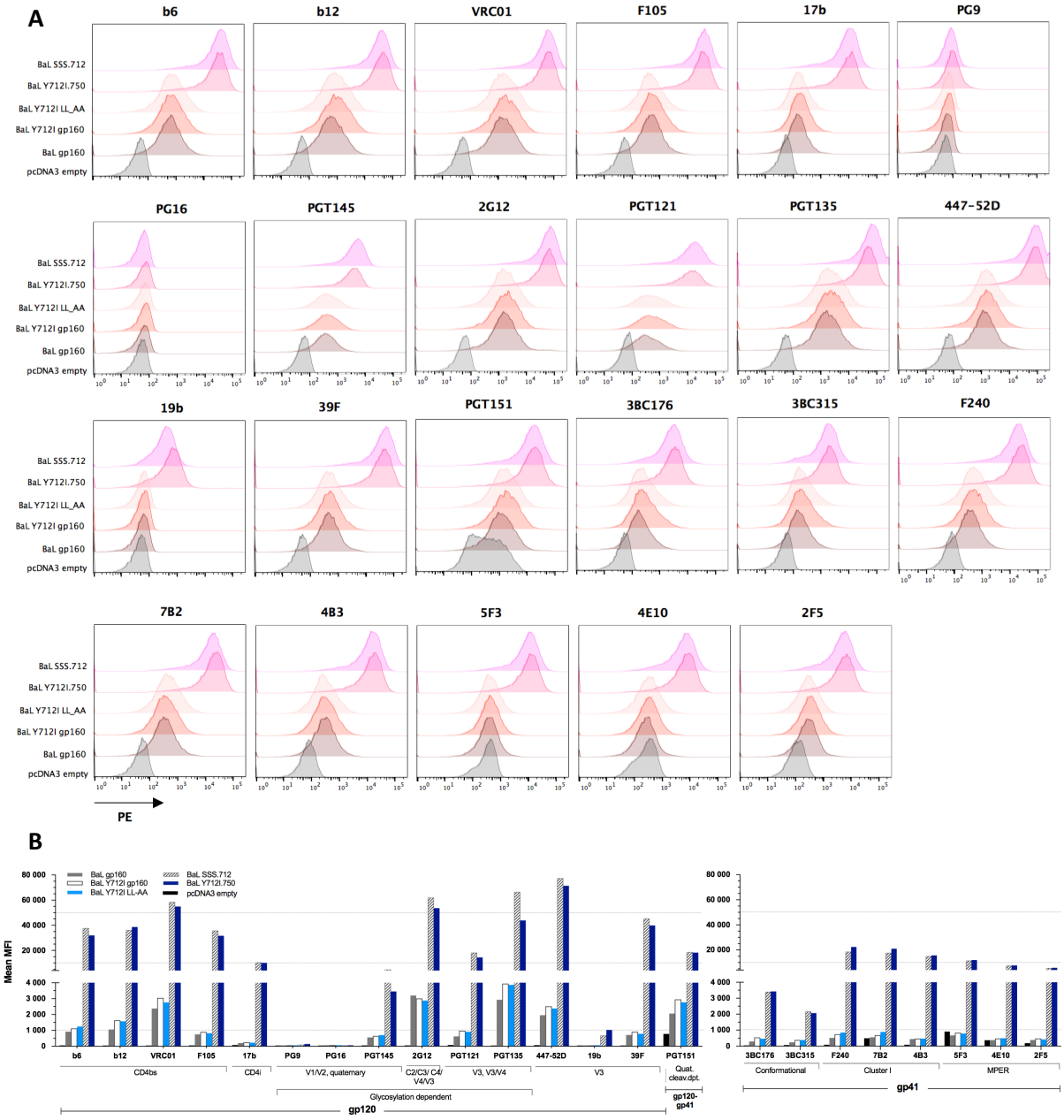


Figure S2. Effect of the endocytosis/recycling motif mutations and CT truncations on BaL Env surface expression. Related to Figure 3. (**A**) Traces overlay of extended BaL constructs FC data from Figure 3. (**B**) Extended mAbs panel from Figure 3.B with mean MFI values plotted. Representative of n ≥ 2 independent experiments.

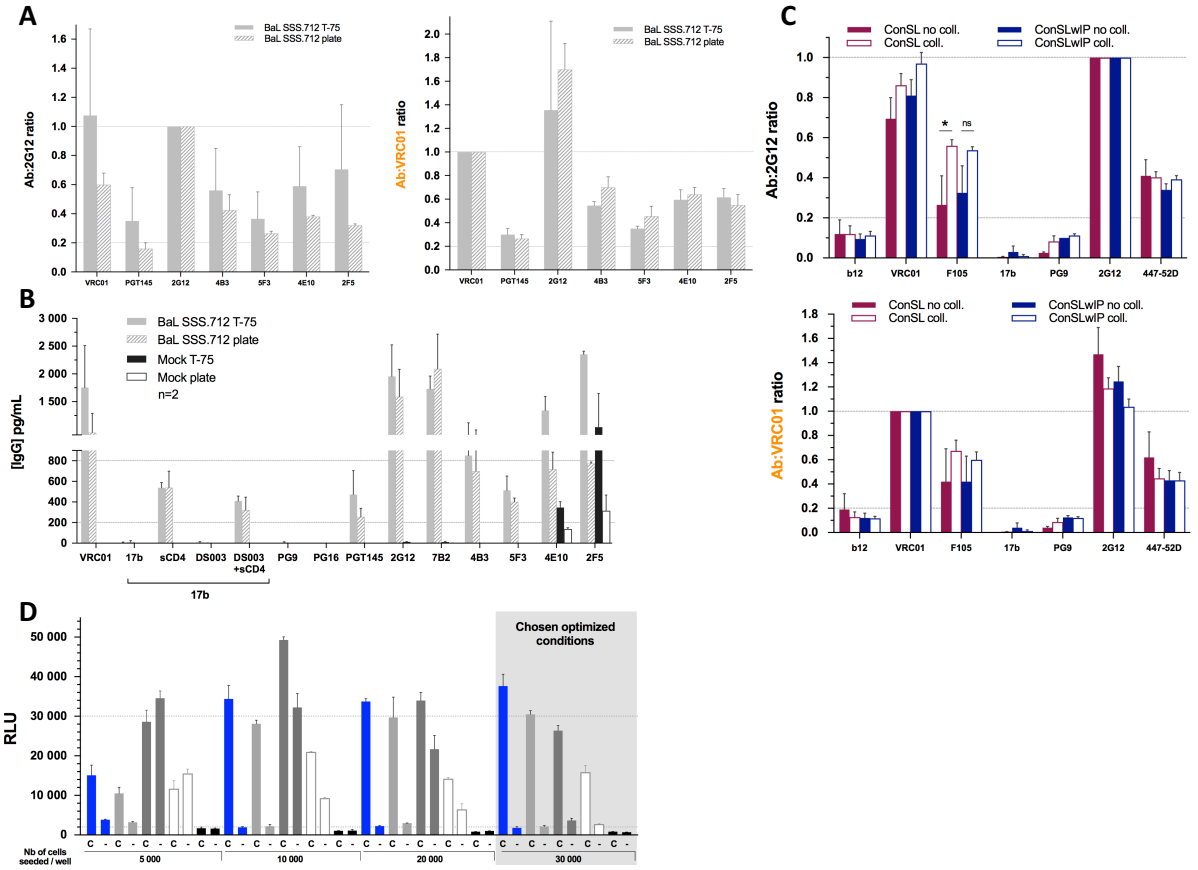


Figure S3. Cell ELISA assay optimization and validation for 293T.17 and human skeletal primary muscle cells. Related to Figure 3. (**A**) Comparison of CE transfection and seeded conditions using BaL SSS.712 expressing 293T.17 cells either transfected in T-75 then seeded onto the ELISA plate or cells directly seeded and transfected onto the plate. Both mAb:2G12 and mAb:VRC01 are shown ($n \geq 2$). (**B**) Same as (**A**) using an extended panel and a standard IgG. DS003 is a microbicide from the International Partnership Microbicide and binds the CD4bs. (**C**) Comparison of collagen coated (coll.) plates versus non coated (no coll.) plates. Both mAb:2G12 and mAb:VRC01 are shown ($n \geq 2$). (**D**) Muscle cells CE optimization using 4 mAbs at 10 µg/mL (2G12 blue, VRC01 light grey, PGT135 dark grey, F240 white) and different cell concentration seeded per well. ConSLIP.712 ('C' on the x axis) was used for optimization along with pcDNA3 empty ('-' on the x axis) vector transfected wells to determine mAbs background. Best conditions are highlighted in grey. Error bars represent mean ± SEM with $n \geq 2$ independent experiments for (**A**), (**B**) and (**C**) and mean ± SD of duplicate wells for (**D**). Unpaired t test *p* values: *<0.05.

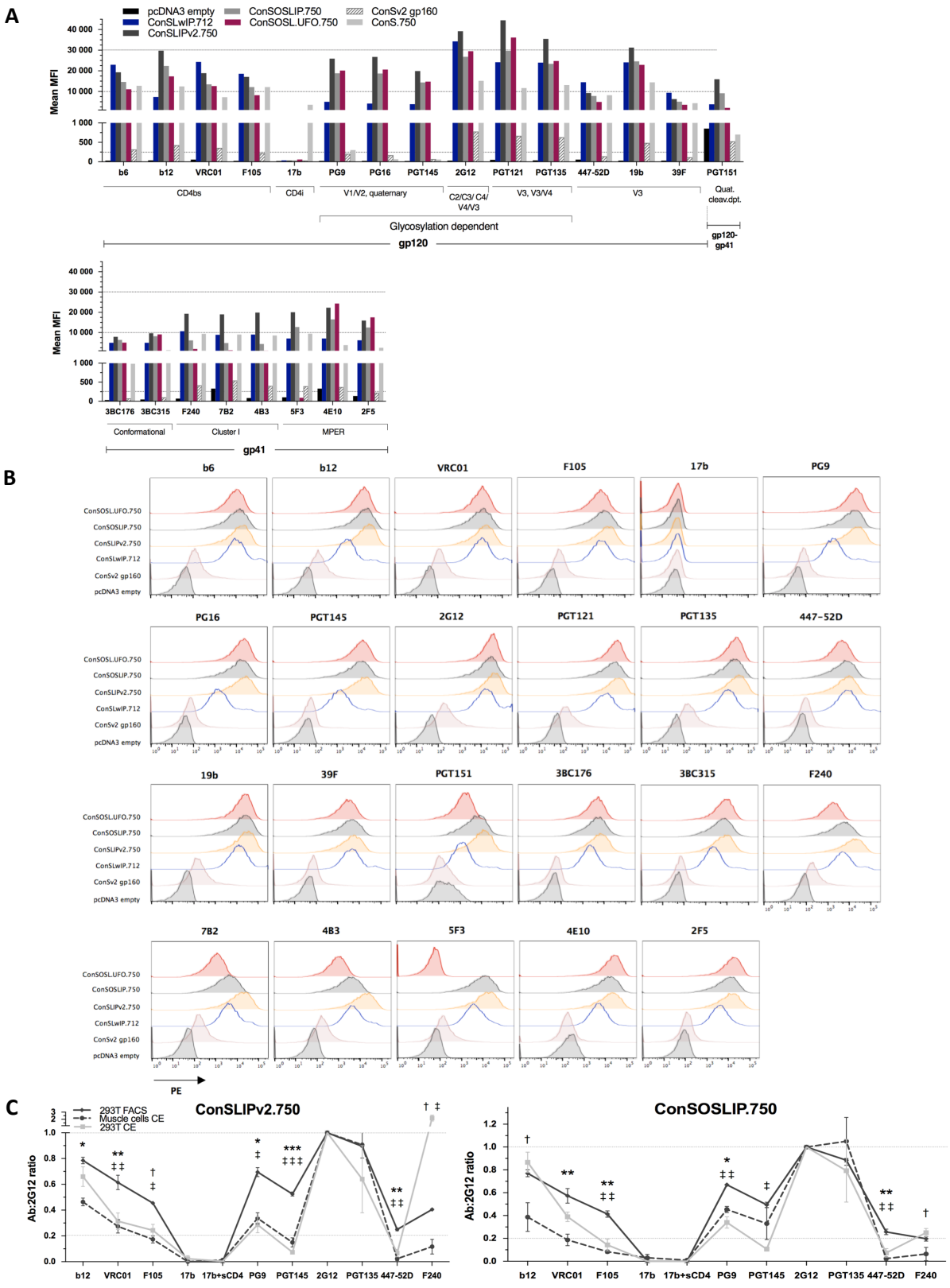


Figure S4. Enhancement of bNAbs binding and subsequent reduction of nNAbs binding by gp41 HR1 stabilization - Flow cytometry and cell ELISA. Related to Figure 4. (**A**) Mean MFI values corresponding to the bar graph of Figure 4.A of Env constructs expressed in 293T.17 and analysed by FC for 23 mAb. Representative of n ≥ 2 independent experiments (for ConSOSL.UFO.750 n ≥ 3). (**B**) Traces overlay of the corresponding experiment expressed in 293T.17 cells. Representative of n ≥ 2 independent experiments (ConSOSL.UFO.750 n ≥ 3). (**C**) Comparison of ConSLIPv2.750 and ConSOSLIP.750 epitope profiles in

muscle cell CE, 293T.17 CE and FC ($n \geq 2$). One-way ANOVA with Sidak's multiple comparisons, *p* values: *<0.05, **<0.01, ***<0.001 where each sign in (**C**) compares: *Muscle Cells CE vs. 293T.17 FC; † Muscle Cells CE vs. 293T.17 CE; ‡ 293T.17 FC vs. 293T CE.

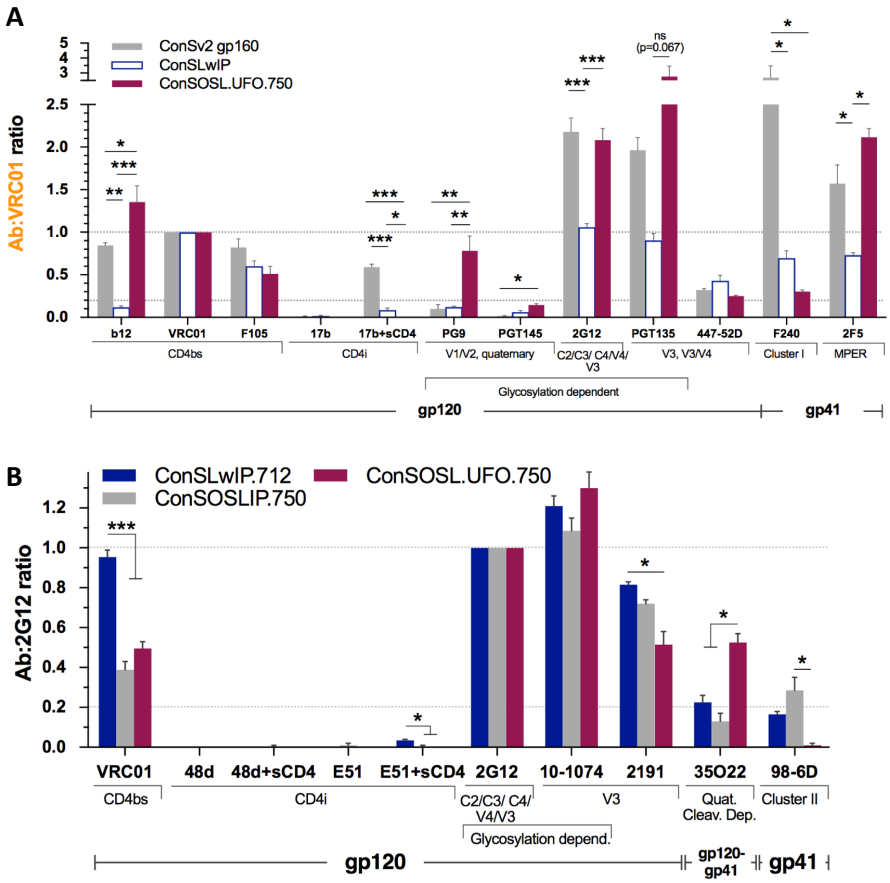


Figure S5. Enhancement of bNAbs binding and subsequent reduction of nNAbs binding by gp41 HR1 stabilization – Cell ELISA. Related to Figure 4. (**A**) 293T.17 CE assessing ConSv2 gp160, ConSLwIP.712 and ConSOSL.UFO.750 using mAb:VRC01 ratio to plot the mean ± SEM values ($n \geq 3$, except ConSv2 gp160 PGT135 $n = 2$). (**B**) Comparison of ConSLwIP.712, ConSOSLIP.750 and ConSOSL.UFO.750 in 293T.17 CE for mAb: VRC01, 48d (+/- sCD4), E51 (+/- sCD4), 2G12, 10-1074, 2191, 35O22 and 98-6D ($n \geq 2$). One-way ANOVA with Sidak's multiple comparisons, p values: *<0.05, **<0.01, ***<0.001.

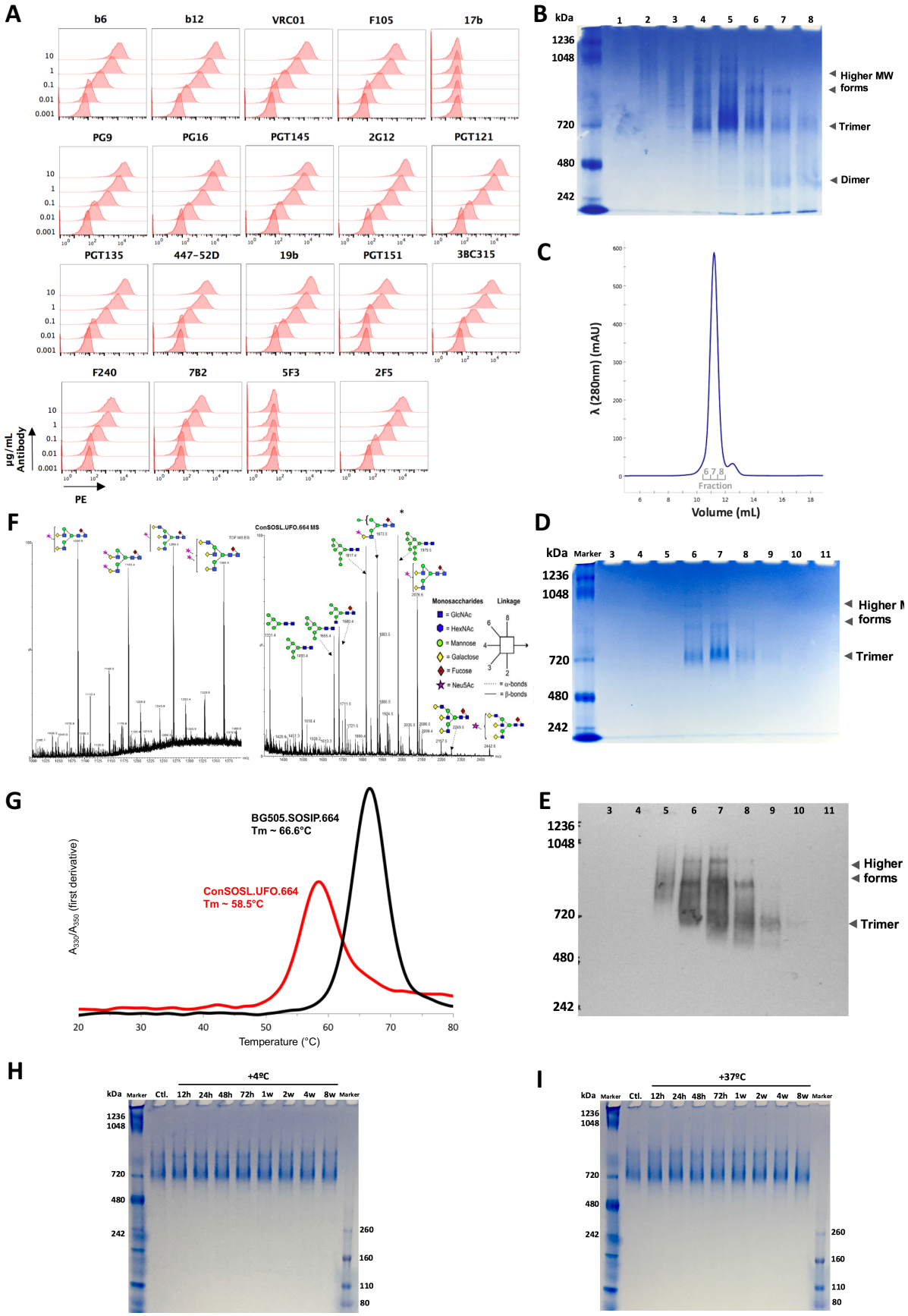


Figure S6. Antibodies titration against ConSOSL.UFO.750 and structural and glycosylation analysis of ConSOSL.UFO.664 Env trimers. Related to Figures 5 and 6. (**A**) Traces corresponding to the mean MFI value plotted in Figure 5. Representative of n = 2 independent experiments. (**B**) Colloidal blue stain of a native

gel running fractions of the SEC purification from Figure 6.A. (**C**) Fractions 4-6 of Figure 6.A were run a second time through a SEC column and the obtained SEC profile is shown. (**D**) Colloidal blue stain of a native gel running fractions of the SEC purification from (**C**). (**E**) Native PAGE western blot of (**D**) using 2G12 (1 µg/mL) for detection. **(B)-(E)** Representative of $n \geq 3$ independent experiments. (**F**) Mass spectrometry spectra of singly (left panel) and doubly charged ions extracted using driftscope. Abundant glycan structures and larger complex glycans are labelled. The m/z=1873 is a hybrid lacking a mannose on 6 arm, but both isomers are present. (**F**) Thermal stability assessment of ConSOSL.UFO.664 (red) using nanoDSF, Tm is indicated. BG505 SOSIP.664 (black) trimer is also plotted for comparison. (**H**) Thermostability of ConSOSL.UFO.664 at +4ºC and (**I**) +37ºC over 8 weeks, representative of n = 1 experiment.

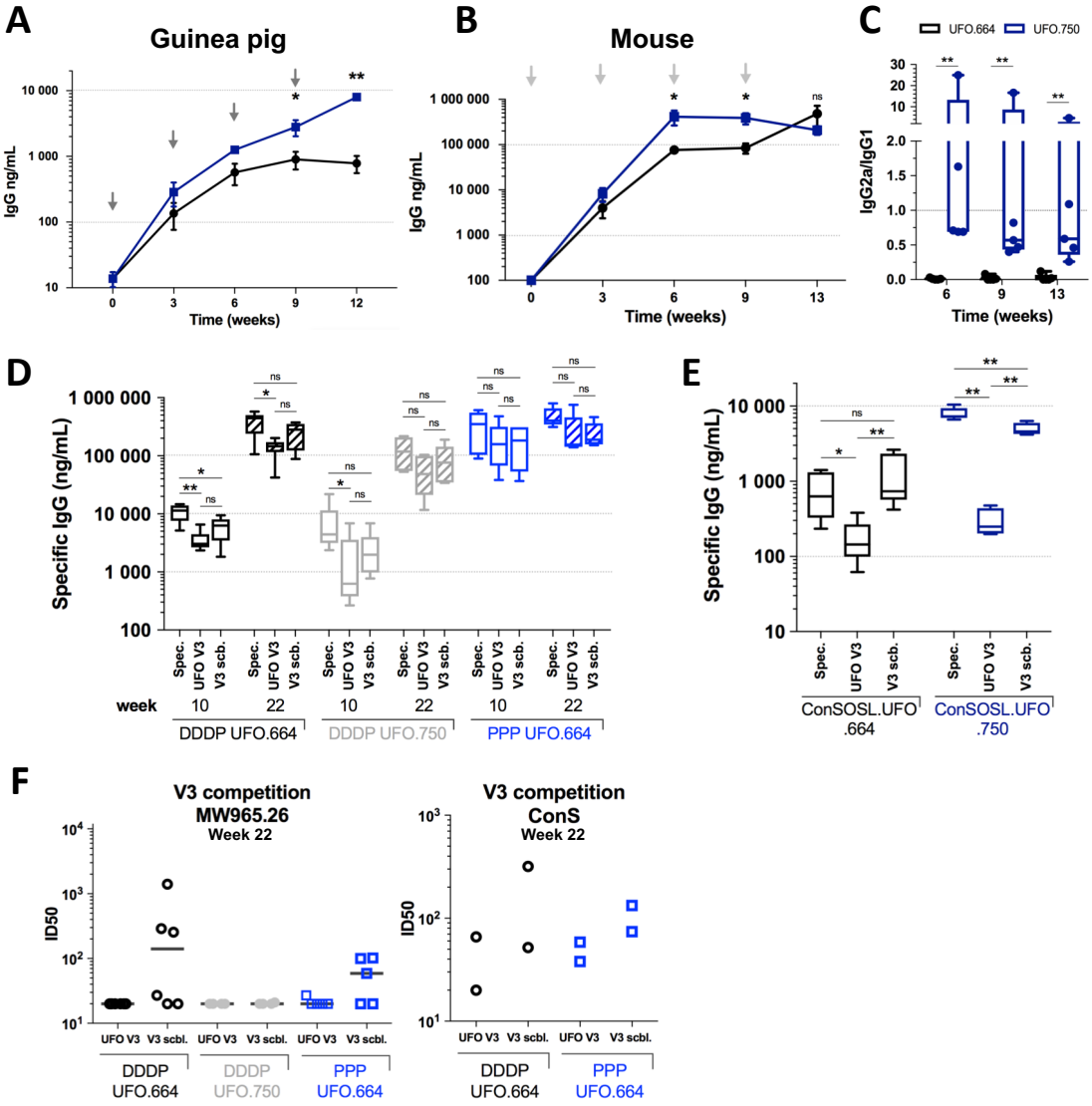
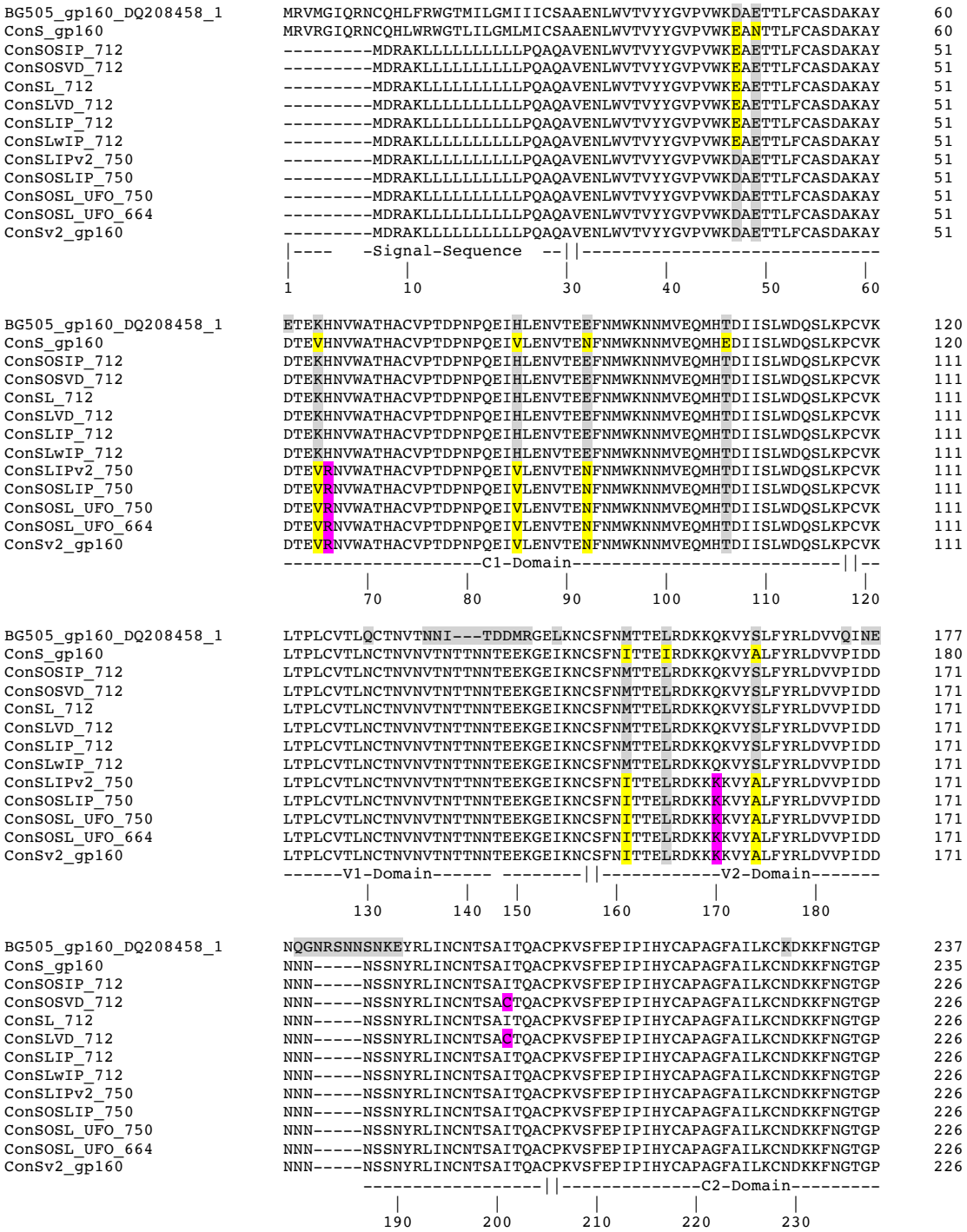
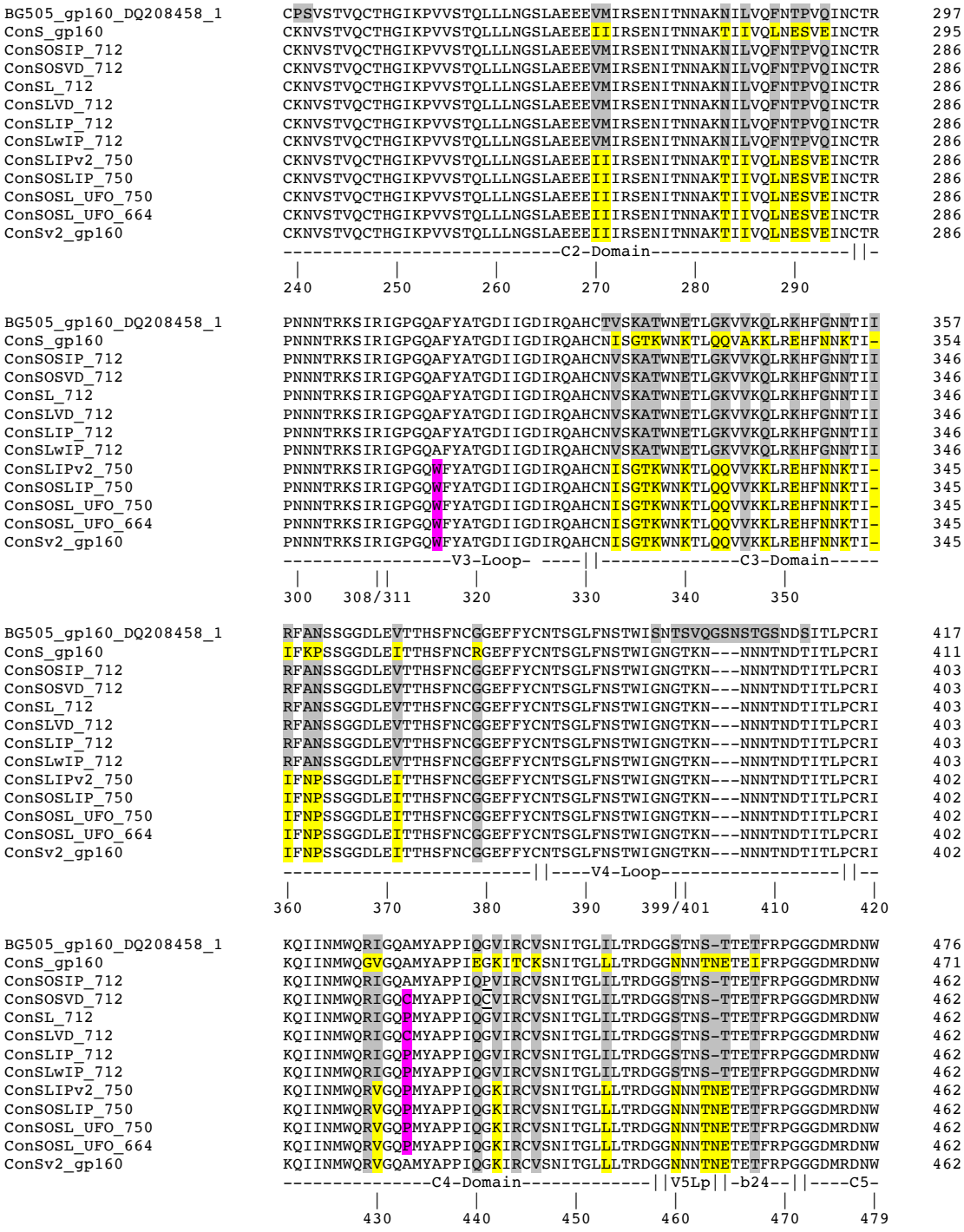


Figure S7. Immunogenicity of ConSOSL.UFO design in Guinea pigs and mice and V3 specific response in rabbits. Related to Figure 7. (**A**) Antigen specific serum IgG binding for DNA immunized Guinea pigs and (**B**) mice with ConSOSL.UFO.750 plasmid (blue) and ConSOSL.UFO.664 plasmid (black). Binding assessed by 9E10 capture ELISA for Guinea pig's sera and by directly coated ConSOSL.UFO.664 trimers for mouse sera. Arrows indicate immunization with DNA IM+EP. For (**A**) time points 0, 3 and 6, n = 6 animals per group, then for time points 9 and 12, n = 5. Mice, n = 5 per group. Error bars represent mean ± SEM values. (**C**) Mouse IgG1 and IgG2a antigen specific responses were assessed by ELISA and the IgG1/IgG2a ratio is shown here. (**D**) Rabbit serum samples from week 10 and 22 and (**E**) Guinea pig's serum samples from week 12 were incubated 30min prior loading onto the ELISA plates with 10ug/mL ConSOSL.UFO V3 cys-cyclised (UFO V3) peptide or MN.3 V3 scrambled linear (V3 scbl.) peptide as a control and the specific binding to ConSOSL.UFO.664 Myc-HIS captured antigen measured. The total specific response is plotted and referred as 'Spec.'. (**F**) Neutralization of HIV-1 MW965.26 and ConS pseudoviruses in TZM-bl assay by rabbit neutralizer sera from week 22 in competition with UFO V3 and V3 scbl. peptides. Data plotted as ID50 values (serum dilution that inhibits by 50% infectivity). Unpaired t-test, p values: *<0.05, **<0.01.

SUPPLEMENTAL DATA

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ConSv2_gp160 PNNNTRKSIRIGPGQWFYATGDIIGDIRQAHCNISGTKWNKTLQQVVKKLREHFNNKTI- 345
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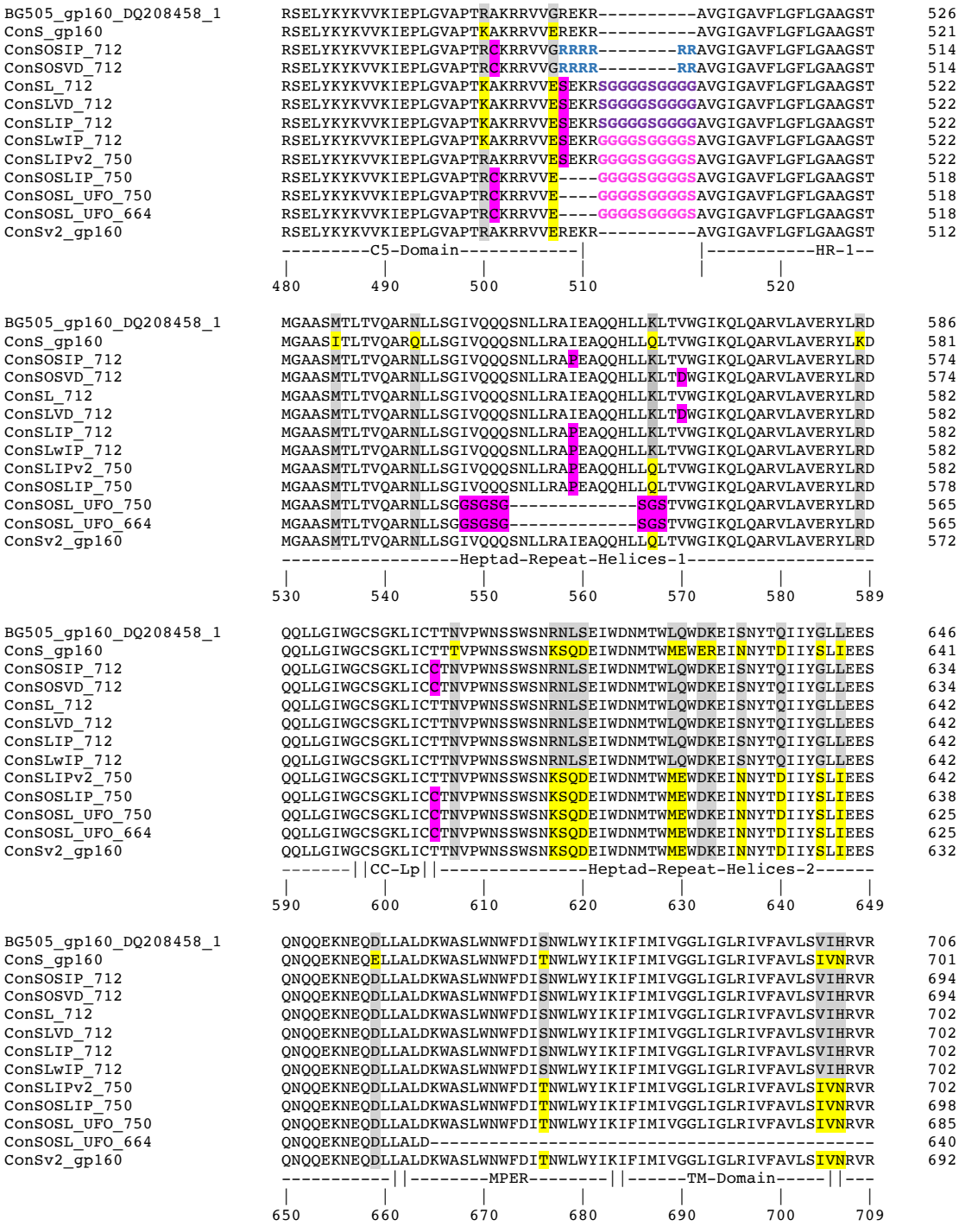
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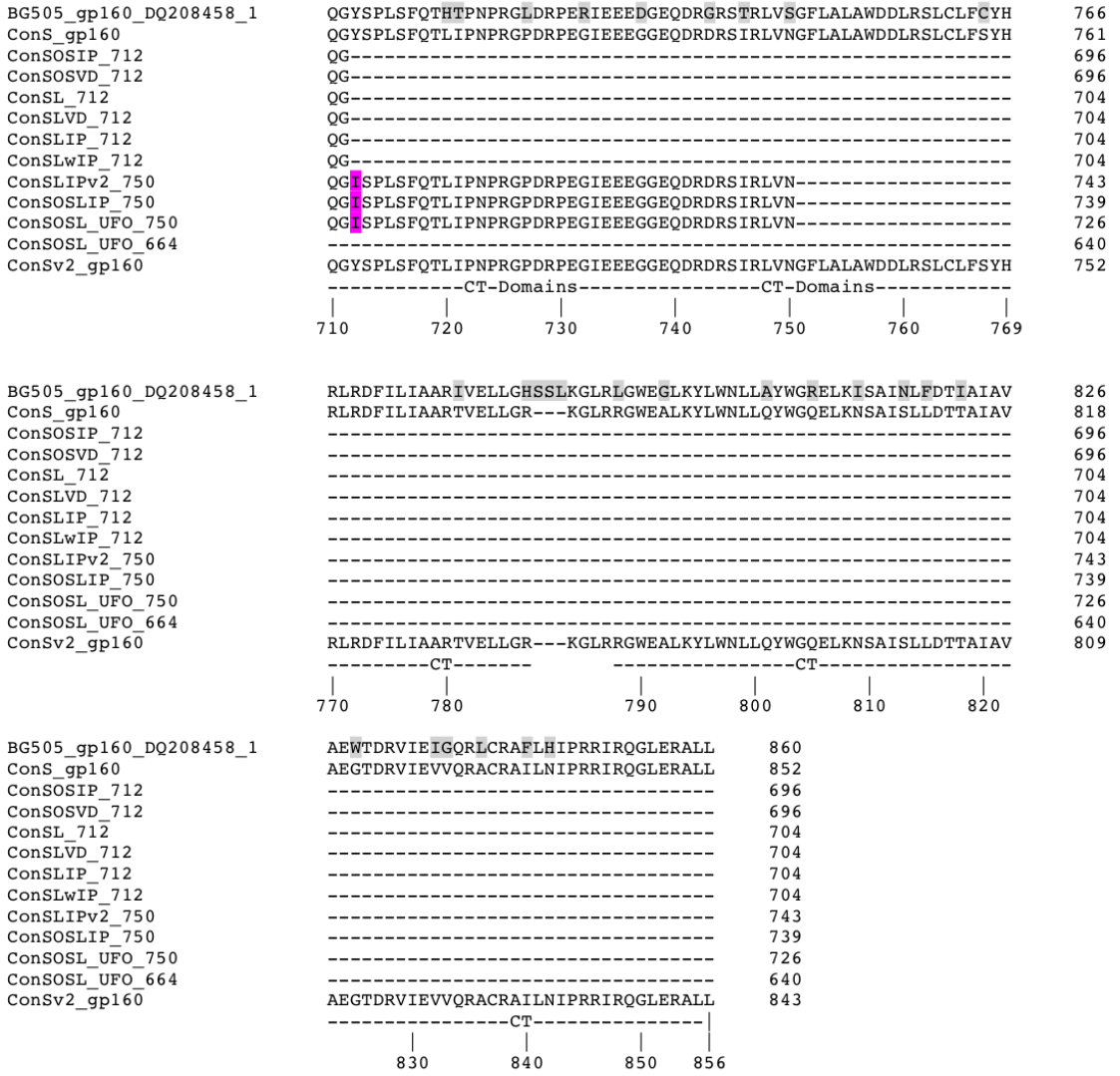
-----------||--------MPER--------||------TM-Domain-----||---
650 660 670 680 690 700 709



B. ConSOSL.UFO.750

MDRAKLLLLLLLLLLPQAQAVENLWVTVYYGVPVWKDAETTLFCASDAKAYDTEVRNVWATHACVPTDPNPQEIVLENVTENFNMWKNNMV
EQMHTDIISLWDQSLKPCVKLTPLCVTLNCTNVNVTNTTNNTEEKGEIKNCSFNITTELRDKKKKVYALFYRLDVVPIDDNNNNSSNYRLI
NCNTSAITQACPKVSFEPIPIHYCAPAGFAILKCNDKKFNGTGPCKNVSTVQCTHGIKPVVSTQLLLNGSLAEEEIIIRSENITNNAKTII
VQLNESVEINCTRPNNNTRKSIRIGPGQWFYATGDIIGDIRQAHCNISGTKWNKTLQQVVKKLREHFNNKTIIFNPSSGGDLEITTHSFNC
GGEFFYCNTSGLFNSTWIGNGTKNNNNTNDTITLPCRIKQIINMWQRVGQPMYAPPIQGKIRCVSNITGLLLTRDGGNNNTNETETFRPGG
GDMRDNWRSELYKYKVVKIEPLGVAPTRCKRRVVEGGGGSGGGGSAVGIGAVFLGFLGAAGSTMGAASMTLTVQARNLLSGGSGSGSGSTV
WGIKQLQARVLAVERYLRDQQLLGIWGCSGKLICCTNVPWNSSWSNKSQDEIWDNMTWMEWDKEINNYTDIIYSLIEESQNQQEKNEQDLL
ALDKWASLWNWFDITNWLWYIKIFIMIVGGLIGLRIVFAVLSIVNRVRQGISPLSFQTLIPNPRGPDRPEGIEEEGGEQDRDRSIRLVN*

Data S1. Sequence alignment and numbering using HXB2 numbering reference. Related to Figure 1. (**A**) Sequences were aligned using Clustal Omega online tool (EMBL-EBI). HXB2 numbering was attributed according to the lanl database using the HXB2 K03455 reference sequence (https://www.hiv.lanl.gov/content/sequence/HIV/REVIEWS/HXB2.html). Highlighted in grey are the amino acid introduced from BG505 (or specific to BG505), in yellow the amino acid reverted to the ConS gp160 sequence (or specific to ConS gp160) while pink highlighted amino acids indicate stabilization substitutions. The optimized cleavage site RRRRRR is coloured in blue and both cleavage site linker $(SG_4)_2$ and $(G_4S)_2$ are coloured in purple and pink respectively. (**B**) ConSOSL.UFO.750 amino acid sequence.