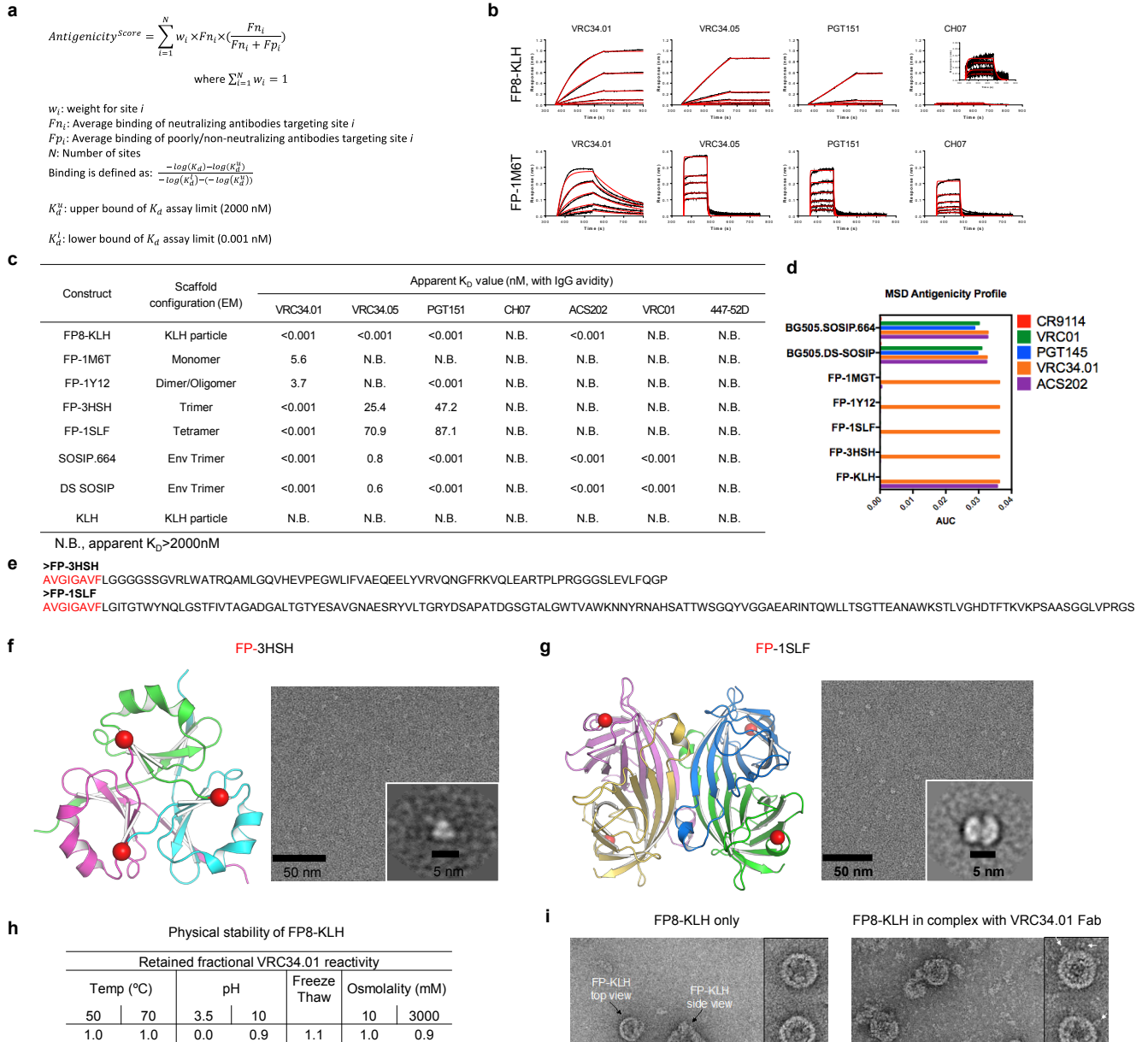


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Epitope-based vaccine design yields fusion peptide-directed antibodies that neutralize diverse strains of HIV-1

Kai Xu^{1,9}, Priyamvada Acharya^{1,2,9}, Rui Kong^{1,9}, Cheng Cheng^{1,9}, Gwo-Yu Chuang¹, Kevin Liu¹, Mark K. Louder¹, Sijy O'Dell¹, Reda Rawi¹, Mallika Sastry¹, Chen-Hsiang Shen¹, Baoshan Zhang¹, Tongqing Zhou¹, Mangaiarkarasi Asokan¹, Robert T. Bailer¹, Michael Chambers¹, Xuejun Chen¹, Chang W. Choi¹, Venkata P. Dandey², Nicole A. Doria-Rose¹, Aliaksandr Druz¹, Edward T. Eng², S. Katie Farney¹, Kathryn E. Foulds¹, Hui Geng¹, Ivelin S. Georgiev³, Jason Gorman¹, Kurt R. Hill¹, Alexander J. Jafari¹, Young D. Kwon¹, Yen-Ting Lai¹, Thomas Lemmin⁴, Krisha McKee¹, Tiffany Y. Ohr¹, Li Ou¹, Dongjun Peng¹, Ariana P. Rowshan¹, Zizhang Sheng^{5,6}, John-Paul Todd¹, Yaroslav Tsybovsky⁷, Elise G. Viox¹, Yiran Wang¹, Hui Wei², Yongping Yang¹, Amy F. Zhou¹, Rui Chen⁸, Lu Yang⁸, Diana G. Scorpio¹, Adrian B. McDermott¹, Lawrence Shapiro^{1,5,6}, Bridget Carragher², Clinton S. Potter², John R. Mascola^{1*} and Peter D. Kwong^{1,5*}

¹Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. ²National Resource for Automated Molecular Microscopy, Simons Electron Microscopy Center, New York Structural Biology Center, New York, NY, USA. ³Vanderbilt Vaccine Center, Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, and Department of Electrical Engineering and Computer Science, Vanderbilt University, Nashville, TN, USA. ⁴Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, CA, USA. ⁵Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY, USA. ⁶Department of Systems Biology, Columbia University, New York, NY, USA. ⁷Electron Microscopy Laboratory, Cancer Research Technology Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD, USA. ⁸GenScript USA, Piscataway, NJ, USA. ⁹These authors contributed equally: Kai Xu, Priyamvada Acharya, Rui Kong, Cheng Cheng. *e-mail: jmascola@nih.gov; pdkwong@nih.gov



Supplementary Figure 1. Characteristics of FP immunogens.

a, Metric of antigenicity. Here we focus on the FP site of vulnerability ($N=1$). VRC34.01, VRC34.05, PGT151, and ACS202 were used as the representative neutralizing antibodies targeting FP, and CH07 was used as the representative poorly/non-neutralizing antibody targeting FP. See (c) for K_D data. **b**, Antigenicity assessment of FP immunogens by BLI method with Octet. Examples of Octet-binding curves for FP8-KLH and FP-1M6T epitope scaffold are shown. **c**, FP-immunogen antigenicity. FP-directed antibodies VRC34.01, VRC34.02, PGT151 and ACS202 were considered neutralizing, whereas CH07 was considered weakly or non-neutralizing. **d**, Immunogen quality control by MSD. **e**, Amino acid sequences for FP scaffolds: FP-3HSH and FP-1SLF. Additional epitope scaffolds are described in Kong et al. 2016. **f**, Structural models of FP-3HSH and FP-1SLF. **g**, Negative-stain EM images (inset shows 2D-class averages). **h**, Physical stability of FP8-KLH. Fractional values refer to VRC34.01 reactivity retained after exposure to various physical extremes as compared to initial reactivity with VRC34.01. Affinity measured by biolayer interferometry. **i**, Binding of VRC34.01 Fab to FP8-KLH illustrated by negative-stain electron microscopy. Examples of micrographs for FP8-KLH (left) and FP8-KLH mixed with VRC34.01 Fab at a molar ratio of 1:10 (right). Right insets, examples of raw particles for FP8-KLH and FP8-KLH in complex with VRC34.01 Fab. White arrows indicate bound Fab fragments. Bottom insets, examples of 2D-class averages. White arrows indicate bound Fab fragments. For (b, f, g and i), $n=3$ independent experiments were performed with similar results.

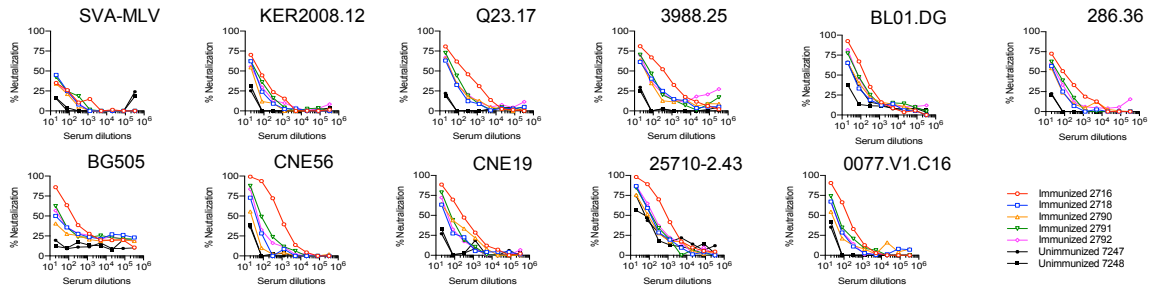
a

Neutralization assessment of 1st-generation murine immune sera

Viruses		Mice sera						ID ₅₀	ID ₈₀
		1868	1882	1883	1884	2586	2602		
SVA-MLV	ID ₅₀	50	23	25	<20	23	<20		
	ID ₈₀	<20	<20	<20	<20	<20	<20		
BG505 WT	ID ₅₀	<20	<20	<20	<20	<20	60		
	ID ₈₀	<20	<20	<20	<20	<20	<20		
BG505 Δ611 glycan	ID ₅₀	108	95	62	89	78	275		
	ID ₈₀	20	<20	<20	<20	<20	22		
BG505 Δ88+Δ611 glycans	ID ₅₀	493	320	239	205	239	575		
	ID ₈₀	65	26	29	26	<20	55	40-100	20-100

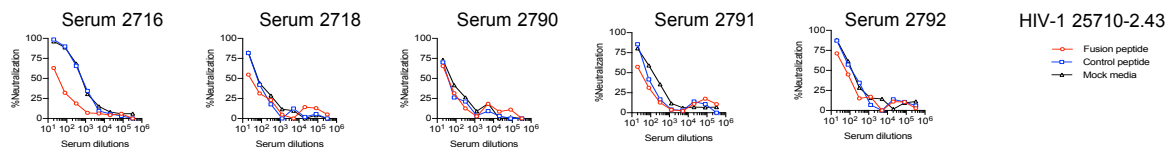
b

Sera neutralization curves on 10-isolate panel with MLV as control



c

FP-competed sera neutralization

Summary of the effect of FP-competition to sera neutralization ID₅₀ on HIV-1 strain 25710-2.43

Serum	Media	Fusion peptide			Control peptide		
	ID ₅₀	ID ₅₀	Fold change	% inhibition	ID ₅₀	Fold change	% inhibition
2716	610	33	18.5	95%	633	1.0	-4%
2718	68	27	2.5	60%	61	1.1	10%
2790	55	38	1.4	31%	36	1.5	35%
2791	141	31	4.5	78%	61	2.3	57%
2792	118	48	2.5	59%	128	0.9	-8%

d

Summary of the effect of FP-competition to NHP sera and control antibody neutralization

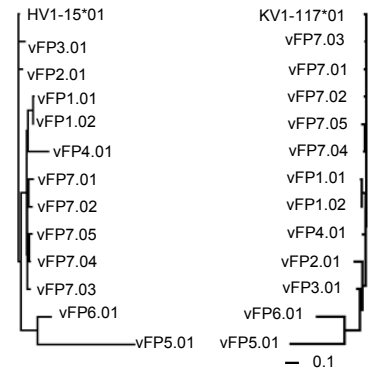
Plasma	Virus	Clade	Media	Fusion peptide			Control peptide		
			ID ₅₀	ID ₅₀	Fold change	% inhibition	ID ₅₀	Fold change	% inhibition
NHP DFIW week 46	Q23.17	A	144	<20	>7.2	>86.1%	145	1.0	-0.7%
	45_01dG5	B	78	<20	>3.9	>74.3%	74	1.1	5.1%
	286.36	C	117	<20	>5.9	>82.9%	122	1.0	-4.3%
	0815.V3.C3	ACD	152	<20	>7.6	>86.8%	161	0.9	-5.9%
Antibody	Virus	Clade	Media	Fusion peptide			Control peptide		
			IC ₅₀	IC ₅₀	Fold change	% inhibition	IC ₅₀	Fold change	% inhibition
VRC34.01	Q23.17	A	0.10	14.10	139.6	99.3%	0.10	1.0	1.9%
	45_01dG5	B	0.31	18.00	58.6	98.3%	0.37	1.2	16.6%
	286.36	C	>50	>50	N/A	N/A	>50	N/A	N/A
	0815.V3.C3	ACD	0.04	1.72	44.1	97.7%	0.03	0.9	-14.7%
VRC01	Q23.17	A	0.12	0.14	1.1	11.9%	0.13	1.1	6.3%
	45_01dG5	B	0.03	0.01	0.6	-78.6%	0.03	1.1	7.4%
	286.36	C	0.46	0.38	0.8	-21.3%	0.48	1.0	4.6%
	0815.V3.C3	ACD	0.04	0.04	1.0	2.5%	0.04	1.1	9.3%

Supplementary Figure 2. Neutralization of immunized sera on 293T-derived HIV-1 Env-pseudotyped viruses in TZM-bl assay. **a**, Neutralization assessment of 1st-generation immune sera from immunized mice in Fig. 1. **b**, Neutralization assessment of 2nd-generation immune sera from immunized mice in Fig. 3c. Serum neutralization curves are shown for 5 immunized and 2 unimmunized control C57/BL6 mice, tested on 10 wildtype HIV-1 Env-pseudotyped viruses (5 with complete glycans around the FP site and 5 naturally missing glycans as shown in Fig. 3c along with ID₅₀ titer). SVA-MLV was assessed as a control. **c**, FP-competed neutralization curves of indicated immunized mice sera on HIV-1 strain 25710-2.43. Sera were pre-incubated with the nine-amino acid FP (sequence AVGIGAVFL) (red), an non-cognate FLAG peptide (blue) or control media (black) before mixing with virus stock. ID₅₀ titers are shown the summary table. ID₅₀ fold change was calculated as ID₅₀[media]/ID₅₀[peptide]. % inhibition of FP or control peptide was calculated as (1-ID₅₀[peptide]/ID₅₀[media])*100. **d**, Neutralization summary for FP competition shown in Fig. 6b. For antibody neutralization, IC₅₀ fold change was calculated as IC₅₀[peptide]/IC₅₀[media]. % inhibition of FP or control peptide was calculated as (1-IC₅₀[media]/IC₅₀[peptide])*100.

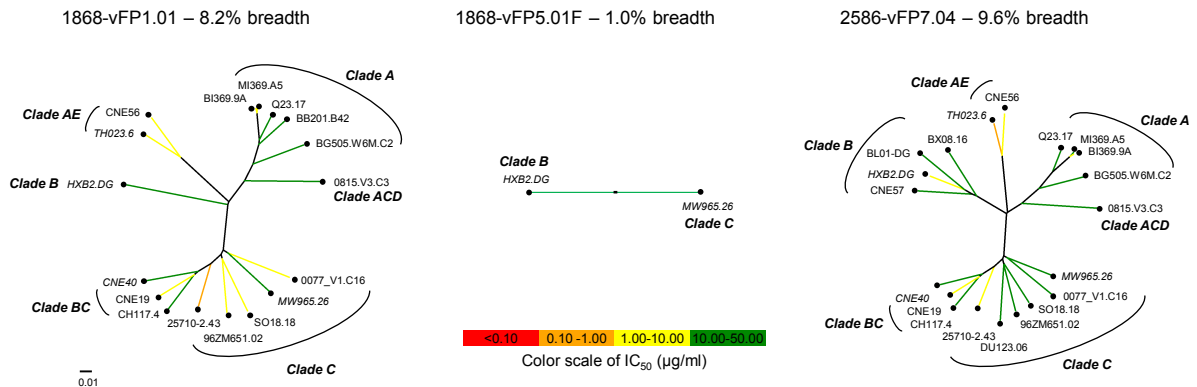
a

Mouse 1868								
Class	Antibody	Heavy			Light			
		HV	HJ	HV identity (%)	CDR H3	KV	KJ	KV identity (%)
vFP1	vFP1.01	HV1-15*01	HJ1*03	94.5	LRNYWYFDV	KV1-117*01	KJ2*01	95.6
	vFP1.02	HV1-15*01	HJ1*03	94.5	LRNYWYFDV	KV1-117*01	KJ2*01	95.9
	vFP2.01	HV1-15*01	HJ2*01	96.5	LKRFYYFDY	KV1-117*01	KJ4*01	93.2
	vFP3.01	HV1-15*01	HJ2*01	95.2	LKRFYYFDY	KV1-117*01	KJ4*01	95.6
	vFP4.01	HV1-12*01	HJ1*03	94.6	LLPKWYFDV	KV1-117*01	KJ2*01	96.9
vFP5	vFP5.01	HV3-6*01	HJ3*01	94.9	EGNYRAY	KV6-23*01	KJ5*01	93.2
vFP6	vFP6.01	HV1-7*01	HJ2*01	93.1	GYVAFHY	KV2-112*01	KJ4*01	97.6
Mouse 2586								
vFP1	vFP7.01	HV1-15*01	HJ1*03	96.5	LRLYGYFDV	KV1-117*01	KJ2*01	99.0
	vFP7.02	HV1-15*01	HJ1*03	96.9	LRLYGYFDV	KV1-117*01	KJ2*01	98.6
	vFP7.03	HV1-15*01	HJ1*03	96.5	LRLYWYFDV	KV1-117*01	KJ2*01	99.3
	vFP7.04	HV1-15*01	HJ1*03	97.9	LRLYWYFDV	KV1-117*01	KJ2*01	98.0
	vFP7.05	HV1-15*01	HJ1*03	96.2	LRLYWYFDV	KV1-117*01	KJ2*01	96.6

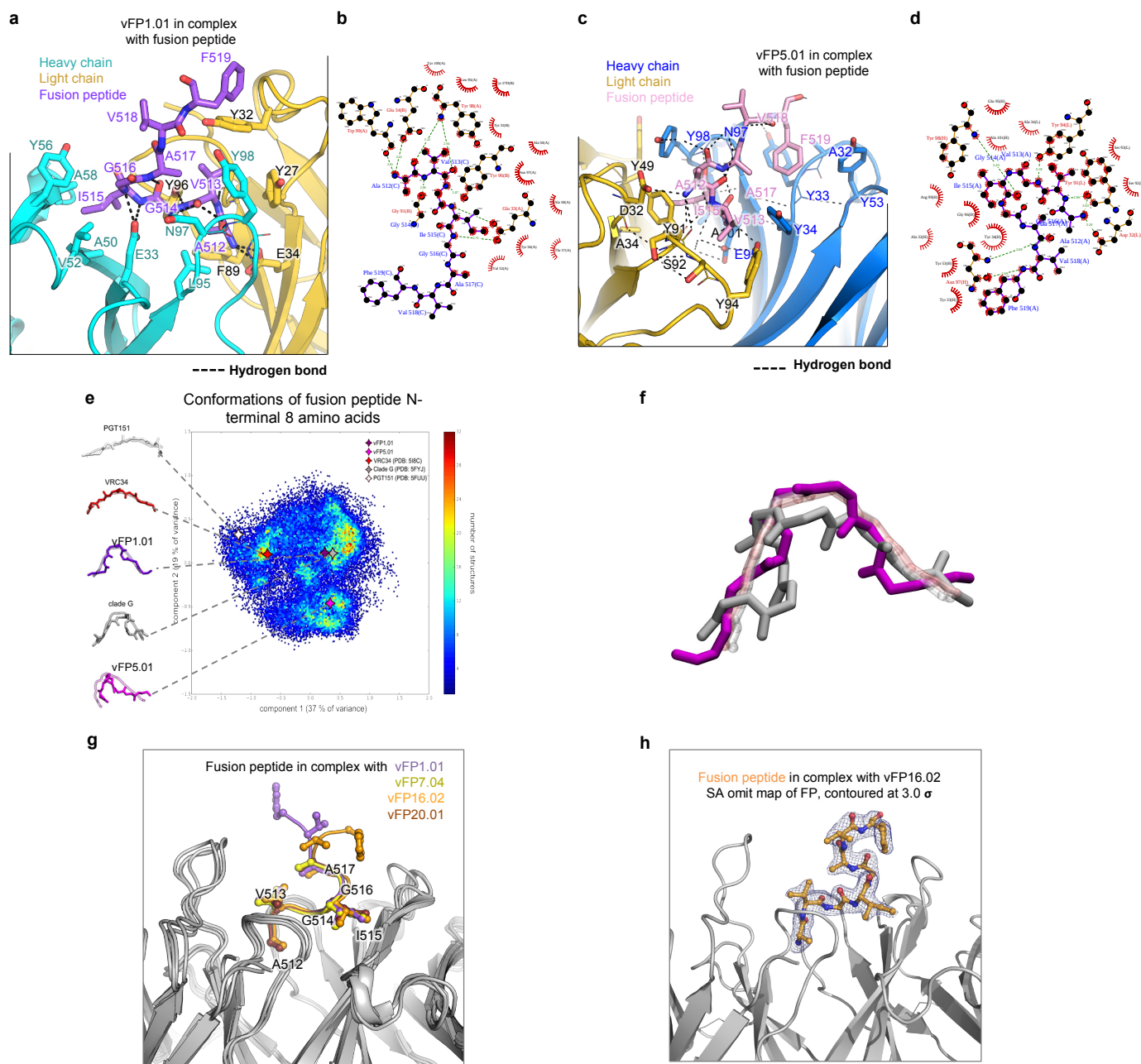
b



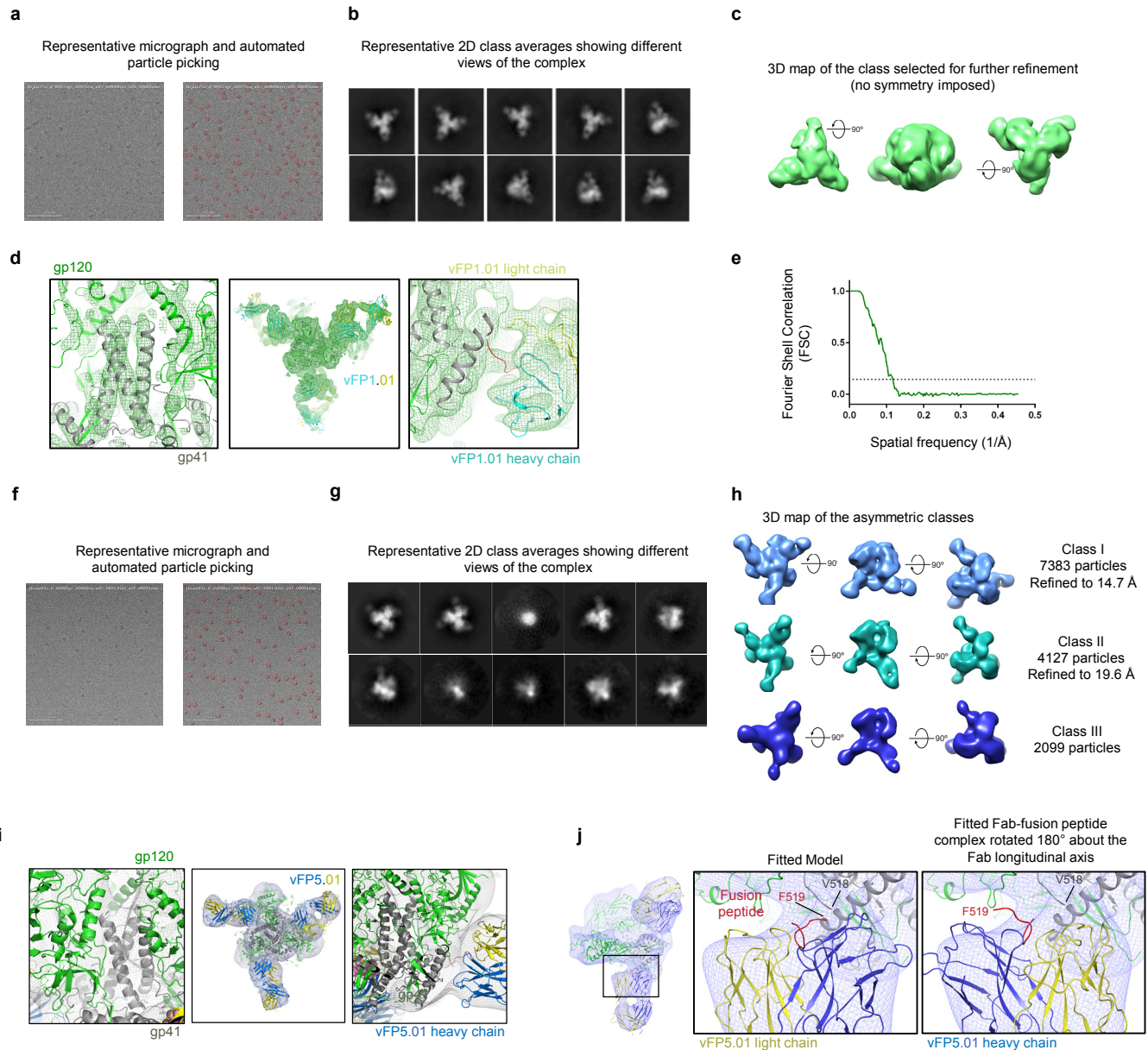
c



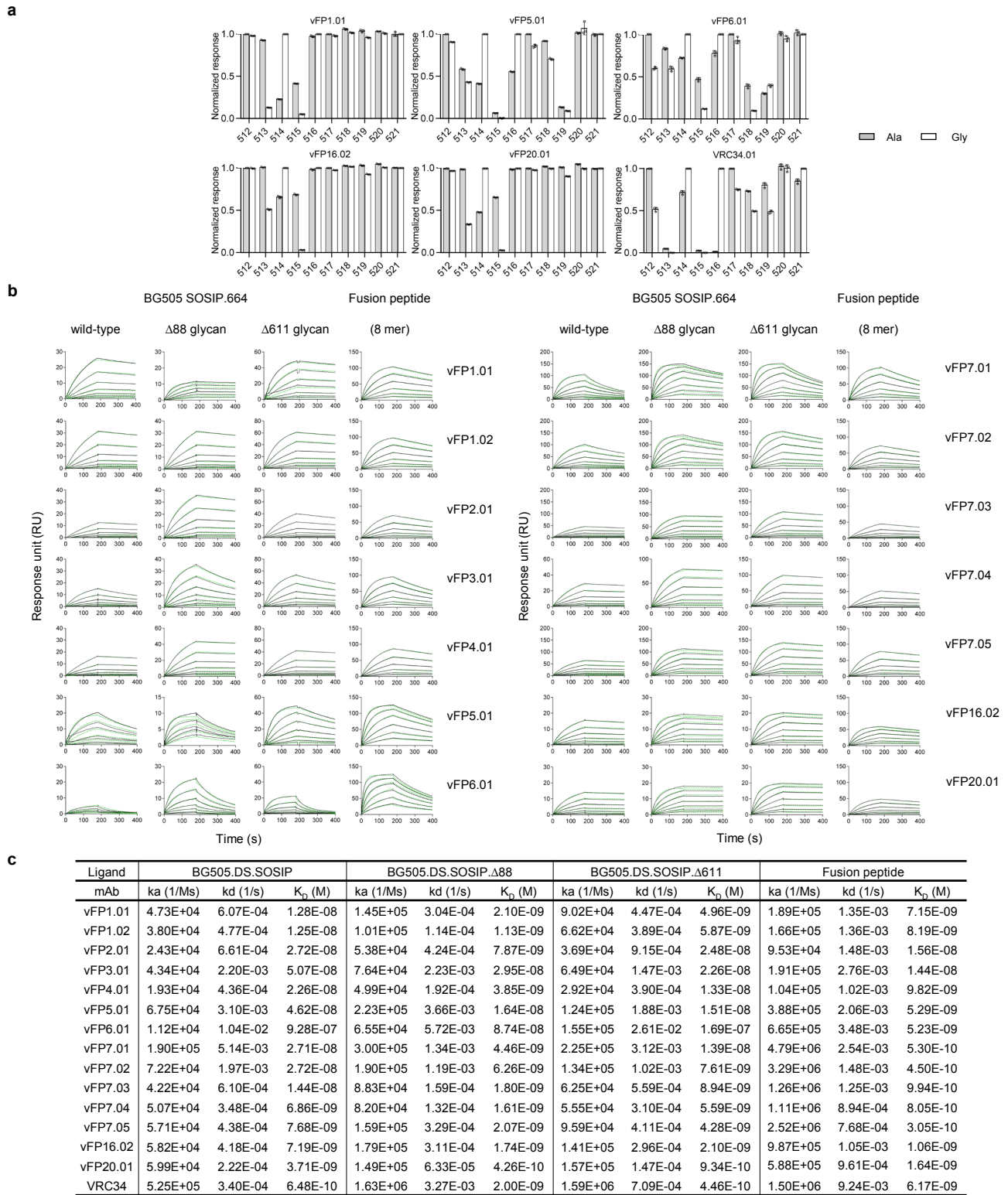
Supplementary Figure 3. First generation vaccine-elicited antibodies targeting FP neutralize up to ~10% of HIV-1 strains. **a**, Genetic characteristics of vaccine-elicited antibodies recognizing both HIV-1 Env and FP. Identity values are based on nucleotide sequence of the indicated heavy or light chain gene. **b**, Heavy and light chain phylogenetic tree. **c**, Neutralization dendrograms tested on 208-isolate panel for vaccine-elicited antibodies vFP1.01, vFP5.01 and vFP7.04, with branches only shown for neutralized strains and colored according to neutralization potency. Strains sensitive to V3-directed or CD4-induced antibodies shown in italics. See Supplementary Table 3a for titers.



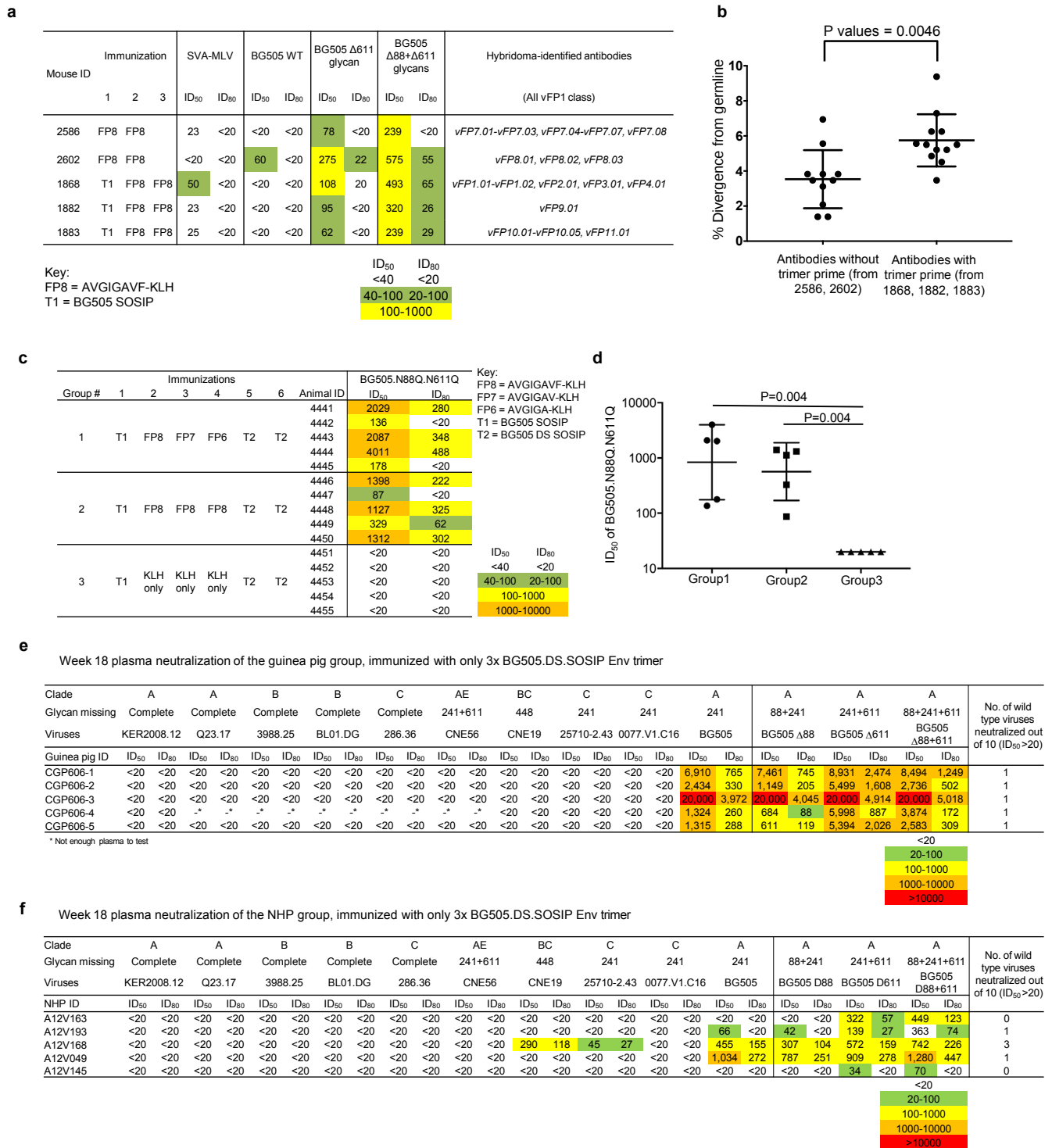
Supplementary Figure 4. Structural details of FP-conformational diversity and recognition by antibody. (a-b) vFP1.01 crystal structure with FP. **a**, Interaction between vFP1.01 and FP (contacting residues are shown as sticks). **b**, Ligplot showing the contact between vFP1.01 and FP. (c-d) vFP5.01 crystal structure with FP. **c**, Interaction between vFP5.01 and FP (contacting residues are shown as sticks). **d**, Ligplot showing the contact between vFP5.01 and FP. **e**, Principal component analysis of FP conformation based on molecular dynamics simulations of fully glycosylated HIV-1-Env trimer. Principal component projections (in transparency) are shown for HIV-1-fusion peptides bound by antibody (or for Clade G Env trimer-5FYJ). Four prevalent clusters of fusion-peptide conformations were observed. Most prevalent was a symmetrical U-shaped conformation, which was recognized by FP1.01 and also observed in the fully glycosylated Env trimer from a Clade G isolate. A J-shaped conformation, recognized by FP5.01, was also highly prevalent, as was the extended linear conformation, recognized by VRC34.01. A related extended conformation was also recognized by antibody PGT151, though the conformational space sampled by the PGT151-bound antibody was substantially less dense than the other antibody-recognized conformations. **f**, Conformational superposition of the peptides analyzed in (e), along with the principal component projection (in transparency). Despite substantial difference in secondary structure, the two conformations share similar global shape. Thus, even though the FP-conformation in 5FYJ is slightly helical (one turn), both peptides adopt a symmetrical "U"-shape conformation. **g**, Superimposition of vFP1-class antibodies in complex with FP (residues 512-519). Antibody variable regions of vFP1-class antibodies, including vFP1.01, vFP7.04, vFP16.02 and vFP20.01, were aligned structurally. **h**, SA omit map of FP, in the crystal structure of vFP16.02 bound complex.



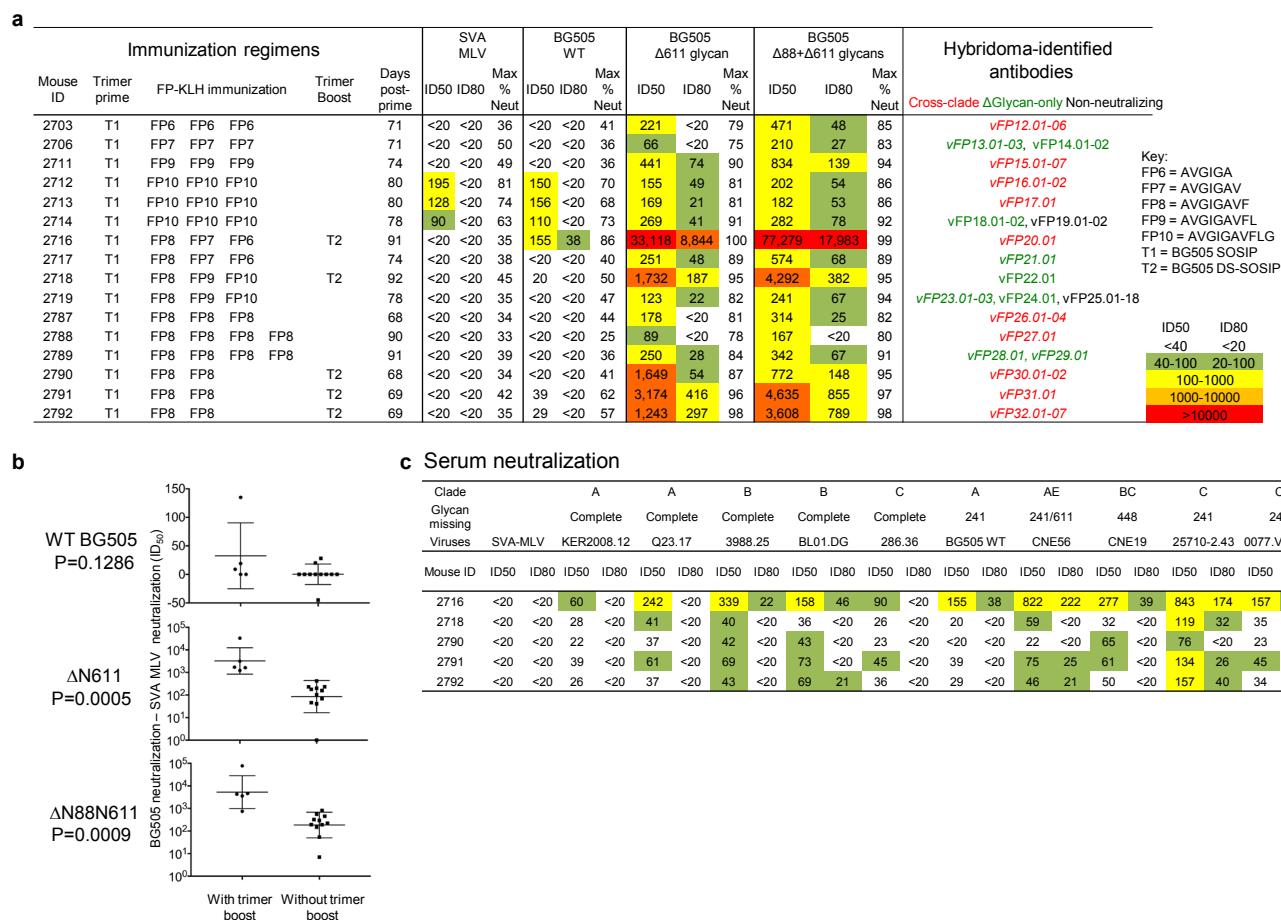
Supplementary Figure 5. Cryo-EM details for reconstructions of up to 8.6 Å resolution. (a-e) Details of cryo-EM structure of vFP1.01 complex with BG505 SOSIP trimer. **a**, Representative micrograph (left) with particles picked with DoGPicker shown in red circles (right). **b**, Representative 2D class averages. **c**, Views of the 3D class selected for refinement. **d**, Snapshot of density and fit of model at different regions of the complex. The panel on the left is shown at a higher map contour than the other two panels to highlight the fit around the gp41 region of the trimer, which is one of the most well-defined regions of the complex. **e**, Fourier shell correlation plot for the 3D refinement ($FSC_{0.143} = 8.6$ Å, indicated by the dotted line). (f-i) Details of cryo-EM structure of vFP5.01 complex with BG505 SOSIP trimer. **f**, Representative micrograph (left) with particles picked with DogPicker shown in red circles. **g**, Representative 2D class averages. **h**, Views of asymmetric 3D classes. **i**, Snapshot of refined Class I density and fit of model at different regions of the complex. **j**, Comparison of the orientation of the vFP5.01 Fab-fusion peptide crystal structure fitted into the cryo-EM map, with its orientation rotated 180° about the longitudinal axis of the Fab. In the fitted model, residue Phe519 of the fusion peptide bound to the Fab in the crystal structure is favorably placed near residue Val518 of the fitted trimer, whereas in the 180°rotated model, Phe519 diverges away from the electron density and gp41 in the fitted trimer. Cryo-EM data collection was performed once for each structure. The represented images in (a, b, f and g) was derived from the analysis of many particles in many images. Additional details on cryo-EM data collection, such as total number of particles, number of images, can be found in Supplementary Table 4.



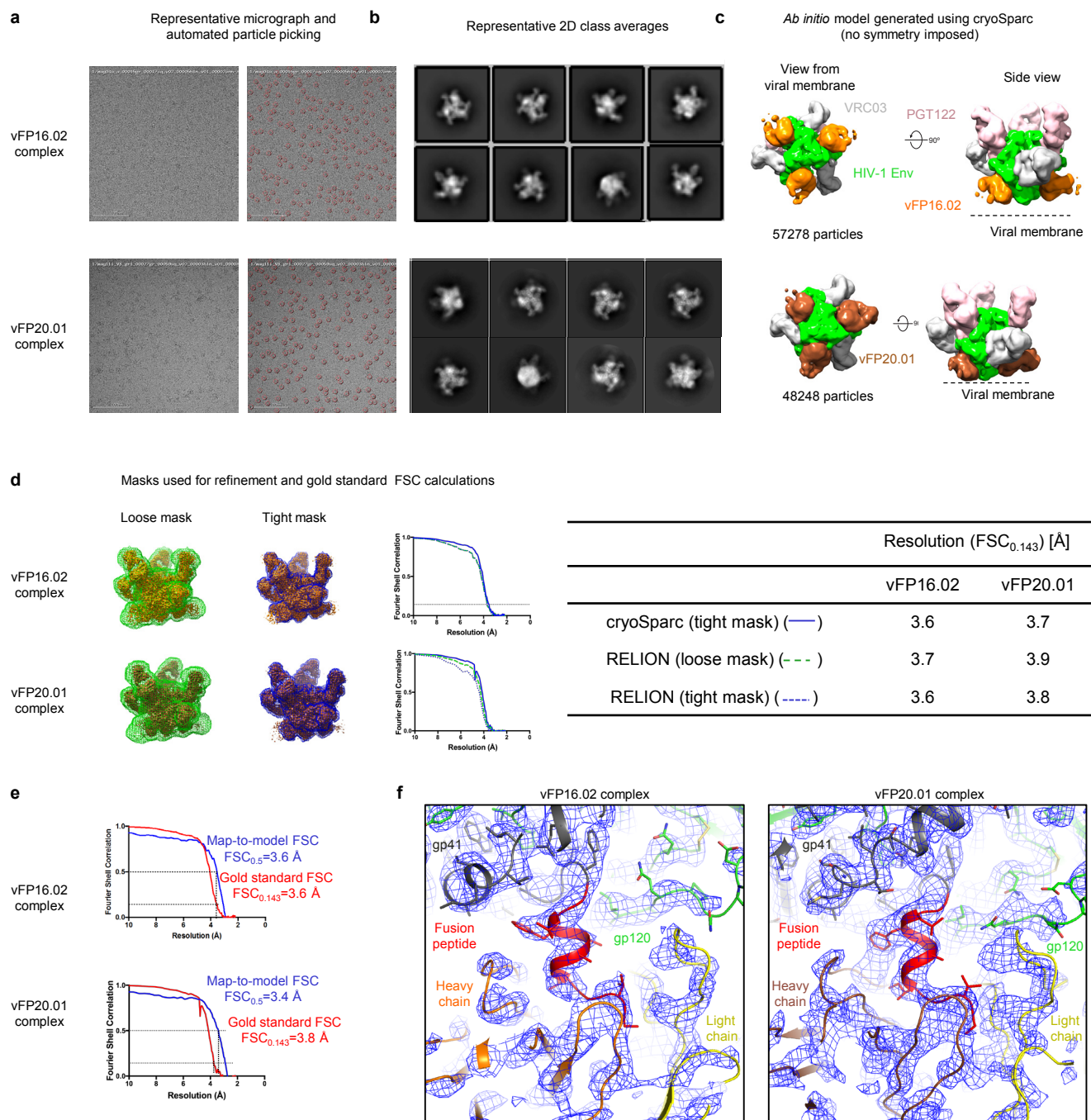
Supplementary Figure 6. Binding of vaccine-elicited antibodies to HIV-1 Env trimers and His-tagged FP. **a**, Bar plots of Ala/Gly scan. Binding of indicated antibodies to alanine (grey bars) and glycine (white bars) mutants are shown, normalized by binding to the wild-type sequence. FP amino acids are shown on x-axis. Experiments were performed in triplicate (n=3 independent experiments), error bars indicating mean with SD are plotted for each residue position in the overlaid scatter dot plot. See Supplementary Table 5 for numerical details and additional vFP1-class antibodies. **b**, SPR sensorgrams of vFP1.01, vFP1.02, vFP2.01, vFP3.01, vFP4.01, vFP5.01, vFP6.01, vFP7.01, vFP7.02, vFP7.03, vFP7.04, vFP7.05, vFP16.02 and vFP20.01 binding to wild type, glycan-deleted BG505.DS-SOSIP trimers and His-tagged FP. Blank-corrected sensorgrams were shown in black and fitted data using a 1:1 Langmuir model of binding were shown in green dashed lines. **c**, SPR constants of all first and second generation vFPs Fabs binding to wild type, glycan-deleted BG505.DS-SOSIP, and FPs.



Supplementary Figure 7. Comparative mouse, guinea pig, and NHP immunization experiments. **a**, FP8-KLH mouse immunizations with or without Env-trimer prime. Sera neutralization and antibody isolation is shown. **b**, Two tailed Mann-Whitney comparing of vFP1-class antibody SHM with and without Env-trimer prime (n=11 for Antibodies without trimer prime, n= 12 for Antibodies with trimer prime, means ± s.d were displayed). **(c-d)** Repeat immunization experiment of mouse ID 2716 with control. **c**, Experimental mouse immunization schema and neutralization on Δ88+611 BG505 virus. **d**, Statistical comparison, by single-tailed Mann-Whitney; Geometric mean and geometric SD were displayed, n= 5 animals. **e**, Week 18 plasma neutralization of a guinea pig comparator group, immunized with 3x BG505 DS-SOSIP Env trimer at week 0, 4 and 16. **f**, Week 18 plasma neutralization of a NHP comparator group, immunized with 3x BG505 DS-SOSIP Env trimer at week 0, 4 and 16.

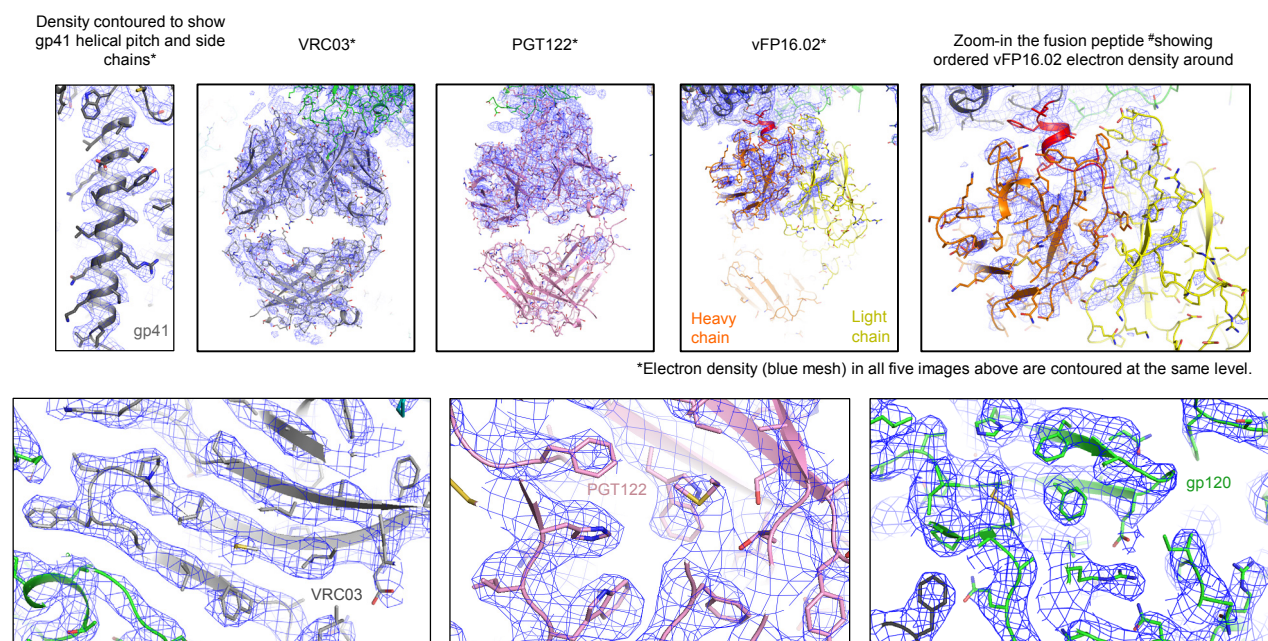


Supplementary Figure 8. Second-generation murine immunization schema and elicited serum neutralization. a, 16 mice and 11 immunization schema (left), with neutralization (middle) and hybridoma-identified antibodies (right). Names for vFP1-class antibodies are italicized and colored according to neutralization properties as indicated. **b**, Trimer boost induced significantly higher serum neutralization titers by two-tailed Mann-Whitney. $n=5$ for “with trimer boost” group and $n=11$ for “without trimer group boost”; mean and SD were displayed for WT BG505 panel, and geometric mean and geometric SD were displayed for the other two panels. **c**, Neutralization by trimer-boosted sera on 10 wildtype isolates, 5 with complete glycans around FP and 5 naturally missing select glycans as specified, with ID50/ID80 colored as in (a). **d**, Neutralization dendrograms. Top row: 208-isolate panel for vaccine-elicited antibodies vFP16.02 and vFP20.01,

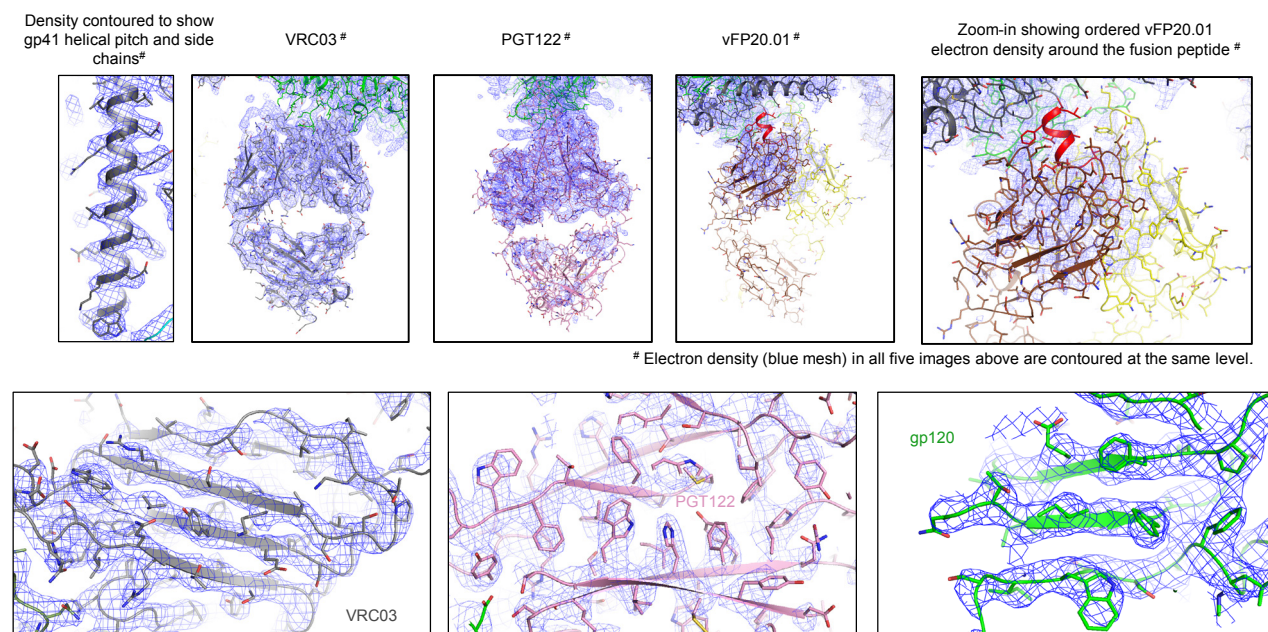


Supplementary Figure 9. Cryo-EM structural details for reconstructions of better than 4 Å resolution. (a-f) Details of cryo-EM structures of vFP16.02-BG505 DS-SOSIP-VRC03-PGT122 and vFP20.01-BG505 DS-SOSIP-VRC03-PGT122 complexes. **a.** Representative micrograph (left) with particles picked with DoGPicker shown in red circles (right). **b.** Representative 2D class averages. **c.** *Ab initio* models generated using cryoSparc. **d.** Refined map in orange with the mask used for the final iteration of cryoSparc refinement (green) and for FSC calculation (blue) shown as mesh. FSC plots and resolution values reported by cryoSparc and RELION according to the gold standard FSC_{0.143} criterion (FSC_{0.143} shown as dotted line). **e.** Gold standard (red) and map-to-model (blue) FSC correlation curves. The map-to-model FSC curve was calculated for protein residues within the complex excluding the antibody constant domains. **f.** Zoomed-in view of the region around the fusion peptide with electron density shown in blue mesh. Cryo-EM data collection was performed once for each structure. The represented images in (a and b) was derived from the analysis of many particles in many images. Additional details on cryo-EM data collection, such as total number of particles, number of images, can be found in Supplementary Table 4.

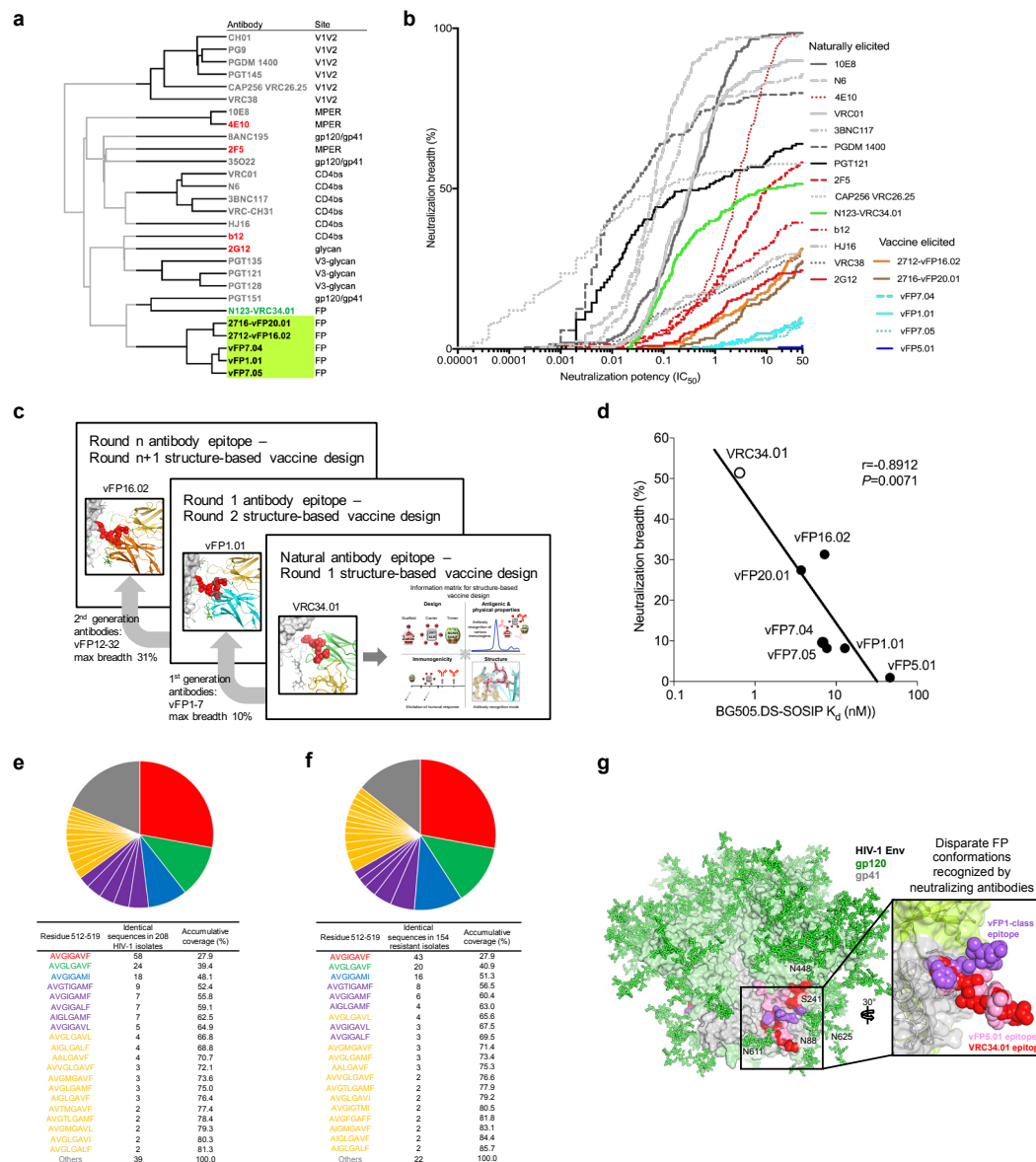
a Representative electron density in vFP16.02.DS.SOSIP-VRC03-PGT122 reconstruction



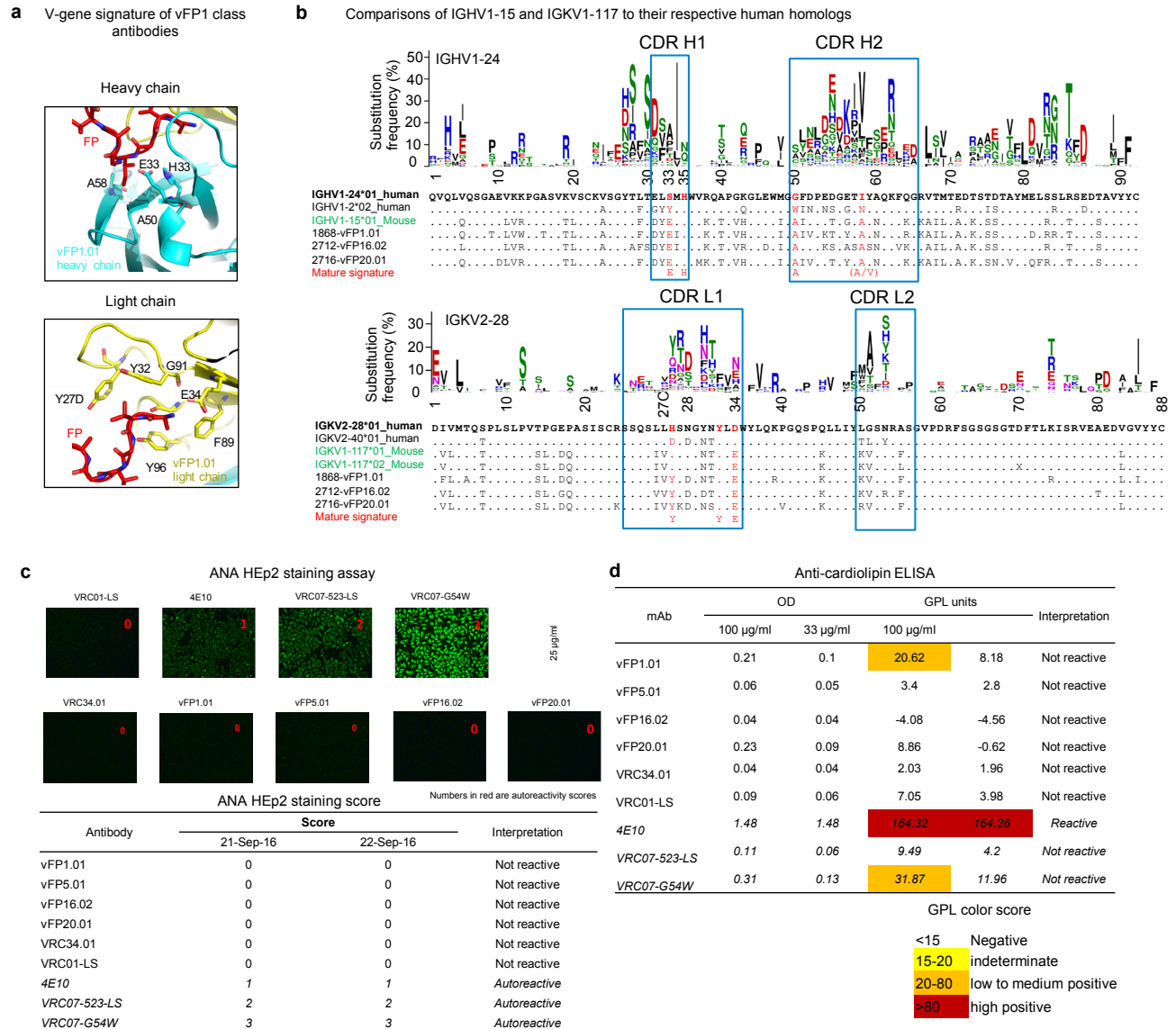
b



Supplementary Figure 10. Cryo-EM reconstructions reveal focused recognition of the HIV-1 fusion peptide by antibodies vFP16.02 and vFP20.01. a, Top panel. Focused recognition of fusion peptide by vFP16.01. Bottom panel. Representative electron density plots from different regions of the vFP16.02-DS.SOSIP-VRC03-PGT122 structure. b, Same as a, but for the vFP20.01-DS.SOSIP-VRC03-PGT122 complex.



Supplementary Figure 11. Iterative epitope-based vaccine development to achieve neutralization of heterologous viral strains. **a**, Dendrogram representing neutralization fingerprints shows vaccine-elicited antibodies to cluster with FP antibodies isolated from HIV-1 infected donors, though as a separate group. **b**, Comparison of HIV-1-neutralization breadth and potency by antibodies isolated from HIV-1 infected donors to vaccine-elicited antibodies. **c**, Schematic showing process of iterative structure-based vaccine design, which involves analysis of elicited antibodies to provide insight into the design and improvement of immunogens and immunization regimens for subsequent cycles of improvement. **d**, Neutralization breadth of FP-directed antibodies versus affinity for stabilized Env trimer. Pearson correlation was displayed, $n=7$ antibodies. **e**, Sequence diversity of the N-terminal eight residues of FP (residue 512 to 519) in the 208-isolate panel, in pie chart format (top) and table format ranked by their prevalence (bottom). The accumulative coverage of the sequence diversity of these five residues is also shown. **f**, Same as (e), but with 154 isolates resistant to neutralization by CD4-induced antibodies, V3-directed antibodies, and F105 antibody. **g**, vFP-defined site of vulnerability allows for target-site conformational diversity.



Supplementary Figure 12. vFP1-class antibodies: V-gene similarity to human homologues and autoreactivity. **a**, V-gene signature of vFP1 class antibodies. Signature residues for heavy chain (top) and light chain (bottom) are showed in sticks and labeled. **b**, Comparisons of IGHV1-15 and IGKV1-117 to their respective human homologs identified by IMGT/V-QUEST and mutation profile of human gene IGHV1-24 and IGKV2-28. Compared to mouse IGHV1-15, human IGHV1-24 contains the H35 signature, and the A50 and A/V58 signatures can be reproduced by somatic hypermutation with high frequencies (See gene-specific substitution profile of IGHV1-24 built from 190 mature human antibody lineages, with one sequence per lineage). This suggested similar key signatures may be reproduced by human antibodies. However, the alignment also showed multiple germline gene positions are diversified between the homolog genes, further investigations are required to understand whether they will affect FP recognition. The mouse IGKV1-117 was aligned to two human kappa chain genes, IGKV2-28 and IGKV2-40. Similar to mouse IGKV1-117, the IGKV2-28 also has a His-Ser-Asn motif in CDR L1. We built a substitution profile for IGKV2-28 using 104 antibody lineages derived from repertoires of three healthy human donors. The substitution profile showed that the key signatures of vFP1 antibodies, Y27d, Y32, and E34 are either germline residue or mutations with high frequency in human IGKV2-28 antibodies, suggesting the signatures observed in the vFP1 antibodies can be obtained with high frequency. V gene positions numbered using Kabat system. **(c-d)** Autoreactivity analysis of FP-directed antibodies. **c**, ANA Hep-2 staining analysis showed none of the five FP-directed antibodies are autoreactive. Control antibodies with known autoreactivity are italicized. The experiment was performed twice (n=2) independently. **d**, Anti-cardiolipin ELISA showed none of the three FP-directed antibodies to be reactive. Control values italicized as in (c). The experiment was performed only once.

a. Amino acid sequence alignment of heavy chain variable region.

CDR regions are defined according to kabat numbering system. We define antibody lineages to be members of the same antibody class if lineage members have the same mode of recognition and utilize similar B cell pathways of development (Kwong and Mascola, 2012).

[illegible]

Chemical Characteristics	Amino Acids
Polar Positive charge	HKR
Polar Negative charge	DE
Polar Neutral	CNQST
Non-polar Neutral	AGILMPV
Nonpolar Aromatic Neutral	FWY

a

Germline nucleotide and amino acid residues are shown in black with the corresponding junctions colored in light blue. Somatic hypermutation events are colored red. Nucleotides deleted by exonuclease trimming are crossed out. The N, P additions occurring at the junctions and the gene assignment are from IMGT Vquest (http://www.imgt.org/IMGT_vquest/share/textes/).

Supplementary Table 2. V(D)J Recombination of vFP lineages. (continued from prior page)

b

1868							
Light chain							
L	vFP1.01	vFP1.02	vFP2.01	vFP3.01	vFP4.01	vFP5.01	vFP6.01
H							
vFP1.01		0	15	15	0	132	93
vFP1.02	0		15	15	0	132	93
vFP2.01	20	20		0	15	143	96
vFP3.01	17	17	3		15	143	96
vFP4.01	41	41	56	58		132	93
vFP5.01	177	177	174	174	179		140
vFP6.01	81	81	63	66	76	173	
Heavy chain							

2706					
Light chain					
L	vFP13.01	vFP13.02	vFP13.03	vFP14.01	vFP14.02
H					
vFP13.01		0	0	65	65
vFP13.02	13		0	65	65
vFP13.03	13	0		65	65
vFP14.01	66	62	62		0
vFP14.02	65	60	60	2	
Heavy chain					

1883						
Light chain						
L	vFP10.01	vFP10.02	vFP10.03	vFP10.04	vFP10.05	vFP11.01
H						
vFP10.01		0	0	0	0	0
vFP10.02	1		0	0	0	0
vFP10.03	3	2		0	0	0
vFP10.04	3	2	0		0	0
vFP10.05	1	0	2	2		0
vFP11.01	31	31	31	31	31	
Heavy chain						

2714				
Light chain				
L	vFP18.01	vFP18.02	vFP19.01	vFP19.02
H				
vFP18.01		0	73	73
vFP18.02	0		73	73
vFP19.01	148	148		0
vFP19.02	148	148	0	
Heavy chain				

2789		
Light chain		
L	vFP28.01	vFP29.01
H		
vFP28.01		0
vFP29.01	17	
Heavy chain		

2719																										
Light chain																										
L	vFP23.01	vFP23.02	vFP23.03	vFP24.01	vFP25.01	vFP25.02	vFP25.03	vFP25.04	vFP25.05	vFP25.06	vFP25.07	vFP25.08	vFP25.09	vFP25.10	vFP25.11	vFP25.12	vFP25.13	vFP25.14	vFP25.15	vFP25.16	vFP25.17	vFP25.18	vFP25.19	vFP25.20	vFP25.21	vFP25.22
H																										
vFP23.01		0	0	65	0	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
vFP23.02	0		0	65	0	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
vFP23.03	0	0		65	0	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
vFP24.01	80	80	80		65	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73	73
vFP25.01	85	85	85	62		8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
vFP25.02	85	85	85	62	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vFP25.03	85	85	85	62	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vFP25.04	85	85	85	62	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vFP25.05	85	85	85	62	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vFP25.06	85	85	85	62	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vFP25.07	85	85	85	62	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
vFP25.08	85	85	85	62	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0
vFP25.09	85	85	85	62	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0
vFP25.10	85	85	85	62	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0
vFP25.11	85	85	85	62	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0
vFP25.12	85	85	85	62	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0
vFP25.13	85	85	85	62	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0
vFP25.14	83	83	83	60	21	21	21	21	21	21	21	21	21	21	21	21	21		0	0	0	0	0	0	0	0
vFP25.15	86	86	86	63	1	1	1	1	1	1	1	1	1	1	1	1	1	1	22		0	0	0	0	0	0
vFP25.16	81	81	81	66	10	10	10	10	10	10	10	10	10	10	10	10	10	10	22	11		0	0	0	0	0
vFP25.17	85	85	85	62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	1	10		0	0	0	0
vFP25.18	85	85	85	62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21	1	10	0		0	0	0
Heavy chain																										

For each mouse with more than one vFP lineage, we performed pairwise comparison of nucleotide differences separating germline versions of heavy (blue) and light (yellow) chain variable regions. Mouse ID is listed in upper left of each table.

a. vFP antibody neutralization against a panel of 208 isolates.

[illegible]

Supplementary Table 3. vFP antibody and sera neutralization.

b. vFP antibody neutralization against a panel of 10 isolates.

IC50											IC80										
HIV strains	KER2008.12	Q23.17	3988.25	BL01.DG	286.36	BG505.W6M.C2	CNE56	CNE19	25710-2.43	0077.V1.C16	KER2008.12	Q23.17	3988.25	BL01.DG	286.36	BG505.W6M.C2	CNE56	CNE19	25710-2.43	0077.V1.C16	
1868-vFP1.01	19.1	3.01	>100	>50	>50	21.9	4.49	2.96	0.756	7.1	>50	>50	>100	>50	>50	>100	39.9	31.7	4.32	44.3	
2586-vFP7.07	28.3	34.6	>50	>50	>50	43.6	9.2	9.18	4.63	>50	>50	>50	>50	>50	>50	>50	40.4	>50	30.3	>50	
2602-vFP8.02	7.35	1.01	1.96	5.86	10.9	2.2	0.715	0.48	0.513	3.39	>50	>50	>50	43.2	>50	22.3	2.92	3.36	2.26	12.1	
2602-vFP8.03	>50	21.3	17.9	>50	18.1	>50	12.5	36.8	18.4	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	
2682-vFP9.01	6.29	6.83	>50	>50	>50	26.9	16	1.32	1.69	18.3	>50	>50	>50	>50	>50	>50	>50	8.97	8.49	>50	
2703-vFP12.01	>50	13.9	5.01	>50	>50	>50	25.9	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	
2703-vFP12.02	>50	11.3	36.1	>50	>50	>50	8.04	7.53	8.13	38.5	>50	>50	>50	>50	>50	>50	>50	30.3	49.3	40.7	
2703-vFP12.04	11.4	4.31	3.58	8.16	35.6	6.96	0.831	3.9	0.796	8.47	>50	>50	>50	41.3	>50	>50	3.47	23.8	4.06	36.1	
2703-vFP12.06	36.1	28.2	5.67	32.6	>50	15.3	8.6	5.96	6.45	>50	>50	>50	>50	>50	>50	49.1	30.5	39.2	23.1	>50	
2711-vFP15.01	45.5	24.5	>50	>50	40.3	44.9	6.07	9.61	2.97	>50	>50	>50	>50	>50	>50	>50	31.1	>50	24.8	>50	
2711-vFP15.02	4.15	1.55	6.68	32.3	20	9.24	2.85	2.38	1.51	13.2	>50	>50	>50	>50	>50	>50	10.4	24.3	5.05	>50	
2711-vFP15.03	3.97	14.4	>50	>50	10.9	21.2	27.6	0.922	0.562	23.8	>50	>50	>50	>50	>50	>50	>50	7.42	2.05	>50	
2711-vFP15.04	19.8	5.16	>50	>50	>50	36.1	3.79	4.66	1.73	23.3	>50	>50	>50	>50	>50	>50	10.3	>50	9.96	>50	
2711-vFP15.05	23	6.39	4.75	>50	13.7	17.9	7.38	3.34	2.09	23.5	>50	>50	>50	>50	>50	>50	15.8	42.4	8.88	>50	
2711-vFP15.07	2.01	5.91	46.2	>50	18.2	>50	6.52	3.82	0.751	6.84	>50	>50	>50	>50	>50	>50	25.4	>50	3.03	44.3	
2712-vFP16.02	1.44	0.84	6.72	3.26	2.67	2.64	1.06	0.203	0.355	1.29	14	13.4	>50	14.3	35	9.31	3.6	0.688	1.21	3.83	
2713-vFP17.01	>50	>50	7.2	12.3	>50	18.9	19.3	4.05	1.46	23.7	>50	>50	>50	>50	>50	>50	>50	>50	13	>50	
2716-vFP20.01	2.84	1.67	1.16	3.91	6.56	2.47	1.82	0.644	0.608	4.39	>50	40.7	>50	23.2	>50	8.15	6.96	3.63	3.39	20.3	
2787-vFP26.02	>50	15.1	8.51	>50	>50	28.5	17.1	23.1	3.95	18.5	>50	>50	>50	>50	>50	>50	>50	>50	22.6	>50	
2787-vFP26.03	3.96	27.7	>50	22.7	>50	39.2	8.43	0.539	0.574	9.46	>50	>50	>50	>50	>50	>50	29.6	3.54	2.37	>50	
2787-vFP26.04	4.25	2.91	8.42	19.8	28.8	6.04	10.6	1.3	1.23	15.2	>50	>50	>50	>50	>50	>50	26.7	>50	8.24	4.49	
2788-vFP27.01	1.84	6.9	9.55	>50	24.8	16.4	11	1.45	1.03	28.2	>50	>50	>50	>50	>50	>50	>50	46.3	11.1	5.41	
2790-vFP30.01	5.48	3.18	9.71	7.39	40.2	1.73	4.17	2.12	2.03	7.28	>50	>50	>50	42.3	>50	5.6	12.1	14.1	8.11	30.3	
2791-vFP31.01	7.05	1.92	14.8	17.3	36.9	21.5	3.92	1.27	1.14	9.7	>50	>50	>50	>50	>50	>50	>50	21.6	7.47	3.5	
2792-vFP32.01	35.9	30.4	>50	35.3	>50	9.38	10.7	2.86	3.13	11.2	>50	>50	>50	>50	>50	32	40.6	17.4	12.7	40.8	
2792-vFP32.03	>50	33.1	>50	2.33	>50	0.751	1.11	7.93	1.27	2.53	>50	>50	>50	>50	>50	2.03	2.62	>50	8.05	11.8	
2792-vFP32.04	34.3	4.75	>50	8.55	>50	17.2	4.46	2.38	1.03	26.7	>50	>50	>50	>50	>50	>50	23	22.3	3.73	>50	
2792-vFP32.06	20.2	44.8	>50	>50	>50	24.6	10.9	2.4	2.42	44.2	>50	>50	>50	>50	>50	>50	49.8	24.9	19.2	>50	
2792-vFP32.07	>50	8.49	>50	2.86	>50	1.67	1.17	10.7	1.14	2.93	>50	>50	>50	>50	>50	6.09	3.66	>50	7	15.4	
N123-VRC34.01	0.028	0.083	0.028	0.048	4.11	0.069	>50	0.033	0.128	0.721	0.138	0.36	0.111	0.193	>50	0.331	>50	1.54	6.22	24.2	

Supplementary Table 3. vFP antibody and sera neutralization.

c. NHP sera neutralization against a panel of 58 isolates with matching FP sequence in the first 8 residues to the immunogens and naturally missing glycans highlighted in gray.

Virus ID	Clade	Naturally missing glycan in FP region				Env trimer only (Wk18)		NHP DF3R		NHP DFPE		NHP DF1W		NHP DF4B		NHP GPV		
		88	241	448	611	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	ID50	ID80	
0439.v5.c1	A					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
3365.v2.c20	A					<20	<20	<20	<20	<20	<20	34	<20	<20	<20	<20	<20	
398-F1_F6_20	A					<20	<20	<20	<20	<20	<20	111	<20	<20	<20	32	<20	
BB201.B42	A					<20	<20	<20	<20	<20	<20	55	<20	<20	<20	24	<20	
BB539.2B13	A					<20	<20	<20	<20	<20	<20	62	<20	<20	<20	<20	<20	
BG505.W6M.C2	A					526	149	129	<20	133	<20	240	31	33	<20	48	<20	
BI369.9A	A					<20	<20	<20	<20	<20	<20	73	<20	<20	<20	<20	<20	
KER2008.12	A					<20	<20	<20	<20	<20	<20	76	<20	<20	<20	<20	<20	
KNH1209.18	A					<20	<20	<20	<20	<20	<20	40	<20	<20	<20	<20	<20	
MB201.A1	A					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
MB539.2B7	A					<20	<20	<20	<20	<20	<20	24	<20	<20	<20	<20	<20	
MI369.A5	A					<20	<20	<20	<20	<20	<20	144	<20	<20	<20	23	<20	
Q23.17	A					<20	<20	<20	<20	35	<20	214	39	<20	<20	40	<20	
Q259.17	A					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
Q842.d12	A					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
QH209.14M.A2	A					<20	<20	<20	<20	<20	<20	182	29	<20	<20	<20	<20	
RW020.2	A					<20	<20	<20	<20	27	<20	100	27	<20	<20	24	<20	
246-F3.C10.2	AC					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
3301.V1.C24	AC					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
3589.V1.C4	AC					<20	<20	<20	<20	<20	<20	21	<20	<20	<20	<20	<20	
0815.V3.C3	ACD					<20	<20	<20	<20	30	<20	228	29	<20	<20	26	<20	
Q168.a2	AD					<20	<20	<20	<20	<20	<20	32	<20	<20	<20	<20	<20	
3988.25	B					<20	<20	<20	<20	<20	<20	45	<20	<20	<20	<20	<20	
45_01dG5	B					<20	<20	<20	<20	<20	<20	64	<20	<20	<20	<20	<20	
B9.6.DG	B					<20	<20	<20	<20	20	<20	23	<20	<20	<20	<20	<20	
Bal.01	B					<20	<20	<20	<20	<20	<20	78	<20	<20	<20	<20	<20	
BL01.DG	B					<20	<20	<20	<20	<20	<20	123	34	<20	<20	<20	<20	
CNE57	B					<20	<20	<20	<20	34	<20	20	<20	<20	<20	37	<20	
HO86.8	B					<20	<20	<20	<20	26	<20	52	<20	<20	<20	<20	<20	
JRFLJB	B					<20	<20	<20	<20	<20	<20	61	<20	<20	<20	<20	<20	
B3JOX002000.0.3.2	BC					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
CH117.4	BC					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
CH119.10	BC					<20	<20	<20	<20	26	<20	36	<20	<20	<20	<20	<20	
CNE15	BC					<20	<20	<20	<20	<20	<20	45	<20	<20	<20	<20	<20	
CNE19	BC					54	<20	<20	<20	93	28	198	20	28	<20	64	<20	
CNE21	BC					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
CNE40	BC					33	<20	22	<20	<20	<20	<20	<20	<20	<20	<20	<20	
286.36	C					<20	<20	<20	<20	<20	<20	154	27	<20	<20	<20	<20	
0921.V2.C14	C					<20	<20	<20	<20	<20	<20	27	<20	<20	<20	<20	<20	
25710.2.43	C					23	<20	<20	<20	100	24	859	244	22	<20	123	40	
3873.V1.C24	C					<20	<20	<20	<20	<20	<20	147	36	<20	<20	<20	<20	
426c	C					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
96ZM651.02	C					<20	<20	<20	<20	44	<20	169	<20	<20	<20	32	<20	
BR025.9	C					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
CAP210.E8	C					<20	<20	<20	<20	23	<20	21	<20	<20	<20	<20	<20	
Ce1176.A3	C					<20	<20	<20	<20	<20	<20	97	<20	<20	<20	<20	<20	
CNE31	C					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
CNE58	C					<20	<20	<20	<20	<20	<20	121	<20	<20	<20	<20	<20	
DU172.17	C					<20	<20	<20	<20	<20	<20	42	<20	<20	<20	<20	<20	
MW965.26	C					92	40	151	54	77	34	130	40	32	20	83	40	
TV1.29	C					<20	<20	<20	<20	<20	<20	28	<20	<20	<20	<20	<20	
ZA012.29	C					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
ZM109.4	C					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
ZM135.10a	C					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
ZM176.66	C					<20	<20	<20	<20	<20	<20	145	32	<20	<20	<20	<20	
ZM214.15	C					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
ZM233.6	C					<20	<20	<20	<20	<20	<20	71	<20	<20	<20	<20	<20	
ZM55.28a	C					<20	<20	<20	<20	<20	<20	102	21	<20	<20	<20	<20	
SVA.MLV	NA					<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
Total number of tested						58	58	58	58	58	58	58	58	58	58	58	58	
Number of neutralized (ID50/80>criteria)						5	2	3	1	12	3	41	13	4	0	12	2	
% neutralized of tested						9%	3%	5%	2%	21%	5%	71%	22%	7%	0%	21%	3%	100-1000
at least % neutralized of total 208						2%	1%	1%	0%	6%	1%	20%	6%	2%	0%	6%	1%	20-100
Geometric mean ID50/80 of neutralized						72	77	75	54	42	28	75	35	28	20	40	40	<20

Supplementary Table 4. Data collection and refinement statistics.

a. Crystal data collection and refinement statistics (molecular replacement)

	vFP1.01:FP	vFP5.01:FP	vFP7.04:FP	vFP16.02:FP	vFP20.01:FP
PDB accession code	5TKJ	5TKK	6CDM	6CDO	6CDP
Data collection					
Space group	P 2 ₁ 2 ₁ 2 ₁	P 2 ₁	P 2 ₁	P 4 ₁ 2 ₁ 2	C 2 2 2 ₁
Cell dimensions					
<i>a</i> , <i>b</i> , <i>c</i> (Å)	76.16, 120.18, 226.18	36.24, 82.23, 72.26	79.57, 61.03, 88.65	141.03, 141.03, 77.64	60.46, 192.31, 87.56
α , β , γ (°)	90.00, 90.00, 90.00	90.00, 90.23, 90.00	90.00, 91.89, 90.00	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å)	50 - 2.12 (2.16 - 2.12)*	50 - 1.55 (1.58 - 1.55)	50 - 2.41 (2.49 - 2.41)	50 - 2.10 (2.17 - 2.10)	50 - 2.46 (2.54 - 2.46)
<i>R</i> _{sym} or <i>R</i> _{merge}	0.093 (0.517)	0.066 (0.468)	0.127 (0.412)	0.108 (0.931)	0.118 (0.423)
<i>I</i> / σ <i>I</i>	20.7 (2.4)	16.1 (2.1)	10.0 (2.0)	30.9 (2.2)	19.8 (2.1)
Completeness (%)	99.6 (97.8)	97.2 (85.2)	99.3 (94.1)	99.8 (98.3)	97.4 (83.2)
Redundancy	7.1 (5.4)	3.5 (2.1)	3.5 (2.8)	13.9 (9.7)	6.4 (4.9)
Refinement					
Resolution (Å)	43.54 - 2.12 (2.16 - 2.12)	25.47 - 1.55 (1.58 - 1.55)	44.3 - 2.41 (2.49 - 2.41)	41.95 - 2.10 (2.17 - 2.10)	48.17 - 2.46 (2.54 - 2.46)
No. reflections	843,512	209,617	113,946	641,724	118,218
<i>R</i> _{work} / <i>R</i> _{free}	0.20 (0.24) / 0.24 (0.29)	0.21 (0.31) / 0.25 (0.39)	0.18 (0.22) / 0.22 (0.28)	0.19 (0.26) / 0.23 (0.31)	0.21 (0.32) / 0.26 (0.39)
No. atoms	14,526	3693	7075	3805	3478
Protein	13,412	3356	6724	3460	3406
Ligand/ion (SO ₄)	35			50	10
Water	1079	337	351	295	62
<i>B</i> -factors (Å ²)	47	38	28	45	55
Protein	47	37	27	44	55
Ligand/ion	56			75	66
Water	43	42	32	49	49
R.m.s. deviations					
Bond lengths (Å)	0.008	0.01	0.005	0.008	0.003
Bond angles (°)	1.01	1.28	0.91	0.98	0.63

One crystal was used for each structure

*Values in parentheses are for highest-resolution shell.

b. Cryo-EM data collection and refinement statistics

	vFP16.02-DS-SOSIP-VRC03-PGT122	vFP20.01-DS-SOSIP-VRC03-PGT122	vFP1.01-DS-SOSIP	vFP5.01-DS-SOSIP
	EMD-7460	EMD-7459		
	6CDI	6CDE	EMD-8420	EMD-8421, EMD-8422
Data collection and processing				
Magnification	22500	130000	22500	22500
Voltage (kV)	300	300	300	300
Electron exposure (e-/Å ²)	67.18	70.70	67.39	68.58
Defocus range (μm)	~-1.0 - -4.0	~-2.0 - -3.0	~-1.8- -4.0	~-1.0- -3.5
Pixel size (Å)	1.07	1.06	1.07	1.07
Symmetry imposed	C3	C3	C3	C1
Initial particle images (no.)	206112	108962	53263	45231
Final particle images (no.)	57278	48248	14931	7383, 41278
Map resolution (Å)	3.6	3.8	8.6	14.7, 19.6
FSC threshold	0.143	0.143	0.143	0.143
Map resolution range (Å)	3.4-8.9	3.4-8.7		
Refinement				
Initial model used (PDB code)	5FYL, 3SE8, 6CDO	5FYL, 3SE8, 6CDP		
Model resolution (Å)	3.7	3.9		
FSC threshold	0.5	0.5		
Model resolution range (Å)	10-3.6	10-3.4		
Map sharpening <i>B</i> factor (Å ²)	-68.00	-69.47		
Model composition				
Non-hydrogen atoms	33165	33057		
Protein residues	3849	3852		
<i>B</i> factors (Å ²)				
Protein	103.84	97.66		
R.m.s. deviations				
Bond lengths (Å)	0.007	0.008		
Bond angles (°)	0.935	1.40		
Validation				
MolProbity score	1.77	1.76		
Clashscore	6.59	6.0		
Poor rotamers (%)	0.45	0.21		
Ramachandran plot				
Favored (%)	94.18	94.01		
Allowed (%)	5.82	5.99		
Disallowed (%)	0.08	0		

Supplementary Table 5. Impact of alanine and glycine mutations at different FP residue positions on binding of FP-directed antibodies.

a

	vFP1.01	vFP1.02	vFP2.01	vFP3.01	vFP4.01	vFP5.01	vFP6.01	vFP7.01	vFP7.02	vFP7.03	vFP7.04	vFP7.05	vFP16.02	vFP20.01	VRC34.01
512	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
513	0.93	0.97	0.69	0.70	0.44	0.59	0.83	0.90	0.97	1.02	1.04	1.02	1.01	0.99	0.05
514	0.24	0.21	0.06	0.04	0.01	0.41	0.72	0.08	0.10	0.04	0.09	0.09	0.66	0.48	0.72
515	0.44	0.44	0.10	0.04	0.05	0.07	0.47	0.25	0.32	0.27	0.29	0.23	0.69	0.66	0.03
516	1.03	1.07	1.01	1.05	0.75	0.56	0.78	0.99	0.99	0.99	1.02	1.00	0.98	0.99	0.01
517	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
518	0.96	1.06	1.03	1.00	1.02	0.92	0.39	1.01	1.03	1.06	1.02	1.06	1.03	1.02	0.73
519	0.93	1.03	0.98	1.02	1.03	0.14	0.30	0.98	1.02	1.04	1.02	1.03	1.03	1.01	0.80
520	0.99	1.06	1.01	1.07	1.05	1.01	1.01	1.03	1.03	1.05	1.01	1.04	1.05	1.05	1.02
521	0.98	1.08	1.02	1.09	1.01	0.99	1.02	1.03	1.02	1.02	1.02	1.00	1.00	1.00	0.85
< 0.25 0.25 - 0.50 0.50 - 0.75 > 0.75															

Binding of indicated antibodies to alanine mutants normalized to binding to the wild-type FP sequence. Experiments were performed in triplicate (n=3 independent experiments).

b

	vFP1.01	vFP1.02	vFP2.01	vFP3.01	vFP4.01	vFP5.01	vFP6.01	vFP7.01	vFP7.02	vFP7.03	vFP7.04	vFP7.05	vFP16.02	vFP20.01	VRC34.01
512	0.98	1.03	0.85	1.09	0.85	0.91	0.60	0.81	0.94	0.88	0.98	0.96	1.00	0.97	0.52
513	0.12	0.09	0.06	0.03	0.04	0.43	0.59	0.13	0.14	0.17	0.30	0.27	0.51	0.34	0.00
514	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
515	0.05	0.03	0.02	0.02	0.01	0.01	0.12	0.04	0.01	0.01	0.01	0.01	0.03	0.03	0.00
516	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
517	0.83	0.96	0.93	0.93	0.96	0.86	0.93	0.94	0.92	0.94	0.97	0.94	0.97	0.98	0.75
518	0.98	1.02	0.96	0.86	1.01	0.71	0.10	0.95	0.98	1.00	0.98	0.99	1.02	1.00	0.49
519	0.95	0.97	0.84	0.95	0.88	0.09	0.39	0.87	0.92	0.90	0.89	0.90	0.92	0.91	0.49
520	0.99	1.06	0.97	1.09	1.03	1.07	0.95	0.97	0.99	1.00	0.98	1.00	1.01	1.00	1.01
521	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
< 0.25 0.25 - 0.50 0.50 - 0.75 > 0.75															

Binding of indicated antibodies to glycine mutants normalized to binding to the wild-type sequence. Experiments were performed in triplicate (n=3 independent experiments).

Supplementary Table 6. Prevalence of FP residue 512-519 by clade.

Clades A/ACD/AD		Clade AC		Clade AE		Clade AG		Clade B		Clades C/BC		Clades D/CD		Clade G	
Residue 512-519	Count	Residue 512-519	Count	Residue 512-519	Count	Residue 512-519	Count	Residue 512-519	Count	Residue 512-519	Count	Residue 512-519	Count	Residue 512-519	Count
AVGIGAVF	19	AVGIGAVF	3	AVGIGAMI	18	AVGLGAVF	8	AVGIGAVF	8	AVGIGAVF	28	AIGLGAMF	6	AVGIMGAVL	2
AVGLGAVF	3	AVGLGAVF	2	AVGIGTMI	2	AVVGLGAVF	2	AVGTIGAMF	8	AVGLGAVF	10	AIGLGALF	4	EVTLGALF	1
AVTMGAVF	2			AVTIGAMI	1	AVGMGALI	1	AVGIGALF	5	AVGLGAVL	4	AIGLGAVF	3	AVGLGAVI	1
AVFGGAFF	2			AVGIGIMI	1	AVGMGALF	1	AVGIGAMF	3	AVGIGAVL	4	AIGMGALF	1	AVGLGAFL	1
AVVELGAVF	1			AVGIGALL	1	AVGLGALF	1	AVGTLGAMF	2	AVGIGAMF	4			AIGMGAVL	1
AVGMGAVF	1					AVLGAFF	1	AVGLGAMF	2	AALGAVF	3			AIGLGTVL	1
AVGMAAVF	1					AIGMGAVF	1	TVGIGALF	1	AVGMGAVF	2				
AVGMAALF	1					AALGAVF	1	DLGLGALF	1	AVGIGAVI	2				
AIGMGAVF	1							AVTLGAVF	1	AVGIGALF	2				
AIGLGAMF	1							AVTLGAMF	1	TVGIGAVF	1				
								AVGMLGAMF	1	AVVGLGAVF	1				
								AVGLGAVF	1	AVGVGAVM	1				
								AVGLGALF	1	AVGTIGAMF	1				
								AVGIVGAMF	1	AVGLGAVI	1				
								AVGIGAVL	1	AVGLGAMI	1				
								AMGIGAMF	1	AVGLGAMF	1				
								AIGIGAVF	1	AVGGIGAVF	1				
								AALGALF	1	AVGGFGAMI	1				
								AAIGALF	1	AALGALF	1				
										AAGIGAVI	1				

Supplementary Table 7. Association of FP-proximal *N*-glycan sequons (N88, N241, N448, and N611) and neutralization based on the 208-isolate panel.

	vFP1.01	vFP7.04	vFP7.05	vFP16.02	vFP20.01	VRC34.01
88	1	1	1	1	1	0.7037
241	0.0122	0.0233	0.0122	0.9382	0.7846	0.7424
448	1	1	1	0.5804	0.6812	0.0327
611	0.4714	0.5504	0.4714	0.3986	0.9479	0.7037

Values displayed are P-values from the two-tailed Fisher's exact test and were corrected for multiple testing using Holm metric. Statistically significant adjusted P-values are highlighted in red and corresponding contingency tables shown below. n=208 HIV-1 strains.

vFP1.01 - 241

	Resistant	Sensitive
Glycan	190	16
Non-glycan	1	1

vFP7.04 - 241

	Resistant	Sensitive
Glycan	183	16
Non-glycan	5	4

vFP7.05 - 241

	Resistant	Sensitive
Glycan	186	13
Non-glycan	5	4

vFP34.01 - 448

	Resistant	Sensitive
Glycan	94	86
Non-glycan	7	21

Supplementary Table 8. vFP antibody neutralization statistics from the 208-virus isolate panel.

IC ₅₀ <50 µg/ml	1868-vFP1.01	1868-vFP5.01	2586-vFP7.04	2586-vFP7.05	2712-vFP16.02	2716-vFP20.01	N123-VRC34.01
Neutralization breadth on 208 virus panel	8.17%	0.96%	9.62%	8.17%	31.3%	27.4%	51.4%
Neutralization breadth on global virus panel	8.33%	0%	8.33%	16.7%	41.7%	41.7%	75.0%
Neutralization breadth against neutralization-resistant isolates	7.69%	0%	8.33%	8.33%	28.9%	25.0%	50.0%
Neutralization breadth on FP8-sequence viruses	20.7%	1.72%	22.4%	17.2%	72.4%	74.1%	94.8%

IC ₅₀ <100 µg/ml	1868-vFP1.01	1868-vFP5.01	2586-vFP7.04	2586-vFP7.05	2712-vFP16.02	2716-vFP20.01	N123-VRC34.01
Neutralization breadth on 208 virus panel	9.62%	1.92%	11.1%	8.65%	36.5%	32.2%	51.92%
Neutralization breadth on global virus panel	16.7%	0%	8.33%	16.7%	41.7%	50.0%	75.0%
Neutralization breadth neutralization-resistant isolates	7.69%	0%	10.3%	8.97%	34.0%	28.9%	50.6%
Neutralization breadth on FP8-sequence viruses	25.9%	3.45%	27.6%	19.0%	81.0%	82.8%	94.8%

IC ₅₀ <500 µg/ml	2712-vFP16.02
Neutralization breadth on 208 virus panel	46.6%
Neutralization breadth on global virus panel	66.7%
Neutralization breadth neutralization-resistant isolates	46.1%
Neutralization breadth on FP8-sequence viruses	96.6%

Neutralization breadth of FP targeting neutralizing antibodies evaluated based on different virus panels: a 208-isolate panel evaluated in this study; the global panel (deCamp et al. 2013 JVI); the neutralization-resistant strains in the 208-isolate panel (154 strains that were not neutralized by antibodies 17b, 48d, F105, 447-52D and 3074); the 58 strains in the 208-isolate panel that have the identical N-terminal 8-mer of the FP as BG505. Breadth statistics for IC₅₀<50 µg/ml are shown at top, statistics for IC₅₀<100 µg/ml are shown in the middle, and statistics for IC₅₀<500 µg/ml are shown at bottom.