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Supplemental Information

HIV-1 VRC01 Germline-Targeting Immunogens Select Distinct Epitope-Specific B Cell Receptors

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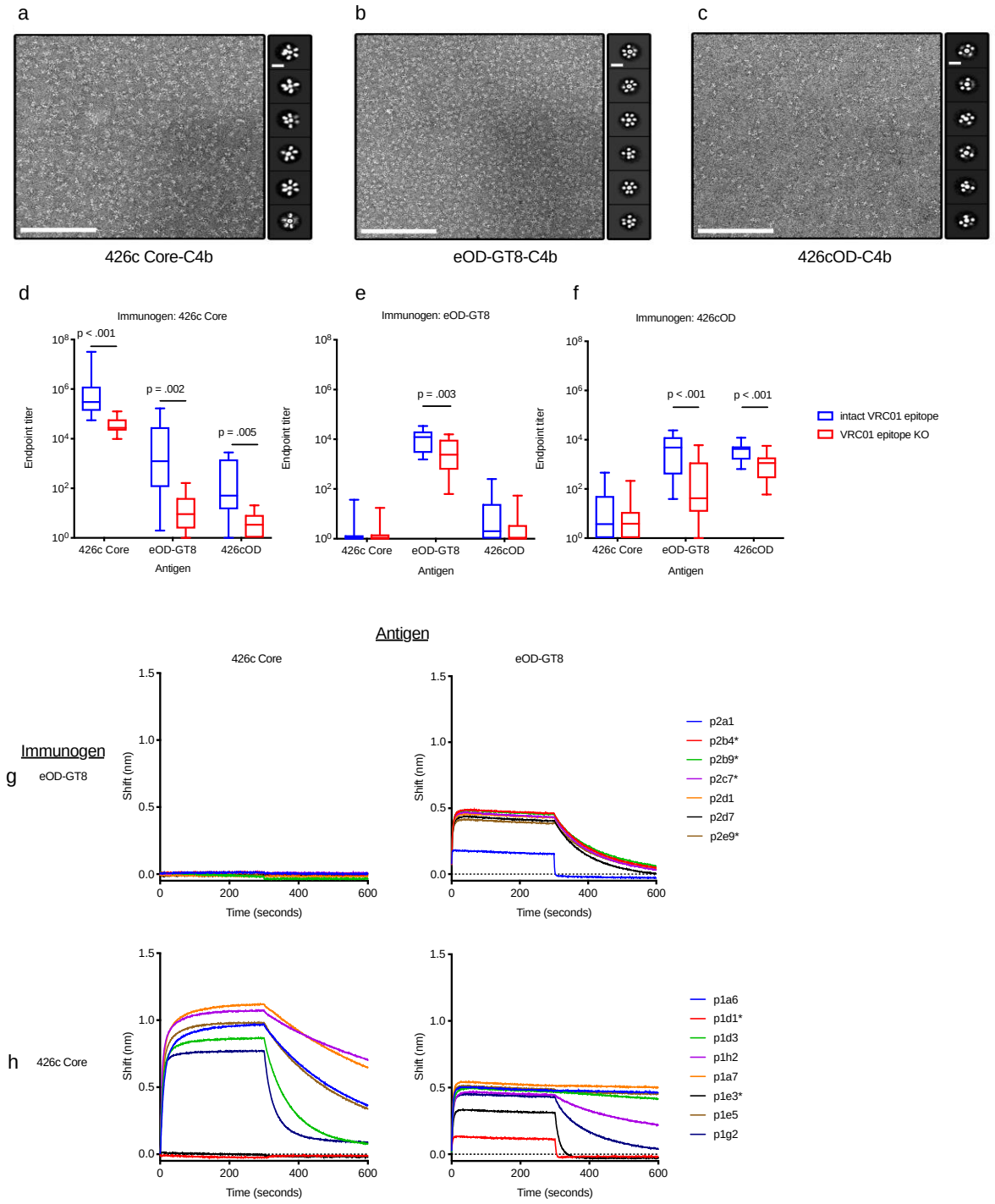
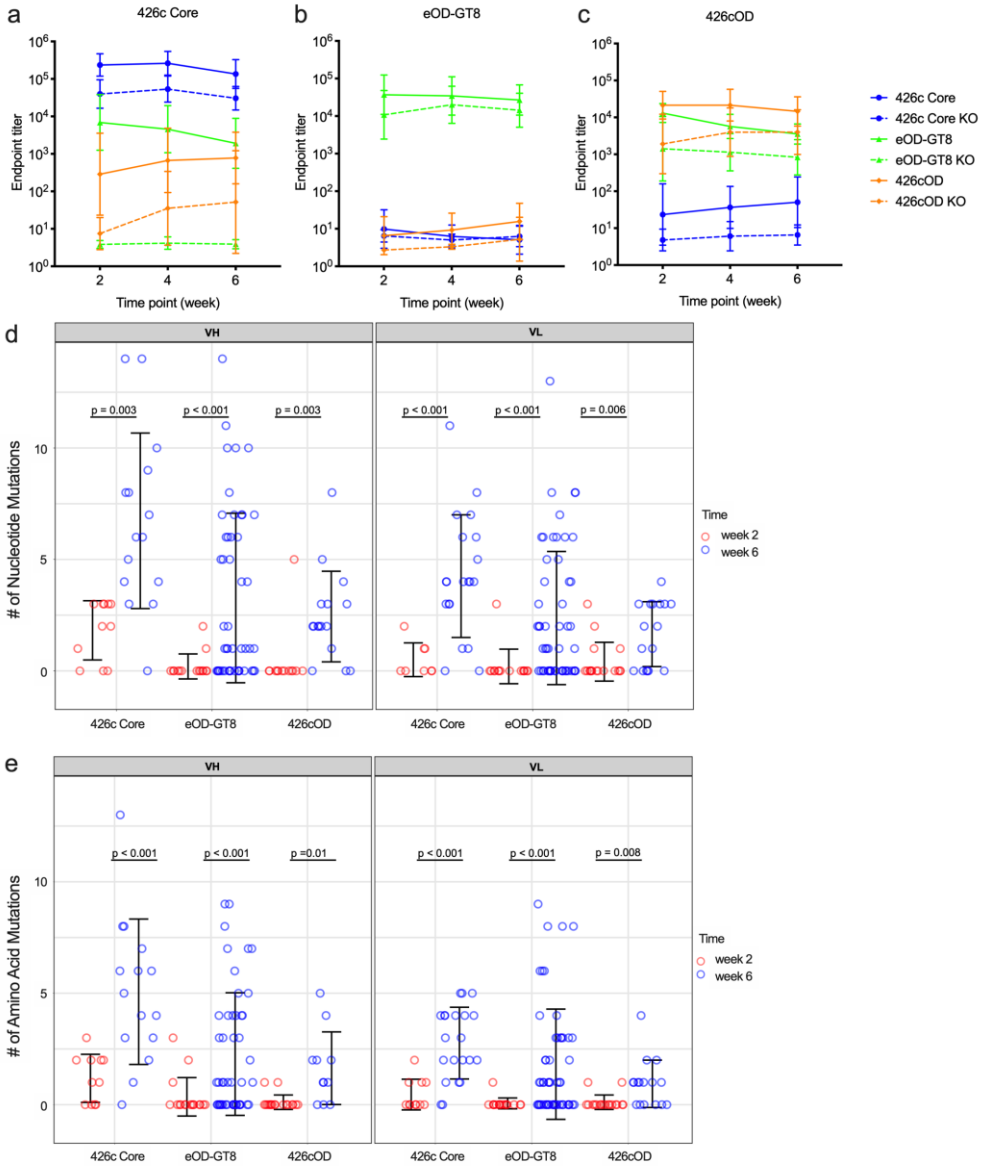
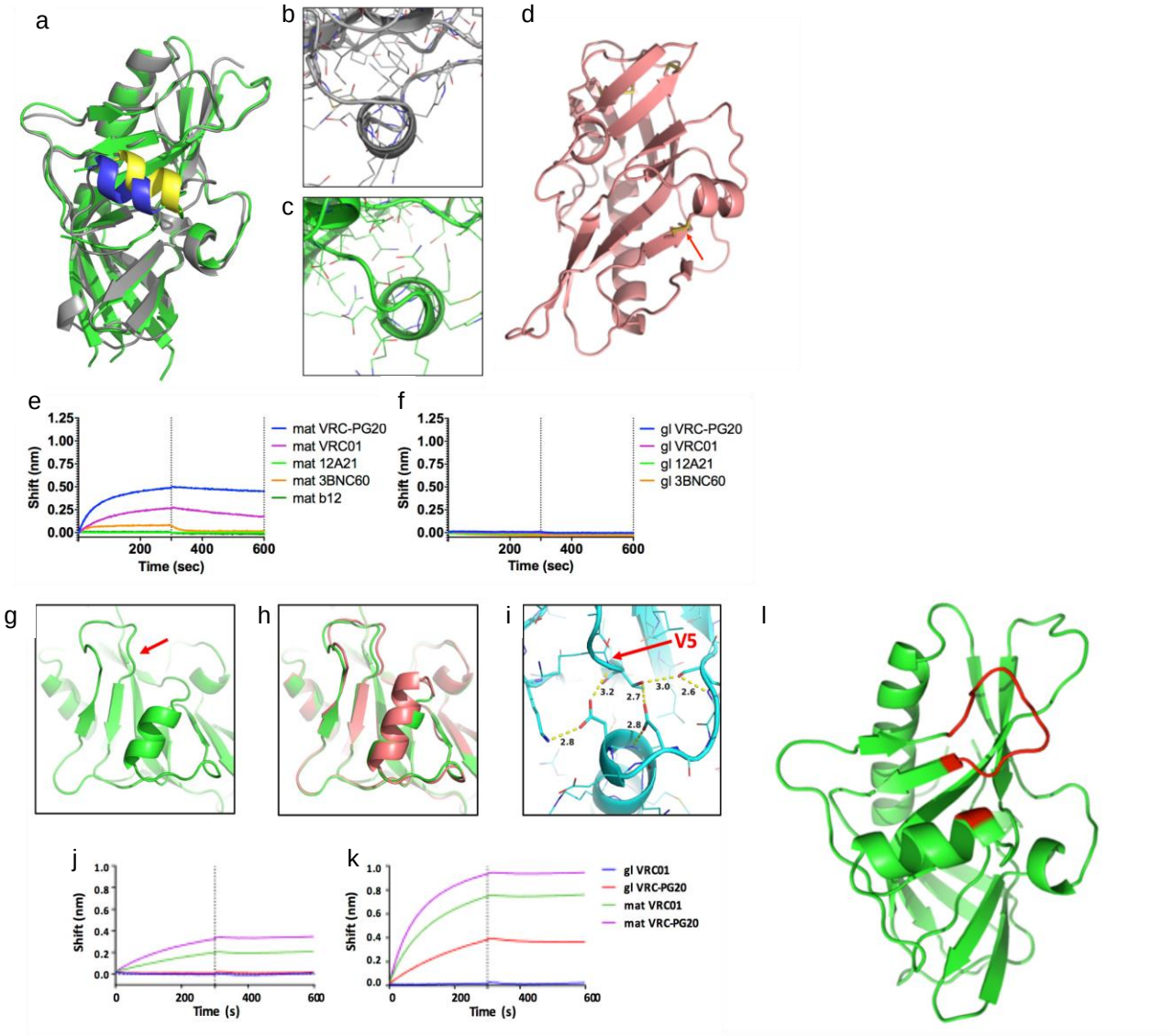


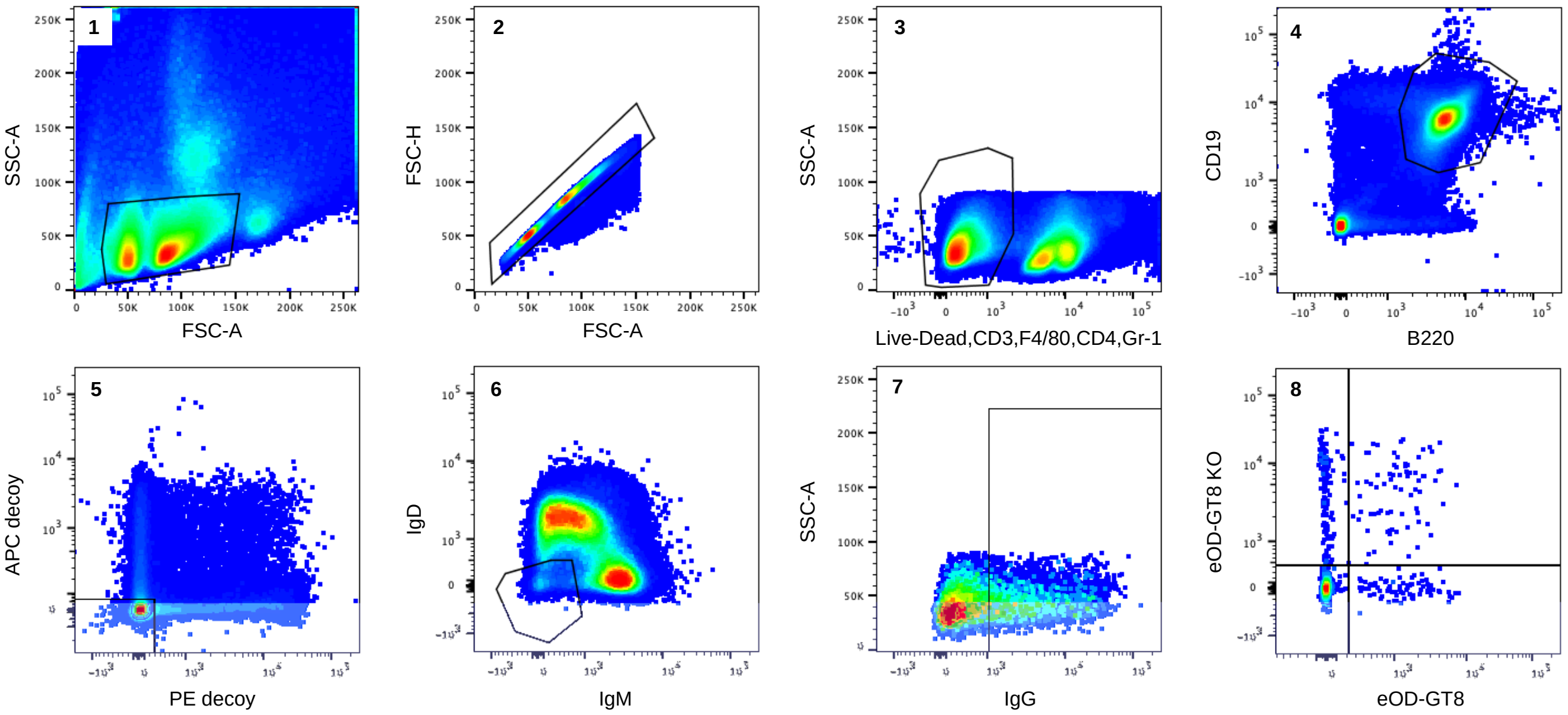
Supplemental Figure 1. Characterization of glVRC01-targeting immunogens *in vivo*, related to figures 3-6. Negative stain electron microscopy was used to visualize multimeric immunogens. Electron micrographs (left) and representative 2D class averages (right) of negatively stained 426c Core-C4b nanoparticles (a), eOD-GT8-C4b nanoparticles (b), and 426cOD-C4b nanoparticles (c). Scale bars: micrographs (200 nm); 2D class averages (100 Å). Plasma antibody endpoint titers, identified using ELISA, 2 weeks post-immunization with 426c Core (n = 14) (d), eOD-GT8 (n = 14) (e) or 426cOD (n = 12) (f) against the indicated proteins. Data is shown as boxplots. P-values were calculated using paired t-tests. BLI was used to evaluate binding of mAbs elicited by eOD-GT8 (g) or 426c Core (h) against eOD-GT8 or 426c Core. BLI binding curves are shown. mAbs with no amino acid mutations as compared to their germline sequences are noted with asterisks. Data are representative of at least 2 independent experiments.



Supplemental Figure 2. Vaccine elicited plasma antibody response and VH/VL mutations over time, related to figure 3 and supplemental tables 5-7.
Animals were immunized with 426c Core, eOD-GT8 or 426cOD. Plasma antibodies were used in ELISA and antigen-specific B cell were single cell sorted and sequenced. Group average endpoint titers of plasma antibodies against 426c Core, eOD-GT8 and 426cOD at weeks 2, 4, and 6 post-immunization with 426c Core (a), eOD-GT8 (b), or 426cOD (c). Plasma from 7 mice was used from each immunization group. Error bars represent standard deviation. The number of nucleotide (d) and amino acid mutations (e) per each VH (left) and VL (right) domain in sequences isolated at week 2 and 6 post immunization. Significance values were calculated with Welch two sample T test. Error bars represent standard deviation. The sequences included in this analysis are in Supplemental Tables 5-7.



Supplemental Figure 3. Generation of the 426c base, related to figure 1 and STAR Methods. (a) Superimposed structures of eOD (gray) and 426c (green) are displayed with the helix position highlighted. The helix environment (looking down helix) in (b) eOD is less polar and more packed than (c) the 426c environment. Modifications to the 426c outer domain were made using RosettaRemodel to adjust the position and structure of the helix, leading to the generation of (d) S2. The red arrow indicates a disulfide bond that was added. The antigenicity of S2 was evaluated using BLI for binding to (e) mature VRC01-class antibodies and (f) glVRC01-class antibodies. (g) The 426c OD V5 loop (arrow) position is shown in relation to the helix. (h) The helix was extended (pink) to bring helix closer to V5 loop than in original structure (green). (i) The hydrogen bond network was extended between helix and V5 loop (arrow) to stabilize the helix. BLI was used to evaluate the S2-D51I (j) and (k) S2-D51 variants against mature and germline VRC01-class antibodies. (l) S2-D51 base or 426c base is shown with the amino acid residue at position 51 highlighted in red.



Supplemental Figure 4. **Gating strategy to sort antigen-specific B cells post-immunization, related to figure 3 and STAR methods.** Axes are labeled with markers used to select population of interest. The flow plots are labeled 1-8 to indicate order of the gates.

SUPPLEMENTAL TABLES

Supplemental Table 1. Amino acid sequences for OD variants, related to figures 1 and 2, supplemental figure 3, and STAR methods. Mutations from original 426c consensus sequence are for S1 and S2 mutants and in bold text.

Variant	Sequence
eOD	DTITLPCRPAPPPHCSSNITGLILTRQGGY ANTVIFRPSGGDWRDIARCQIAGTVVSTQL FLNGSLAEEEVVIRSEDWRDNAKSICVQLA TSVEIACTGAGHCAISRAKWANTLKQIASK LREQYGAKTIIFKPSSGGDPEFVNHSFNCG GEFFYCASTQLFASTWF
426c core	TISNATIMLPCRPAPPPHCKSDITGLLLLRD GGDTTDNTEIFRPGGGDMRDNWRCQIAGT VVSTQLLLNGSLAEEEIVIRSKNLRDNAKII CVQLNKSVEIVCTGAGYCNISGRNWSEAV NQVKKKLKEHFPHKNISFQSSSGGDLEITT HSFNCGGEFFYCNTSGLFND
S2	TISNATIMLPCRPAPPPHCKS**N**ITGLLLLRD GGDTTDNTEIFRP**S**GGDMR**CAE**R**LG**I**PSS**V VSTQLLLNGSLAEEEIVIRSKNLRDNAKIIC VQL**Q**KSVEIVCTGAGYC**Q**ISGRNWSEAVN QVKKKLKEHFPHKNISFQSSSGGDLEITTHS FNCGGEFFYCNTSGLF**Q**D
S2-D51I	TISNATIMLPCRPAPPPHCKS**N**ITGLLLLRD GGDTTDNTEIFRP**S**GGD**EIAQ**RC**MERLG**I**P **SS**VVSTQLLLNGSLAEEEIVIRSKNLRDNAK **T**ICVQL**Q**KSVEIVCTGAGYC**Q**ISGRNWSEA VNQVKKKLKEHFPHKNISFQSSSGGDLEIT THSFNCGGEFFYCNTSGLF**Q**D
S2-D51 (426c base)	TISNATIMLPCRPAPPPHCKS**N**ITGLLLLRD GGDTTDNTEIFRP**S**GGD**EDAQ**RC**MERLG**I **PSS**VVSTQLLLNGSLAEEEIVIRSKNLRDNA K**T**ICVQL**Q**KSVEIVCTGAGYC**Q**ISGRNWSE AVNQVKKKLKEHFPHKNISFQSSSGGDLEI TTHSFNCGGEFFYCNTSGLF**Q**D

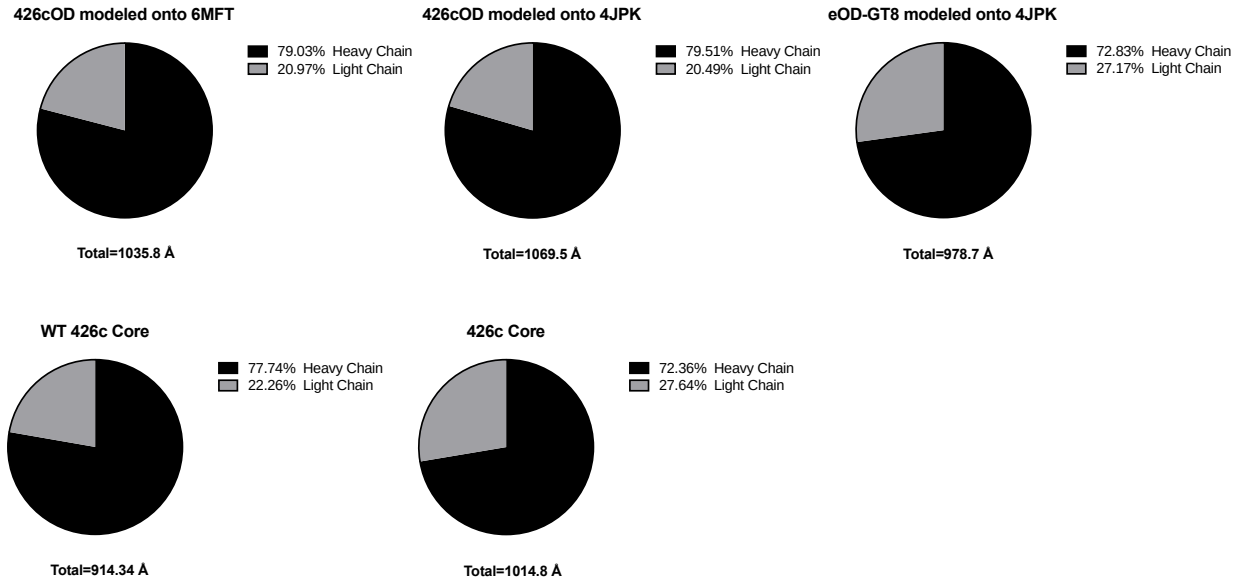
Supplemental Table 2. Data collection and refinement statistics for 426cOD with VRC01Fab, related to figure 2.

	426cOD/VRC01Fab
Data collection	
Space group	P43
Cell dimensions	
a, *b*, *c* (Å)	137.367, 137.367, 66.188
α, *β*, *γ* (°)	90, 90, 90
Resolution (Å)	50 – 3.42 (3.48 – 3.42)*
R_{sym} or R_{merge}	1.117 (0.930)*
I/sI	25.57 (1.69)*
Completeness (%)	100 (100)*
Redundancy	6.7 (6.5)*
$CC_{1/2}$	0.992 (0.661)*
Refinement	
Resolution (Å)	48.57 – 3.421 (3.544 – 3.421)*
No. reflections	15984 (854)*
R_{work}/R_{free}	21.04/26.07 (27.32/33.36)
No. atoms	4587
Protein	597
Water	60
Ligand	104
B-factors (Å^2)	59.24
Protein	58.73
Water	26.02
Ligand	100.17
R.m.s deviations	
Bond lengths (Å)	0.004
Bond angles (º)	0.66
Ramachadran Favored %	91.33
Ramachadran Outliers %	0.17
MolProbity all-atoms clashscore	5.65
PDB ID	**6VLW**

* Statistics for the highest-resolution shell are shown in parentheses.

Supplemental Table 4, related to figure 2. Buried surface area (BSA) of glVRC01 Heavy and Light chains upon binding to 426cOD (2 models), WT 426c Core PDB ID (6MFT), eOD-GT8 (modeled onto 4JPK), 426c Core (5FEC) calculated by PDBePISA (https://www.ebi.ac.uk/pdbe/pisa/).

	glVRC01 Heavy chain BSA (Å²)	glVRC01 Light chain BSA (Å²)	Total BSA (Å²)
426cOD modeled onto 6MFT	818.6	217.2	1035.8
426cOD modeled onto 4JPK	850.4	219.1	1069.5
WT 426c Core (PDB 6MFT)	710.8	203.5	914.3
eOD-GT8 modeled onto 4JPK	712.8	265.9	978.7
426c Core (PDB 5FEC)	734.3	280.5	1014.8



Supplemental Table 6. Summary of the immunization groups and number of B cells sorted fromeach group, related to STAR methods.

Immunogen	Group	Adjuvant	Harvest time point	Number of animals	Number of B cells sorted	# of heavy chain sequences	# of light chain sequences
426c Core	a	GLA-LSQ	Week 2	5	166	84	26
	b	poly (I:C)	Week 2	5	56	5	25
	c	GLA-LSQ	Week 6	4	329	56	126
	d	GLA-LSQ	Week 6	4	na	na	na
eOD-GT8	a	poly (I:C)	Week 2	4	79	30	39
	b	GLA-LSQ	Week 2	4	41	18	20
	c	GLA-LSQ	Week 6	4	147	135	75
	d	GLA-LSQ	Week 6	4	64	32	39
426cOD	a	poly (I:C)	Week 2	4	294	59	97
	b	GLA-LSQ	Week 2	4	28	8	13
	c	GLA-LSQ	Week 6	4	111	39	79
	d	GLA-LSQ	Week 6	4	na	na	na

na indicates not sorted.

Supplemental Table 7. Primers for 426cOD-base and 426cOD cloning and mutagenesis, related to STAR methods.

426cOD primers for pETCON vector cloning	F	AGGCGGAGGGTCGGCTTCGCATATGACCATAAGTAATGCGACTATCATGCTCC
	R	TTTGTTCGGAACCTCCACCCTCGAGGTCTTGAAAGAGTCCTGACGTGTTACAA
426cOD primers for random site mutagenesis	F	AGTGGTGGAGGAGGCTCTGGTGGAGGCGGTAGCGGAGGCGGAGGGTCGGCTTCGCATATG
	R	GATCTCTATTACAAGTCCTCTTCAGAAATAAGCTTTTGTTCGGAACCTCCACCCTCGAG
426cOD-base primers for T462I, D463N mutagenesis	F	CGCGACGGTGGAGATACCATTAATAATACCGAGATTTTC
	R	GAAAATCTCGGTATTATTAATGGTATCTCCACCGTCGCG
426cOD-base primers for R480W mutagenesis	F	AGACGAGGACGCTCAATGGTGTATGGAACGG
	R	CCGTTCCATACACCATTGAGCGTCCTCGTCT
426cOD-base primers for N276D, R278S mutagenesis	F	GAAGAAATTGTAATTCGGAGTAAGGATCTCAGCGATAACGCCAAAACAATTTGCGTG
	R	CACGCAAATTGTTTTGGCGTTATCGCTGAGATCCTTACTCCGAATTACAATTTCTTC
426cOD-base primers for R273W mutagenesis	F	AGCCTTGCTGAGGAAGAAATTGTAATTTGGAGTAAGGATCTC
	R	GAGATCCTTACTCCAAATTACAATTTCTTCCTCAGCAAGGCT
426cOD primers for D368R, E370A mutagenesis	F	CAGTCCTCCAGCGGAGGTCGCCTTGCAATTACAACCCACTCTTT
	R	AAAGAGTGGGTTGTAATTGCAAGGCGACCTCCGCTGGAGGACTG
426cOD primers for D279A mutagenesis	F	CAAATTGTTTTGGCGTTAGCGCTGAGATCCTTACT
	R	AGTAAGGATCTCAGCGCTAACGCCAAAACAATTTG