Supplemental information

A

mAbs	CRF250 virus									
	293T	N156A	N160A	T+Kif	T+Swain	293S	N156A + Kif	N156A + Swain	N160A + Kif	N160A + Swain
CAP256.UCA	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
CAP256.24	0.61620	>10	>10	0.02663	0.22750	0.01948	>10	>10	>10	8.24000
CAP256.I1	>10	>10	>10	>10	0.65650	7.42400	>10	>10	>10	>10
CAP256.I2	0.25330	>10	>10	0.03669	0.01299	0.04167	>10	>10	>10	>10
CAP256.01	0.81370	>10	>10	0.49330	0.07001	0.04539	7.54500	>10	>10	3.23700
CAP256.06	4.43500	>10	>10	2.55400	0.86700	0.74530	>10	>10	>10	>10
CAP256.14	0.00732	0.03417	2.33700	0.41860	0.00357	0.03118	0.00844	0.04419	>10	0.18470
CAP256.16	0.00659	0.06437	0.30340	0.06966	0.00126	0.00837	0.00339	0.01427	>10	0.22340
CAP256.17	0.00195	0.01159	0.01181	0.12340	0.00172	0.01960	0.00504	0.00349	0.27280	0.00734
CAP256.18	0.00763	0.04331	0.27180	0.34320	0.00262	0.02116	0.01335	0.00557	1.19600	0.26820
CAP256.15	0.00851	0.67270	0.69350	0.01611	0.00466	0.00579	0.00783	0.34430	0.44960	0.14200
CAP256.11	0.09417	1.73000	1.96100	0.71830	0.02131	0.13030	0.23090	4.41300	9.46000	1.24200
CAP256.32	0.03913	9.25400	1.13600	0.11790	0.01528	0.04761	0.10920	>10	4.36000	0.36610
CAP256.02	0.13150	7.92800	0.88780	0.12140	0.01768	0.00963	0.05843	>10	2.43200	0.09984
CAP256.10	0.04967	1.74400	0.68260	0.03851	0.00960	0.00081	0.06691	1.09400	2.63900	0.33890
CAP256.19	0.01509	0.53690	0.61040	0.18070	0.00630	0.02043	0.02537	0.66830	1.33100	0.28770
CAP256.23	0.79230	>10	>10	0.04830	0.08678	0.00607	>10	>10	>10	>10
CAP256.05	0.04527	6.90000	>10	0.05427	0.00266	0.00137	0.34780	6.43600	>10	>10
CAP256.13	9.80000	>10	1.91900	>10	6.79300	>10	>10	>10	>10	0.39040
CAP256.12	2.51100	>10	0.09352	>10	1.66900	>10	>10	>10	1.81800	0.27670
CAP256.07	0.02076	5.38400	0.03522	2.14300	0.02516	0.36170	0.37000	>10	0.00612	0.00242
CAP256.03	0.00001	0.00016	0.00001	0.00245	0.00001	0.00001	0.00001	0.00022	0.00001	0.00001
CAP256.25	0.00001	0.00038	0.00001	0.000193	0.00001	0.00075	0.00001	0.00002	0.00001	0.00001
CAP256.04	0.00263	0.12200	0.00007	0.00443	0.00003	0.00010	0.00026	0.01432	0.00261	0.00004
CAP256.09	0.00042	0.29580	0.003381	0.01830	0.00008	0.00499	0.00670	0.25190	0.01227	0.00700
CAP256.20	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
CAP256.26	0.00294	0.11010	0.01228	0.01630	0.00055	0.00386	0.00016	0.00927	0.00011	0.00002
CAP256.27	0.00008	0.01544	0.00072	0.00166	0.00007	0.00235	0.00720	0.05847	0.00274	0.00047
CAP256.08	0.00029	0.09290	0.00327	0.00906	0.00008	0.00437	0.00118	0.07351	0.00074	0.00020
CAP256.21	0.25300	>10	>10	>10	0.01270	>10	>10	>10	>10	8.61900
CAP256.31	0.02433	8.49800	1.55300	0.14070	0.00126	0.21960	0.21060	3.80300	0.88110	0.13390
CAP256.30	0.00010	1.04100	0.12010	0.10570	0.00004	0.05376	0.06245	0.05427	0.25090	0.02747
CAP256.28	0.00005	0.32160	0.00586	0.00946	0.00003	0.00201	0.00317	0.01915	0.00096	0.00004
CAP256.29	0.00005	0.43260	0.00467	0.00244	0.00001	0.00769	0.00225	0.01818	0.00150	0.00051
CAP256.22	0.00038	0.24360	0.00076	0.00859	0.00002	0.00306	0.00117	0.01858	0.00140	0.00018
PGT145	0.01206	>10	>10	4.60100	0.00670	0.00015	>10	>10	>10	>10
PGT128	0.00749	0.00606	0.00530	0.00079	0.00564	0.00201	0.00159	0.00509	0.00061	0.00400

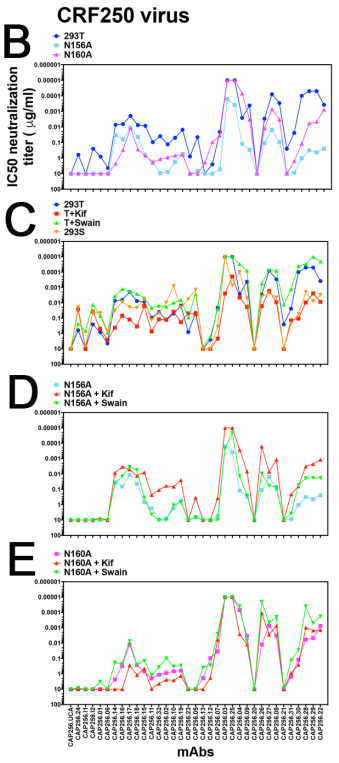


Figure S1. CAP256 mAbs showed varied neutralizing activity of CRF250 WT virus and its glycan eliminated variants and glycovariants. Related to Figure 1.

A. Neutralizing activity of CAP256 mAbs against CRF250 WT virus (293T cells), CRF250 glycan eliminated variants (N156A and N160A) and CRF250 virus glycovariants. The glycovariants are grown in 293T cells in the presence of glycoside inhibitors kifunensine (Kif: ER α - mannosidase I inhibitor) or swainsonine (Swain: Golgi α-mannosidase II inhibitor) or in 293S cells that lack an N-acetyl glucosaminyl transferase enzyme. The Abs were tested in a concentration range (0.00001-10 μg/ml) and the numerical values indicate IC50 neutralization titers using TZM-bl target cells. The information is color-coded with deep red colors indicating more potent neutralizing activity. The bnAbs PGT145 and PGT128 were used as controls.

B-E. Trends in glycan-dependent neutralization by CAP256 mAbs. **B.** Neutralization of CRF250 WT virus (grown in 293T cells) and two glycan eliminated virus variants (N156A and N160A). **C.** Neutralization of CRF250 WT virus or virus grown in the presence of glycoside inhibitors, kifunensine and swainsonine and the virus produced in 293S cells by CAP256 mAbs. **D.** Neutralization of CRF250 N156A virus grown in 293T cells alone or in combination with kifunensine or swainsonine by CAP256 mAbs **E.** As for (C) but for CRF250 N160A virus

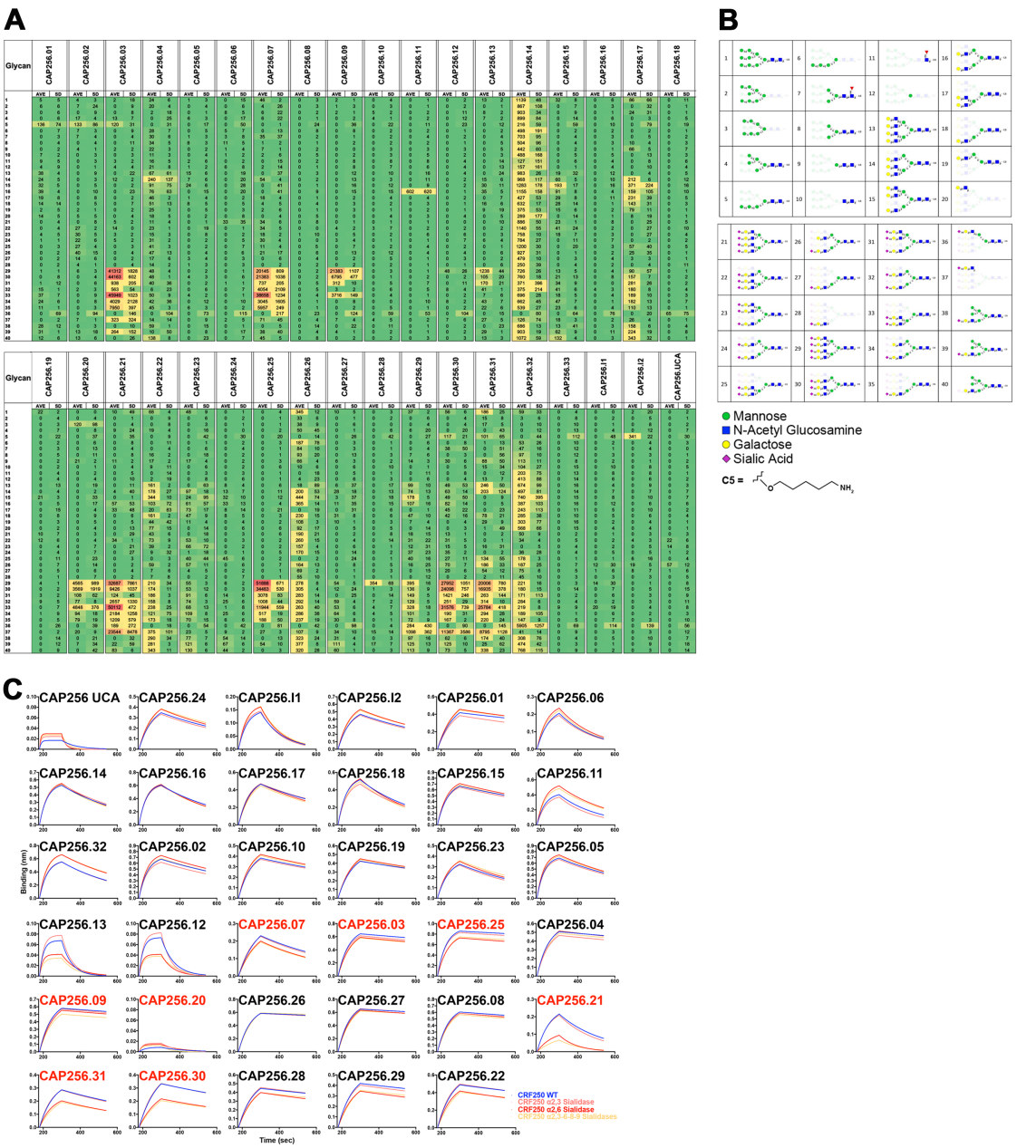


Figure S2. CAP256 mAb members display binding to α2,6 sialic acid bearing glycans on glycan microarrays and soluble HIV Env trimer protein. Related to Figure 1 and Figure 5.

A. The reactivity of CAP256 mAbs was assessed to a variety of glycan on glycan microarray and the data were recorded as fluorescence intensity (a.u.). The binding activity of mAbs is represented as average fluorescence intensity.

B. Types of glycans used in the assay and the symbols for individual monosaccharides units are shown.

C. Octet kinetics of CAP256 mAbs binding to WT CRF250 SOSIP.664 trimer and desialylated forms. Octet binding of CAP256 mAbs to CRF250 WT trimer and α2,3 or α2,6 or a combination of α2,3,6,8,9-sialidase-treated trimers to specifically cleave terminally linked sialic acid residues. MAbs at 10μg/ml

concentration were captured onto an anti-human IgG-Fc sensor (AHC: ForteBio.) to achieve binding of at least 1 response unit (1 RU). The IgG immobilized biosensor was immersed in the 100nM trimer solution with trimer as the analyte and the binding curves are shown indicating the association (120 s; 180-300) and dissociation (240 s; 300-540) of Ab-trimer interaction. The CAP256 mAbs that displayed binding to α2,6 sialic acid-containing glycans on glycan microarray are shown in red.

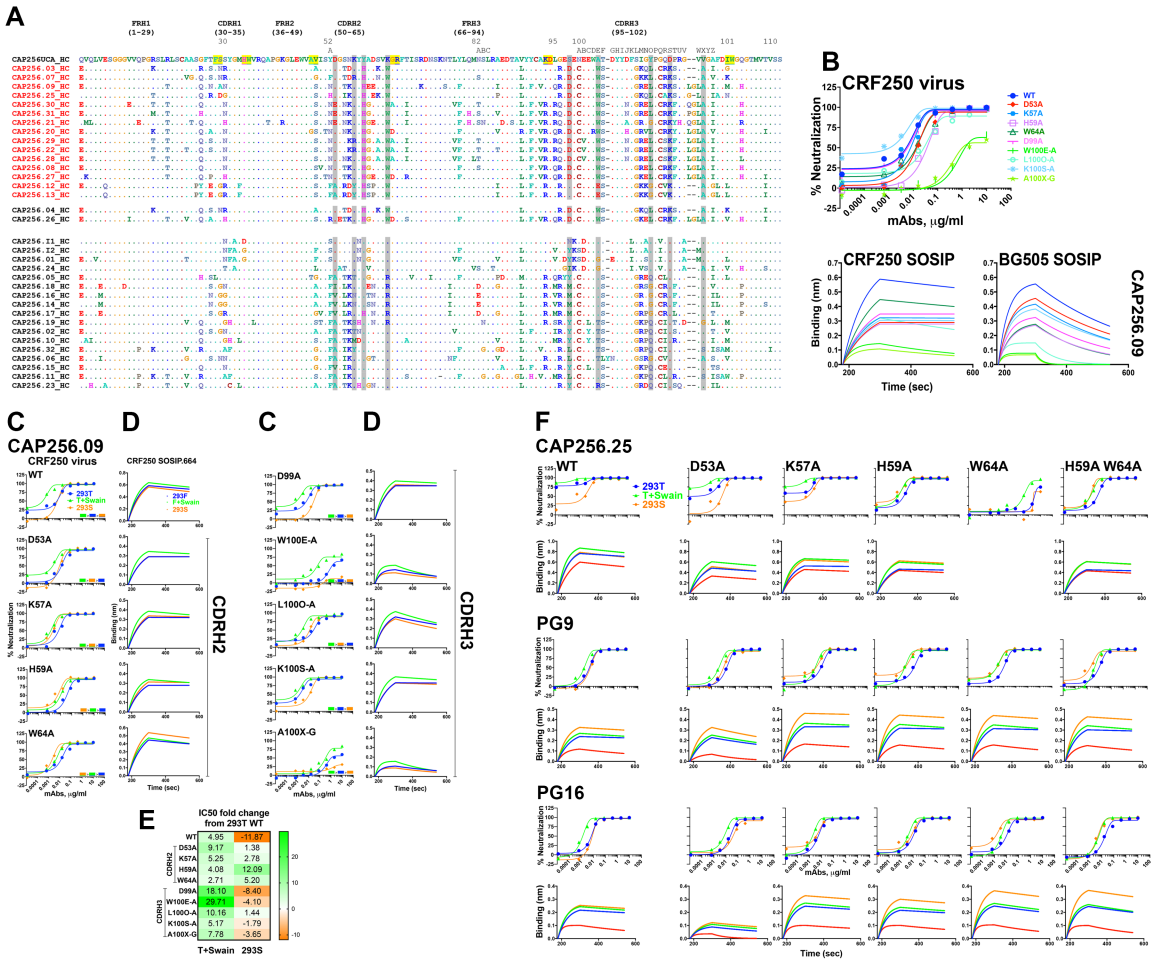


Figure S3. Germline encoded and somatic mutated CDRH2 heavy chain residues are important for binding and neutralization of CRF250 Env glycovariants. Related to Figure 2.

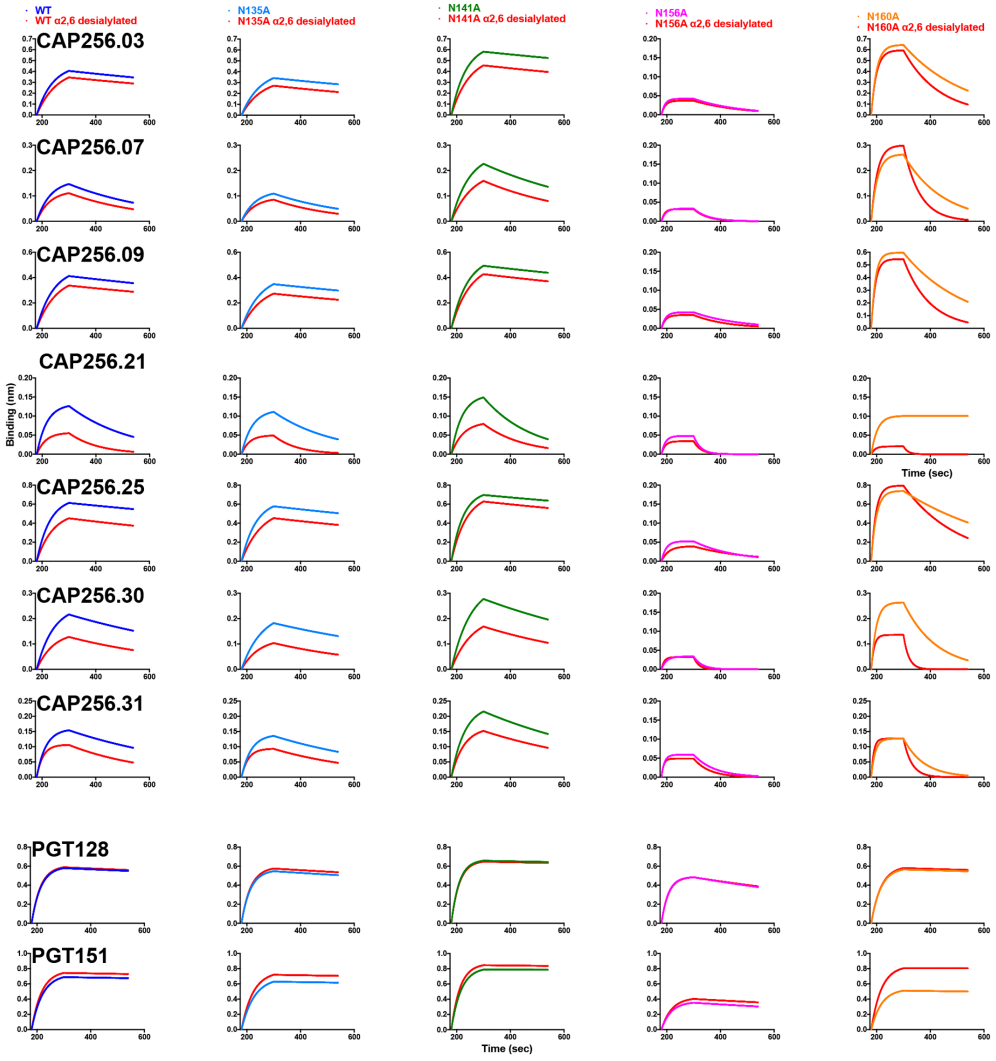
A. Amino acid sequence alignment of the heavy chain variable regions of CAP256 mAbs with the unmutated common ancestor Ab (CAP256 UCA). The amino acid residues are labeled according to the Kabat numbering scheme. The antibody CDR and FR junctions are underlined and highlighted (yellow). The Ab lineage members that showed both reactivity to the α2,6 sialic acid-containing glycans on the glycan microarray and/or binding sensitivity to α2,6 sialidase treated CRF250 trimer are highlighted in red and include the broadest Ab member in this Ab family, CAP256.25 Ab. The remaining CAP256 Ab members did not show either binding to glycans on the glycan microarray or reduced binding to CRF250 trimer upon α2,6 sialidase treatment and are shown in black. Sequence analysis revealed 9 amino acid positions (CDRH2 = 4 and CDRH3 = 5) in the heavy chain variable region that could potentially differentiate glycan-reactive CAP256 Ab sub-lineage mAbs from non-glycan reactive mAbs. CAP256.04 and CAP256.26 mAbs were exceptions that shared sequence similarity with the glycan-reactive Ab members but failed to display either binding

to glycans on the array or show sensitivity to the α2,6 sialidase treated CRF250 trimer binding.

B. Neutralization of CRF250 virus and octet binding kinetics of CRF250 and BG505 SOSIP.664 soluble trimer proteins by CAP256.09 antibody (WT) and its 9 heavy chain single amino acid substituted Ab variants.

C-F. Neutralization of CRF250 glycovariant viruses and binding of CRF250 trimer glycovariants by CAP256.09, CAP256.25, PG9 and PG16 mAbs. **C.** Neutralization of CRF250 WT (293T) and glycan-modified viruses (293T + swainsonine and 293S cells) by CAP256.09 WT Ab and 9 variants and **D**. Octet binding kinetics of 2G12 Ab-purified soluble SOSIP.664 trimer glycovariants for CAP256.09 WT Ab and the 9 variants. The CRF250 glycovariant viruses and trimers were grown either in the presence of Golgi α-mannosidase II inhibitor swainsonine (Swain; at 25 μM final concentration) or in 293S cells that lack N-acetyl glucosaminyl transferase enzyme. The neutralization IC50 values for the WT CRF250 virus and its two glycovariants are denoted by rectangles just above the X-axis (293T = blue, 293T + Swain = green and 293S = orange) and show the change in neutralization capacity of each Ab variant. **E.** Fold change in neutralization IC50 for CAP256.09 and the 9 Ab variants for 293T + swain and 293S grown CRF250 virus compared to the 293T grown virus. A small decrease in neutralizing ability for the W64A CDRH2 Ab variant and increase for two of the CDRH3 variants (D99A and W100E-A) against the swainsonine-treated virus as compared to the WT CAP256.09 Ab is noted. The neutralizing ability of all the four CDRH2 Ab variants (D53A, K57A, H59A and W64A) increased substantially for 293S cell grown CRF250 virus, indicating that the substitution of these germline and somatic mutated residue with alanine leads to enhanced interaction with Env trimer with smaller high mannose glycan (lacking complex-type glycans). **F.** The CAP256.25, PG9 and PG16 and their CDRH2 Ab mutants were tested for neutralization and octet binding of the CRF250 virus or SOSIP.664 trimer glycovariants. As for CAP256.09 Ab variants, the CAP256.25, PG9 and PG16 CDRH2 Ab variants showed improved binding to the 293S produced trimers when the glycan-specific Ab somatic mutations are removed.

A CRF250 SOSIP.664



B

CRF250 SOSIP.664

Antibodies	WT				WT α,26 desialylated				N135A				N135A α,26 desialylated				N141A				N141A α,26 desialylated			
	Response	KD (M)	kon(1/Ms)	kdis(1/s)	Response	KD (M)	kon(1/Ms)	kdis(1/s)	Response	KD (M)	kon(1/Ms)	kdis(1/s)	Response	KD (M)	kon(1/Ms)	kdis(1/s)	Response	KD (M)	kon(1/Ms)	kdis(1/s)	Response	KD (M)	kon(1/Ms)	kdis(1/s)
CAP256.03	0.4139	6.12E-09	1.09E+05	6.64E-04	0.3485	8.44E-09	8.66E+04	7.31E-04	0.3445	8.37E-09	9.10E+04	7.62E-04	0.2761	1.21E-08	8.35E+04	1.01E-03	0.5837	3.65E-09	1.19E+05	4.36E-04	0.4569	6.25E-09	9.48E+04	5.93E-04
CAP256.07	0.1566	2.60E-08	1.12E+05	2.91E-03	0.1202	3.06E-08	1.16E+05	3.55E-03	0.1175	3.08E-08	1.08E+05	3.33E-03	0.0909	4.06E-08	1.09E+05	4.41E-03	0.2321	2.44E-08	8.72E+04	2.13E-03	0.1611	4.34E-08	6.63E+04	2.88E-03
CAP256.09	0.4199	5.89E-09	1.03E+05	6.09E-04	0.3425	7.45E-09	8.80E+04	6.55E-04	0.3517	7.02E-09	9.23E+04	6.48E-04	0.2778	9.68E-09	8.31E+04	8.04E-04	0.4934	4.58E-09	1.08E+05	4.94E-04	0.4281	6.40E-09	9.24E+04	5.91E-04
CAP256.21	0.1407	2.84E-08	1.51E+05	4.28E-03	0.0615	6.36E-08	1.43E+05	9.09E-03	0.1238	3.16E-08	1.39E+05	4.37E-03	0.0551	7.51E-08	1.54E+05	1.16E-02	0.1611	4.27E-08	1.31E+05	5.57E-03	0.0867	6.18E-08	1.08E+05	6.69E-03
CAP256.25	0.619	3.52E-09	1.32E+05	4.65E-04	0.4581	7.42E-09	1.06E+05	7.85E-04	0.5805	4.68E-09	1.20E+05	5.59E-04	0.4539	8.34E-09	8.54E+04	7.12E-04	0.6991	2.49E-09	1.50E+05	3.72E-04	0.625	4.11E-09	1.16E+05	4.77E-04
CAP256.30	0.2264	1.50E-08	9.78E+04	1.47E-03	0.1355	2.36E-08	9.25E+04	2.18E-03	0.1865	1.73E-08	7.94E+04	1.38E-03	0.1097	2.80E-08	8.70E+04	2.44E-03	0.2816	1.73E-08	8.34E+04	1.44E-03	0.1732	2.67E-08	7.47E+04	1.99E-03
CAP256.31	0.1742	1.07E-08	1.83E+05	1.95E-03	0.1224	1.27E-08	2.61E+05	3.31E-03	0.1514	1.17E-08	1.74E+05	2.03E-03	0.1085	1.15E-08	2.46E+05	2.84E-03	0.2283	1.44E-08	1.21E+05	1.75E-03	0.1641	1.44E-08	1.32E+05	1.90E-03
PGT128	0.5893	1.09E-09	1.95E+05	2.13E-04	0.6007	1.06E-09	2.00E+05	2.12E-04	0.5613	1.65E-09	1.99E+05	3.27E-04	0.5877	1.50E-09	1.94E+05	2.91E-04	0.6695	3.72E-10	2.29E+05	8.52E-05	0.6575	3.87E-10	2.25E+05	8.70E-05
PGT151	0.7002	4.84E-10	1.65E+05	8.01E-05	0.7553	4.68E-10	1.79E+05	8.39E-05	0.6352	6.21E-10	1.40E+05	8.70E-05	0.7266	5.37E-10	1.55E+05	8.31E-05	0.7956	5.82E-11	1.64E+05	1.07E-05	0.8548	2.28E-10	2.04E+05	4.64E-05

	N156A				N156A α,26 desialylated				N160A				N160A α,26 desialylated			
	Response	KD (M)	kon(1/Ms)	kdis(1/s)	Response	KD (M)	kon(1/Ms)	kdis(1/s)	Response	KD (M)	kon(1/Ms)	kdis(1/s)	Response	KD (M)	kon(1/Ms)	kdis(1/s)
CAP256.03	0.0506	1.21E-08	4.82E+05	5.81E-03	0.0436	1.23E-08	4.61E+05	5.65E-03	0.6745	1.44E-08	3.07E+05	**4.40E-03**	0.6203	2.59E-08	2.94E+05	**7.60E-03**
CAP256.07	0.037	6.04E-08	3.09E+05	1.87E-02	0.0359	4.76E-08	4.17E+05	2.00E-02	0.287	2.78E-08	2.49E+05	**6.94E-03**	0.3075	8.80E-08	1.89E+05	**1.67E-02**
CAP256.09	0.0509	1.54E-08	3.91E+05	6.02E-03	0.0421	2.10E-08	3.87E+05	8.13E-03	0.6481	1.05E-08	4.18E+05	**4.37E-03**	0.5721	2.79E-08	3.68E+05	**1.03E-02**
CAP256.21	0.052	1.81E-07	2.11E+05	3.82E-02	0.0371	1.46E-07	2.49E+05	3.63E-02	0.0934	<1.0E-12	2.91E+05	**<1.0E-07**	0.021	7.67E-07	7.81E+04	**5.99E-02**
CAP256.25	0.0633	1.93E-08	3.43E+05	6.62E-03	0.0455	2.27E-08	2.22E+05	5.03E-03	0.7696	7.37E-09	3.37E+05	**2.48E-03**	0.8308	1.39E-08	3.55E+05	**4.93E-03**
CAP256.30	0.0353	1.99E-07	1.61E+05	3.21E-02	0.0342	1.80E-07	2.90E+05	5.20E-02	0.2903	2.78E-08	3.00E+05	**8.34E-03**	0.1394	1.79E-07	2.84E+05	**5.07E-02**
CAP256.31	0.0679	2.44E-08	5.22E+05	1.27E-02	0.0544	2.54E-08	6.73E+05	1.71E-02	0.1392	3.56E-08	3.72E+05	**1.33E-02**	0.1337	6.40E-08	5.12E+05	**3.28E-02**
PGT128	0.5142	3.91E-09	2.56E+05	1.00E-03	0.5118	3.71E-09	2.48E+05	9.19E-04	0.5713	7.80E-10	1.86E+05	1.45E-04	0.5858	8.08E-10	1.82E+05	1.47E-04
PGT151	0.379	3.45E-09	1.83E+05	6.31E-04	0.424	2.82E-09	1.74E+05	4.89E-04	0.5198	5.54E-10	1.74E+05	9.62E-05	0.8095	<1.0E-12	1.77E+05	**<1.0E-07**

Figure S4. Octet binding kinetics of the CAP256 mAbs to the WT CRF250 trimer and its V2 apex single glycan removed variants shows varied sensitivity to α2,6 sialidase treatment. Related to Figure 4.

A. Octet binding curves (association: 120 s; (180-300) and dissociation: 240 s; (300-540)) for CAP256 Abs with WT CRF250 trimer and its individual V2 apex

glycan eliminated variants (N135A, N141A, N156A and N160A), purified by 2G12 Ab affinity chromatography. Like the PGT145 Ab-purified WT CRF250 trimer and its desialylated form (Figure S4), the binding of CAP256 Abs generally showed a similar pattern of somewhat reduced binding to the α2,6 desialylated form of 2G12 Ab-purified CRF250 WT trimer, indicating trimer populations enriched by both the Abs likely had comparable α2,6 sialic acid-bearing glycoforms around the apex. The binding response of CAP256 mAbs with the CRF250 N156A glycan removed variant, which showed the most dramatic reduction in the binding, is fixed to 0.2 nanometer (nm) binding response for clarity. A High-mannose glycan specific Ab, PGT128, and a complex-type tri or tetra-antennary glycan specific Ab, PGT151 that only binds to the cleaved Env trimers, were used as controls. The binding response of PGT151 Ab with the N156A and N160A glycan eliminated trimer variants was reduced as compared to the WT CRF250 trimer suggesting the lack of these individual glycans may potentially affect trimer assembly.

B. Binding response, dissociation constants (KD), antibody on-rate (kon(1/Ms)) and off-rate (kdis(1/s)) or “koff” of the CAP256 mAbs with WT CRF250 SOSIP.664 trimer, its individual V2 apex glycan eliminated variants and their α2,6 desialylated versions.

A

Virus/mutant	CAP256.01	CAP256.02	CAP256.03	CAP256.04	CAP256.05	CAP256.06	CAP256.07	CAP256.08	CAP256.09	CAP256.10	CAP256.11	CAP256.12	CAP256.13	CAP256.14	CAP256.15	CAP256.16	CAP256.17	CAP256.18	CAP256.19	CAP256.20
CRF250 WT	0.81370	0.13150	<0.00001	0.00263	0.04527	4.43500	0.02076	0.00029	0.00042	0.04967	0.09417	2.51100	9.80000	0.00732	0.00851	0.00659	0.00195	0.00763	0.01509	>10
R166K	>10	>10	2.40600	>10	>10	>10	>10	3.00500	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
R166T	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
R166S	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
K169R	>10	3.72900	<0.00001	0.01339	5.07800	>10	0.52230	0.01552	0.05940	1.22700	1.86700	>10	>10	1.27400	1.36200	6.02500	0.29010	>10	0.30190	>10
K169Q	>10	>10	0.15710	4.46900	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	0.62860	>10
K169E	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
R166S-K169E	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10

Virus/mutant	CAP256.21	CAP256.22	CAP256.23	CAP256.24	CAP256.25	CAP256.26	CAP256.27	CAP256.28	CAP256.29	CAP256.30	CAP256.31	CAP256.32	CAP256.I1	CAP256.I2
CRF250 WT	0.25300	0.00038	0.79230	0.61620	<0.00001	0.00294	0.00008	0.00005	0.00005	0.00010	0.02433	0.03913	>10	0.25330
R166K	>10	>10	>10	>10	0.78410	6.40500	4.02800	>10	>10	>10	>10	>10	>10	>10
R166T	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
R166S	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
K169R	9.63500	0.00326	>10	>10	<0.00001	0.00994	0.00186	0.00138	0.00294	0.00653	0.29460	1.30500	>10	>10
K169Q	2.64000	0.08101	>10	>10	0.08030	9.09700	4.77900	0.09437	0.04221	0.06539	1.01600	5.04900	>10	>10
K169E	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
R166S-K169E	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10

PGT151	PGT128
0.00702	0.00749
0.00692	0.00710
0.00656	0.00759
0.00629	0.00624
0.01089	0.01375
0.00647	0.00898
0.00917	0.00908
0.00769	0.02715

B

Virus/mutant	CAP256.25 WT	D53A	K57A	H59A	W64A	H59A-W64A
CRF250 WT	<0.00001	0.00004	<0.00001	0.00140	4.02800	0.00483
R166K	0.78350	6.08500	0.49510	>10	>10	>10
R166T	>10	>10	>10	>10	>10	>10
R166S	>10	>10	>10	>10	>10	>10
K169R	<0.00001	0.00002	<0.00001	0.00141	0.66390	0.12340
K169Q	0.07991	0.35830	0.02634	>10	>10	>10
K169E	>10	>10	>10	>10	>10	>10
R166S-K169E	>10	>10	>10	>10	>10	>10

C

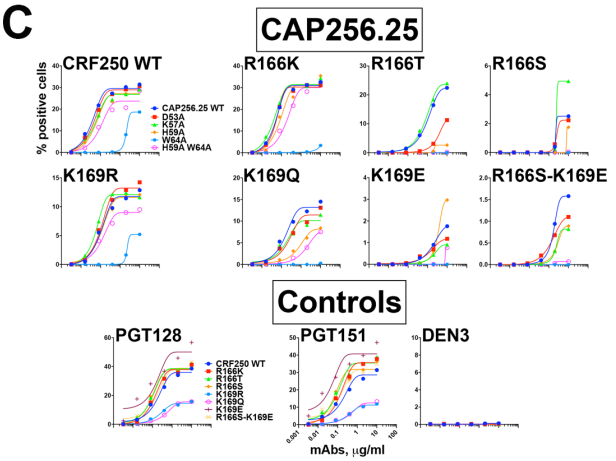


Figure S5. Sialic acid-binding HC residues of CAP256 mAbs are critical for neutralization and binding of CRF250 Env variants that mimic escape mutations in the CAP256 donor virus. Related to Figure 6.

A. Neutralizing activity of CAP256 mAbs against CRF250 WT virus and R166- and K169-variants corresponding to escape mutations in the CAP256 superinfecting virus (CAP256.SU: donor virus that led to the CAP256 Ab lineage). The IC50 neutralization titers for each Ab-virus combination are shown as numerical values with the intensity of red colors representing the Ab potency. The neutralizing activity of the control bnAbs, PGT128 and PGT151 remained largely unchanged with V2 apex Env substitutions.

B. The neutralizing activity of CAP256.25 WT Ab and its CDRH2 Ab variants against R166 and K169 substituted Env virus variants.

C. Flow cytometry binding of CAP256.25 Ab and its CDRH2 variants to cell-surface expressed CRF250 Env trimer variants. The CRF250 WT Env and its R166 and K169 V2 region-substituted variants were expressed on the surface of 293T cells as membrane-bound trimers. The CAP256.25 WT Abs and its CDRH2 Ab variants were evaluated for binding to Env trimer mutants using 5-fold titration decreasing concentration steps of Ab beginning at 10ug / ml. Binding was detected by a PE-conjugated anti-human-Fc secondary Ab and is represented as % positive cells. The Abs PGT128, PGT151 and DEN3 were controls for the assay. The PGT128 and PGT151 control Abs revealed that most of the Env trimer variants displayed comparable surface expression of well-cleaved trimers, except a relatively low expression level for two CRF250 Env K169 variants, K169R and K169Q.

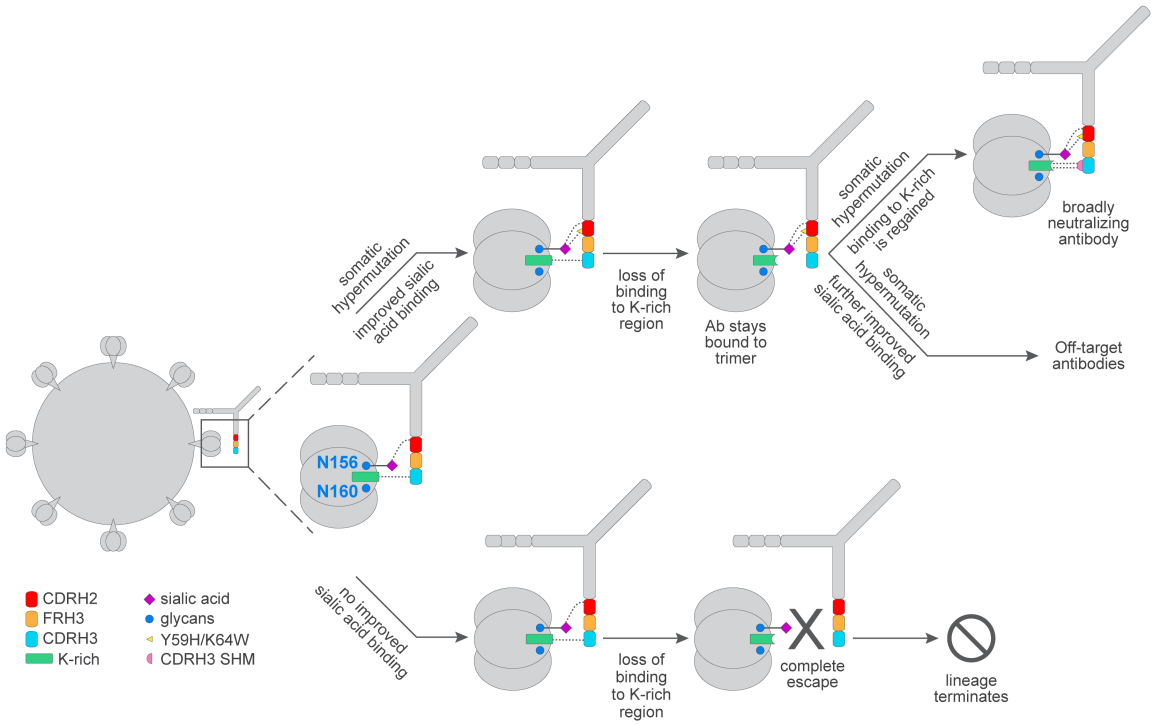


Figure S6. Proposed virus and B-cell co-evolutionary pathways that lead to CAP256 and PG9 V2 apex bnAbs. Related to Figure 1-6.

The schema shows a proposal for how a V2 apex bnAb response may be generated in HIV natural infection. Upon initial infection, a viral envelope sequence that has affinity for the V2 apex bnAb germline precursors, presented as a trimeric protein spike on the HIV virion surface (grey sphere), is proposed to select and trigger an appropriate precursor B-cell expressing Ab with a long CDRH3 loop (Antibody; Y-shape). The CDRH3 loop (shown in cyan) rich in anionic residues incorporates a germline D-gene encoded motif (YYD) to interact with the lysine-rich region (K-rich: shown as green rectangle) of the V2 apex core epitope by penetrating between glycans N156 and N160 (shown as blue spheres). Germline-encoded CDRH2 residues D53 and K57 contact the α2,6 terminally linked sialic acid (shown as purple diamond) likely on the N156 Env glycan. The V2 apex bnAb precursors further accumulate two somatic mutations (SHM) in the adjacent CDRH2 residues, Y59H and K64W (represented as yellow triangle), which further strengthen the interaction with a terminal sialic acid (indicated in dotted line). The circulating virus initiates an escape pathway by mutating the positively charged residues in the strand C core epitope to loosen interaction with the CDRH3 anionic residues of the antibody. The germline B-cells that have not accumulated the CDRH2 somatic mutations lose cognate interaction with the escaping virus and this B-cell lineage terminates but the B-cells with additional sialic acid-specific somatic mutations are able to maintain interaction, albeit at lower affinity. These B cells can now accumulate further SHM in the antibody variable regions that allow V2 apex bnAbs to accommodate potential virus escape mutations in the strand-C K-rich region. Thus, Env glycan recognition by V2 apex bnAb precursors early in the antibody developmental pathway is proposed to help these Abs to tolerate potential escape mutations in

the protein part of the epitope and helps them to deal with the variations in the core epitope of escaping virus that enables them to broaden coverage across a range of diverse HIV isolates. Some Abs appear to mature towards enhanced sialic acid binding at the expense of broader recognition and become “off-target antibodies”.