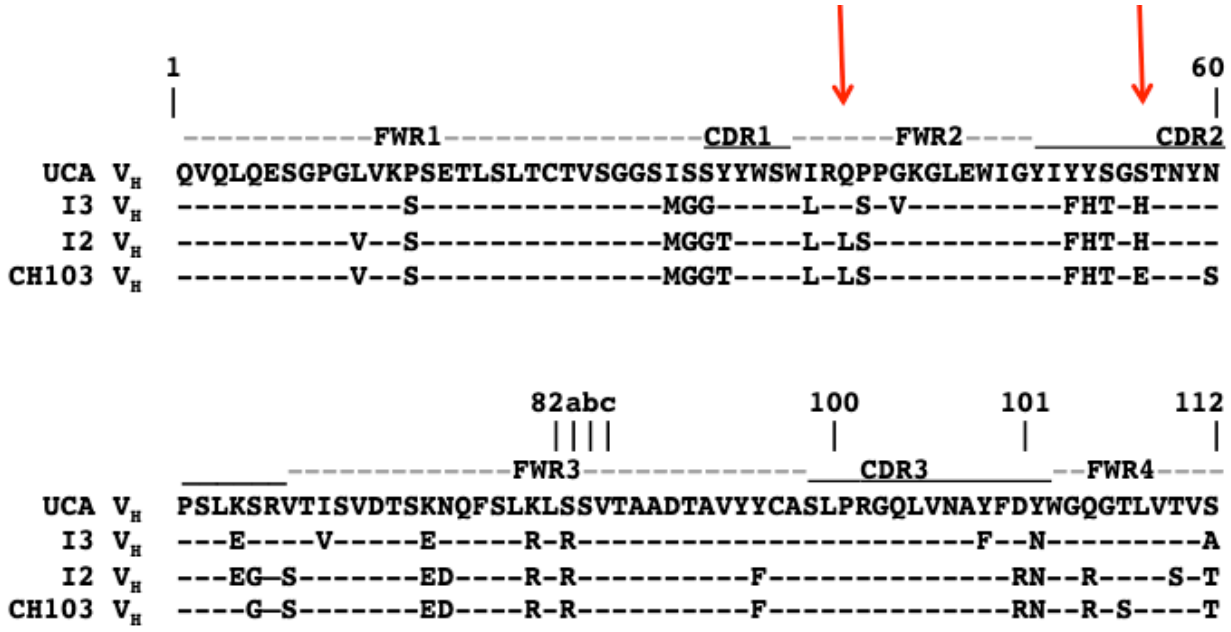
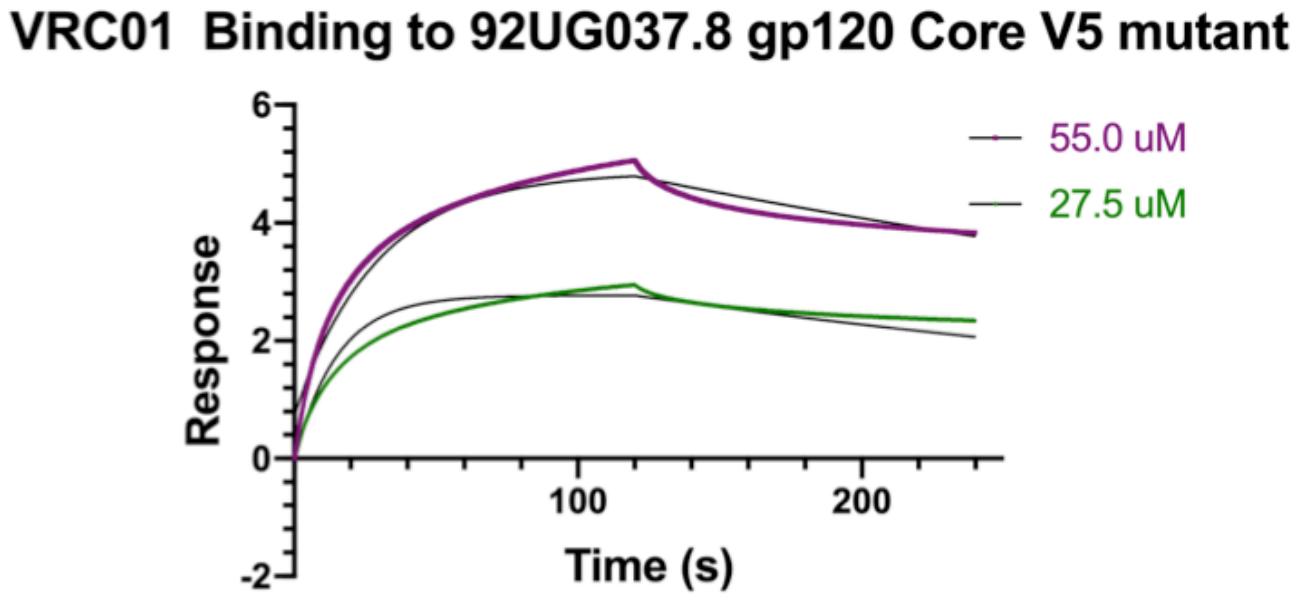
Supplementary Table 1. Binding of CH103 lineage Fabs to gp120 core variants.

	CH505 T/F gp120 core WT		
	K_D (μM)	**k_{on}** ($x10^3$ $M^{-1}s^{-1}$)	**k_{off}** ($x10^{-1}$ s^{-1})
I3.2	1.06 ± 0.09	21.32 ± 0.59	0.24 ± 0.004
I32M	0.98 ± 0.06	14.34 ± 0.57	0.14 ± 0.003
I33M	1.86 ± 0.11	29.61 ± 1.12	0.55 ± 0.009
I3.1	1.98 ± 0.34	24.25 ± 1.42	0.25 ± 0.005
UCA WT* **	2.40 ± 0.04	87.16 ± 2.84	2.10 ± 0.06
VRC01* **	1.16 ± 0.03	1.13 ± 0.02	1.30 ± 0.017 x 10^{-2}
	CH505 T/F gp120 core V5 ETF mutant*		
	K_D (μM)	**k_{on}** ($x10^3$ $M^{-1}s^{-1}$)	**k_{off}** ($x10^{-1}$ s^{-1})
I3.2	>100	---	---
I32M	15.51 ± 4.11	18.63 ± 8.90	2.52 ± 0.62
I33M	3.70 ± 0.18	34.69 ± 0.78	1.28 ± 0.09
I3.1	23.74 ± 2.77	12.98 ± 1.14	3.05 ± 0.09
UCA WT	NB	NB	NB
VRC01* **	1.44 ± 0.04	0.40 ± 0.01	5.75 ± 0.11 x 10^{-3}
	92UG037.8 gp120 core WT		
	K_D (μM)	**k_{on}** ($x10^3$ $M^{-1}s^{-1}$)	**k_{off}** ($x10^{-1}$ s^{-1})
I3.2	>100	---	---
I32M	41.89 ± 8.26	10.66 ± 1.86	4.40 ± 0.29
I33M	16.17 ± 2.21	4.87 ± 0.49	0.78 ± 0.03
I3.1	3.28 ± 0.83	24.67 ± 1.18	0.95 ± 0.02
UCA WT	NB	NB	NB
VRC01	8.11 ± 0.88	0.53 ± 0.03	4.29 ± 0.21 x 10^{-2}
	92UG037.8 gp120 core V5 mutant**		
	K_D (μM)	**k_{on}** ($x10^3$ $M^{-1}s^{-1}$)	**k_{off}** ($x10^{-1}$ s^{-1})
UCA WT	52.23 ± 6.37	81.22 ± 0.71	4.20 ± 0.15
UCA $Q39_HL$	25.10 ± 2.43	11.03 ± 0.76	2.61 ± 0.07
UCA $S56_HE$	6.98 ± 0.44	4.39 ± 0.21	0.31 ± 0.01
UCA $Q39_HL$, $S56_HE$	2.20 ± 0.30	7.94 ± 0.55	0.25 ± 0.04
VRC01	2.27 ± 0.16	1.06 ± 0.03	2.41 ± 0.09 x 10^{-2}

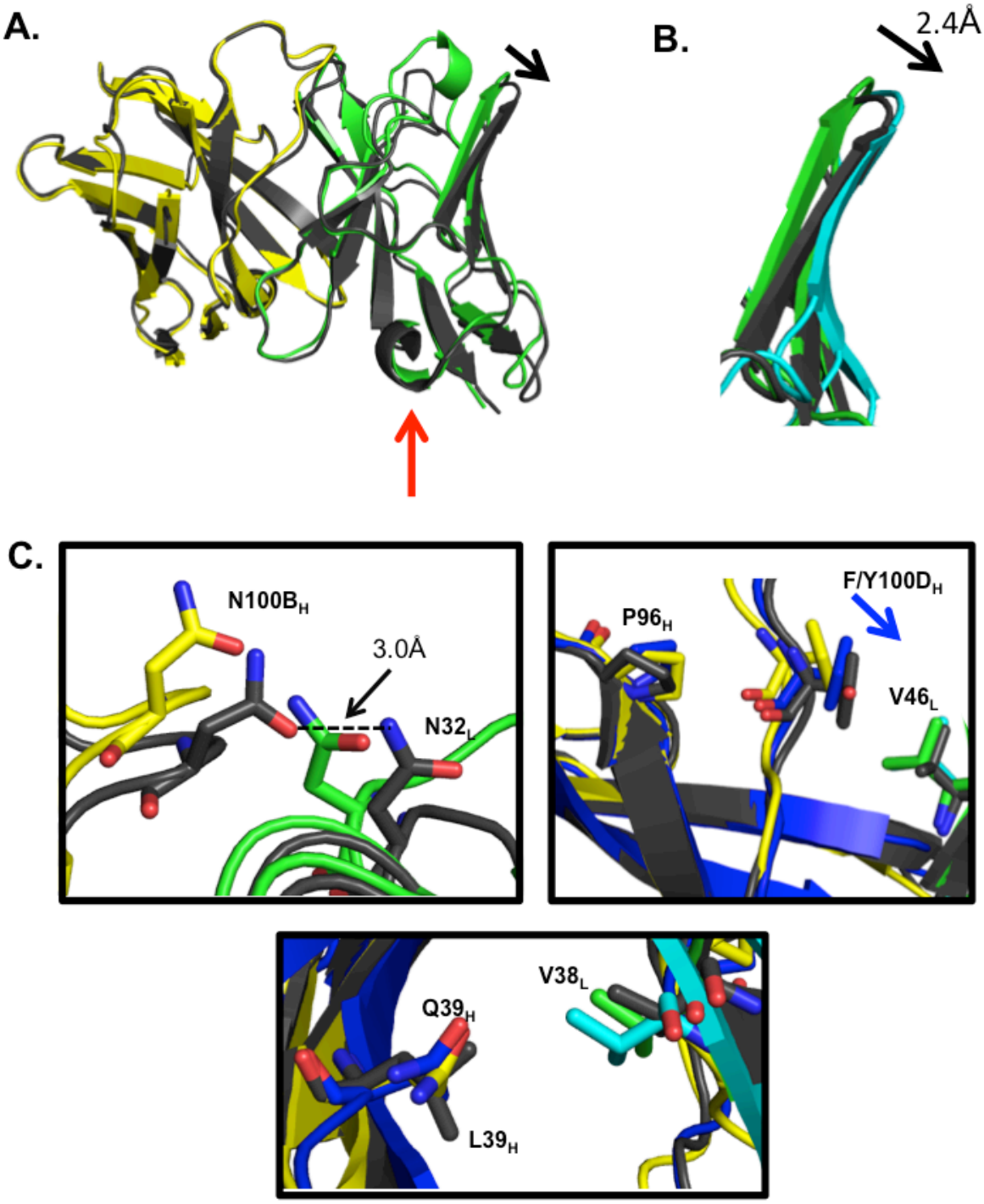
*A three residue "ETF" insertion was added to the V5 loop of wild-type CH505 T/F gp120 core.
**V5 loop residues "GNINES" of wild-type 92UG037.8 gp120 core were replaced with "KNNT" of CH505 T/F virus Env.
***Published elsewhere [22].
NB denotes no binding detected.
Dashes indicate combinations not tested.



Supplementary Figure 1. Sequence alignments of CH103 Fab heavy chain sequences. Complementarity determining region (CDR) loops and the framework region (FWR) for each chain are indicated. Conserved residues with respect to the UCA are marked by a dash. Sites mutated in this study are indicated with a red arrow.



Supplementary Figure 2. Representative curves from biolayer interferometry for a non-CH103 lineage CD4bs bnAb, VRC01. The VRC01 Fab was immobilized onto an anti-human Fab-CH1 biosensor, and gp120 core constructs were introduced at three or more different concentrations, ranging from low micromolar to high micromolar. Fits and affinities derived as described in Methods.



Supplementary Figure 3. Superpositions of mutant I33M, chimeric I3.1, and the CH103 bnAb. (A) Overall superposition of mutant Fab I33M onto the CH103 bnAb. The domains are colored: I33M V_H (yellow) and V_L (green), and CH103 (gray). Black arrows indicate regions where there is a conformational shift. Red arrow indicates the absence of a shift. (B) Zoomed in view of the DE loops of I33M (green), I3.1 (cyan), and CH103 (gray). The degree of shift is given. (C) Zoomed in views of residues that were mutated are shown as sticks for the three Fabs. Domains for the different Fabs are colored as in (A) and (B). I3.1 is excluded from the top left panel for clarity. Hydrogen bond distances are given.