

HIV-1 Vaccine-elicited Antibodies Reverted to Their Inferred Naive Germline Reveal Associations between Binding Affinity and *in vivo* Activation

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Supplementary Figures:

Name	Sequence (5' - 3')	
	Heavy chain	Light chain
	Forward	
GEBT383	CCTGTGGTTCTTCTCCTCCTG	CTCCTCTCCTCCTCACTCTCC
GEBT391	TCACCATGAAGTTTGGGCTGAG	GCTCCTGCTGCTCTGGC
GEBT393	CCATGAAGTTTGGGCTGAG	GGTAAGGAAGGAGAACAGTAGGA
GEBT403	CCCCAGAGCACAGCACCTCA	TCCTCCTCACCCCTTCTCATT
GEBT404	GGCCTGAGCTGGGTTTTCC	TTAATTGTGTGGACATCTGTT
GEBT412	GGCTGAGCTGGGTTTTCT	CCTGCTGCTCTGGCTCCC
GEBT414	CATTTCTGTCTCCACCACGATG	ATGAGGGTCCCCGTTGAGC
	Reverse	
GEBT383	CTCCCTGCACCTGCTCCTG	GCCCTGTTCTGATTCTGA
GEBT391	GAGGTTCCCTGGGGAAGTGT	GTCCCCTTCTACCGACACC
GEBT393	GTTCCCTGGGGAAGTGT	TTGGGATGCAGAGGTGAC
GEBT403	TAATATTTCTATATAATT	GAAGGAAGCAGCAGCCACAGA
GEBT404	GATGACGTCCCACATCCTGACA	GACTTTCAAATCTGACTGAGTTT
GEBT412	GAAGATGACGTCCCACATCC	CTTCTACCGACACCATCAGAG
GEBT414	TCCACCTCCCATGGTTAGGTCC	GGTGCAGAGGTGACTTCAT

Table S1 . PCR primers used for genomic sequence amplification

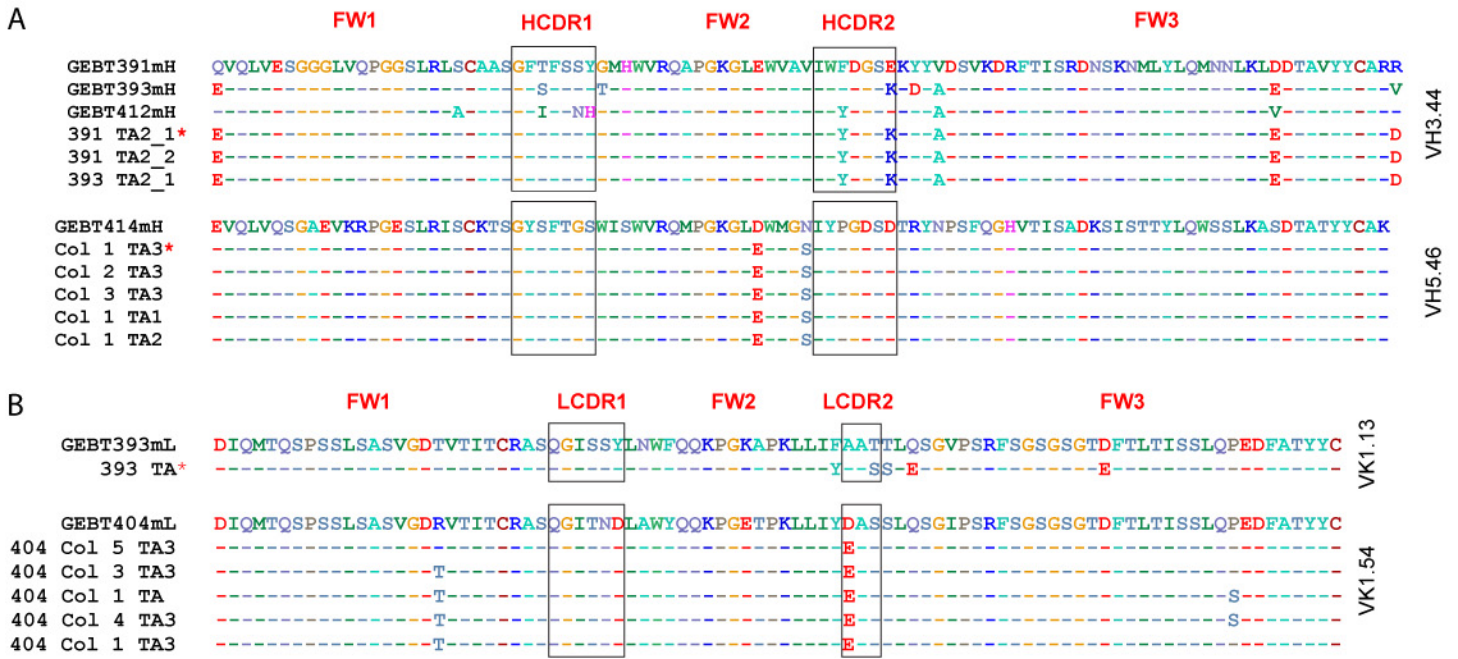


Figure S1 . Unique VH gene sequences (A) and VL gene sequences (B) obtained from NHP F128 T cell genomic DNA amplification and TA cloning. The first sequence in each gene family is the mature version and the sequences that follow are the TA clones. The closest germline genes as currently named in IMGT are shown on the right. The “*” indicates the clone’s sequence which was used in the construction of the igL antibody. Sequences from 404 were analyzed but not used because the published database was the same except for one change and we decided to use it. Col - refers to colony.

Heavy chain amino acid alignment

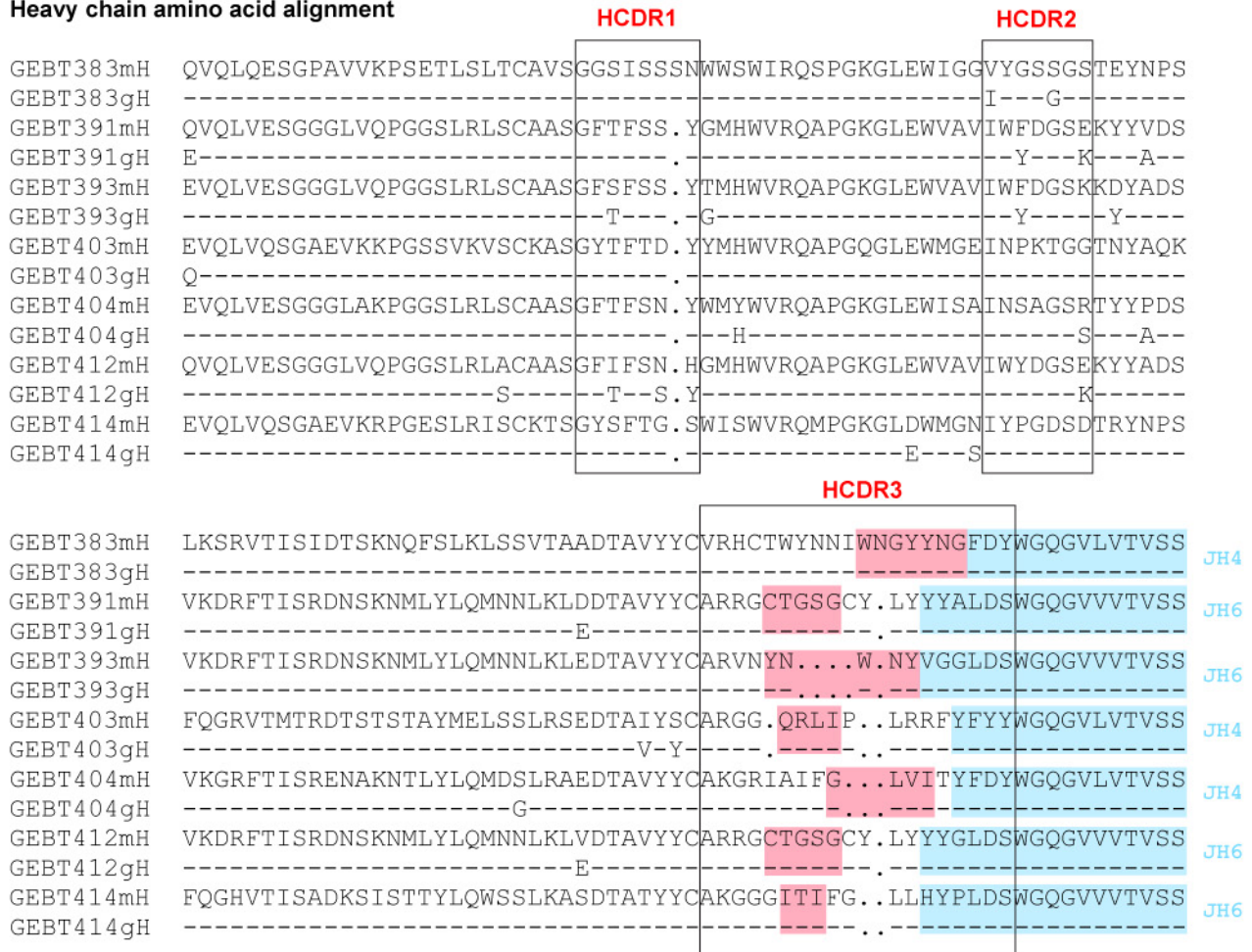


Figure S2. Amino acid alignment of the antibodies. mH (mature heavy chain) is shown on top with the corresponding gH (germline heavy chain) compared below with only the changes highlighted. DH and JH regions are boxed in red and blue respectively.

Light chain amino acid alignment

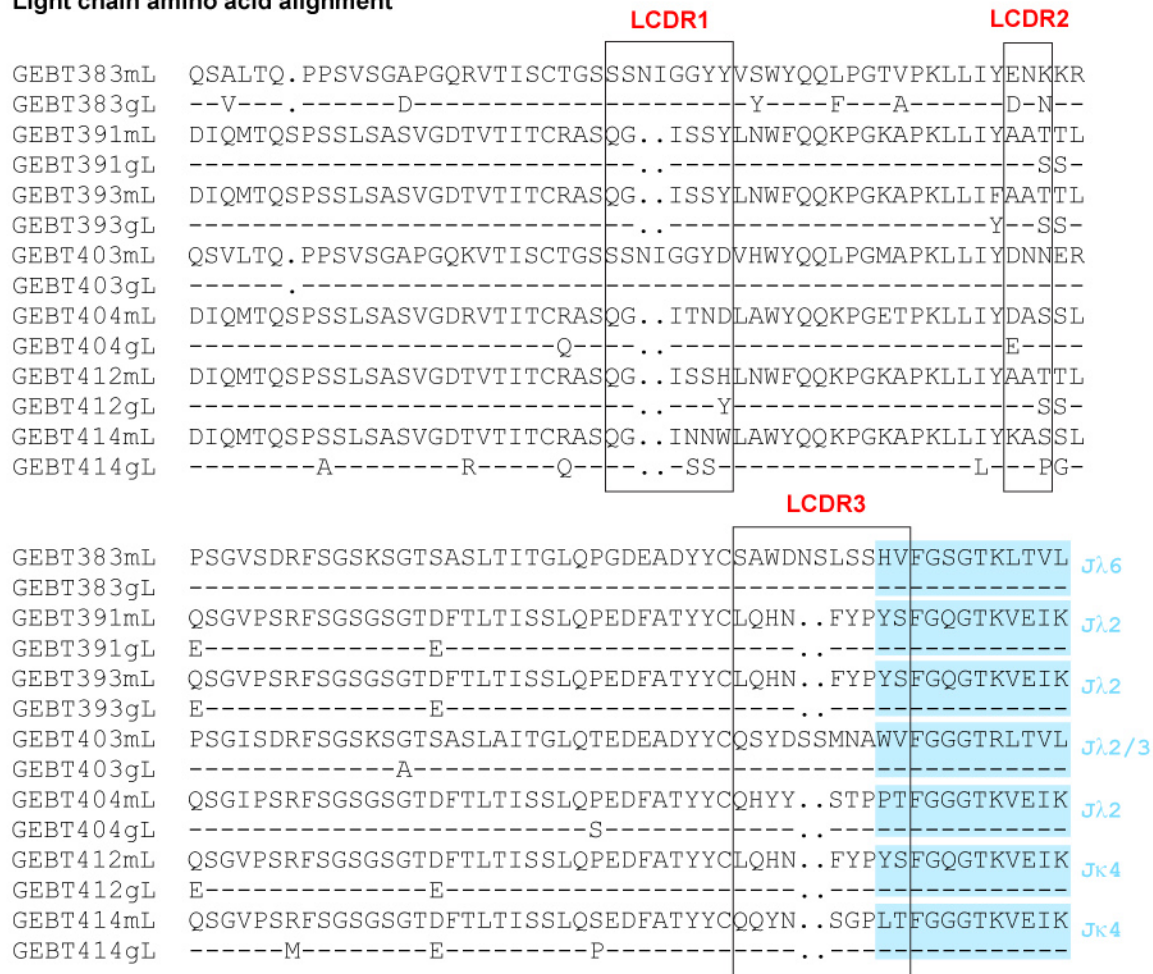


Figure S3 . Amino acid alignment of the antibodies. mL (mature light chain) is shown on top with the corresponding gL (germline light chain) compared below with only the changes highlighted. JL regions are boxed blue.

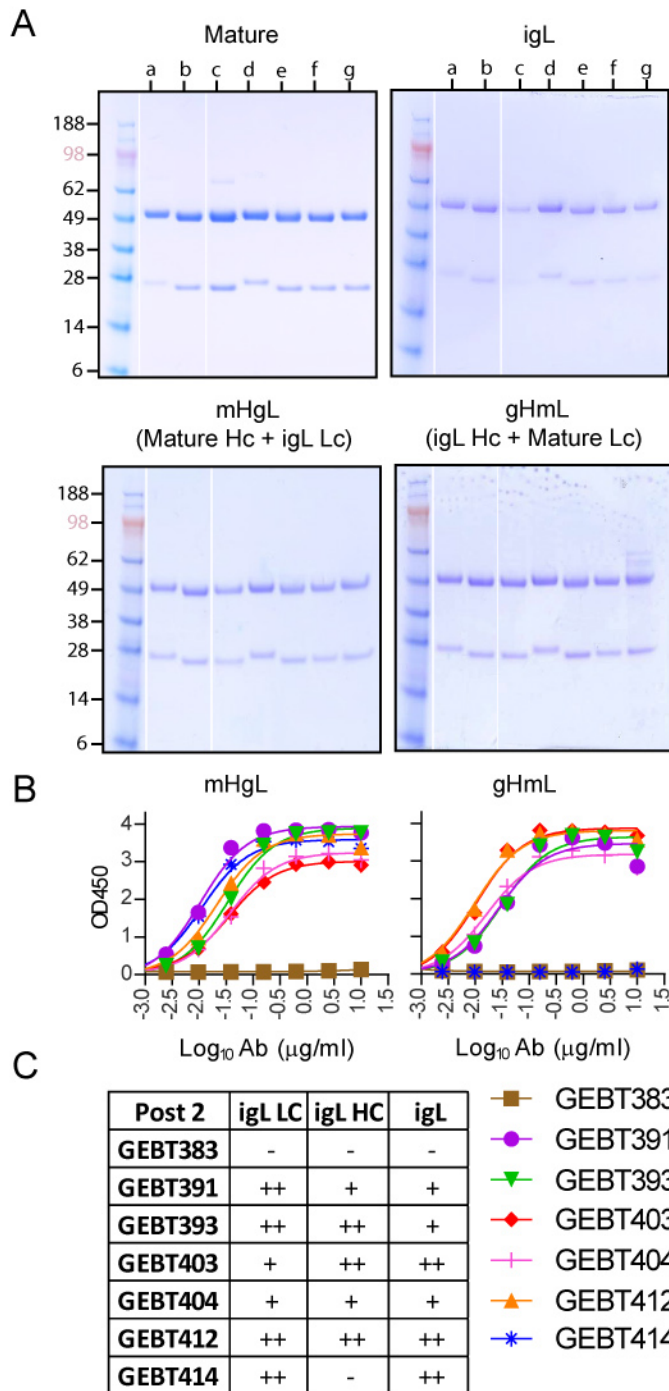


Figure S4. Reducing gels of all antibodies in the study and chimeric antibody binding to YU2 gp140-F.
 (A) Heavy and light chains of mature, igL and chimeric antibodies (a-g) on reducing gels. (B) Chimeric IgG binding to YU2 gp140-F by ELISA. (C) Chimeric antibody binding to gp140-F is depicted as a table.

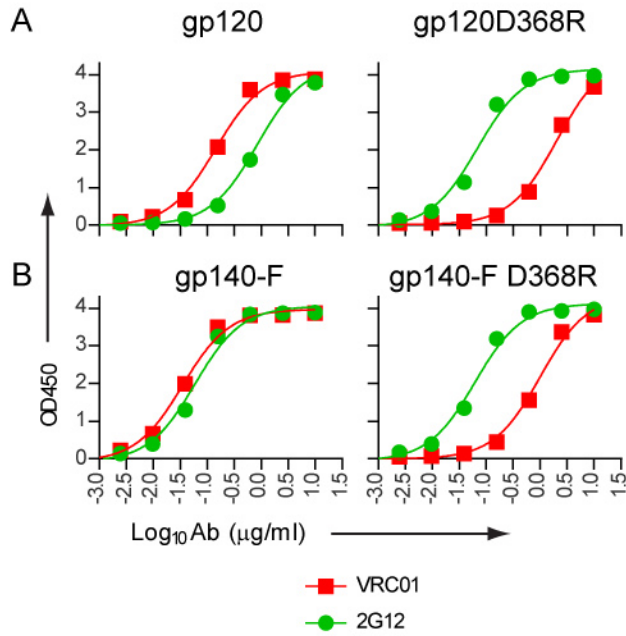


Figure S5. ELISA binding curves of the positive control antibodies. VRC01 and 2G12 antibodies binding to gp120 (A), gp140-F (B) and to the corresponding D368R mutants.

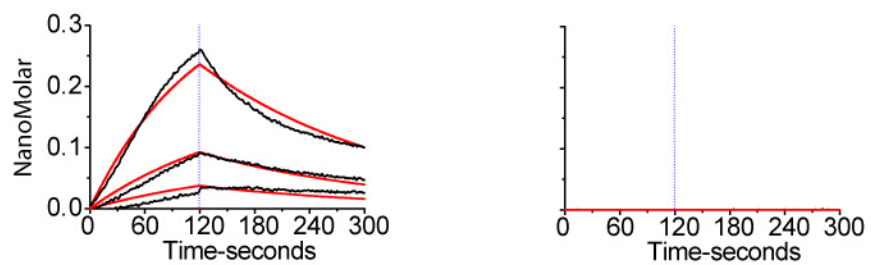


Figure S6. Binding analysis of GEBT 383.

GEBT383 antibody binds to His-Tag (left) and not to the control protein without the His-tag (right).

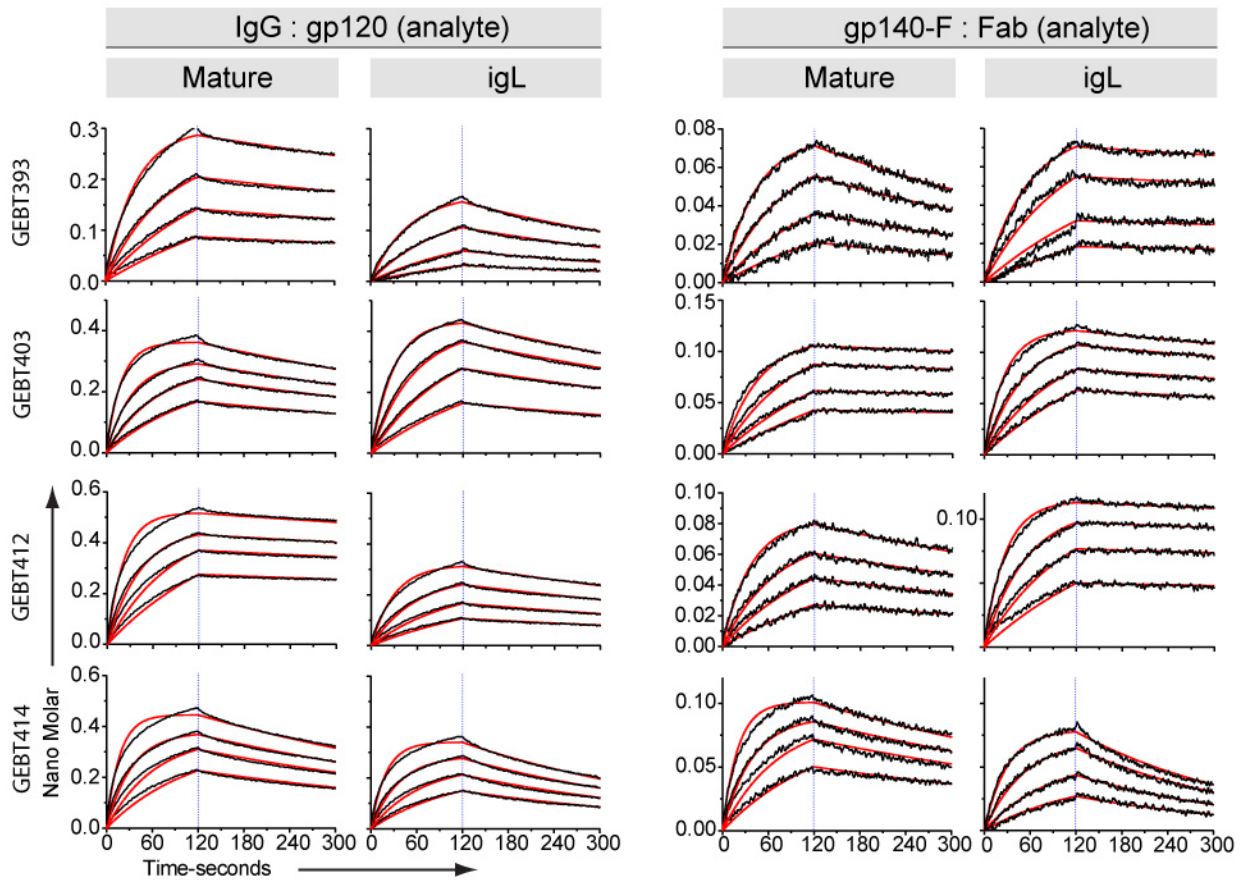


Figure S7. BLI binding curves of the antibodies. Mature and igL IgG binding to gp120 (two panels on left), and the same antibodies as Fabs binding to gp140-F (two panels on right).

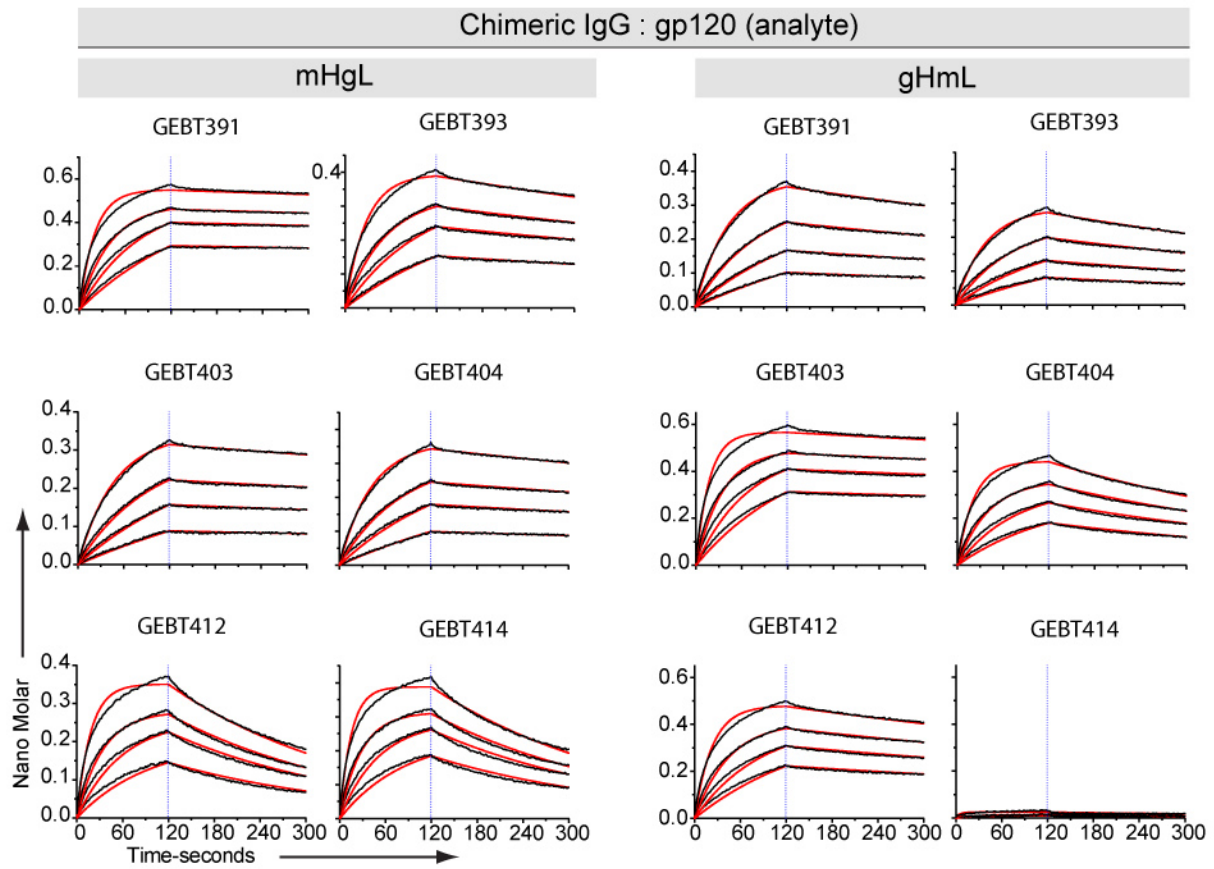


Figure S8. BLI binding curves of the antibodies. Chimeric IgG binding to gp120. mHgL - mature heavy chain and igL light chain. gHmL - igL heavy chain and mature light chain.

A

BLI	kD (nM)	kon(1/Ms)	kdis(1/s)	kD (nM)	kon(1/Ms)	kdis(1/s)
	IgG : gp120 (analyte)					
	IgL			Mature		
GEBT391	118	1.72E+04	2.04E-03	6	9.21E+04	5.58E-04
GEBT393	146	1.76E+04	2.57E-03	15	5.74E+04	8.32E-04
GEBT403	11	1.34E+05	1.47E-03	13	1.14E+05	1.50E-03
GEBT404	56	4.95E+04	2.79E-03	43	9.08E+04	3.91E-03
GEBT412	104	2.84E+04	2.95E-03	11	8.38E+04	8.85E-04
GEBT414	63	4.86E+04	3.05E-03	24	1.13E+05	2.74E-03
Fab : gp140-F (analyte)						
IgL			Mature			
GEBT391	86	1.78E+04	1.53E-03	2	1.61E+05	3.31E-04
GEBT393	101	2.11E+04	2.14E-03	5	7.07E+04	3.65E-04
GEBT403	2	1.71E+05	3.06E-04	5	1.16E+05	5.86E-04
GEBT404	32	9.85E+04	3.10E-03	7	1.22E+05	9.08E-04
GEBT412	55	2.61E+04	1.43E-03	2	1.47E+05	2.28E-04
GEBT414	36	1.20E+05	4.24E-03	9	1.94E+05	1.75E-03
Chimeric IgG : gp120 (analyte)						
gHmL			mHgL			
GEBT391	42	2.26E+04	9.47E-04	4	4.98E+04	2.08E-04
GEBT393	59	2.43E+04	1.42E-03	24	2.06E+04	4.96E-04
GEBT403	5	5.76E+04	3.12E-04	77	5.19E+04	4.01E-03
GEBT404	50	4.47E+04	2.23E-03	26	3.64E+04	9.59E-04
GEBT412	22	4.31E+04	9.35E-04	31	2.32E+04	7.12E-04
GEBT414	LB	-	-	61	6.33E+04	3.88E-03

B

SPR	kD (nM)	kon(1/Ms)	kdis(1/s)	kD (nM)	kon(1/Ms)	kdis(1/s)
	Fab : gp140-F (analyte)					
	IgL			Mature		
GEBT391	30	9.53E+05	2.83E-02	27	6.19E+04	1.64E-03
GEBT393	43	9.09E+04	3.90E-03	12	2.06E+05	2.57E-03
GEBT403	2	2.57E+05	4.58E-04	7	2.28E+05	1.59E-03
GEBT404	17	1.23E+05	2.09E-03	7	2.62E+05	1.81E-03
GEBT412	16	1.77E+05	2.79E-03	2	2.84E+05	4.57E-03
GEBT414	9	1.34E+05	1.25E-03	6	1.21E+05	7.76E-04

Figure S9. Binding affinity (kD), association rates (kon) and dissociation rates (koff) of the antibodies. (A) IgG, Fabs and chimeric IgGs by BLI and (B) Fabs by SPR. The values shown in green are a repetition of data shown in Figure 3. LB - Low binding.

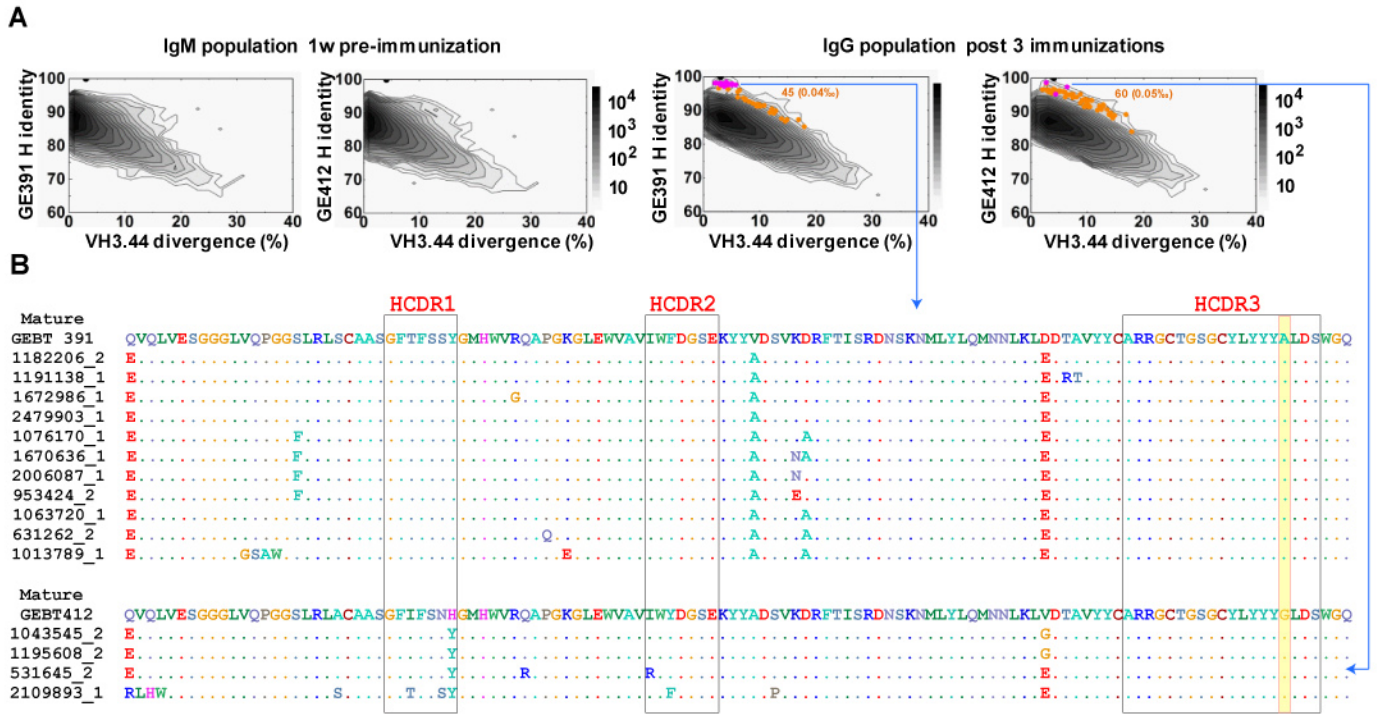


Figure S10: Germline gene usage of the VH chains derived from the unbiased antibody repertoire 3 weeks after the 3rd inoculation during F128 immunization. The distribution is plotted at the gene family level with the percent repertoire (%) labeled. (A) Identity/divergence analysis of heavy chain repertoires for CD4bs-directed mAbs isolated from F128 GE391 and GE412 is shown. The heavy chain population is plotted as a function of sequence identity to the template mAb and sequence divergence from putative germline V genes, with the template mAb labeled as a black dot on the plot partially visible at the top left corner of the plot. Color coding denotes sequence density. Sequences with an HCDR3 identity 95% or greater to the template mAb are shown in orange. The purple dots refer to matched sequences shown in B. (B) Alignments of sequences that show 100% HCDR3 identity to GE391 and GE412. The alignments were generated by the sequence alignment editor software, BioEdit.

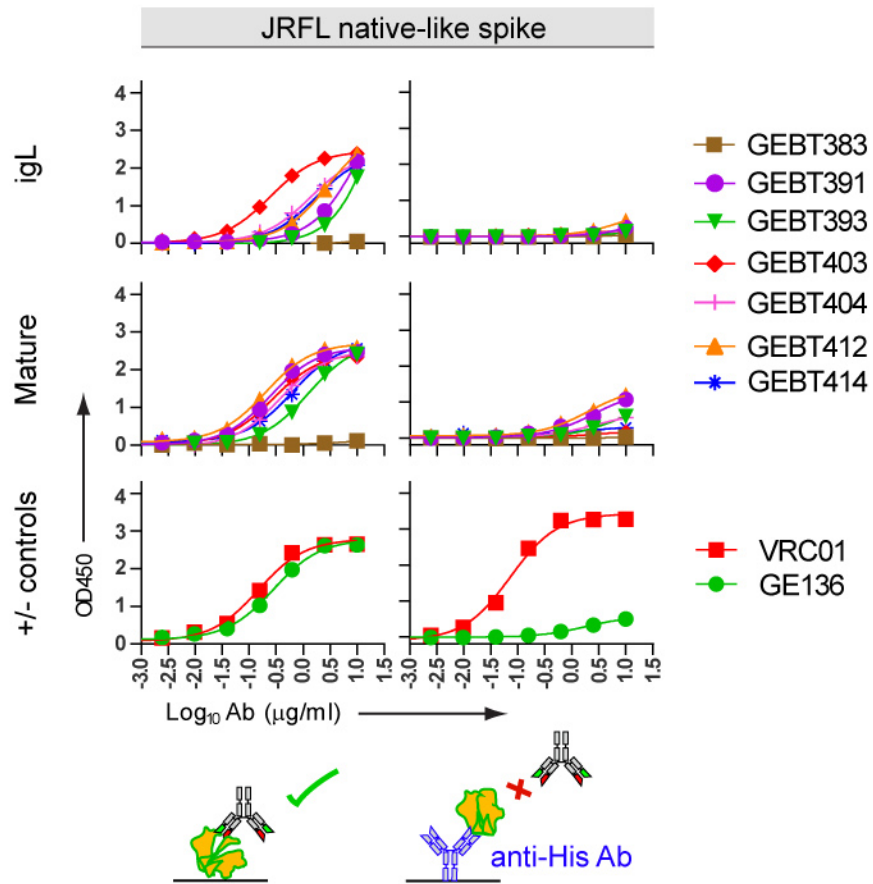


Figure S12. ELISA binding curves of igL and mature antibodies to JRFL-SOSIP native like spike that is either coated directly (left panels) or captured on a plate by anti-His antibody (right panels).

		Mature		igL	
		HxB2.DG.SG3	MN.3.SG3	HxB2.DG.SG4	MN.3.SG4
IgG	GEBT383	>50	15.1	>50	11.3
	GEBT391	1.36	0.223	>50	>50
	GEBT393	5.10	45.0	>50	>50
	GEBT403	0.455	0.269	0.216	0.211
	GEBT404	3.57	18.1	>50	17.5
	GEBT412	0.735	0.682	12.8	3.05
	GEBT414	>50	>50	>50	>50
Fab	GEBT383	>50	>50	>50	16.8
	GEBT391	0.924	0.004	>50	>50
	GEBT393	13.7	1.11	>50	>50
	GEBT403	0.931	1.20	0.997	>50
	GEBT404	5.53	0.469	>50	49.9
	GEBT412	4.51	>50	>50	>50
	GEBT414	>50	2.29	16.4	2.62
		mHgL		gHmL	
Chimeric IgG	GEBT383	>50	>50	>50	8.44
	GEBT391	0.669	0.256	>50	>50
	GEBT393	8.59	12.0	>50	>50
	GEBT403	0.380	0.320	0.510	>50
	GEBT404	6.86	>50	22.6	>50
	GEBT412	5.57	>50	14.0	0.668
	GEBT414	>50	>50	>50	13.0
+ve Control	VRC01	0.014	0.067		
Color shading represents potency as follows:					
		<0.100	0.100-1.00	1.00-10.0	>10.0

Figure S13. IC-50 neutralization values are shown for IgG, Fabs and Chimeric IgG.