

Supporting Online Material for

High Resolution Longitudinal Study of HIV-1 Env Vaccine-elicited B Cell Responses to the Virus Primary Receptor Binding Site Reveals Affinity Maturation and Clonal Persistence

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This PDF file includes:

Figures S1 to S3 and Tables S1

Figure S1. Genetic characterization of Env and CD4bs-specific Ig light chain sequences.

Figure S2. Characterization of clonal lineage persistence by next generation sequencing (NGS).

Figure S3. Binding specificity and genetic characterization of the selected variant clones from the GEAT361 lineage.

Table S1. Neutralization profiles (IC_{50} value, $\mu\text{g/ml}$) of vaccine-elicited CD4bs-specific mAbs

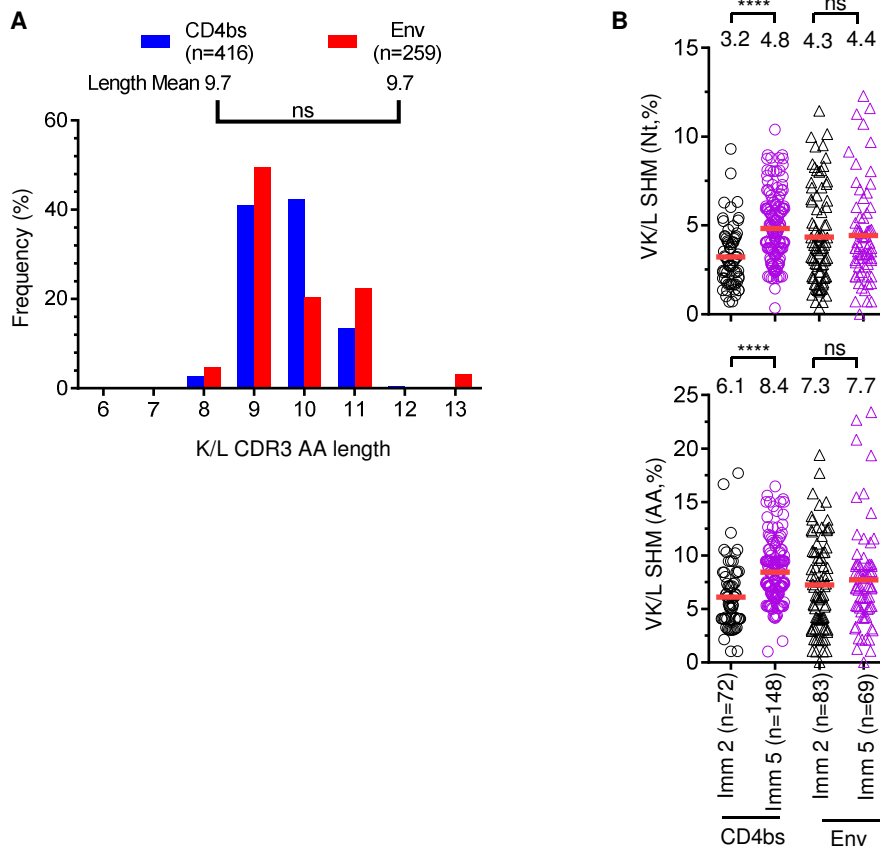


Figure S1. Genetic characterization of Env and CD4bs-specific Ig light chain sequences. (A) Characterization of light chain CDR3 length. Statistical differences of CDR3 length between the CD4bs- and Env-specific Ig repertoires were evaluated using Mann-Whitney test with ns = nonsignificant. **(B)** Light chain V-gene SHMs of CD4bs-specific Ig repertoire increase during the course of immunization of animal F125. SHM differences of sequences at Imm 2 and Imm 5 were evaluated statistically using one-way ANOVA with **** $p < 0.0001$ and ns = nonsignificant.

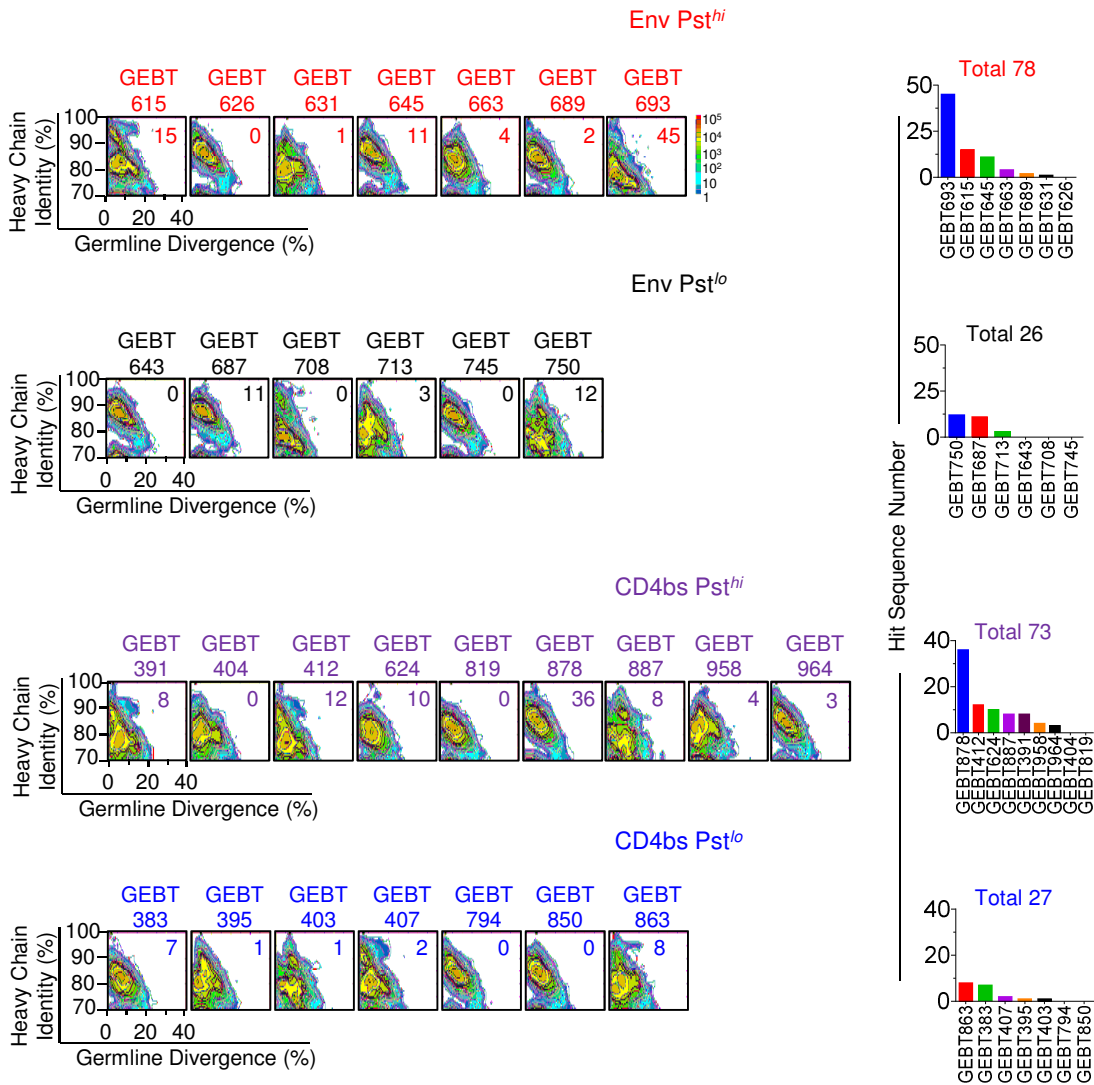


Figure S2. Characterization of clonal lineage persistence by next generation sequencing (NGS). Single-cell sorted representative heavy chain VDJ sequences from animal F128 Imm 2 were used to query against the same animal Imm 5 NGS data set (J Immunol 192: 3637-3644). **Left**, heat map plots show sequence identity (Y-axis) versus germline divergence (X-axis). The number at the top right corner in each heat map corresponds to the number of raw reads with at least 95% identity to the query sequence, which are label in red, black, purple and blue for Env Pst^{hi}, Env Pst^{lo}, CD4bs Pst^{hi}, and CD4bs Pst^{lo}, respectively (left panel). **Right**, total hit number is summarized from the heat map panel.

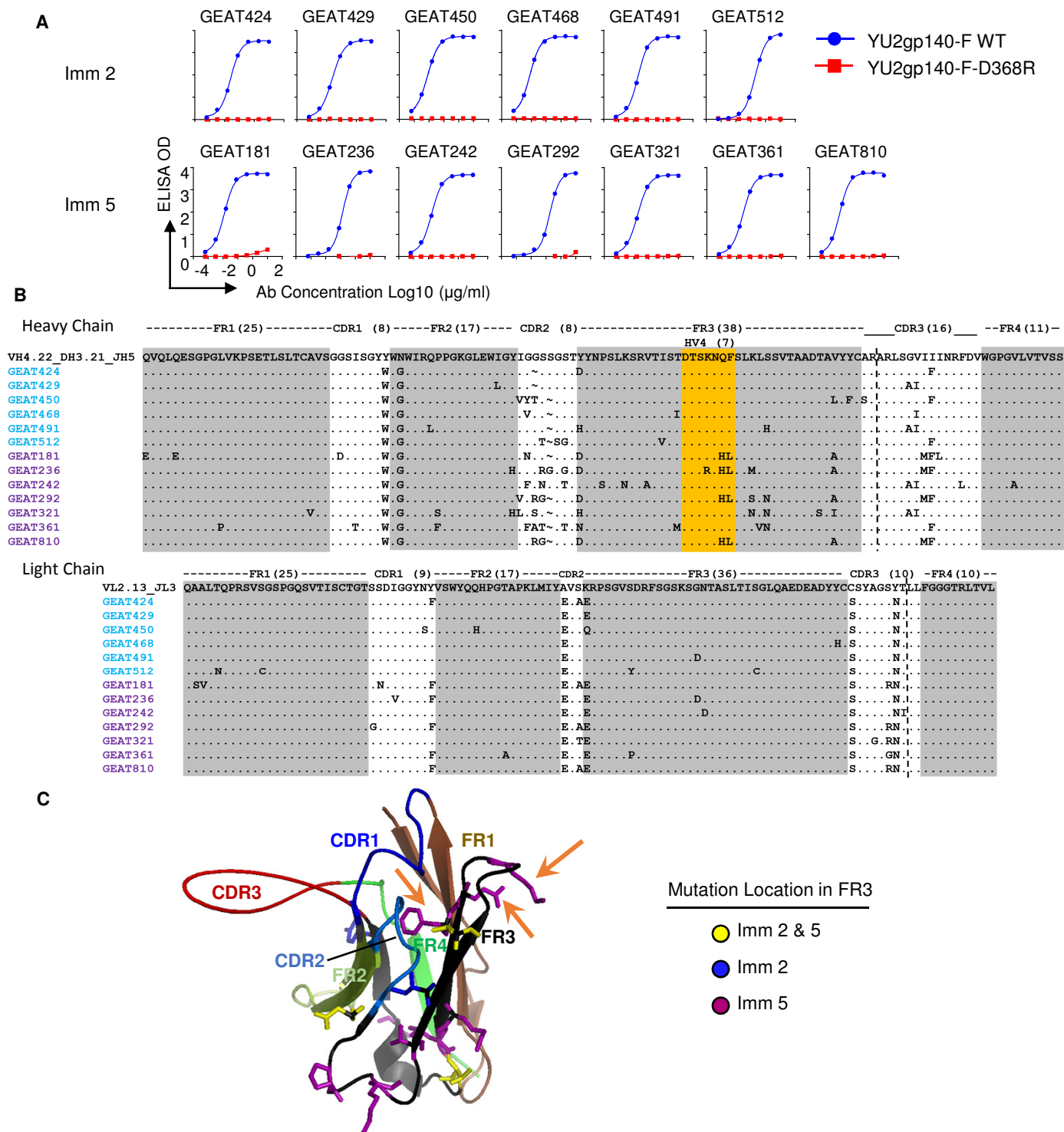


Figure S3. Binding specificity and genetic characterization of the selected variant clones from the GEAT361 lineage. (A) Binding specificity of the GEAT361 lineage mAb clones from Imm 2 and Imm 5 to wildtype trimer YU2gp140-F (blue circle) or mutant trimer YU2gp140-F-D368R (red square). (B) Accumulated mutations within subdomains of Ig heavy and light chains of selected variant clones during the immunization course. Each subdomain of the GEAT361 lineage clones was aligned with the corresponding germline sequence for heavy chain (upper panel) and light chain (lower panel). The clones from Imm 2 and Imm 5 are highlighted in blue and purple, respectively. The framework (FRs) regions are boxed in gray and amino acid residues with variation from the germline precursor sequence are depicted. The TCR HV4-like region within the FR3 is highlighted in orange. A dashed line separates the V-gene (on the left) from (D)J-genes (on the right). The number in parenthesis represents the subdomain length. (C) Accumulated mutations within subdomains of GEAT361 lineage heavy chain variable domain modeled using GE136 Fab (PDB ID code 4KTD) as template. Heavy chain subdomains, including CDR1 (navy), CDR2 (cyan), CDR3 (red), FR1 (brown), FR2 (lemon), FR3 (black) and FR4 (green) are marked. The side chains of the residues divergent from the assigned germline sequence observed in clones from both Imm 2 and Imm 5, Imm 2 only and Imm 5 only are labeled in yellow, blue and purple, respectively. Most of the mutations are distal from the CDRs, while some of the mutations in the HV4-like region within FR3 are close to the CDRs. Note the TCR HV4-like loop region within the FR3, with residues mutated from the germline version indicated by arrows, is in the same plane with the antigen binding loops including CDRs1,2, and 3.

Table S1. Neutralization profiles (IC₅₀ value, µg/ml) of vaccine-elicited CD4bs-specific mAbs

Animal	Imm. time point	Persistence (Pst ^{hi} or ^{lo} or Newly emerging)	CD4bs mAb	Clade B						Clade A	Clade C	Clade B			Tier 1 geomean	Tier 1 breadth		
				HXBc2	MN	SF162	SS1196	BaL.26	JRCFSF	DJ263.8	MW965.26	JRFL	89.6	YU2				
				Tier 1						Tier 2								
F125	Imm 2	<i>lo</i>	GEAT422	0.48	>50	10.9	ND	>50	ND	>50	>50	>50	>50	>50	>50	17.9	33	
		<i>hi</i>	GEAT424	2.18	1.69	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	22.1	25
		<i>hi</i>	GEAT429	1.32	0.19	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	15.8	25
		<i>lo</i>	GEAT442	>50	>50	>50	>50	>50	>50	>50	25.4	0.47	>50	>50	>50	>50	25.6	25
		<i>hi</i>	GEAT450	0.66	0.24	14.2	ND	>50	ND	>50	>50	25.2	>50	>50	>50	>50	7.2	67
		<i>hi</i>	GEAT468	0.55	0.22	>50	ND	>50	ND	>50	>50	>50	>50	>50	>50	>50	9.5	33
		<i>hi</i>	GEAT482	0.79	0.51	47.7	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	16.7	38
		<i>hi</i>	GEAT491	2.14	0.64	>50	>50	>50	>50	>50	15.9	>50	>50	>50	>50	>50	17	38
		<i>hi</i>	GEAT493	3	8.99	>50	>50	>50	>50	>50	>50	2.68	>50	>50	>50	>50	19.7	38
		<i>hi</i>	GEAT512	1.21	0.14	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	15.1	25
		<i>lo</i>	GEAT524	>50	>50	>50	ND	>50	ND	>50	>50	>50	>50	>50	>50	>50	50	0
		<i>lo</i>	GEAT532	2.58	>50	44.3	ND	>50	ND	>50	>50	>50	>50	>50	>50	>50	29.9	33
		<i>hi</i>	GEAT615	2.26	3.82	32.6	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	23.3	38
		<i>hi</i>	GEAT718	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	50	0
F128	Imm 2	<i>hi</i>	GEBT391	0.2	0.1	1.7	43	>50	>50	13.2	2.24	>50	>50	>50	>50	4.3	75	
		<i>lo</i>	GEBT392	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	50	0	
		<i>lo</i>	GEBT393	2.91	0.73	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	20.7	25	
		<i>lo</i>	GEBT403	0.07	0.04	1.23	>50	>50	>50	>50	>50	>50	>50	>50	>50	5.6	38	
		<i>hi</i>	GEBT404	0.55	0.05	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	12	25	
		<i>hi</i>	GEBT412	0.41	0.59	4.13	11.3	49.6	>50	18.4	>50	>50	>50	>50	>50	8.4	75	
		<i>hi</i>	GEBT414	3.93	>50	29.2	>50	>50	>50	44.4	>50	>50	>50	>50	>50	33.5	38	
		<i>hi</i>	GEBT624	1.52	>50	6.94	>50	>50	>50	>50	>50	>50	>50	>50	>50	25.2	25	
		<i>hi</i>	GEBT878	1.09	4.62	25.1	>50	>50	>50	>50	>50	>50	>50	>50	>50	21.1	38	
		<i>hi</i>	GEBT900	0.77	0.15	7.3	>50	>50	>50	>50	>50	>50	>50	>50	>50	11.3	38	
		<i>hi</i>	GEBT909	2.76	>50	30.7	>50	>50	>50	>50	>50	>50	>50	>50	>50	32.8	25	
		<i>hi</i>	GEBT916	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	50	0	
		<i>hi</i>	GEBT962	5.15	>50	20.1	>50	>50	>50	>50	>50	>50	>50	>50	>50	33.6	25	
F125	Imm 5	New	GEAT117	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	50	0	
		New	GEAT134	0.26	0.09	2.79	40.7	>50	>50	4.41	0.07	>50	>50	>50	>50	2.6	75	
		<i>hi</i>	GEAT139	0.46	12.4	5.7	>50	>50	>50	>50	>50	>50	>50	>50	>50	17.8	38	
		New	GEAT144	0.48	>50	8.52	>50	>50	>50	1.97	>50	>50	>50	>50	>50	15	38	
		New	GEAT156	0.08	0.4	0.9	6.4	>50	>50	0.43	>50	>50	>50	>50	>50	3.2	63	
		<i>hi</i>	GEAT181	0.51	0.23	11.7	24.6	>50	>50	8.05	0.08	>50	>50	>50	>50	3.9	75	
		<i>hi</i>	GEAT236	1.19	0.05	7.07	>50	>50	>50	12.6	>50	>50	>50	>50	>50	8.7	50	
		<i>hi</i>	GEAT242	1.1	0.44	31.6	>50	>50	>50	16	0.23	>50	>50	>50	>50	7.2	63	
		<i>hi</i>	GEAT282	1.61	0.45	3.74	>50	>50	>50	>50	>50	>50	>50	>50	>50	13.1	38	
		<i>hi</i>	GEAT292	0.42	0.1	6.69	13.5	>50	>50	15.7	>50	>50	>50	>50	>50	7.2	63	
		<i>hi</i>	GEAT301	2.05	0.86	>50	>50	>50	>50	10.6	>50	>50	>50	>50	>50	16.6	38	
		<i>hi</i>	GEAT304	0.75	0.5	>50	>50	>50	>50	6.63	0.05	>50	>50	>50	>50	5.5	50	
		<i>hi</i>	GEAT321	0.53	0.07	6.91	>50	>50	>50	6.01	>50	>50	>50	>50	>50	7.5	50	
		<i>hi</i>	GEAT361	1.59	0.45	9.78	>50	>50	>50	16	0.05	>50	>50	>50	>50	5.4	63	
		<i>hi</i>	GEAT375	0.86	0.05	>50	>50	>50	>50	5.45	>50	>50	>50	>50	>50	9.6	38	

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	<i>hi</i>	GEAT810	0.52	0.23	6.82	19.6	>50	>50	>50	>50	>50	>50	>50	10	50
	<i>hi</i>	GEBT424	>50	>50	>50	ND	>50	ND	>50	>50	>50	>50	>50	50	0
	New	GEBT428	1.38	>50	39.6	ND	>50	ND	>50	>50	>50	>50	>50	26.4	33
	New	GEBT433	1.51	0.1	>50	ND	>50	ND	>50	>50	>50	>50	>50	9.8	33
	New	GEBT442	0.27	0.04	7.33	2.9	17.6	>50	0.37	0.004	>50	>50	>50	0.9	88
	<i>hi</i>	GEBT462	8.56	>50	>50	ND	37.1	ND	>50	>50	>50	>50	>50	35.5	33
	<i>hi</i>	GEBT464	0.45	0.06	4.11	ND	>50	ND	>50	>50	>50	>50	>50	4.9	50
	New	GEBT467	7.12	0.36	>50	ND	>50	ND	>50	1.34	>50	>50	>50	8.7	50
F128	Imm 5	New	GEBT474	0.69	0.1	5.1	ND	>50	ND	>50	>50	>50	>50	5.9	50
	<i>hi</i>	GEBT478	1.97	0.82	7.2	>50	>50	>50	36	2.43	>50	>50	>50	10.3	63
	<i>hi</i>	GEBT501	1.98	6.24	>50	ND	>50	ND	>50	>50	>50	>50	>50	20.6	33
	<i>hi</i>	GEBT506	1.93	3.16	>50	>50	>50	>50	>50	>50	>50	>50	>50	23.6	25
	New	GEBT523	3.08	2.75	14.7	ND	>50	ND	>50	>50	>50	>50	>50	15.8	50
	New	GEBT536	11.7	>50	44.9	ND	>50	ND	>50	>50	>50	>50	>50	38.6	33
	<i>hi</i>	GEBT550	0.86	>50	7.41	>50	>50	>50	30.5	>50	>50	>50	>50	22.3	38
	<i>hi</i>	GEBT557	0.52	0.38	3.17	>50	44.7	>50	>50	0.67	>50	>50	>50	6.3	63
	<i>hi</i>	GEBT585	0.97	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	30.6	13
*		VRC01	0.02	0.02	0.13	0.25	0.03	0.08	0.08	0.04	0.02	0.53	0.2	0.05	100

Note: * human bNAbs antibody; ND: no determined; IC₅₀ (µg/ml):

<1 1-50