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Supplemental information

Adjuvants influence the maturation of VRC01-like antibodies during immunization

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Figure S1, Plasma antibody responses elicited by HXB2.WT.Core, Related to Figure 2

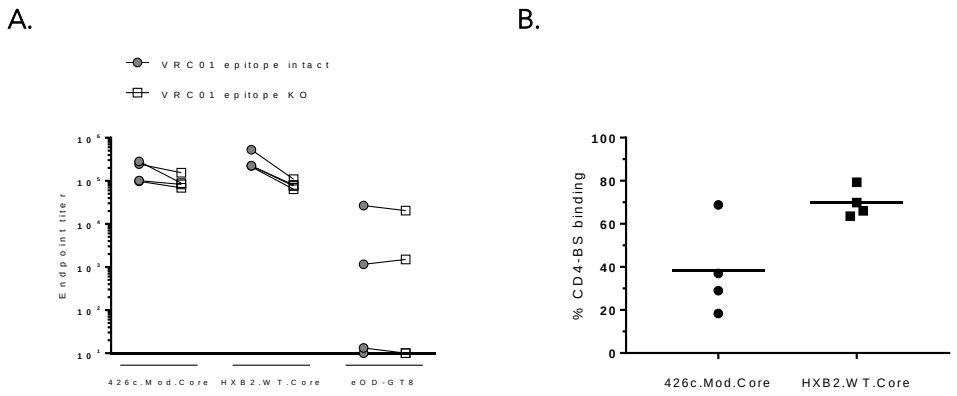


Figure S1. Plasma antibody responses elicited by HXB2.WT.Core. Related to Figure 2. (A) Mice were immunized with HXB2.WT.Core Ferritin adjuvanted with GLA-LSQ, and the plasma antibody responses were evaluated 2 weeks later against the indicated Env proteins (closed symbols) and their corresponding CD4-BS KO versions (open symbols). Each symbol represents one mouse, and lines connect data from the same mouse. (B) Percentages of plasma antibodies binding to the CD4-BS of the indicated proteins (derived from the results in panel (A)). Horizontal lines represent mean values of the indicated groups.

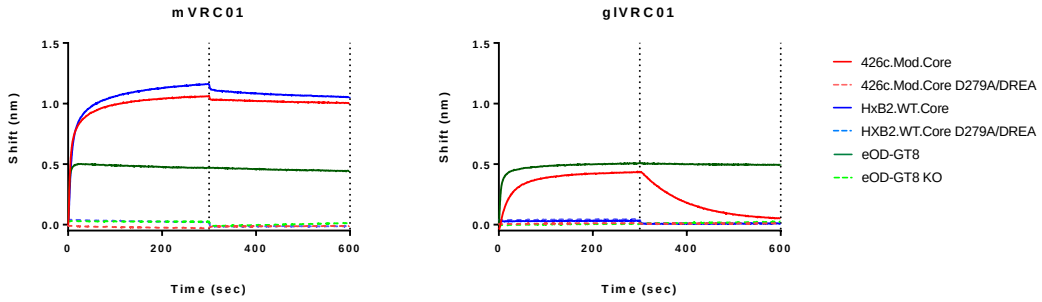
Figure S2, Information on the VRC01-like mAbs isolated following the boost immunization with HxB2.WT.Core, Related to Figure 5 and Table 1

mAb code	HC, #aa changes	LC, #aa changes	HC V-gene	HC J-gene	LC V-gene	LC J-gene	Adjuvant
MLK-002	15	8	Homsap IGHV1-2*02 F	Homsap IGHJ1*01 F	Musmus IGKV8-30*01 F	Musmus IGKJ1*01 F	Poly(I:C)
MLK-004	9	2	Homsap IGHV1-2*02 F	Homsap IGHJ1*01 F	Musmus IGKV8-30*01 F	Musmus IGKJ1*01 F	
MLK-005	7	3	Homsap IGHV1-2*02 F	Homsap IGHJ1*01 F	Musmus IGKV8-30*01 F	Musmus IGKJ1*01 F	
MLK-006	5	4	Homsap IGHV1-2*02 F	Homsap IGHJ1*01 F	Musmus IGKV8-30*01 F	Musmus IGKJ1*01 F	
MLK-008	8	4	Homsap IGHV1-2*02 F	Homsap IGHJ1*01 F	Musmus IGKV8-30*01 F	Musmus IGKJ2*01 F	GLA-LSQ
MLK-009	11	5	Homsap IGHV1-2*02 F	Homsap IGHJ1*01 F	Musmus IGKV8-30*01 F	Musmus IGKJ2*01 F	
MLK-010	8	6	Homsap IGHV1-2*02 F	Homsap IGHJ1*01 F	Musmus IGKV8-30*01 F	Musmus IGKJ2*01 F	
MLK-012	0	0	Homsap IGHV1-2*02 F	Homsap IGHJ1*01 F	Musmus IGKV8-30*01 F	Musmus IGKJ2*01 F	
MLK-014	7	2	Homsap IGHV1-2*02 F	Homsap IGHJ1*01 F	Musmus IGKV8-30*01 F	Musmus IGKJ2*01 F	
MLK-015	9	3	Homsap IGHV1-2*02 F	Homsap IGHJ1*01 F	Musmus IGKV8-30*01 F	Musmus IGKJ2*01 F	
MLK-016	7	4	Homsap IGHV1-2*02 F	Homsap IGHJ1*01 F	Musmus IGKV8-30*01 F	Musmus IGKJ2*01 F	

Figure S2. Information on the VRC01-like mAbs isolated following the boost immunization with HxB2.WT.Core. Related to Figure 5 and Table 1. Eleven VRC01-like mAbs were generated from animals in the Poly(I:C) and GLA-LSQ groups following their immunization with the HxB2.WT.Core Ferritin. The Env-recognition properties of these mAbs is presented in Figure 5. Their neutralizing potentials are presented in Table 1B.

Figure S3, mVRC01 and glVRC01 mAbs binding curves, Related to Figure 5 and Table 1

A.



B.

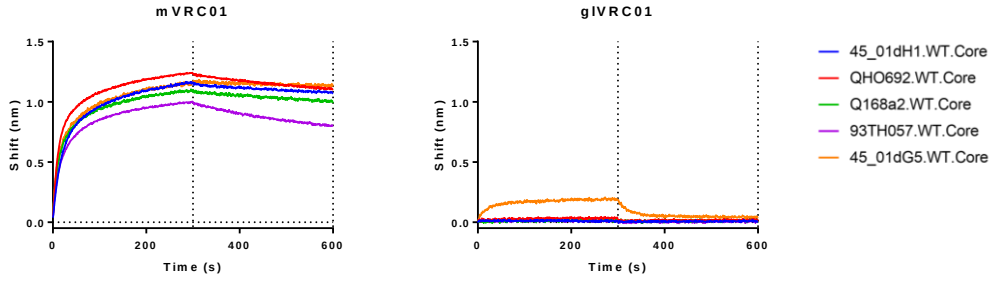


Figure S3. Related to Figure 5 and Table 1. mVRC01 and glVRC01 mAbs binding curves. Binding properties of mVRC01 and glVRC01 to the indicated soluble monomeric Envs (A) and five heterologous WT Cores (B) are shown as controls. Dotted lines indicate end of association and dissociation phases.

Table S1, All primers and cycling conditions used to amplify heavy and light chain, Related to Figure 3

PCR Step	Annealing temp.	Primer name	5′-3′ sequence	Reference
Igκ 1st	50°C	Forward		Tiller et al. J Immunol Met 2009
		5′ L-Vκ_3	TGCTGCTGCTCTGGGTTCCAG	
		5′ L-Vκ_4	ATTWTCAGCTTCCTGCTAATC	
		5′ L-Vκ_5	TTTTGCTTTTCTGGATTYCAG	
		5′ L-Vκ_6	TCGTGTTKCTSTGGTTGTCTG	
		5′ L-Vκ_6,8,9	ATGGAATCACAGRCYCWGGT	
		5′ L-Vκ_14	TCTTGTTGCTCTGGTTYCCAG	
		5′ L-Vκ_19	CAGTTCCTGGGGCTCTTGTTGTTC	
		5′ L-Vκ_20	CTCACTAGCTCTTCTCCTC	
		Reverse		
		3′ mCκ	GATGGTGGGAAGATGGATACAGTT	
Igκ 2nd	45°C	Forward		
		5′ mVkappa *	GAYATTGTGMTSACMCARWCTMCA	
		Reverse		
		3′ BsiWI P-mJK01	GCCACCGTACGTTTGATTTCCAGCTTGGTG	
		3′ BsiWI P-mJK02	GCCACCGTACGTTTTATTTCCAGCTTGGTC	
		3′ BsiWI P-mJK03	GCCACCGTACGTTTTATTTCCAACTTTGTC	
		3′ BsiWI P-mJK04	GCCACCGTACGTTTCAGCTCCAGCTTGGTC	
IgH 1st	56°C	Forward		
		5' Mouse Leader	CTCTTCCTCCTGTCAGTAACTGAAGGTGTCC	-
		Reverse		
		3′ KI Rev	TGAGGAGACGGTGACCAGGGTGCC	-
IgH 2nd	60°C	Forward		
		5' gIVRC01 *	CAGGTGCAGCTGGTGCAGTCTGG	Jardine et al. Science 2015
		Reverse		
		3′ KI Rev	TGAGGAGACGGTGACCAGGGTGCC	-

Table S3, VH/VL sequences of the VRC01-like antibodies isolated after the boost immunization. Amino acid sequences are aligned to the V gene from which they are derived. For sequences where the PCR product did not contain the 5′ end, the sequence is shown beginning at CDR1, Related to Figure 5

VRC01glHC	
Ab name	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWVRQAPGQGLEWMGWINPNSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGKNSDYNWDFQH
MLK-002	----------.....D..IN.....RH......R.....NIVF......T...A.I.F.....D.....
MLK-004	----------.....D..IN....T.....KN.....T.....D..F.....
MLK-005	----------.....D..IN.....KH.....I.....D.....
MLK-006	----------.....A.....D...N.....R.....I..K.....
MLK-008	----------.....VN.....TN.....Q.....A.....V.....D.....
MLK-009	----------.....D.FIN.....L.....R.A.....S.G...S.....D.....
MLK-010	----------E.....VN.....TN.....Q.....A.....L.....D.....
MLK-012	----------.....
MLK-014	----------.....D..VN.....TN.....Q.....A.....
MLK-015	----------.....T.....D...N.M.....L.....YR.A.....G.....S.....
MLK-016	----------.....VN.....TN.....Q.....A.....V.....

IGKV8-30*01	
Ab name	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYS
MLK-002	----------....HD...T.D.D.....A.....D.....H..Q
MLK-004	----------.....D.....S.....K
MLK-005	----------.....D.....D.D.....K
MLK-006	----------.....I....D.E.S.....K
MLK-008	----------.....D.N.....S.....S.....EA
MLK-009	----------.....D.E.....P.....F.....I.....EF
MLK-010	----------.....N...N.D.N.....S.....S.....EA
MLK-012	----------.....EA
MLK-014	----------.....D.N.....EA
MLK-015	----------.....E.....Y.....I.....E.
MLK-016	----------.....D.N.....S.....S.....EA