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Supplemental information

Broad and ultra-potent cross-clade neutralization of HIV-1 by a vaccine-induced CD4 binding site bovine antibody

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Table S1: Primers used for producing chimeric bovine- human full antibodies. Related to STAR Methods (Single cell cDNA synthesis, RT-PCR and cloning)

Primer code	Sequence 5'-3'		PCR Reaction
odp 2569	ATGAACCCACTGTGGACCCTC	Forward	H1
odp 2570	AGAACTCAGAGGGTAGACTTTCGG	Reverse	H1
odp 3667	CTTTCGGGGCTGTGGTGGAGGC	Reverse	H1
odp 3668	**GAATTC**GMAGGTGCAGCTGCRGGAGTC	Forward (EcoRI)	H2
odp 2568	**GCTAGC**TGAGGAGACGGTGACCAGGAG	Reverse (NheI)	H2
odp 3670	CACCATGGCCTGGTCCCCTCTG	Forward	L1
odp 3671	GACCCCAGACTCACCATCTC	Forward	L1
odp 3672	AGGGCTGCGGGCTCAGAAGGCAGC	Forward	L1
odp 3673	CTGCCCCTCCTCACTCTCTGC	Forward	L1
odp 3674	GGAACCTTTCCTGCAGCTC	Forward	L1
odp 3675	GCTTGCTTATGGCTCAGGTC	Forward	L1
odp 2573	ATGTCCACCATGGCCTGGTCC	Forward	L1
odp 2574	CTTGTTGCCGTTGAGCTCCTC	Reverse	L1
odp 2571	**GAATTC**GCAGGCTGTGCTGACTCAG	Forward (EcoRI)	L2
odp 3677	**CCTAGG**ACGACKGTCAGTGTGGTSCC	Reverse (AvrII)	L2
odp 2781	CTCAACTCTACGTCTTTGTTTC	Forward	Sequencing

Restriction enzyme sites are in bold and the nucleotides inserted to keep the frame reading in the expression vector are in Italic and underlined. H: heavy chain, L: light chain. Reaction number shows whether the primer was used in nester RT-PCR reaction 1 or reaction 2.

Table S2: Primers used for site directed mutagenesis of full length AD8 gp160 Env. Related to STAR Methods (Generation of HIV-1 AD8 pseudovirus mutant)

Primer code	Sequence 5'-3'		Mutation
odp 3770	cacaagaagtagtattgg**C**aaatgtgacaga	Forward	E87A
odp 3771	attttctgtcacattt**G**ccaatactacttc	Reverse	E87A
odp 3772	taaagccatgtgta**GC**attaaccccactctgtg	Forward	K121A
odp 3773	acacagagtggggttaa**TGC**tacacatggc	Reverse	K121A
odp 3774	aagactatgcacttttttataga**GC**tgatgtagtaccaatag	Forward	L179A
odp 3775	tcattatctattggtactacatct**GC**tctataaaaaagtgcatag	Reverse	L179A
odp 3776	ttgatgtagtaccaatag**C**taatgataatactagctatagg	Forward	D185A
odp 3777	acctatagctagtattatcatta**G**ctattggtactaca	Reverse	D185A
odp 3778	tataggttgataaattgt**GC**tacctcaaccattacacagg	Forward	N197A
odp 3779	tgtgtaatggttgaggta**GC**acaatttatcaacctatagc	Reverse	N197A
odp 3782	tcaactcaactgctgtta**GC**tggcagtctagc	Forward	N262A
odp 3783	ttcttctgctagactgcca**GC**taacagcagttgag	Reverse	N262A
odp 3784	agaggtagtaattagatctagt**GC**tttcacagacaatgc	Forward	N276A
odp 3785	ttgcattgtctgtgaaa**GC**actagatctaattactacctc	Reverse	N276A
odp 3786	tctagtaatttcacag**C**caatgcaaaaaacataatagtac	Forward	D279A
odp 3787	atgtttttgcattg**G**ctgtgaaattactagatctaattactac	Reverse	D279A
odp 3788	atttcacagacaatgca**GC**aaacataatagtacagttg	Forward	K282A
odp 3789	aactgtactattatgtt**TGC**tgcattgtctgtgaaattac	Reverse	K282A
odp 3790	ttcacagacaatgcaaaa**GC**cataatagtacagttg	Forward	N283A
odp 3791	ttcaactgtactattat**GGC**ttttgcattgtctgtg	Reverse	N283A
odp 3792	agtatacatatagga**G**caggaagagcattttatac	Forward	P313A
odp 3793	tgttgtataaaatgctcttcctg**C**tcctatatgtatac	Reverse	P313A
odp 3794	aggagatataagacaa**AA**acattgcaacattagtagaac	Forward	A329K
odp 3795	ttgttctactaatgtt**TT**aatgtgcttgtcttatatctcc	Reverse	A329K
odp 3796	aagacaagcacattgca**C**cattagtagaacaaaatgg	Forward	N332T
odp 3797	tgttattccattttgttctactaatg**G**tgcaatgtgcttgtcttatatc	Reverse	N332T
odp 3800	aataaaacaatagtctttaat**GC**atcctcaggaggggaccc	Forward	Q363A
odp 3801	acaatttctgggtcccctcctgaggat**GC**attaaagactattgttttattatt ccc	Reverse	Q363A
odp 3802	aacaatagtctttaatcaa**G**cctcaggaggggacccagaaattg	Forward	S364A
odp 3803	ttctgggtcccctcctgagg**C**ttgattaaagactattgttttattattcc	Reverse	S364A
odp 3804	aatagtctttaatcaatcc**G**caggaggggacccagaaattgtaatgc	Forward	S365A
odp 3805	aatttctgggtcccctcctg**C**ggattgattaaagactattgttttattattcc	Reverse	S365A
odp 3806	tagtctttaatcaatcctcag**C**aggggacccagaaattgtaatgc	Forward	G366A
odp 3807	attacaatttctgggtcccct**G**ctgaggattgattaaagac	Reverse	G366A
odp 3808	tagtctttaatcaatcctcaggag**C**ggacccagaaattgtaatgc	Forward	G367A
odp 3809	tgcattacaatttctgggtcc**G**ctcctgaggattgattaaagac	Reverse	G367A
odp 3810	ttaatcaatcctcaggaggggg**C**cccagaaattgtaatgcac	Forward	D368A
odp 3811	aactgtgcattacaatttctggg**G**cccctcctgaggattg	Reverse	D368A

Table S2: Continued.

Primer code	Sequence 5'-3'		Mutation
odp 3812	aatcaatcctcaggaggggac**G**cagaaattgtaatgcacag	Forward	P369A
odp 3813	aactgtgcattacaatttctg**C**gtcccctcctgagg	Reverse	P369A
odp 3814	aatcaatcctcaggaggggacccag**C**aattgtaatgcacag	Forward	E370A
odp 3815	taaaactgtgcattacaatt**G**ctgggtcccctcctgagg	Reverse	E370A
odp 3816	aatcctcaggaggggacccagaa**GC**tgtaatgcacagttttaattgtgg	Forward	I371A
odp 3817	aattaaaactgtgcattaca**GC**ttctgggtcccctcc	Reverse	I371A
odp 3818	atcaatcctcaggaggggacccagaaattg**C**aatgcacagttttaattgtgg	Forward	V372A
odp 3819	ttcccctccacaattaaaactgtgcatt**G**caatttctgggtcccctcc	Reverse	V372A
odp 3820	acccagaaattgta**GC**gcacagttttaattgtggaggg	Forward	M373A
odp 3821	ttcccctccacaattaaaactgtgc**GC**tacaatttctggg	Reverse	M373A
odp 3822	atgacactatcacactcccatgt**GC**aataaaacaaattataaacatgtg	Forward	R419A
odp 3823	atgtttataatttgttttatt**GC**acatgggagtgtgatagtgtc	Reverse	R419A
odp 3824	atattacagggctgatatta**G**caagagatggtggaaataacc	Forward	T455A
odp 3825	ttgtggttatttccaccatctcttg**C**taatatcagccctg	Reverse	T455A
odp 3826	tacagggctgatattaacaagag**C**tggtggaaataaccacaataatgatac	Forward	D457A
odp 3827	ttgtggttatttccacca**G**ctcttgttaatatcagccctg	Reverse	D457A
odp 3828	taccgagacctttagacctg**C**aggaggagatatgagggac	Forward	G471A
odp 3829	ttgtccctcatatctcctcct**G**caggtctaaaggtctcgg	Reverse	G471A
odp 3830	agacctttagacctggag**C**aggagatatgagggacaattgg	Forward	G472A
odp 3831	attgtccctcatatctcct**G**ctccaggtctaaaggtctcgg	Reverse	G472A
odp 3832	acctttagacctggaggag**C**agatatgagggacaattggag	Forward	G473A
odp 3833	ttctccaattgtccctcatatct**G**ctcctccaggtctaaaggtctcgg	Reverse	G473A
odp 3834	tagacctggaggaggag**C**tatgagggacaattggagaagtg	Forward	D474A
odp 3835	ttctccaattgtccctcata**G**ctcctcctccaggtctaaagg	Reverse	D474A
odp 3836	ttagacctggaggaggagat**GC**gagggacaattggagaagtg	Forward	M475A
odp 3837	ttctccaattgtccctc**GC**atctcctcctccaggtctaaagg	Reverse	M475A
odp 3838	tggaggaggagatatg**GC**ggacaattggagaagtg	Forward	R476A
odp 3839	acttctccaattgtcc**GC**catatctcctcctccaggtctaaagg	Reverse	R476A
odp 1377	ggtacataatgtttgggccac	Forward	Sequencing
odp 1379	gctgttaaatggcagtctagc	Forward	Sequencing
odp 1441	ctactgtaattcaacacaactg	Forward	Sequencing

Nucleotides to be mutated are shown in capital letters and bold font.

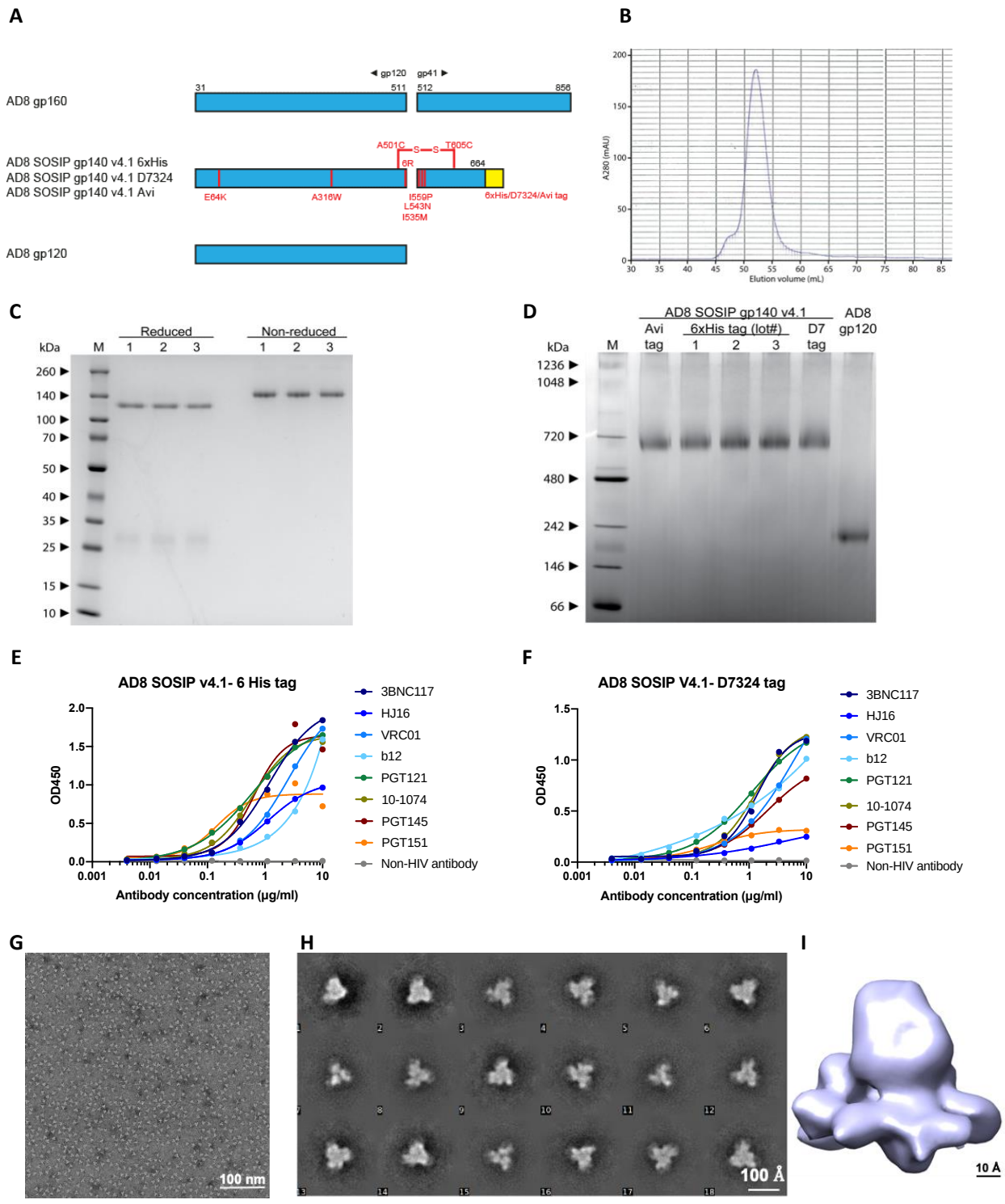

Figure S1: Design and characterization of AD8 SOSIP trimers. (A) Linear representation of mature AD8 gp160, SOSIP gp140 v4.1 (with either a 6xHis, D7324, or Avi tag at the C-terminus at position 664), and gp120. All Envs were expressed with their wild-type signal peptide. SOSIP v4.1 mutations were introduced as previously described [ref v4.1 paper SW de Taeye 2015 Cell]. (B) SEC profile of 2G12-purified AD8 SOSIP gp140 v4.1 6xHis run on a Superdex S200 16/600 column.

(C) SDS-PAGE analysis using an 8-16% Tris-glycine gel of 3 separate lots (numbered 1-3) of 2G12/SEC purified 6xHis-tagged AD8 SOSIP gp140 v4.1. Proteins were running with or without reducing agent. Lane M was loaded with Spectra Multicolor Broad Range Protein Ladder. (D) BN-PAGE analysis using a 4-16% Bis-Tris NativePAGE gel. Trimeric AD8 SOSIP gp140 v4.1 with Avi, 6xHis (3 separate lots), or a D7324 tag were analysed as well as monomeric AD8 gp120. Lane M was loaded with NativeMark Unstained Protein Standard. For both (C) and (D), gels were stained with Coomassie Blue. Capture ELISA on AD8 SOSIP v4.1 His tag (E) and D7324 tag (F) using human bNAbs. G) Negative staining using 1% Uranyl Acetate on freshly glow discharged carbon coated copper grids. Images were taken on FEI Talos L120C microscope. Pixel size: 1.9Å 73000X magnification. (H) 2D classes showing different views and (I) 3D volume map for AD8-SOSIP. Related to Figure 2.

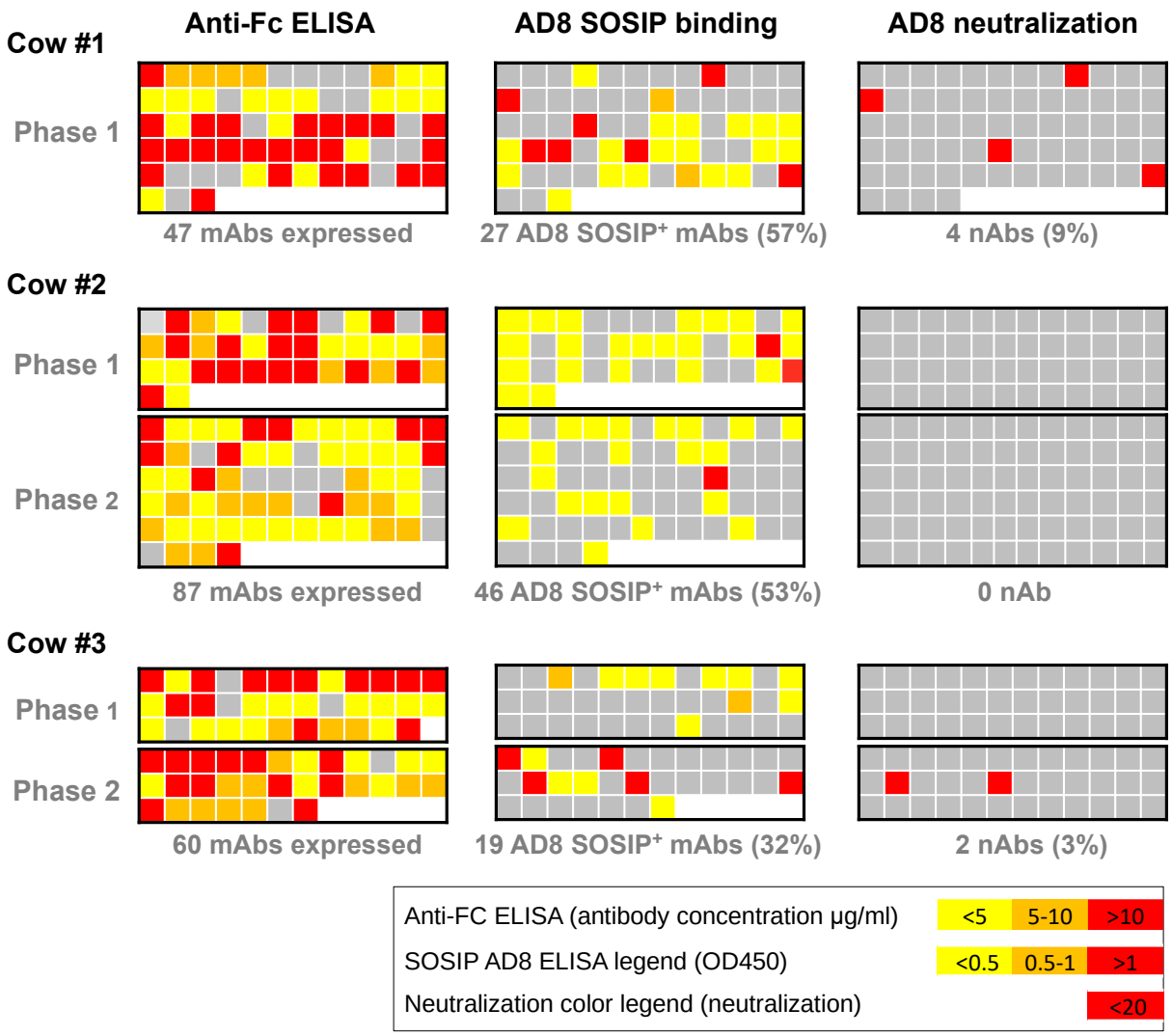

Figure S2. Workflow of isolating anti-HIV bovine antibodies. Amplified heavy chains were their light chain or with MEL-2129 antibody light chain and tested for expression (anti-Fc ELISA), antigen binding was assessed in capture ELISA using AD8 SOSIP gp140 v4.1 and autologous neutralization (AD8 pseudovirus) was investigated using TZM-bl cells. Related to Figure 2.

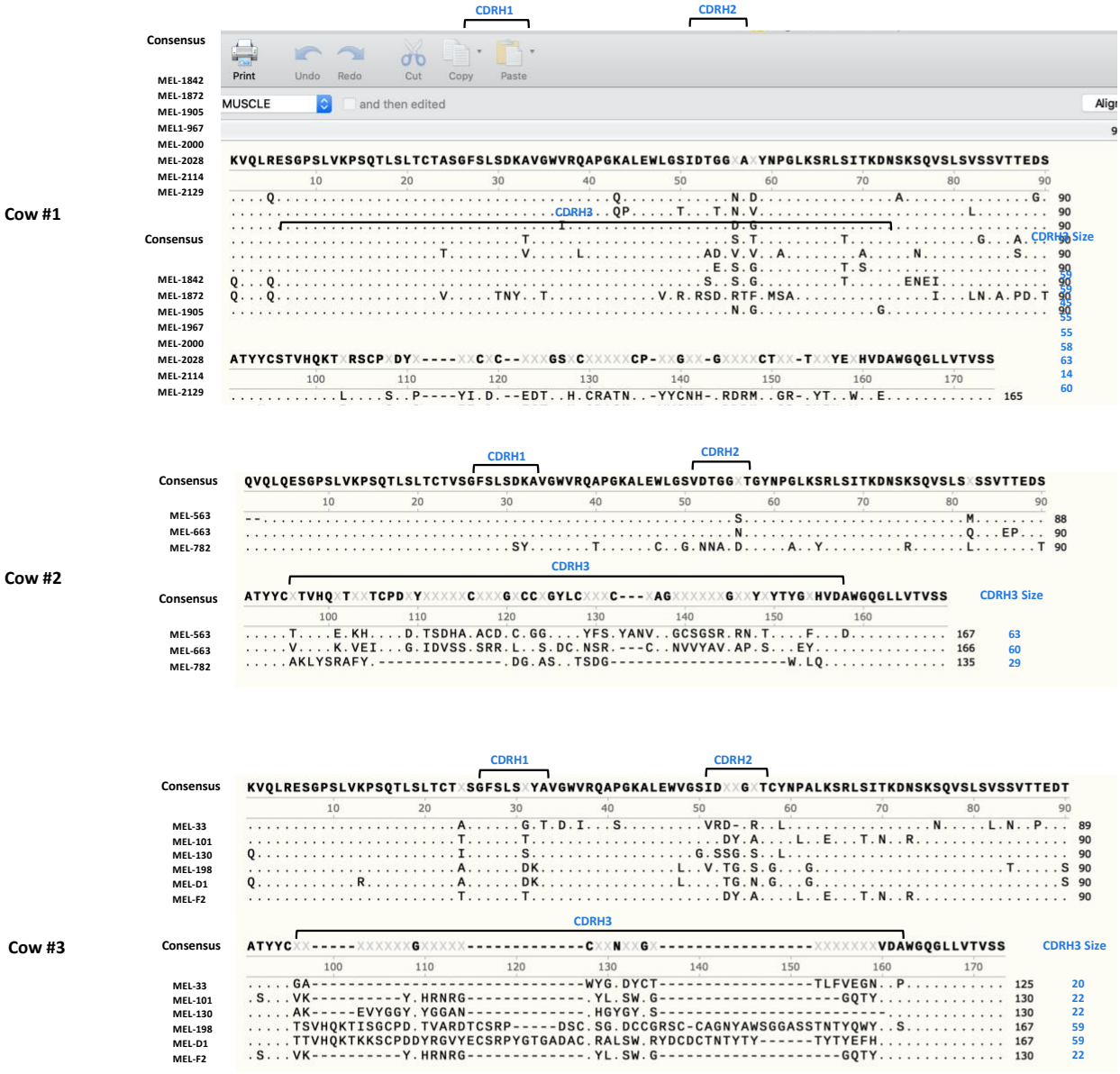

Figure S3. Amino acid alignment of heavy chain sequences of isolated monoclonal antibodies. Related to Figure 2.

Geometric mean IC50

Clade	n	VRC01		NC-Cow1		MEL-1842		MEL-1872		MEL-2129	
		Breadth	IC50	Breadth	IC50	Breadth	IC50	Breadth	IC50	Breadth	IC50
A	7	NA	NA	100%	0.040	100%	0.011	100%	0.005	86%	0.215
B	12	100%	0.0117	50%	0.094	100%	0.004	100%	0.004	100% *	0.072
C	19	NA	NA	42%	0.081	32%	0.018	37%	0.021	21%	0.087
AE	5	NA	NA	100%	0.216	80%	0.154	60%	0.008	20%	0.001
BC	2	NA	NA	50%	0.219	0%	-	0%	-	0%	-
AC	1	NA	NA	0%	-	0%	-	100%	2.830	0%	-
G	1	100%	0.073	100%	0.118	100%	0.069	100%	0.007	100%	0.257
	47										

IC50 (µg/ml) <0.01 0.01-1 1-20

* 11 clade B tested.

Geometric mean IC80

Clade	n	VRC01		NC-Cow1		MEL-1842		MEL-1872		MEL-2129	
		Breadth	IC80	Breadth	IC80	Breadth	IC80	Breadth	IC80	Breadth	IC80
A	7	NA	NA	100%	0.185	100%	0.070	100%	0.028	71%	1.030
B	12	92%	0.346	42%	1.029	100%	0.030	100%	0.031	64% *	0.047
C	19	NA	NA	37%	0.181	26%	0.035	32%	0.053	21%	0.710
AE	5	NA	NA	60%	0.164	40%	0.022	60%	0.032	20%	0.006
BC	2	NA	NA	50%	0.934	0%	-	0%	-	0%	-
AC	1	NA	NA	0%	-	0%	-	0%	-	0%	-
G	1	100%	1.146	100%	0.997	100%	2.005	100%	0.122	100%	10.015
	47										

IC80 (µg/ml) <0.01 0.01-1 1-20

* 11 clade B tested.

Figure S4. Categorization of neutralization activity of bovine bNAbs against clade A, B, C, AE, BC, AC and G HIV viruses. Values in red color show low IC_{50} and IC_{80} and better neutralization while, those in yellow color show high IC_{50} and IC_{80} values and less neutralization activity. Neutralizatoin assays were performed in duplicates with two independent biological replicates. Related to Figure 3.

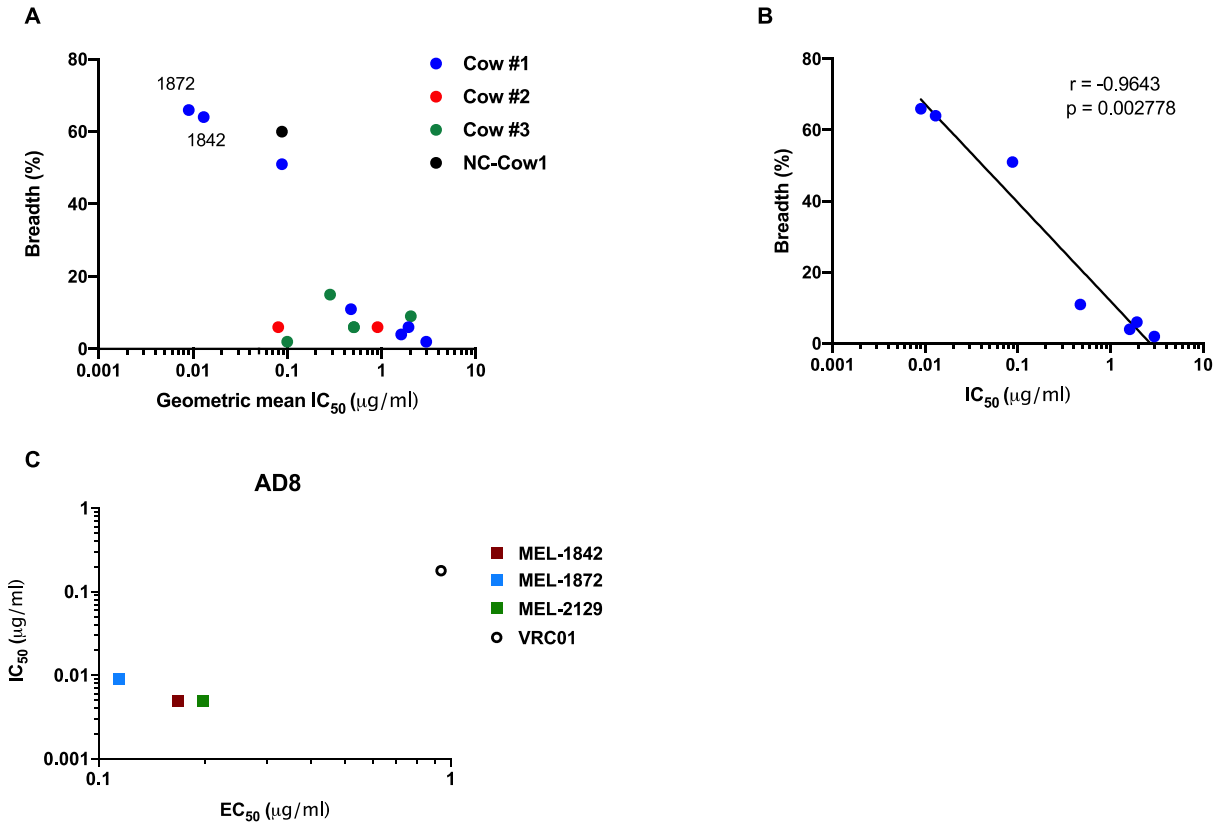

Figure S*5*. Correlation of neutralization and Env binding in isolated monoclonal antibodies. A) Correlations between the neutralizing breadth and neutralization activity (IC_{50}) of AD8 SOSIP binding antibodies, B) Correlations between the neutralizing breadth and neutralization activity (IC_{50}) of bNAbs from cow #1, C) Correlation between IC_{50} and EC_{50} of bNAbs of cow#1 against AD8 strain. Related to Figure 2 and 3.

IC50

	MEL-1842	MEL-1872	MEL-2129	VRC01	b12	PGT121
WT	0.004	0.004	0.006	0.183	0.263	0.016
E87A	0.006	0.005	0.009	0.429	0.469	0.020
K121A	0.003	0.004	0.005	0.272	0.212	0.014
L179A	0.003	0.003	0.005	0.099	0.192	0.007
D185A	0.003	0.002	0.004	0.132	0.294	0.006
N197A	0.003	0.002	0.003	0.082	0.030	0.014
N262A	0.005	0.003	0.004	0.118	0.189	0.010
N276A	0.005	0.004	0.006	0.035	0.238	0.017
D279A	0.002	0.003	0.011	> 4	0.528	0.012
K282A	0.002	0.003	0.002	0.114	0.176	0.011
N283A	0.005	0.005	0.011	0.196	0.171	0.018
P313A	0.004	0.004	0.005	0.173	0.243	0.016
N332T	0.005	0.004	0.005	0.132	0.465	0.110
Q363A	0.004	0.003	0.007	0.161	0.386	0.013
S364A	0.004	0.003	0.016	0.063	0.070	0.012
G366A	0.014	0.029	0.015	0.129	1.285	0.010
E370A	0.005	0.003	0.005	0.183	0.446	0.066
I371A	0.008	0.293	3.620	0.167	0.225	0.011
V372A	0.004	0.003	0.006	0.215	2.460	0.014
M373A	0.004	0.003	0.005	0.231	0.183	0.016
R419A	0.003	0.003	0.005	0.152	0.268	0.009
T455A	0.005	0.004	0.105	0.125	0.143	0.009
G471A	> 4	> 4	> 4	0.657	3.004	0.039
G472A	0.002	0.002	0.004	0.173	0.369	0.013
G473A	0.003	0.002	0.007	0.104	0.158	0.009
D474A	0.009	0.003	0.056	0.302	0.366	0.014
M475A	0.004	0.004	0.006	0.196	0.242	0.024
R476A	0.003	0.001	0.002	0.101	0.186	0.017

IC80

	MEL-1842	MEL-1872	MEL-2129	VRC01	b12	PGT121
WT	0.020	0.014	0.021	0.514	0.919	0.060
E87A	0.017	0.015	0.024	1.642	3.234	0.063
K121A	0.013	0.013	0.020	0.702	0.744	0.058
L179A	0.009	0.007	0.014	0.429	2.020	0.022
D185A	0.007	0.008	0.013	0.570	1.303	0.030
N197A	0.007	0.006	0.008	0.250	0.165	0.035
N262A	0.012	0.007	0.013	0.585	0.498	0.024
N276A	0.012	0.012	0.023	0.107	0.732	0.044
D279A	0.006	0.007	0.058	> 4	1.477	0.054
K282A	0.009	0.004	0.010	0.297	0.594	0.027
N283A	0.013	0.016	0.048	0.527	0.672	0.064
P313A	0.012	0.011	0.024	0.699	0.733	0.042
N332T	0.016	0.011	0.016	0.462	0.722	1.840
Q363A	0.018	0.009	0.028	0.414	1.681	0.075
S364A	0.015	0.012	0.069	0.185	0.215	0.034
G366A	0.054	0.117	0.071	0.331	> 4	0.028
E370A	0.037	0.015	0.027	0.932	0.717	0.033
I371A	0.057	> 4	> 4	0.751	0.954	0.039
V372A	0.014	0.010	0.021	0.584	> 4	0.054
M373A	0.013	0.011	0.020	0.712	0.582	0.044
R419A	0.010	0.009	0.023	0.451	1.817	0.032
T455A	0.031	0.100	1.201	0.344	0.688	0.040
G471A	> 4	> 4	> 4	> 4	> 4	0.115
G472A	0.007	0.004	0.012	0.423	> 4	0.038
G473A	0.011	0.009	0.037	0.603	0.741	0.028
D474A	0.060	0.027	0.829	2.830	2.910	0.045
M475A	0.011	0.009	0.018	0.619	0.643	0.094
R476A	0.028	0.014	0.013	0.427	1.289	0.059

Figure S6. Heatmap of relative neutralization activity of monoclonal antibodies against AD8 Env mutants. The colors refer to the changes in IC_{50} and IC_{80} ranging from 0.001 ug/ml (yellow) to 4ug/ml (red). Values in yellow color show low IC_{50} and better neutralization while, those in red color show high IC_{50} values and less neutralization activity. White indicates values of >4, meaning that the IC_{50} could not be achieved for the viruses with that particular mutation to Env. PGT121 (V3-glycan epitope) and b12 and VRC01 (CD4bs epitope) were included for comparison. Neutralization assays were performed in duplicates with two independent biological replicates. Average IC_{50} values were used for drawing heatmaps. Related to Figure 5.