Table S1. Antibody libraries and selected binders

Ig isotype	Display	Format	Size ($x10^6$)	Antibodies	Binding/ neutralization
IgG	yeast	scFv	1	no	
IgG+IgM	yeast	scFv	5	yes	yes/weak
IgG+IgM	yeast	scFab	1	yes	yes/weak
IgG+IgM	phage	scFv	100	no	
IgG	phage	Fab	150	no	
IgM	phage	Fab	250	no	
IgG+IgM	phage	Fab	100	m66 m65	yes/yes yes/no

Table S2. Neutralization of TZM-bl cell infection by IgG1 2F5, m66 and m66.6

Virus isolate	Clade	2F5	m66	m66.6
0260.v5.c36	A	>50	>50	>50
0330.v4.c3	A	14.6	>50	>50
0439.v5.c1	A	4.43	>50	>50
3415.v1.c1	A	43.9	>50	>50
3718.v3.c11	A	3.88	>50	>50
398-F1_F6_20	A	0.280	>50	>50
BB201.B42	A	2.92	>50	33.6
BB539.2B13	A	0.136	>50	32.7
BI369.9A	A	0.249	>50	10.8
BS208.B1	A	1.10	>50	>50
KER2008.12	A	6.98	>50	>50
KER2018.11	A	2.01	>50	23.8
KNH1209.18	A	2.24	>50	>50
MB201.A1	A	0.436	>50	22.1
MB539.2B7	A	2.49	>50	>50
MI369.A5	A	1.44	>50	33.7
MS208.A1	A	1.10	>50	20.6
Q168.a2	A	7.83	>50	>50
Q23.17	A	10.8	>50	>50
Q259.17	A	16.1	>50	>50
Q461.e2	A	13.4	>50	>50
Q769.d22	A	0.609	>50	>50
Q769.h5	A	>50	>50	>50
Q842.d12	A	>50	>50	>50
QH029.14M.A2	A	>50	>50	>50
RW020.2	A	7.55	>50	>50
UG037.8	A	0.202	>50	>50
3301_V1_C24	AC	>50	>50	>50
3589_V1_C4	AC	6.99	>50	19.8
6540.v4.c1	AC	40.0	>50	>50
0815_V3_C3	ACD	7.37	>50	>50
6095_V1_C10	ACD	0.147	>50	>50
3468_V1_C12	AD	3.51	>50	>50
CNE3	AE	6.79	>50	24.9
CNE5	AE	9.70	>50	14.1
CNE55	AE	1.49	>50	>50
CNE56	AE	0.974	>50	23.9
CNE59	AE	0.029	26.4	0.704
TH966.8	AE	0.182	>50	21.6
TH976.17	AE	0.131	>50	28.3
235-47	AG	>50	>50	>50
242-14	AG	1.06	>50	35.0
250-4	AG	2.93	>50	>50
251-18	AG	30.5	>50	>50
255-34	AG	>50	>50	>50
257-31	AG	5.46	>50	10.6
263-8	AG	>50	>50	>50
266-60	AG	8.04	>50	>50
269-12	AG	>50	>50	>50
271-11	AG	13.2	>50	>50
278-50	AG	4.27	>50	>50
280-5	AG	4.30	>50	>50
928-28	AG	1.04	>50	30.8
DJ263.8	AG	>50	>50	>50
T253-11	AG	4.27	>50	11.8
T33-7	AG	10.0	>50	19.9

3988.25	B	>50	>50	>50
5768.04	B	0.139	>50	7.33
6101.10	B	>50	>50	>50
6535.3	B	4.90	>50	43.5
7165.18	B	1.35	>50	15.5
89.6.DG	B	1.514	>50	>50
AC10.0.29	B	0.975	>50	>50
ADA.DG	B	0.271	>50	5.16
Bal.01	B	4.13	>50	>50
BaL.26	B	3.16	>50	>50
BG1168.01	B	1.35	>50	>50
BL01.DG	B	0.010	>50	45.0
BR07.DG	B	0.675	>50	50.000
CAAN5342.A2	B	11.6	>50	>50
HO86.8	B	0.049	>50	1.154
HT593.1	B	0.172	5.28	35.7
HXB2.DG	B	0.040	>50	2.21
JRCSF.JB	B	9.45	>50	>50
JRFL.JB	B	7.84	>50	37.3
MN.3	B	<0.023	>50	3.09
PVO.04	B	>50	>50	>50
QH0515.01	B	0.3	>50	22.4
QH0692.42	B	2.22	>50	>50
REJO4541.67	B	0.300	>50	>50
RHPA4259.7	B	23.2	>50	>50
SC422661.8	B	1.34	>50	17.1
SF162.LS	B	2.47	>50	49.0
SS1196.01	B	25.3	>50	>50
THRO4156.18	B	>50	>50	>50
TRJO4551.58	B	>50	>50	>50
TRO.11	B	>50	>50	>50
WITO4160.33	B	2.29	>50	18.3
YU2.DG	B	>50	>50	>50
CNE10	B'	1.10	>50	38.5
CNE12	B'	5.02	>50	>50
CNE14	B'	5.68	>50	>50
CNE4	B'	1.77	>50	33.4
CNE57	B'	1.09	>50	28.6
CH038.12	BC	>50	>50	>50
CH070.1	BC	>50	>50	>50
CH117.4	BC	>50	>50	>50
CH181.12	BC	>50	>50	>50
CNE15	BC	>50	>50	>50
CNE7	BC	1.13	>50	28.2
CNE40	B'C	>50	>50	>50
286.36	C	>50	4.38	>50
288.38	C	>50	>50	>50
0013095-2.11	C	>50	>50	>50
001428-2.42	C	>50	>50	>50
0077_V1_C16	C	>50	>50	>50
00836-2.5	C	>50	>50	>50
16055-2.3	C	>50	>50	>50
16845-2.22	C	>50	>50	>50
25710-2.43	C	>50	>50	>50
25711-2.4	C	44.8	>50	>50
25925-2.22	C	>50	>50	>50
26191-2.48	C	>50	>50	>50
3168_V4_C10	C	23.1	>50	>50
3637_V5_C3	C	>50	>50	>50
3873_V1_C24	C	>50	>50	>50

6322_V4_C1	C	>50	>50	>50
6471_V1_C16	C	>50	>50	>50
6631_V3_C10	C	>50	>50	>50
6644_V2_C33	C	0.219	>50	>50
6785_V5_C14	C	>50	>50	>50
96ZM651_02	C	>50	>50	>50
BR025.9	C	>50	>50	>50
CAP210.E8	C	>50	>50	>50
CAP244.D3	C	>50	>50	>50
CAP45.G3	C	>50	>50	>50
CNE30	C	>50	>50	>50
CNE31	C	>50	>50	>50
CNE53	C	>50	>50	>50
CNE58	C	>50	>50	>50
DU123.06	C	>50	>50	>50
DU151.02	C	>50	>50	>50
DU156.12	C	>50	>50	>50
DU172.17	C	>50	>50	>50
DU422.01	C	>50	>50	>50
MW965.26	C	>50	>50	>50
SO18.18	C	>50	>50	>50
TV1.29	C	3.29	>50	40.4
TZA125.17	C	>50	>50	>50
TZBD.02	C	>50	>50	>50
ZA012.29	C	>50	>50	>50
ZM106F.PB9	C	>50	>50	>50
ZM109F.PB4	C	>50	>50	>50
ZM135M.PL10a	C	>50	>50	>50
ZM146.7	C	>50	>50	>50
ZM176.66	C	>50	>50	>50
ZM197M.PB7	C	35.7	>50	>50
ZM214M.PL15	C	>50	>50	>50
ZM215F.PB8	C	>50	>50	>50
ZM233M.PB6	C	>50	>50	>50
ZM249M.PL1	C	>50	>50	>50
ZM53M.PB12	C	>50	>50	>50
ZM55F.PB28a	C	>50	>50	>50
3326_V4_C3	CD	19.6	>50	>50
3337_V2_C6	CD	4.63	>50	>50
247-23	D	1.95	>50	31.8
3016.v5.c45	D	0.765	>50	>50
57128.vrc15	D	>50	>50	>50
6405.v4.c34	D	7.51	>50	>50
A03349M1.vrc4a	D	>50	>50	>50
NKU3006.ec1	D	1.10	>50	>50
UG021.16	D	>50	>50	>50
UG024.2	D	0.029	>50	6.29
X2088_c9	G	>50	>50	>50
SIVmac251.30.SG3	NA	>50	>50	>50
SVA.MLV	NA	>50	>50	>50

*Values are the concentrations (μg/ml) at which relative luminescence units (RLUs) were reduced 50% compared to virus control wells (no test sample)

Table S3. Binding of IgG1 m66, m66.6, and 2F5 to gp41 MPER alanine scanning mutants. The association (k_a) and dissociation (k_d) rate constants were measured by Biacore and the equilibrium dissociation constant K_D was calculated as k_d/k_a. The mutants were based on a wild type MPER peptide encompassing gp41 residues 657-670 (EQELLELDKWASLWGGTETSQVAPA).

	m66			m66.6			2F5		
MPER Peptide	k_a (1/Ms x10^5)	k_d (1/s x10^{-2})	K_D (M x10^{-9})	k_a (1/Ms x10^5)	k_d (1/s x10^{-2})	K_D (M x10^{-9})	k_a (1/Ms x10^5)	k_d (1/s x10^{-2})	K_D (M x10^{-9})
E657A	9.95 ± 0.05	5.19 ± 0.03	52.2 ± 0.4	8.09 ± 0.06	6.52 ± 0.05	80.6 ± 0.9	5.76 ± 0.02	0.84 ± 0.00	14.65 ± 0.09
Q658A	8.71 ± 0.05	5.16 ± 0.03	59.2 ± 0.5	6.60 ± 0.05	4.82 ± 0.04	73.0 ± 0.8	4.87 ± 0.02	0.81 ± 0.00	16.57 ± 0.11
E659A	7.02 ± 0.04	4.57 ± 0.03	65.1 ± 0.6	5.63 ± 0.04	4.53 ± 0.03	80.5 ± 0.8	3.92 ± 0.01	0.85 ± 0.00	21.61 ± 0.09
L660A	na	na	na	na	na	na	5.39 ± 0.01	0.86 ± 0.00	15.94 ± 0.06
L661A	5.76 ± 0.07	10.9 ± 0.1	189 ± 3.0	8.20 ± 0.10	9.70 ± 0.20	118.0 ± 3.0	6.64 ± 0.03	4.16 ± 0.02	62.65 ± 0.41
E662A	8.21 ± 0.00	5.37 ± 0.00	65.36 ± 0.0	6.46 ± 0.04	5.02 ± 0.04	77.7 ± 0.8	4.72 ± 0.02	1.89 ± 0.01	39.96 ± 0.23
L663A	na	na	na	na	na	na	5.15 ± 0.02	6.55 ± 0.03	127.18 ± 0.76
D664A	na	na	na	na	na	na	na	na	na
K665A	na	na	na	na	na	na	na	na	na
W666A	na	na	na	na	na	na	na	na	na
WT	6.94 ± 0.04	4.04 ± 0.02	58.2 ± 0.4	4.97 ± 0.04	3.38 ± 0.03	68.0 ± 0.8	3.97 ± 0.00	0.76 ± 0.00	19.24 ± 0.00
S668A	1.00 ± 0.01	7.15 ± 0.04	712.0 ± 5.0	6.66 ± 0.06	5.84 ± 0.05	88.0 ± 1.0	5.39 ± 0.01	0.84 ± 0.00	15.57 ± 0.06
L669A	11.32 ± 0.05	7.76 ± 0.04	68.6 ± 0.5	8.35 ± 0.05	6.85 ± 0.04	82.0 ± 0.7	4.74 ± 0.01	3.75 ± 0.01	79.11 ± 0.27
W670A	3.40 ± 0.04	5.10 ± 0.06	150.0 ± 3.0	2.59 ± 0.03	5.32 ± 0.06	205.0 ± 3.0	3.52 ± 0.01	0.63 ± 0.00	17.88 ± 0.06

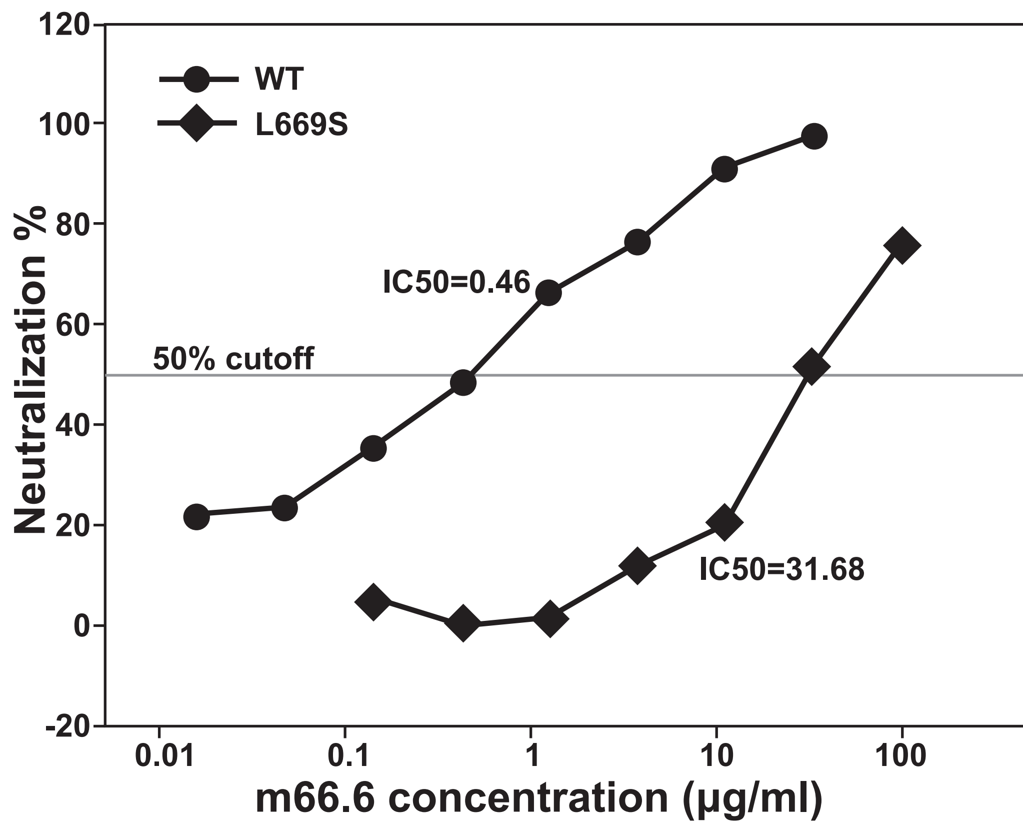


Fig. S1. Neutralization of the L669S mutant virus by IgG1 m66.6 compared to neutralization of wild type virus. Inhibition of the L669S mutant (highly sensitive to 2F5 neutralization) and the WT viruses by m66.6 was measured on TZM-bl cells.

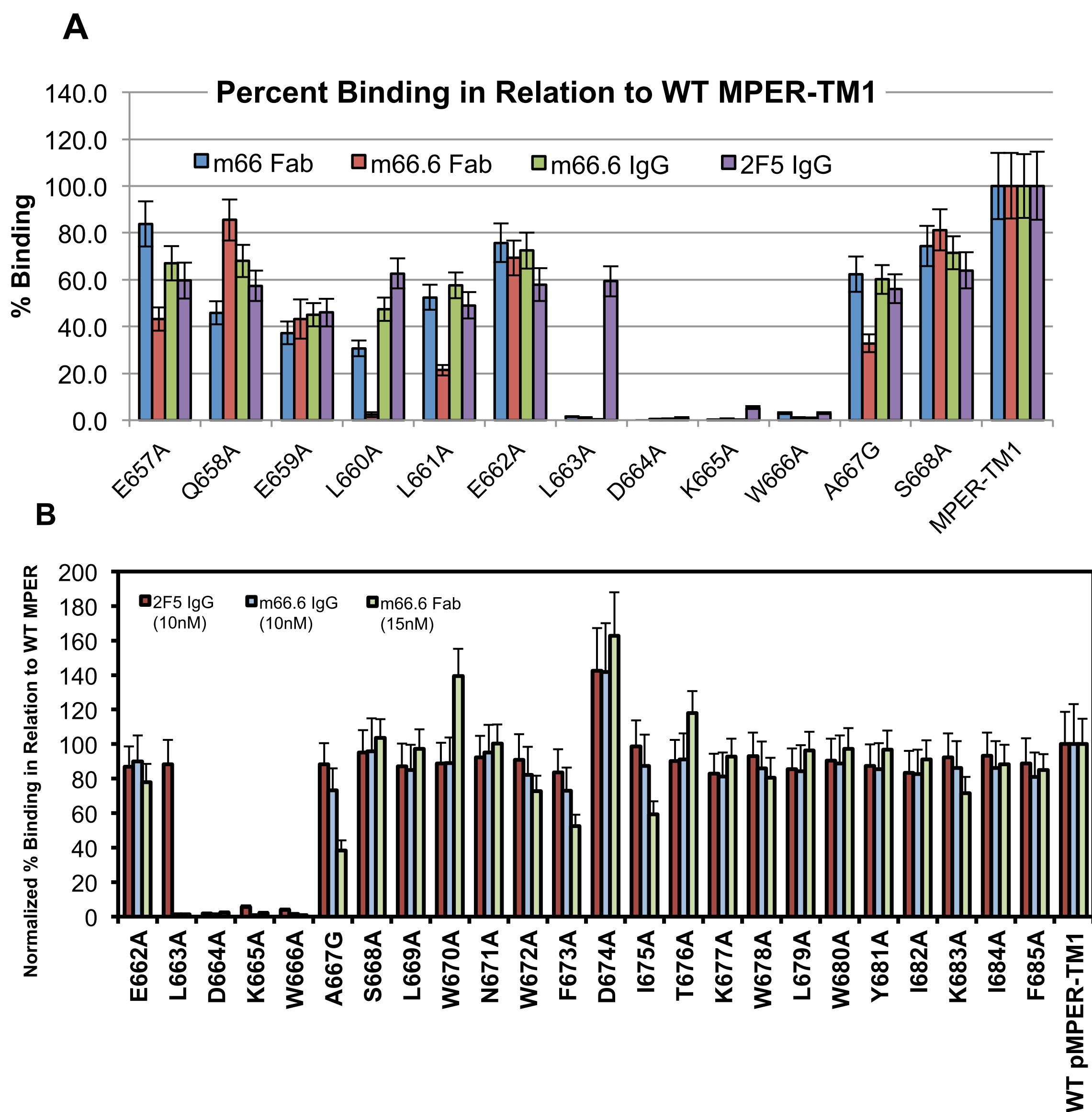


Figure S2. Binding profiles of 2F5 IgG, m66.6 IgG and m66.6 Fab to Ala-scan MPER mutants. Ab binding to Ala- or Gly-substitutions at (A) MPER positions 657-668 or (B) MPER positions 662-685.

COS-7 cells were transfected with plasmid MPER-TM1, which expresses the HA tag (DVPDYA) followed by a gp41JRFL fragment covering the C-terminal half of the C-heptad repeat, the MPER, the transmembrane region and the N-terminal 27 residues of the C-terminal domain. After 48 hours incubation cell vesicles were prepared by passing a cell suspension 30 times through a 22-gauge needle, then sonicating it (2 x 15 sec) with a Virsonic sonicator (VirTis). Microwells of high-binding microtiter plates were coated with 20-30 g total cell vesicles diluted in tris-buffered saline (TBS), or with positive control antigens (recombinant 50 ng HIV-1MN gp41 and 200 ng 2F5 peptide) or negative control antigens (1 ug ovalbumin; 2% w/v bovine serum albumin (BSA), 2% w/v non-fat dried milk, cell lysate prepared for mock transfected cells), then blocked with TBS containing 2% w/v BSA. After 6 washes with TBS containing 0.1% v/v Tween 20 (TBS/Tween), wells were incubated with 10 nM m66.6 IgG 2F5 IgG or 17/9 IgG, or 15 nM m66.6 Fab, diluted in 5% w/v NFDM/TBS/Tween, for 2 hrs at RT. After 6 washes, the wells were incubated with secondary Abs conjugated to HRP. Bound 2F5 and m66.6 IgG were detected with Protein A/G-HRP (1:4000), m66.6 Fab with goat-anti-human (Fab)-HRP (1:1500), and 17/9 MAb with goat-anti-mouse IgG-HRP (1:1000) diluted in 5% NFDM/TBS/Tween. Plates were washed 6 times as before, and bound HRP was detected by addition of ABTS solution (400 ng/ml 2'2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid in citrate/phosphate buffer containing 0.03% (v/v) H2O2)). Absorbance was measured using a Versamax microplate reader (Molecular Devices) and reported as the absorbance at 405 nm - 490 nm.

Results for all Abs are reported as % of binding in relation to WT MPER after normalizing for protein expression by binding of MAb 17/9 to HA tag peptide at the N-terminus of each MPER protein, using the formula: [(AbsbNt MAb MPER mutant)/(AbsbNt MAb WT MPER))/ ((Abs17/9 MAb MPER mutant)/(Abs17/9 MAb WT MPER)]. 95% confidence intervals are shown (1.96 x standard error of the mean (SEM)). (Note the increased binding to D674 has not been observed in our previous experiments, and is probably due to experimental error). Results for the positive and negative control antigens are not shown, but were as expected.

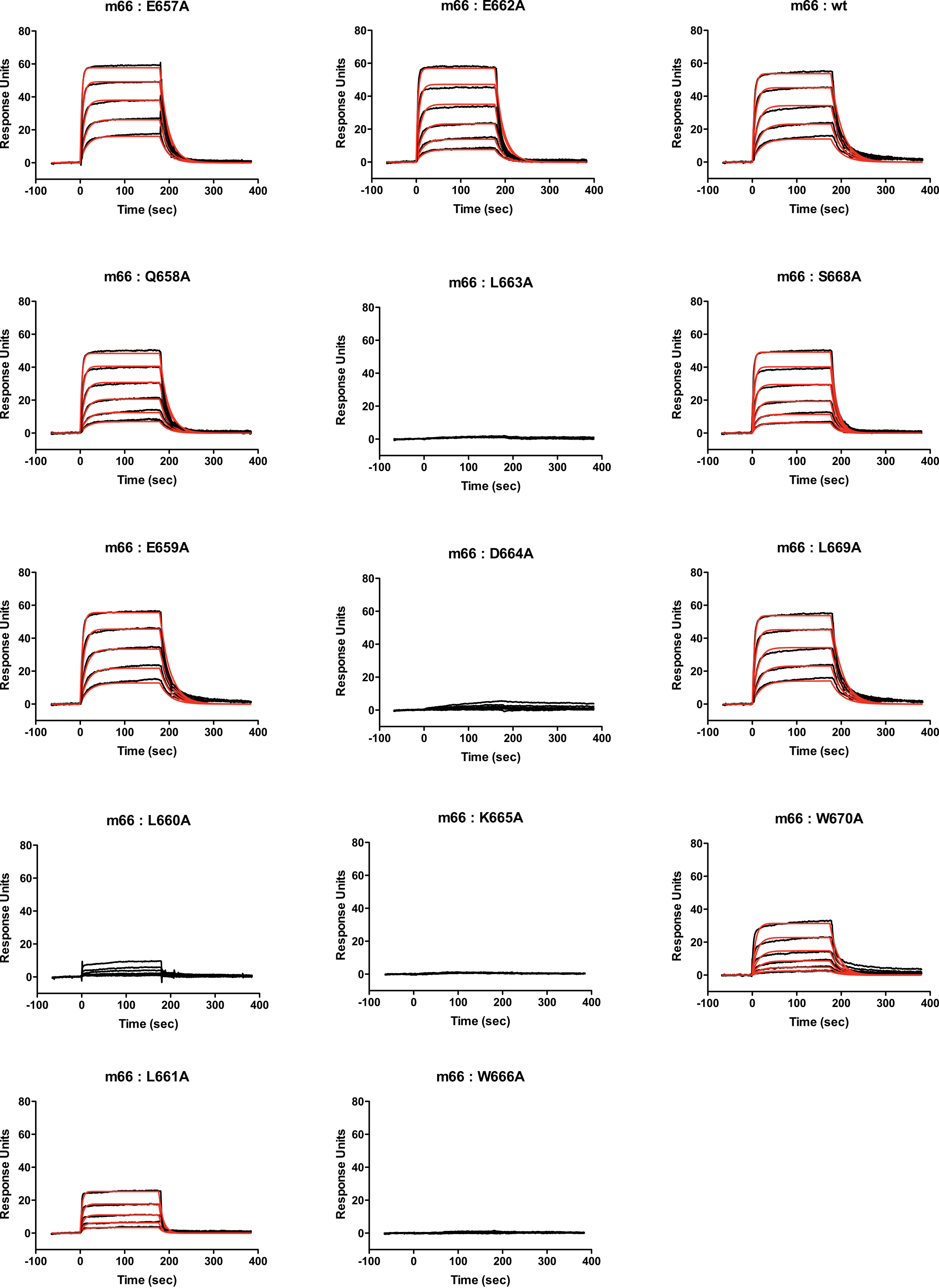


Fig. S3 (A). Biacore kinetics curves for binding of m66.

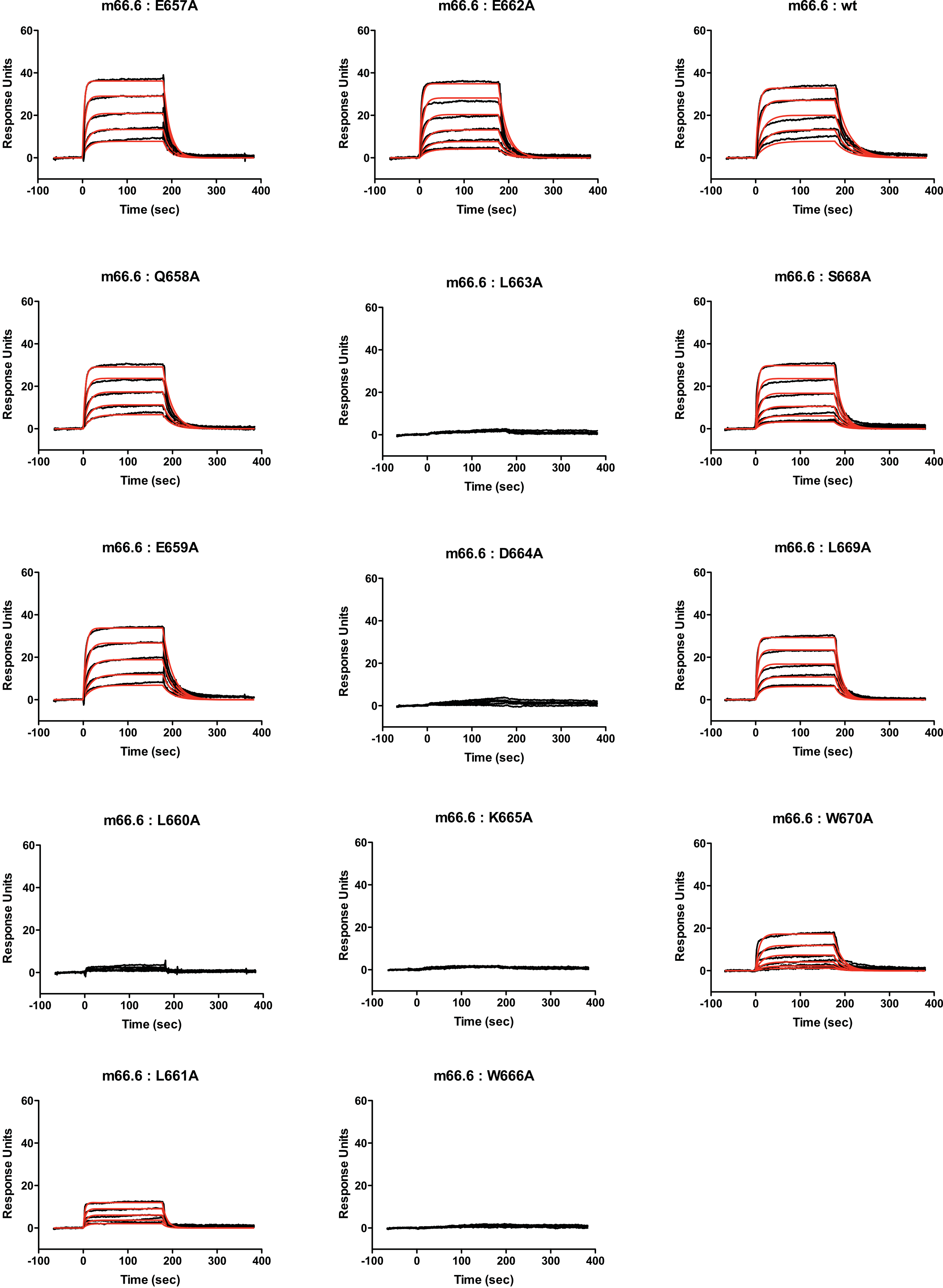


Fig. S3 (B). Biacore kinetics curves for binding of m66.6.

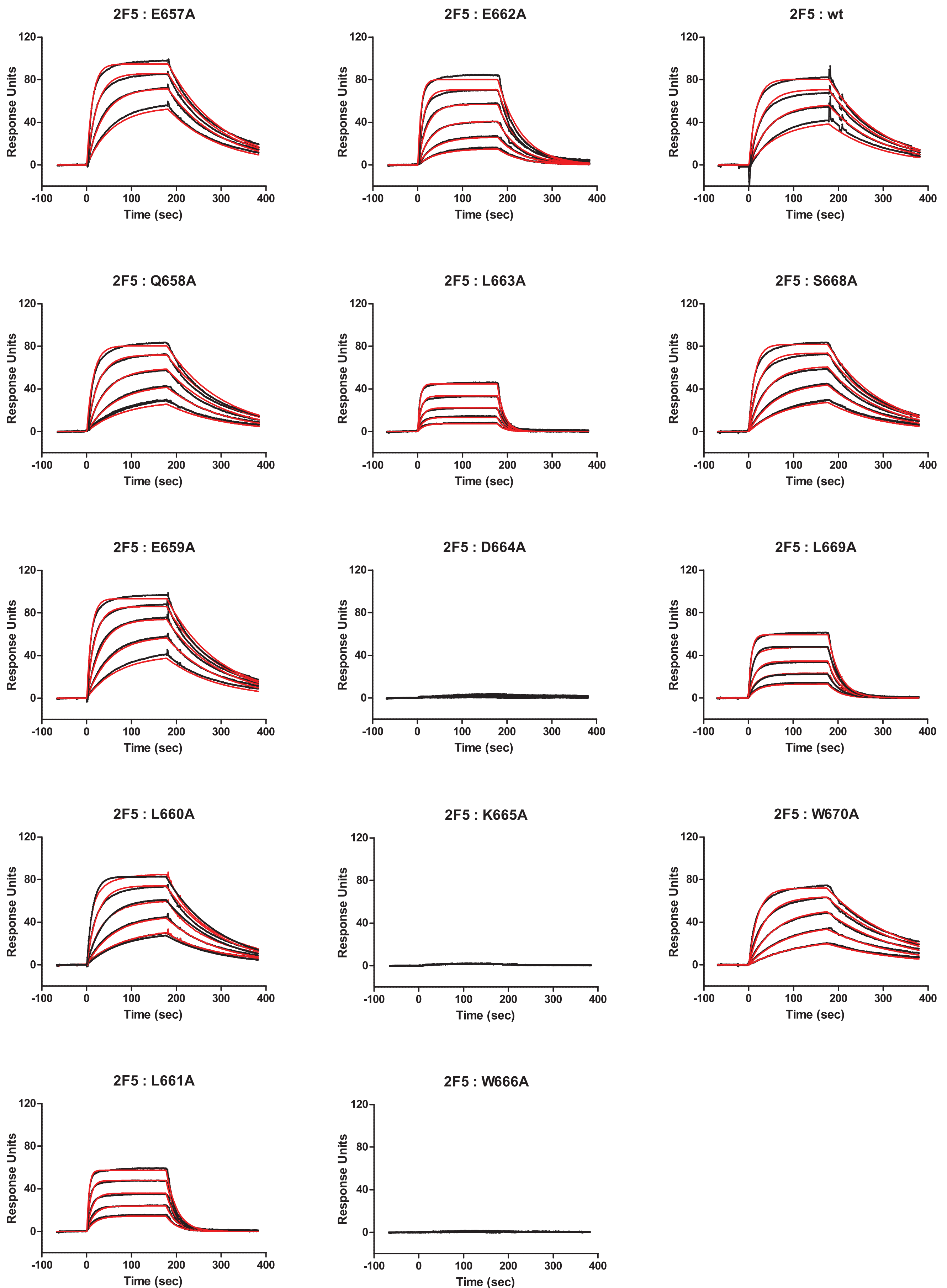
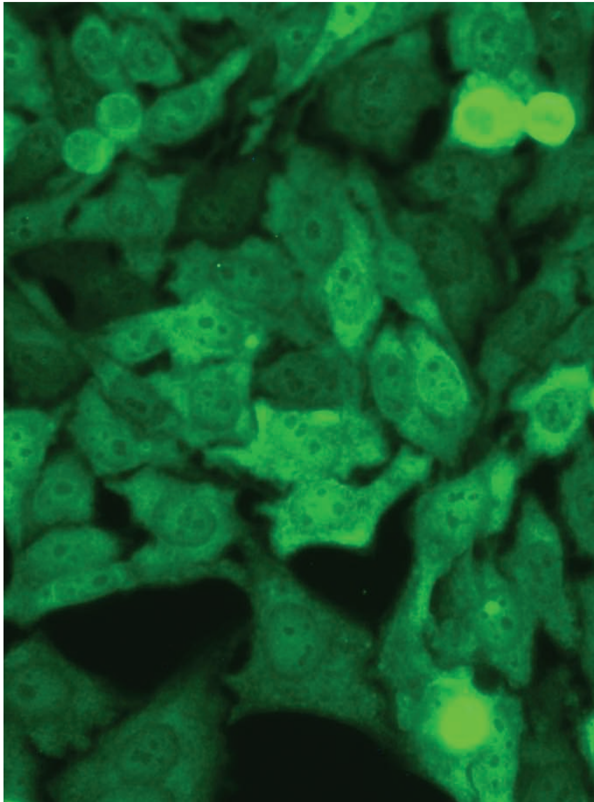
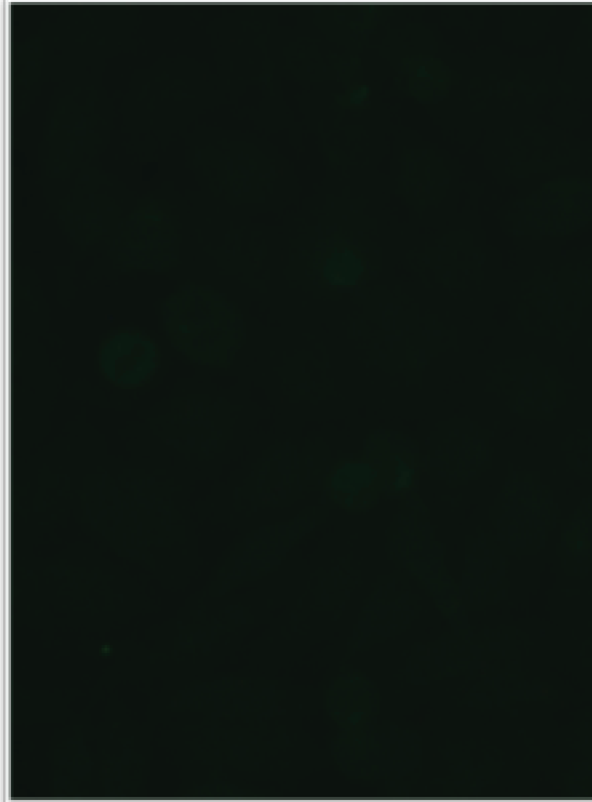


Fig. S3 (C). Biacore kinetics curves for binding of 2F5.

Fig. S3: SPR analysis of binding of m66 (A), m66.6 (B) and 2F5 (C) to alanine mutants of a peptide containing gp41 residues 657EQELLELDKWASLW670 linked to a C-terminal C9 tag. IgGs were directly immobilized to Biacore CM5 sensor chips to surface densities of ~4000 RUs, and alanine mutant peptides based on the wild type MPER-C9 peptide were flowed over at 30 ml/min as analyte. Twofold serial dilutions of peptides ranging from 250 nM to 15.6 or 7.9 nM were used. For 2F5 binding to peptides E657A, Q658A, L660A, A667 (WT), S668A, and W670A, concentrations are from 125 nM to 15.6 or 7.9 nM. In all cases, sensograms were fit with a 1:1 Langmuir model.





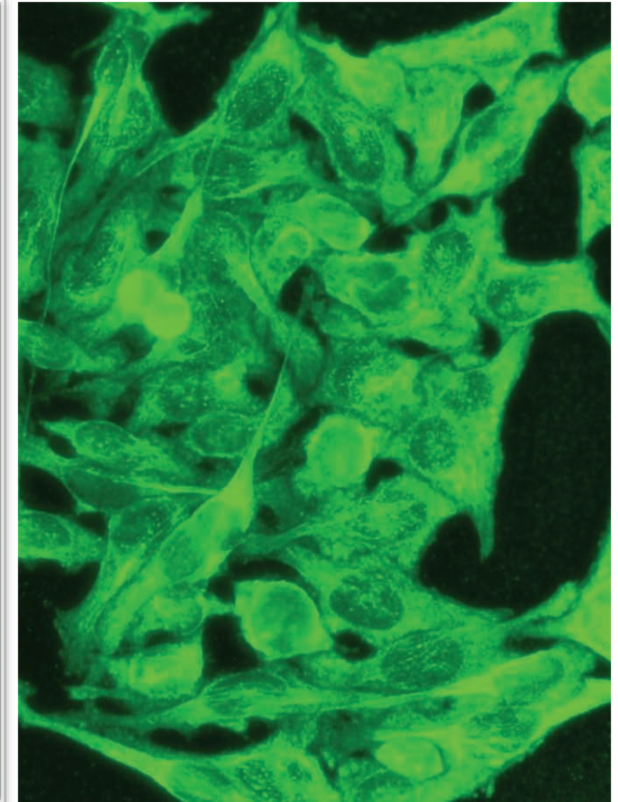


Fig. S4 Reactivity of m66.6 with self-antigens. Binding of m66.6 and 2F5 to HIV-1 negative human epithelial cell line Hep-2 cells. Another HIV-1-specific Ab, 17b, was used as negative control. Reactivity to HIV-1 negative human epithelial HEp-2 cells was determined by indirect immunofluorescence on HEp-2 slides using Evans Blue as a counterstain and FITC-conjugated goat anti-human IgG (Zeus Scientific, Raritan N.J.). Slides were photographed on a Nikon Optiphot fluorescence microscope.

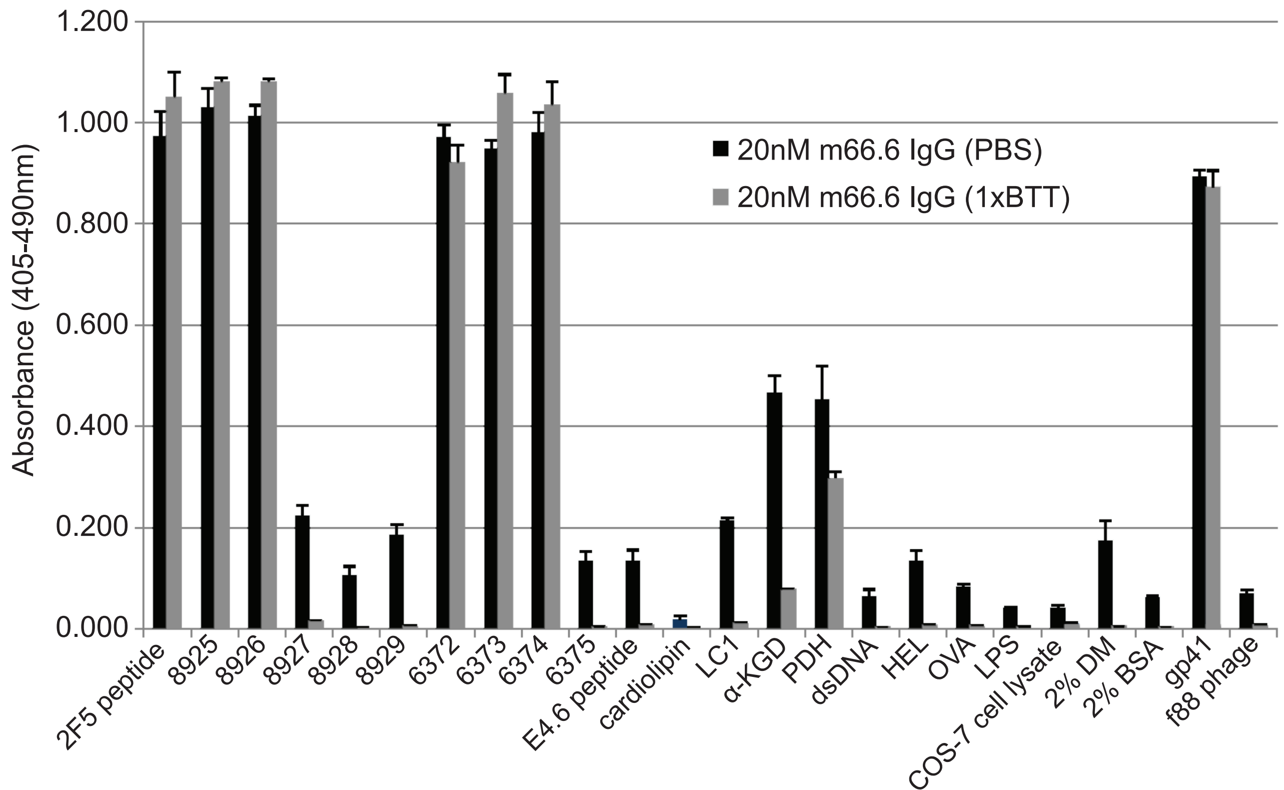


Fig. S5. Reactivity of m66.6 with self and non-self antigens in BSA as measured by ELISA. Microplate wells were coated with antigens as described in Singh et al., (2011) then blocked with 2%BSA/1xTBS (200μL per well) for 1hr at 37°C and then washed 2x with 1xTBS/0.1%Tween, 1x with 1xTBS. 20nM of IgG1 m66.6 was added, diluted in either 1xBTT (1%BSA/1xTBS/0.1%Tween) or 1xPBS and incubated at room temperature (RT) for 2hr. Plates were washed 5x with Tris-buffered saline (TBS) containing 0.1%Tween, and 1x with TBS. Secondary Ab Protein-A/G-HRP conjugate was diluted 1:4000 in 1xBTT or 1xPBS and incubated at RT for 45min., then washed as above. Absorbance was recorded at 405-490nm. [Singh, H., Henry, K.A., Wu, S.S.T., Chruscincki, A., Utz, P.A. and Scott, J.K. Reactivity profiles of broadly neutralizing anti-HIV-1 antibodies are distinct from pathogenic autoantibodies, 2011 AIDS, 25:1247-1257.]