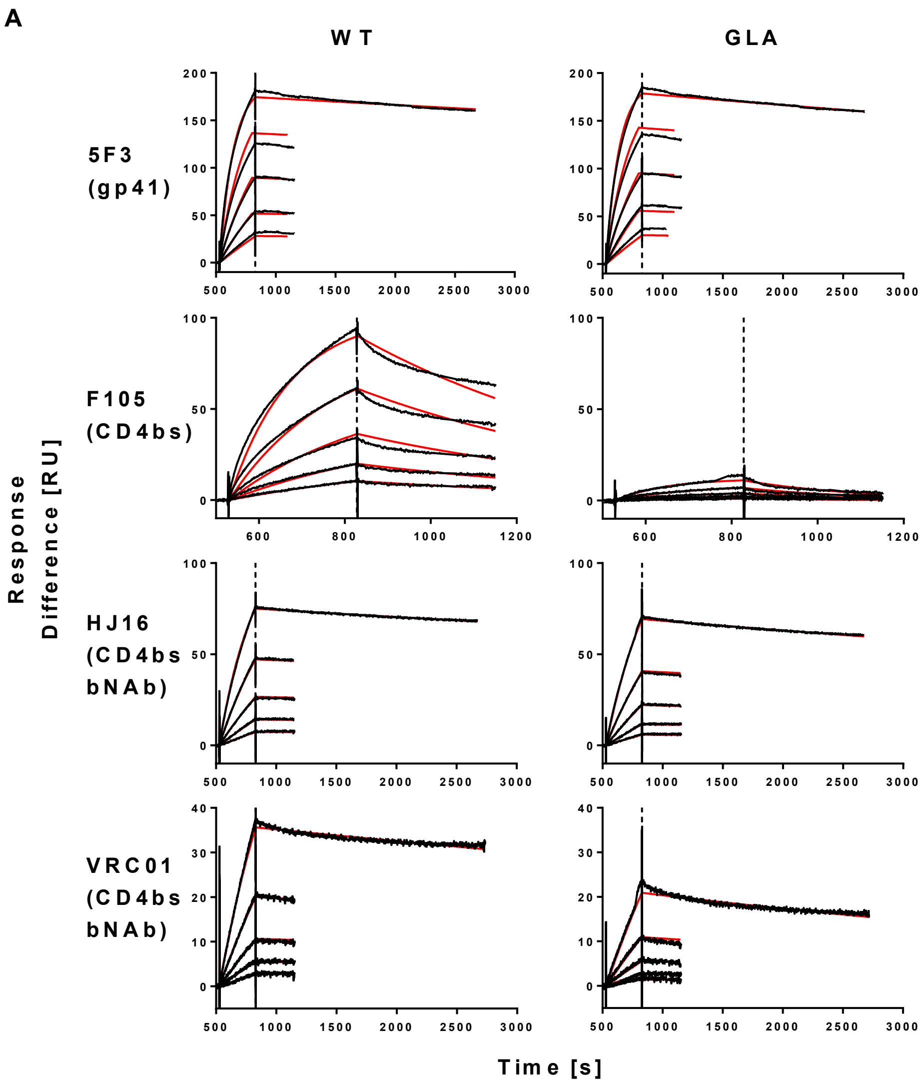
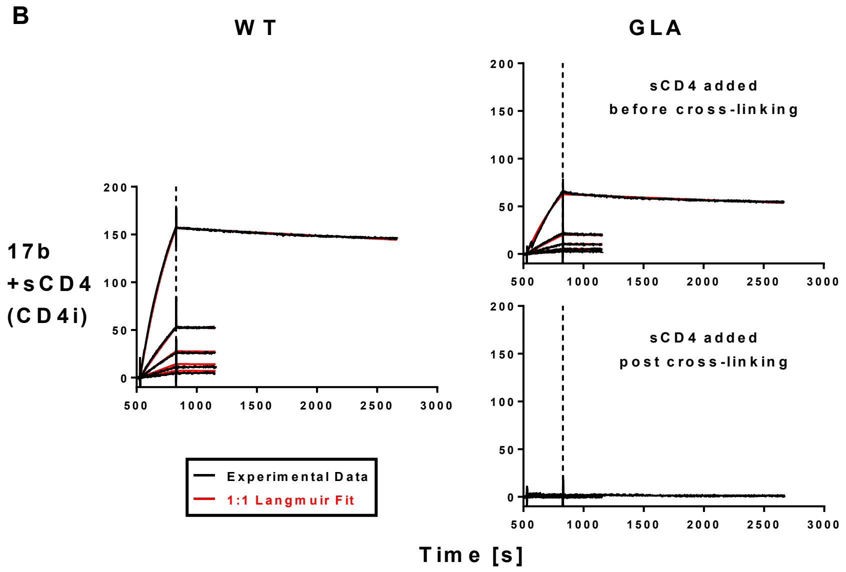


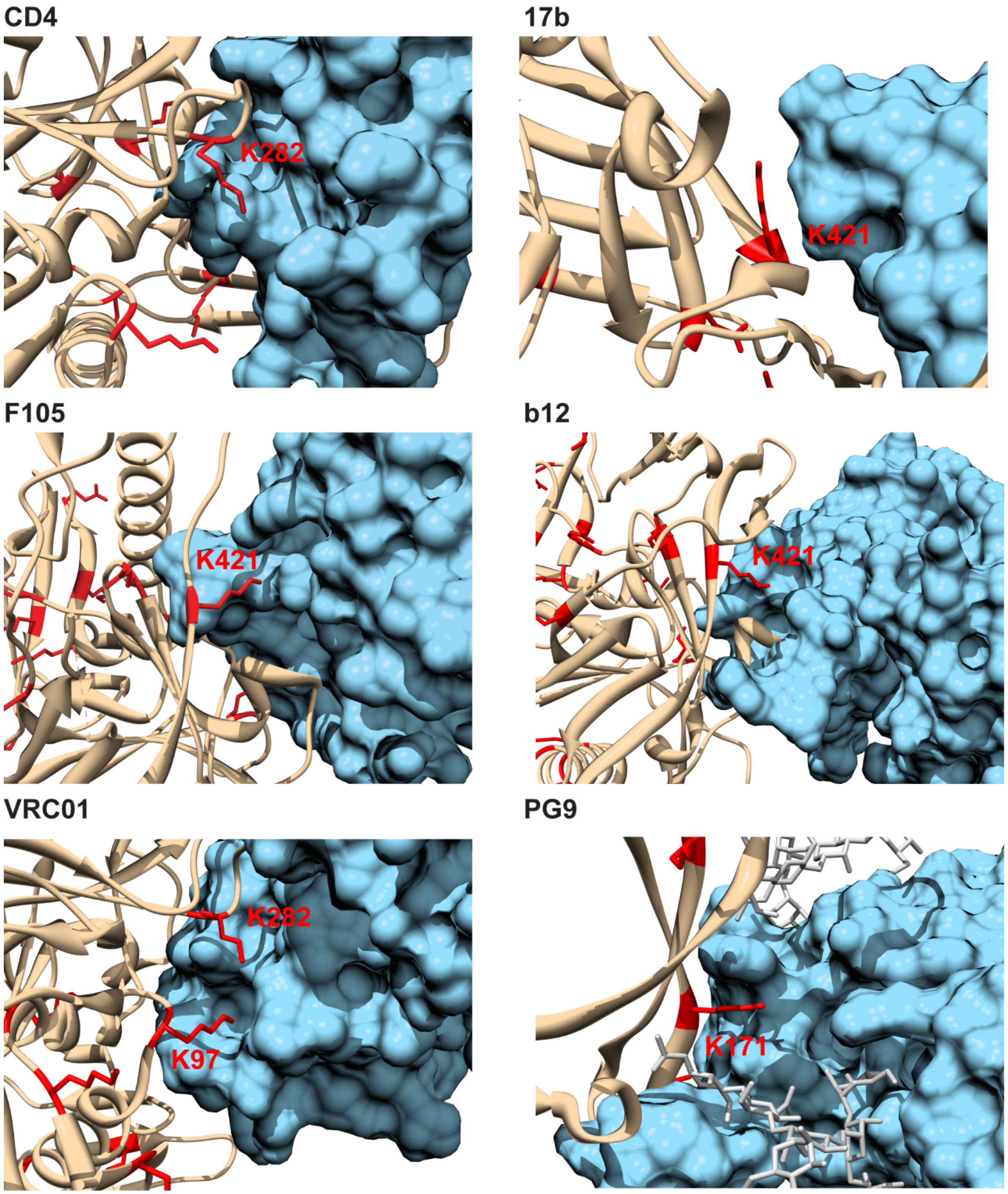


C

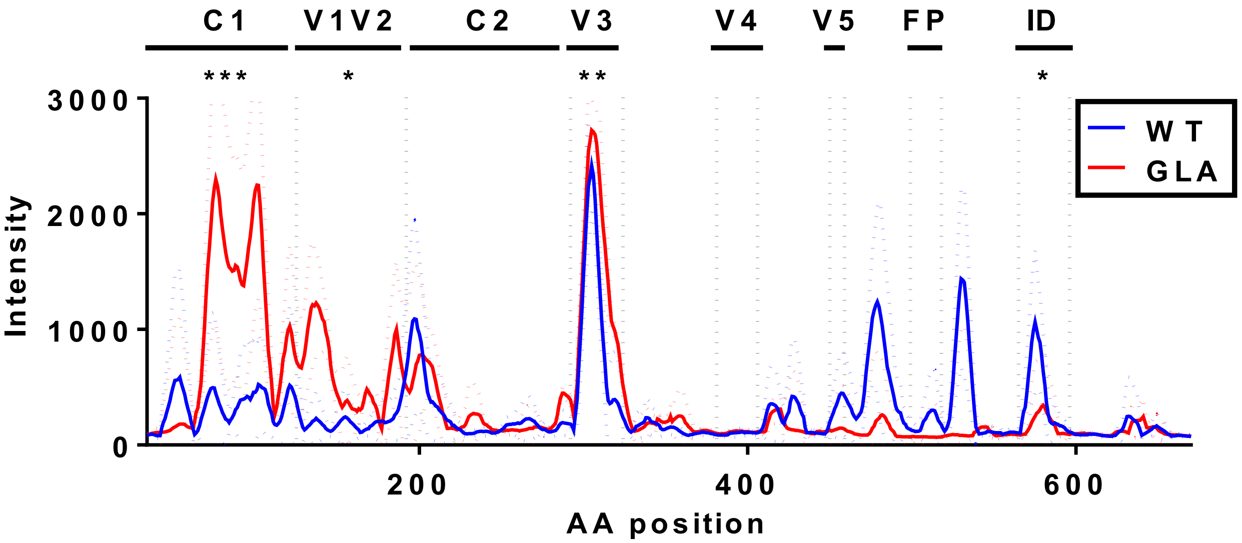
	WT					GLA				
	KD (nM)	Rmax (RU)	ka (1/Ms)	kd (1/s)	Chi²	KD (nM)	Rmax (RU)	Ka (1/Ms)	Kd (1/s)	Chi²
HJ16	3.96	116	13.7	5.44E-05	0.17	8.68	139	9.25	8.02E-05	0.206
VRC01	12	91.7	6.44	7.73E-05	0.182	69.8	134	2.29	1.60E-04	0.204
F105	67.7	129	22	1.49E-03	5.47	190	20.5	19.4	3.68E-03	0.212
5F3	0.154	190	268	4.11E-05	15.8	0.21	192	288	6.05E-05	16.2
17b*	3.92	273	11.2	4.40E-05	2.21	9.04	122	9.49	8.58E-05	0.751

* gp140 was pre-incubated with sCD4 before cross-linking

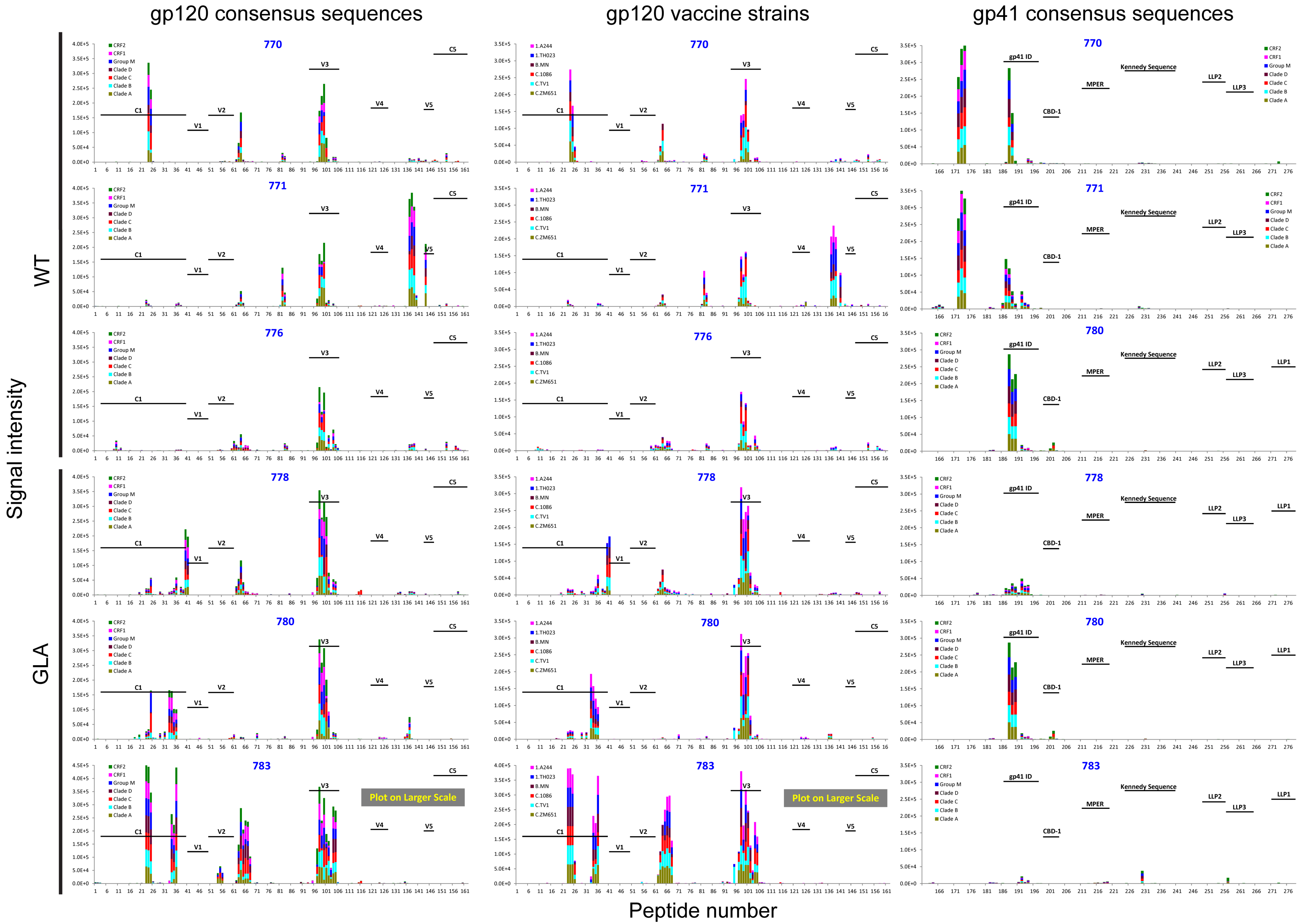
Supplementary Fig. 1: SPR analysis of the antigenicity of GLA cross-linked gp140. A+B: Titration series of unmodified (WT) or cross-linked (GLA) gp140 were passed over mAbs as indicated. Black curves show experimental data, red lines indicate results of a 1:1 langmuir analysis and vertical black lines show the end of the gp140 injection. B: sCD4 was added to gp140 either before (top right) or after (bottom right) cross-linking and samples were analysed as in A. C: Table shows the results from the 1:1 Langmuir analysis.



Supplementary Fig. 2: Modification sites within mAb epitopes. Ribbon representations of gp120 or gp120 fragments (fawn) are shown in complex with blue surface representations of CD4 or mAbs as indicated. Potential modification sites (lysine residues) are shown in red and residue numbers of lysines interacting with mAbs are labelled in red according to HXB2 numbering. Lysine residues were identified based on the sequence of strain CN54 except for PG9, in which case the sequence of strain UG37 was used consistent with the experiments performed in this study. PG9 interacts with a second lysine of UG37 (K168), which is shielded from view by PG9 in this orientation.



Supplementary Fig. 3: Homologous peptide scanning of week 24 serum responses. Dotted curves indicated SD of 4 animals tested, dotted vertical lines indicated borders of epitopes.



Supplementary Fig. 4: Heterologous peptide scanning of week 24 serum responses.

Supplementary Table 1: TZM-bl assay neutralization data

Group	Animal	Week	DJ263.8	TH023.6	SF162.LS	MN-3	Bx08.16	BaL.26	BZ167.12	MW965.23	TV1.21	ZM109F.PB4	ZM197M.PB7	T271-11	25710-2.43	Du422.1	ZM53M.PB12	AC10.29	Q23.17	MuLV
		Tier (Clade)	1B (A)	1B (A/E)	1A (B)	1A (B)	1B (B)	1B (B)	1B (B)	1A (C)	1B (C)	1B (C)	1B (C)	1B (C)	1B (C)	2 (C)	2 (C)	2 (B)	2 (A)	Neg
WT	770	-1	<10	<10	<10	<10	<10	<10	<10	11	11	<10	<10	<10	<10	<10	13	<10	<10	<10
		14	64	ND	362	ND	27	19	ND	2842	27	17	14	ND	ND	10	11	11	<10	<10
		24	27	356	99	154	18	17	<10	1592	18	22	16	15	14	15	11	11	<10	<10
	771	-1	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
		14	23	ND	344	ND	32	34	ND	2298	10	<10	<10	ND	ND	<10	<10	<10	<10	<10
		24	23	262	248	63	51	38	15	2865	16	18	20	37	<10	12	<10	<10	<10	<10
	772	-1	<10	<10	<10	<10	<10	<10	<10	16	11	11	11	<10	<10	<10	14	<10	<10	<10
		14	112	ND	532	ND	38	31	ND	4003	39	18	20	ND	ND	13	13	<10	<10	10
		24	32	793	230	304	18	13	14	4267	18	21	13	49	<10	<10	<10	<10	<10	<10
	773	-1	11	<10	<10	<10	<10	<10	<10	12	15	13	19	<10	<10	12	14	<10	<10	12
		14	78	ND	522	ND	25	26	ND	3113	21	<10	11	ND	ND	<10	<10	<10	<10	<10
		24	23	543	338	373	22	26	15	3002	11	<10	13	23	<10	<10	<10	<10	<10	<10
	774	-1	<10	<10	<10	<10	11	<10	<10	13	12	12	17	<10	<10	12	13	<10	<10	<10
		14	53	ND	552	ND	34	24	ND	2824	14	<10	14	ND	ND	<10	10	<10	<10	<10
		24	22	172	276	277	42	37	19	4394	16	20	15	20	<10	<10	<10	<10	<10	<10
	775	-1	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	11	<10	<10	<10	10	<10	<10	<10
		14	208	ND	1696	ND	193	213	ND	7932	127	24	122	ND	ND	34	13	17	<10	28
		24	22	1189	313	300	38	32	23	3879	18	18	10	23	<10	<10	<10	<10	<10	<10
	776	-1	12	<10	<10	<10	10	<10	<10	17	20	18	27	<10	<10	19	23	20	<10	18
		14	29	ND	228	ND	21	25	ND	2034	<10	<10	17	ND	ND	<10	<10	<10	<10	<10
		24	11	190	166	342	12	11	12	1492	<10	11	<10	12	<10	<10	<10	<10	<10	<10
	777	-1	<10	<10	<10	<10	11	<10	<10	11	18	12	20	<10	<10	17	18	15	<10	13
		14	268	ND	896	ND	104	85	ND	8863	40	21	28	ND	ND	16	23	20	<10	13
		24	47	158	488	99	74	50	17	7852	16	19	10	61	<10	<10	<10	<10	<10	<10

GLA	**778**	-1	<10	<10	<10	<10	<10	<10	<10	11	<10	<10	11	<10	<10	<10	<10	<10	<10	<10
		14	102	ND	472	ND	17	21	ND	5486	40	10	13	ND	ND	<10	<10	<10	<10	<10
		24	130	2734	671	526	38	39	28	8886	34	26	<10	91	<10	<10	<10	<10	<10	<10
	779	-1	<10	<10	<10	<10	<10	<10	<10	11	<10	<10	13	<10	<10	<10	<10	12	<10	<10
		14	86	ND	247	ND	15	22	ND	2982	<10	<10	<10	ND	ND	<10	<10	<10	<10	<10
		24	46	518	331	579	37	38	21	4394	<10	14	<10	27	<10	<10	<10	<10	<10	<10
	780	-1	25	<10	<10	<10	15	10	<10	19	17	20	24	<10	<10	20	24	29	<10	19
		14	1061	ND	2311	ND	107	83	ND	>21,870	125	31	<10	ND	ND	<10	<10	<10	<10	<10
		24	462	15009	3832	351	202	187	64	>21,870	104	99	18	424	23	<10	<10	<10	<10	<10
	781	-1	<10	<10	<10	<10	<10	<10	<10	10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
		14	186	ND	861	ND	75	81	ND	5419	28	16	16	ND	ND	<10	<10	<10	<10	<10
		24	70	787	1779	2050	112	139	174	10572	38	24	13	62	<10	<10	<10	<10	<10	<10
	782	-1	17	<10	<10	<10	14	10	<10	20	19	21	31	<10	<10	19	22	28	<10	25
		14	69	ND	736	ND	23	86	ND	2998	18	<10	11	ND	ND	<10	<10	<10	<10	<10
		24	40	436	700	16	38	29	10	4769	13	27	<10	45	10	<10	<10	<10	<10	<10
	783	-1	<10	<10	<10	<10	12	<10	<10	<10	12	<10	14	<10	<10	10	13	11	<10	<10
		14	336	ND	1136	ND	56	81	ND	13182	38	18	<10	ND	ND	<10	<10	<10	<10	<10
		24	354	2028	4070	298	321	306	334	>21,870	128	61	11	175	13	<10	<10	<10	<10	<10
	784	-1	<10	<10	<10	<10	10	<10	<10	14	14	<10	12	<10	<10	17	<10	13	<10	<10
		14	71	ND	874	ND	16	34	ND	6726	15	<10	<10	ND	ND	<10	<10	<10	<10	<10
		24	158	627	397	73	64	40	64	11404	104	32	11	178	13	11	<10	<10	<10	<10
	785	-1	23	18	<10	<10	10	<10	<10	16	26	22	27	<10	17	26	18	30	<10	16
		14	217	ND	1081	ND	33	72	ND	>21,870	67	<10	<10	ND	ND	<10	<10	<10	<10	<10
		24	160	4374	1157	424	69	99	20	11740	129	20	<10	134	<10	<10	<10	<10	<10	<10

Supplementary Table 2: A3R5 assay neutralization data

Group	Animal	Week	Ce0393_C3.LucR.T2A.ecto	CAP45.2.00.G3.LucR.T2A.ecto	Ce1086_B2.LucR.T2A.ecto	DU151.2.LucR.T2A.ecto	Ce2010_F5.LucR.T2A.ecto	Du422.1.LucR.T2A.ecto	Ce0393_C3.LucR.T2A.ecto
		Tier (Clade)	Tier 1A Clade C	Tier 1B Clade C	Tier 2 Clade C	Tier 2 Clade C	Tier 2 Clade C	Tier 2 Clade C	Tier 1A Clade C
WT	770	-1	14	<10	16	28	21	22	14
		14	493	119	80	83	63	100	493
		24	742	153	137	96	79	121	742
	771	-1	<10	<10	<10	20	<10	15	<10
		14	240	67	82	10	39	10	240
		24	427	220	139	71	47	67	427
	772	-1	14	<10	22	25	22	23	14
		14	451	131	113	10	10	76	451
		24	827	182	164	77	67	132	827
	773	-1	17	<10	25	21	22	20	17
		14	170	190	96	10	10	10	170
		24	374	376	263	76	43	37	374
	774	-1	20	<10	26	29	21	15	20
		14	73	32	169	10	10	10	73
		24	256	120	71	45	43	50	256
	775	-1	16	<10	27	21	15	17	16
		14	504	408	262	142	76	163	504
		24	1042	186	143	56	46	111	1042
	776	-1	30	25	42	41	48	42	30
		14	143	10	91	10	10	10	143
		24	522	384	97	36	28	49	522
	777	-1	18	13	36	36	32	40	18
		14	208	118	117	10	10	10	208
		24	614	281	191	43	89	90	614

GLA	**778**	-1	<10	<10	18	12	13	18	<10
		14	131	35	64	10	10	10	131
		24	463	134	108	35	47	59	463
	779	-1	17	<10	23	33	29	25	17
		14	719	40	106	10	10	10	719
		24	1763	159	113	93	53	79	1763
	780	-1	30	35	40	29	39	30	30
		14	580	514	113	10	10	116	580
		24	1870	142	129	118	51	139	1870
	781	-1	15	10	20	20	20	20	15
		14	123	95	91	10	10	10	123
		24	572	262	111	67	37	45	572
	782	-1	42	38	62	51	58	41	42
		14	551	10	10	10	10	10	551
		24	1063	107	187	84	89	202	1063
	783	-1	14	12	28	23	14	13	14
		14	182	181	10	10	10	10	182
		24	1789	291	49	35	40	43	1789
	784	-1	11	<10	16	21	<10	17	11
		14	346	73	117	10	34	76	346
		24	1096	170	103	64	47	83	1096
	785	-1	34	27	39	36	41	37	34
		14	514	151	10	10	10	10	514
		24	2049	308	112	78	58	152	2049