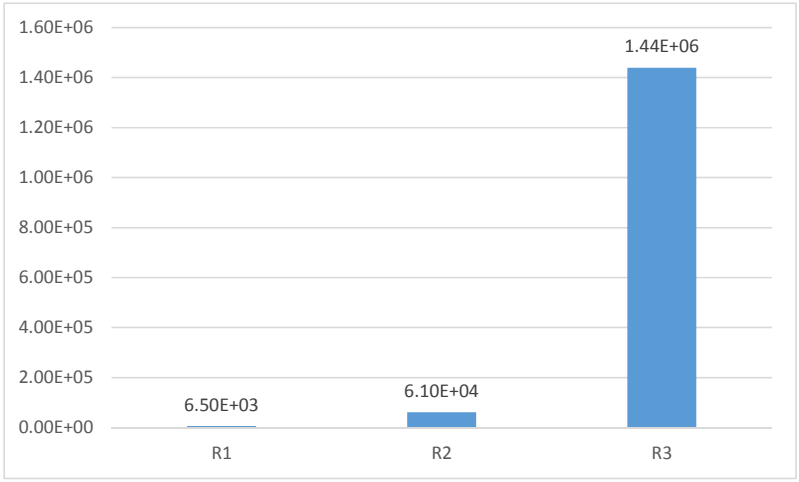
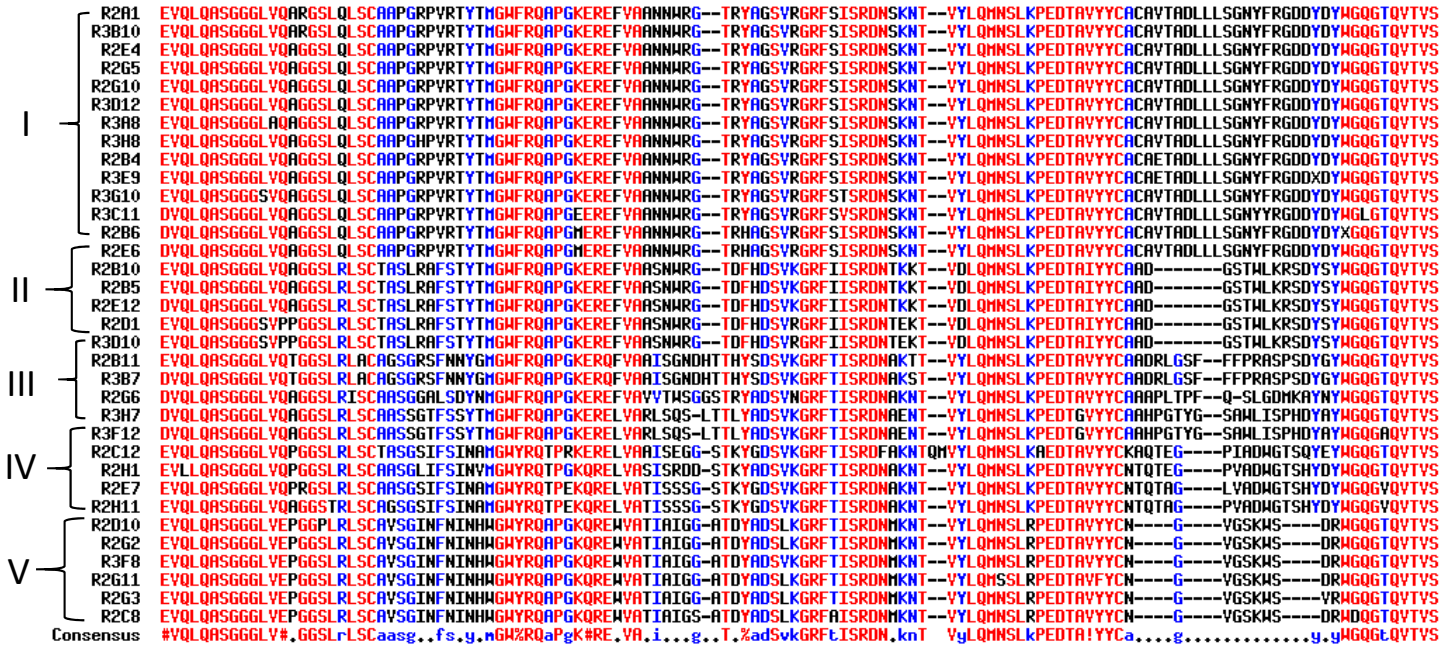


Fig S1. Biopanning against EBOV GP. The titer of eluted phage was determined at end of each round of biopanning. Each panning round used the same number of inputted phage (~$5x10^{11}$).



FigS2. Thirty four potential GP sdAb sequences selected from the second and third rounds of panning against EBOV GP. R2 signifies sequences selected in round 2 while R3 sequences were isolated from round 3.

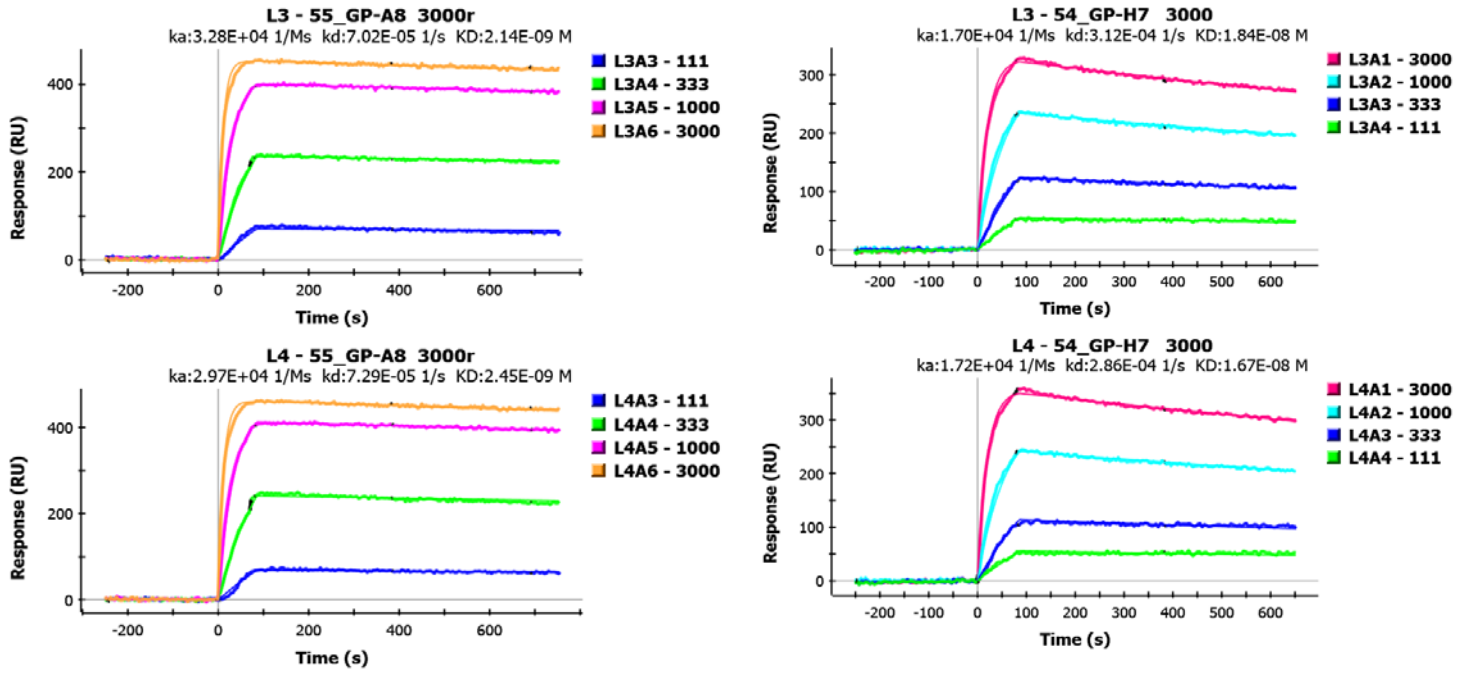


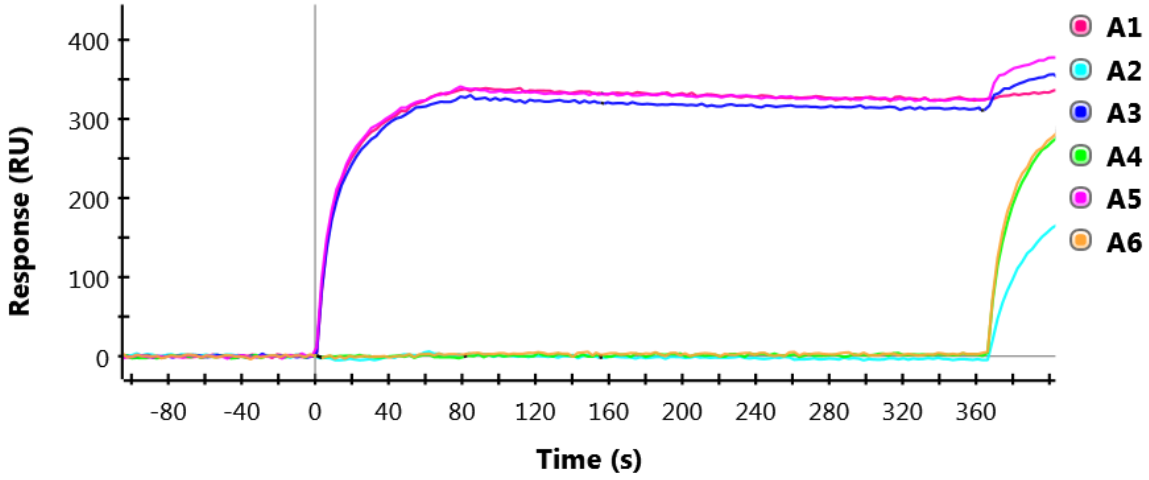
Fig. S3. Measurements of KD using SPR biosensor. The surface was coated with EBOV GP first and a serial dilution of purified sdAbs (3000, 1000, 333, and 111 nM) were then injected. Duplicate data is shown above for EBOV-GP-A8 on the left and EBOV-GP-H7 on the right.

IMGT number	EBOV-GP-H7	EBOV-GP-G6	EBOV-GP-A8
1	D	D	E
2	V	V	V
3	Q	Q	Q
4	L	L	L
5	Q	Q	Q
6	A	A	A
7	S	S	S
8	G	G	G
9	G	G	G
10	-	-	-
11	G	G	G
12	L	L	L
13	V	V	A
14	Q	Q	Q
15	A	A	A
16	G	G	G
17	G	G	G
18	S	S	S
19	L	L	G
20	R	R	Q
21	L	I	L
22	S	S	S
23	C	C	C
24	A	A	A
25	A	A	A
26	S	S	P
27	S	G	G
28	G	G	R
29	T	A	P
30	F	L	V
31	-	-	-
32	-	-	-
33	-	-	-
34	-	-	-
35	S	S	R
36	S	D	T
37	Y	Y	Y
38	T	N	T
39	M	M	M
40	G	G	G
41	W	W	W
42	F	F	F
43	R	R	R
44	Q	Q	Q
45	A	A	A
46	P	P	P
47	G	G	G
48	K	K	K
49	E	E	E
50	R	R	R
51	E	E	E
52	L	F	F
53	V	V	V

54	A	A	A
55	R	V	A
56	L	V	N
57	S	T	N
58	Q	W	W
59	S	S	-
60	-	-	-
61	-	-	-
62	-	G	-
63	L	G	R
64	T	S	G
65	T	T	T
66	L	R	R
67	Y	Y	Y
68	A	A	A
69	D	D	G
70	S	S	S
71	V	V	V
72	K	N	R
73	-	-	-
74	G	G	G
75	R	R	R
76	F	F	F
77	T	T	S
78	I	I	I
79	S	S	S
80	R	R	R
81	D	D	D
82	N	N	N
83	A	A	S
84	E	K	K
85	N	N	N
86	T	T	T
87	V	V	V
88	Y	Y	Y
89	L	L	L
90	Q	Q	Q
91	M	M	M
92	N	N	N
93	S	S	S
94	L	L	L
95	K	K	K
96	P	P	P
97	E	E	E
98	D	D	D
99	T	T	T
100	G	A	A
101	V	V	V
102	Y	Y	Y
103	Y	Y	Y
104	C	C	C
105	A	A	A
106	A	A	C
107	H	A	A

108	P	P	V
109	G	L	T
110	T	T	A
111	Y	P	D
111A	G	F	L
111B	S	Q	L
111C	A	S	L
112D	W	-	S
112E	-	-	G
112D			N
112C	L	L	Y
112B	I	G	F
112A	S	D	R
112	P	M	G
113	H	K	D
114	D	A	D
115	Y	Y	Y
116	A	N	D
117	Y	Y	Y
118	W	W	W
119	G	G	G
120	Q	Q	Q
121	G	G	G
122	T	T	T
123	Q	Q	Q
124	V	V	V
125	T	T	T
126	V	V	V
127	S	S	S
128	S	S	S

Fig. S4. Table of IMGT number and corresponding amino acid at each position for clones EBOV-GP-A8, EBOV-GP-H7 and EBOV-GP-G6. CDR regions are highlighted in red for CDR1, blue for CDR2, and green for CDR3.



SdAb	% Inhib. vs A8
EBOV-GP-H7	68
EBOV-GP-D1	82
EBOV-GP-B5	80
EBOV-GP-B11	87
EBOV-GP-G6	97
EBOV-GP-E7	0
EBOV-GP-C12	0

Fig S5. Binding competition determination. First an SPR chip was coated with EBOV GP. Next EBOV-GP-A8 was flowed over three lanes (lanes 1, 3, and 5); buffer only was flowed over lanes 2, 4, and 6. Finally various anti-EBOV GP sdAbs were flowed over pairs of lanes: one lane blocked by pre-binding of EBOV-GP-A8 and one unblocked (buffer only in the previous step). To determine inhibition we compared sdAb binding to the two surfaces. The graph above shows data for EBOV-GP-H7 (red and light blue), EBOV-GP-D1 (dark blue and green), and EBOV-GP-B5 (pink and orange). The adjoining table shows the calculated percent inhibition versus A8 for the 3 sdAbs shown on the graph above and sdAbs determined by performing additional tests.

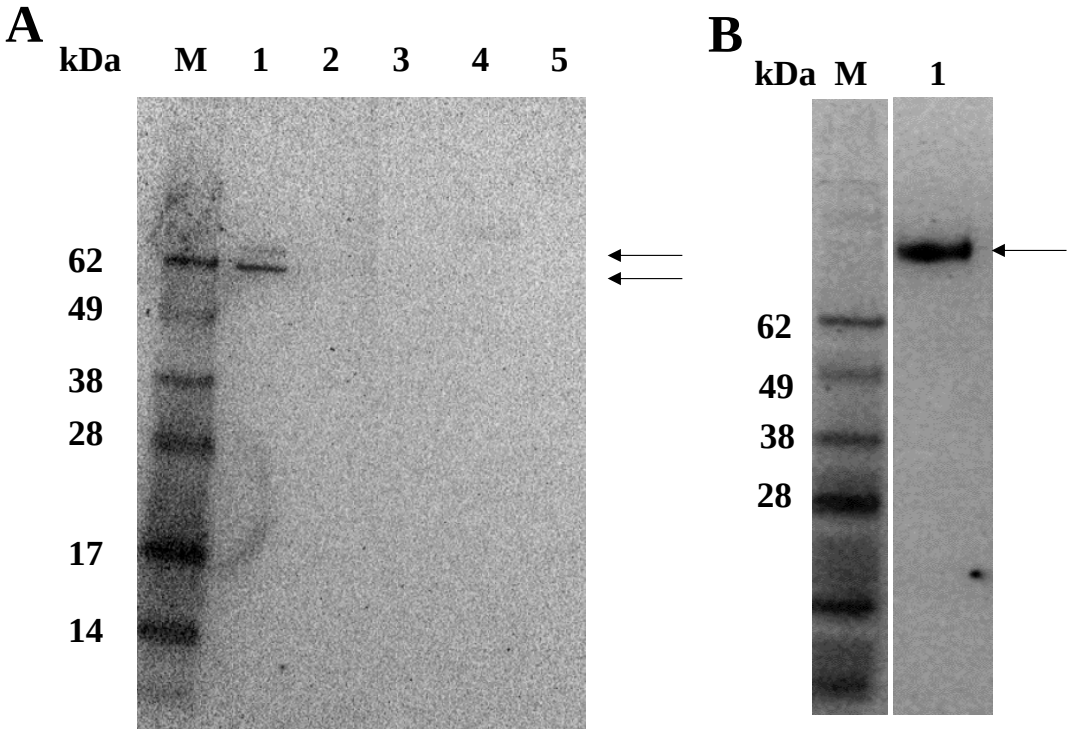


Fig. S6. Western blotting analysis of GP and VLP binding specificity and cross reactivity for G6-neg+-GS3K. A. Lane 1 represents EBOV VLPs, where as GP indicated by arrows. Lanes 2 and 3 represent the SUDV VLPs and MARV VLP respectively. Lanes 4 and 5 represent EBOV GP and MARV GP, respectively. Lane M is the size marker. B. The binding of A8-fneg+-GS3K to EBOV GP. Lane 1 represents EBOV GP as indicated by the arrow.