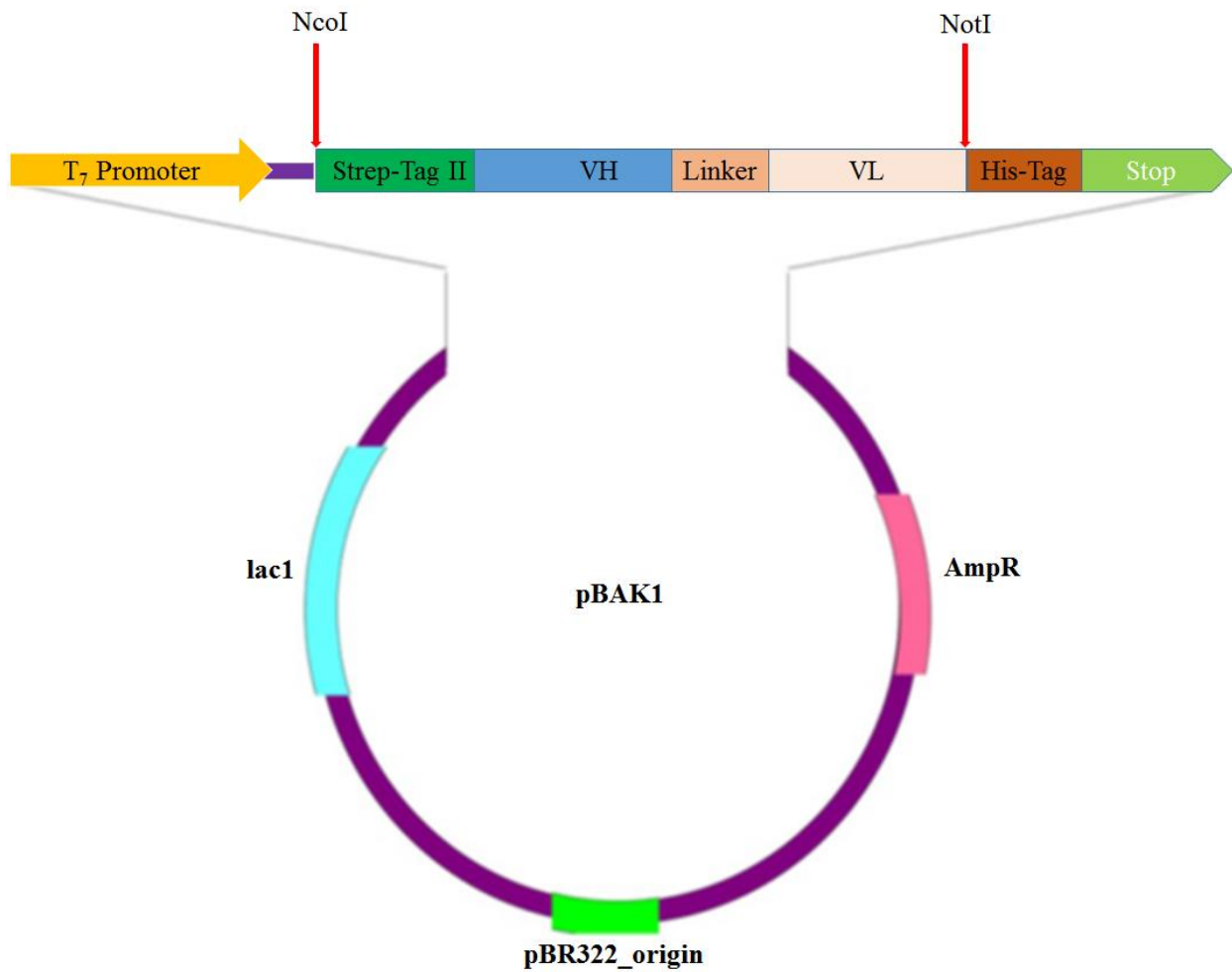


S1 Fig. Alignment of the derived aminoacids sequences of the randomly chosen scFvs from linked antibody library and complementary determining regions (CDRs). FRs and CDRs are determined by the IMGT information system. Diversity was found predominantly in the CDR regions. A normal 20 amino acid linker [(G4S)4] joins the VH and VL chains. Alignments were **colour coded** according to residue property groups. AVFPMILW-red, DE-blue, RK-magenta, STYHCNGQ-green, others-grey.

Symbols in the alignment are as follows: (*) indicates where there is a conserved amino acid; (:) indicates an amino acid position with conserved similarity; (.) indicates a semi-conserved amino substitution has occurred; (-) indicates spaces introduced to optimise the alignment.



S2 Fig. Schematic diagram of the pBAK1 (*anti-EbovGP-his-ScFv*) expression vector. The position of the enzymatic cleavage is indicated by the *red arrow*. The gene encoding anti-EbovGP-his-ScFv protein was inserted into the pBAK1 vector under the control of the T7lac promoter, in frame with a strep tag II.

S1 Table
Nucleotide sequences of primers used.

Primer name	Primer sequence (5' – 3')
	VH primers
MVH_F1	CGAGAAGACCGGCAGCGGTGGGGCAGAGCTTGTGAAGCCA
MVH_F2	CGAGAAGACCGGCAGCGGTGGAGGAGGCTTGATGCAACCT
MVH_F3	CGAGAAGACCGGCAGCGGTGGACCTGAGCTGGAGATGCCT
MVH_F4	CGAGAAGACCGGCAGCGGTGGACCTGGCCTGGTGAGACCT
MVH_F5	CGAGAAGACCGGCAGCGGTGGGGGAGGCTTAGTGAAGCCT
MVH_F6	CGAGAAGACCGGCAGCGGTGGGGCAGAGCTTGTGAAGCCA
MVH_F7	CGAGAAGACCGGCAGCGGTGGAGGGGGCTTGGTACAGCCT
MVH_F8	CGAGAAGACCGGCAGCGGTGGGGCAGAGCTTGTGAGGTCA
MVH_R1	GGAGCCGCCGCCGCCGAGAACACCACCACC <u>GGATCC</u> ACCACCACCCGAG GAAACGGTGACCGTGGT
MVH_R2	GGAGCCGCCGCCGCCGAGAACACCACCACC <u>GGATCC</u> ACCACCACCCGAG GAGACTGTGAGAGTGGT
MVH_R3	GGAGCCGCCGCCGCCGAGAACACCACCACC <u>GGATCC</u> ACCACCACCCGCA GAGACAGTGACCAGAGT
MVH_R4	GGAGCCGCCGCCGCCGAGAACACCACCACC <u>GGATCC</u> ACCACCACCCGAG GAGACGGTGACTGAGGT
	VL primers
MVL_F1	GGCGGCGGCGGCTCCGGTGGTGGT <u>GGATCC</u> GCAATCATGTCTGCATCTCC
MVL_F2	GGCGGCGGCGGCTCCGGTGGTGGT <u>GGATCC</u> GCCTCCCTATCTGTATCTGTG
MVL_F3	GGCGGCGGCGGCTCCGGTGGTGGT <u>GGATCC</u> GCCTCCCTATCTGCATCTGTG
MVL_F4	GGCGGCGGCGGCTCCGGTGGTGGT <u>GGATCC</u> CTCACTTTGTCGGTTACCATT
MVL_F5	GGCGGCGGCGGCTCCGGTGGTGGT <u>GGATCC</u> TCAGCCTCTTTCTCCCTGGGA
MVL_F6	GGCGGCGGCGGCTCCGGTGGTGGT <u>GGATCC</u> CTCCTCCCTGAGTGTGTCAGCA
MVL_F7	GGCGGCGGCGGCTCCGGTGGTGGT <u>GGATCC</u> CTCCTCCCTGCCTGTCAGTCTT
MVL_F8	GGCGGCGGCGGCTCCGGTGGTGGT <u>GGATCC</u> CTCCTCCCTGCCTGTCAGTCTT
MKR	AGAACACTCATTCCTGTTGAAGCTCTTGACAATGGGTGAAGTTG
MKR_Not I	AGT <u>GCGGCCG</u> CAGAACACTCATCCTGTTGAAGCTCTTGACAATGGGTGAAGT TG
	Strep Tag II
KzSTREP II	CGAATTCCACCATGGCC TGG AGC CAT CCG CAG TTC GAG AAG ACC GGC AGC GG
	T7 promoter
RDT7	CTATAGAAGG GTAATACGACTCACTATAGGGCGAATTCCACCATGGCC

S2 Table**Cross-reactivity profiles of anti-EBOV GP scFvs from ELISA result.**

scFv	GP _{EBOV}	GP _{SUDV}	GP _{RESTV}	GP _{BDBV}	GP _{TAFV}	GP _{MARV}
3-2	+	+	-	-	+	+
4-2	+++	+++	++	++	++	+++
10-3	-	-	-	-	+	+
13-1	+	-	-	-	-	+
16-1	+	+	-	-	++	+
18-1	+	+	++	++	++	+
22-1	++	+++	++	++	++	+++
27-5	-	-	-	-	-	-
35-2	++	++	+	-	+	++
39-1	++	++	-	+	+	++
Mouse anti-Zaire ebola antibody 6D8	+++					
Mouse anti-Sudan ebola virus antibody		+++				
Rabbit anti-Reston GP polyclonal antibody			+++			
Rabbit anti-Bundibugyo GP polyclonal antibody				+++		
Rabbit anti-MARV GP polyclonal antibody						+++
Rabbit anti-Tai Forest virus GP IgG					+++	
XfPilB21	-	-	-	-	-	-

†: GPs of each virus species were used as antigens. *: scFv reactivity was evaluated based on ELISA OD450. +++: OD>1.0; ++: OD ≥ 1.0; +: 0.3 < OD < 1.0; -: OD ≤ 0.3. XfPilBscFv21: Negative control. Mouse anti-Zaire ebola antibody 6D8, Mouse anti-Sudan ebola virus antibody, Rabbit anti-Reston GP polyclonal antibody, Rabbit anti-Bundibugyo GP polyclonal antibody, Rabbit anti-MARV GP polyclonal antibody, and Rabbit anti-Tai Forest virus GP IgG: Positive controls.

S3 Table 3

% CV & Bias around calculated EC-50. Accuracy and precision around the calculated EC-50 values are within acceptable level.

scFv4-2 vs GP _{EBOV}				scFv4-2 vs GP _{SUDV}			scFv4-2 vs GP _{MARV}		
µg/mL	Mean Absorbance	%CV of calculated conc	%BIAS	Mean Absorbance	%CV of calculated conc	%BIAS	Mean Absorbance	%CV of calculated conc	%BIAS
30	2.748	21.0	1.2	2.121	5.8	-5.1	2.847	31.8	-1.9
20	2.709	34.3	10.4	2.030	9.0	4.7	2.816	4.1	2.4
15	2.667	3.9	0.1	1.916	1.7	5.1	2.756	13.1	5.9
10	2.523	11.5	4.4	1.613	2.4	-5.4	2.523	8.4	-0.4
5	1.735	3.3	-0.4	1.117	4.9	2.2	1.627	7.2	0.3
1	0.143	1.6	8.5	0.170	2.0	-3.7	0.067	1.8	-0.4
0.1	0.049	97.9	3.8	0.006	7.1	-0.2	0.0	6.3	18.1
0.01	0.034	Range?	Range?	0.005	12.7	776.8	0.0	6.0	1019.1

S4 Table. % CV & Bias around calculated EC-50. Accuracy and precision around the calculated EC-50 values are within acceptable level.

scFv4-2 vs GP _{BDBV}				scFv4-2 vs GP _{RESTV}			scFv4-2 vs GP _{TAFV}		
µg/ml	Mean Absorbance	%CV of calculated conc	%BIAS	Mean Absorbance	%CV of calculated conc	%BIAS	Mean Absorbance	%CV of calculated conc	%BIAS
30	1.286	4.2	-0.5	1.349	10.1	1.0	2.065	6.4	-2.6
20	1.199	13.0	1.2	1.266	15.0	1.3	1.918	16.4	4.8
15	1.087	5.1	0.3	1.167	0.6	0.6	1.765	5.7	3.9
10	0.832	3.4	0.1	0.930	5.5	0.1	1.426	2.7	-6.2
5	0.328	0.3	-0.05	0.424	0.2	-0.1	0.984	0.8	3.7
1	0.020	4.9	5.4	0.033	5.5	9.8	0.179	1.5	-5.9
0.1	0.009	54.4	10.9	0.012	17.5	12.0	0.011	4.6	14.7
0.01	0.008	Range?	Range?	0.008	0	5755.6	0.002	7.0	402.7

S5 Table

% CV & Bias around calculated EC-50. Accuracy and precision around the calculated EC-50 values are within acceptable level.

scFv22-1 vs GP _{EBOV}				scFv4-2 vs GP _{SUDV}			scFv4-2 vs GP _{MARV}		
µg/ml	Mean Absorbance	%CV of calculated conc	%BIAS	Mean Absorbance	%CV of calculated conc	%BIAS	Mean Absorbance	%CV of calculated conc	%BIAS
30	2.711	6.3	0.2	1.937	23.9	3.2	1.636	12.8	0.8
20	2.497	13.2	4.0	1.763	4.3	0.3	1.592	8.2	2.3
15	2.261	6.2	0.263	1.593	5.6	0.3	1.507	9.0	0.2
10	1.835	5.3	-5.0	1.301	0.9	0.368	1.269	6.5	0.5
5	1.235	5.4	5.5	0.749	1.7	-0.697	0.530	9.0	-0.1
1	0.184	0.8	-18.0	0.124	3.6	5.3	0.020	4.2	18.2
0.1	0.035	12.0	16.0	0.020	23.4	-16.0	0.006	16.2	18.6
0.01	0.059	73.8	3133.1	0.012	0	307.5	0.001	Range?	Range?

S6 Table.

% CV & Bias around calculated EC-50. Accuracy and precision around the calculated EC-50 values are within acceptable level.

scFv4-2 vs GP _{BDBV}				scFv4-2 vs GP _{RESTV}			scFv4-2 vs GP _{TAFV}		
µg/ml	Mean Absorbance	%CV of calculated conc	%BIAS	Mean Absorbance	%CV of calculated conc	%BIAS	Mean Absorbance	%CV of calculated conc	%BIAS
30	1.838	9.1	-5.1	1.872	8.2	-1.4	1.508	11.2	-4.6
20	1.688	3.0	0.3	1.706	2.1	1.2	1.366	3.1	7.9
15	1.465	1.0	3.6	1.513	12.3	1.9	1.141	1.9	1.0
10	0.813	0.4	-3.0	1.134	0.8	-1.0	0.800	1.7	-6.4
5	0.231	1.5	10.4	0.521	1.0	0.3	0.445	14.3	5.0
1	0.027	9.4	2.7	0.060	9.6	18.7	0.069	11.8	8.9
0.1	0.017	Range?	Range?	0.019	1.8	4.3	0.023	85.0	-24.9
0.01	0.004	Range?	Range?	0.008	Range?	Range?	0.012	Range?	Range?

S7 Table

Apparent affinity and maximal binding of EBOVGP scFvs to GP_{ZEBOV}, GP_{SUDV}, GP_{RESTV}, GP_{BDBV}, GP_{TAFV} and GP_{MARV}.

GP	scFv4-2		scFv22-1	
	<i>K_{d,app}</i> (μg)	<i>B_{max}</i> signal (10 ⁵)	<i>K_{d,app}</i> (μg)	<i>B_{max}</i> signal (10 ⁵)
ZEBOV	4.8 ± 1.5	3.5 ± 0.3	9.3 ± 1.2	3.7 ± 0.2
SUDV	6.6 ± 0.9	2.8 ± 0.1	10.8 ± 1.6	2.8 ± 0.2
RESTV	12.2 ± 3.4	2.1 ± 0.2	17.4 ± 4.2	3.2 ± 0.4
BDBV	15.1 ± 4.8	2.2 ± 0.3	38 ± 23	4.6 ± 1.8
TAFV	8.2 ± 0.8	2.8 ± 0.1	19.3 ± 4.3	2.7 ± 0.3
MARV	5.9 ± 1.8	3.8 ± 0.3	10.8 ± 3.8	2.5 ± 0.3

The values for apparent affinity (*K_{d,app}*) and maximal binding (*B_{max}*, shown as relative absorbance units) were derived from the ELISAs with GP_{ZEBOV}, GP_{SUDV}, GP_{RESTV}, GP_{BDBV}, GP_{TAFV} and GP_{MARV}. Values are averages (and ranges) of three experiments.