

The following are supplemental materials and will be published online only

EbovGPscFv18-1	MAWSPHQFEKTGSGDVMLVESGGLVQSGGSMLSCVASGITFG-NYWMNRVRQSPKEKGLEWVAE
EbovGPscFv35-2	MAWSPHQFEKTGSGDVKLQESGPGLVKPSQSLSLTCSVTGYSITSGYWNWIRQFPGNKLEWMGY
EbovGPscFv10-3	MAWSPHQFEKTGSGEVQLVESGGGLVQPGGSLSLSCAASGFTFT-DYYMSWVVRQPPGKALEWLGF
EbovGPscFv4-2	MAWSPHQFEKTGSG-----GAELVKGASVKLSCATASGFKNIK-DDYMHWVKQRPEQGLEWIGW
EbovGPscFv27-5	MAWSPHQFEKTGSGQVQLQQSGAELVKPGASVKSCTATGFNIK-DYYIHWVKQRTEQGLEWIGR
EbovGPscFv13-1	MAWSPHQFEKTGSGQVQLQQSGAELVKPGASVKSCKASGYFT-EYTIHWVKQRSQQGLEWIGW
EbovGPscFv39-1	MAWSPHQFEKTGSGEVQLQQSGAELMKPGASVKLSCCATSYFT-GYWIEWVKQRPGRGRGLEWIGE
EbovGPscFv16-1	MAWSPHQFEKTGSG-----GAEVRSGASVKLSCATASGFKNIK-DDYMHWVKQRPEQGLKWIWG
EbovGPscFv3-2	MAWSPHQFEKTGSGQVQLQQPGAELVKPGASVKSCKASGYFT-SYWIMHWVKQRPGRGRGLEWIGR
EbovGPscFv22-1	MAWSPHQFEKTGSGQVQLQQPGTELVKPGASVKLSCCKASGYFT-SYWIMHWVKQRPGQQGLEWIGN

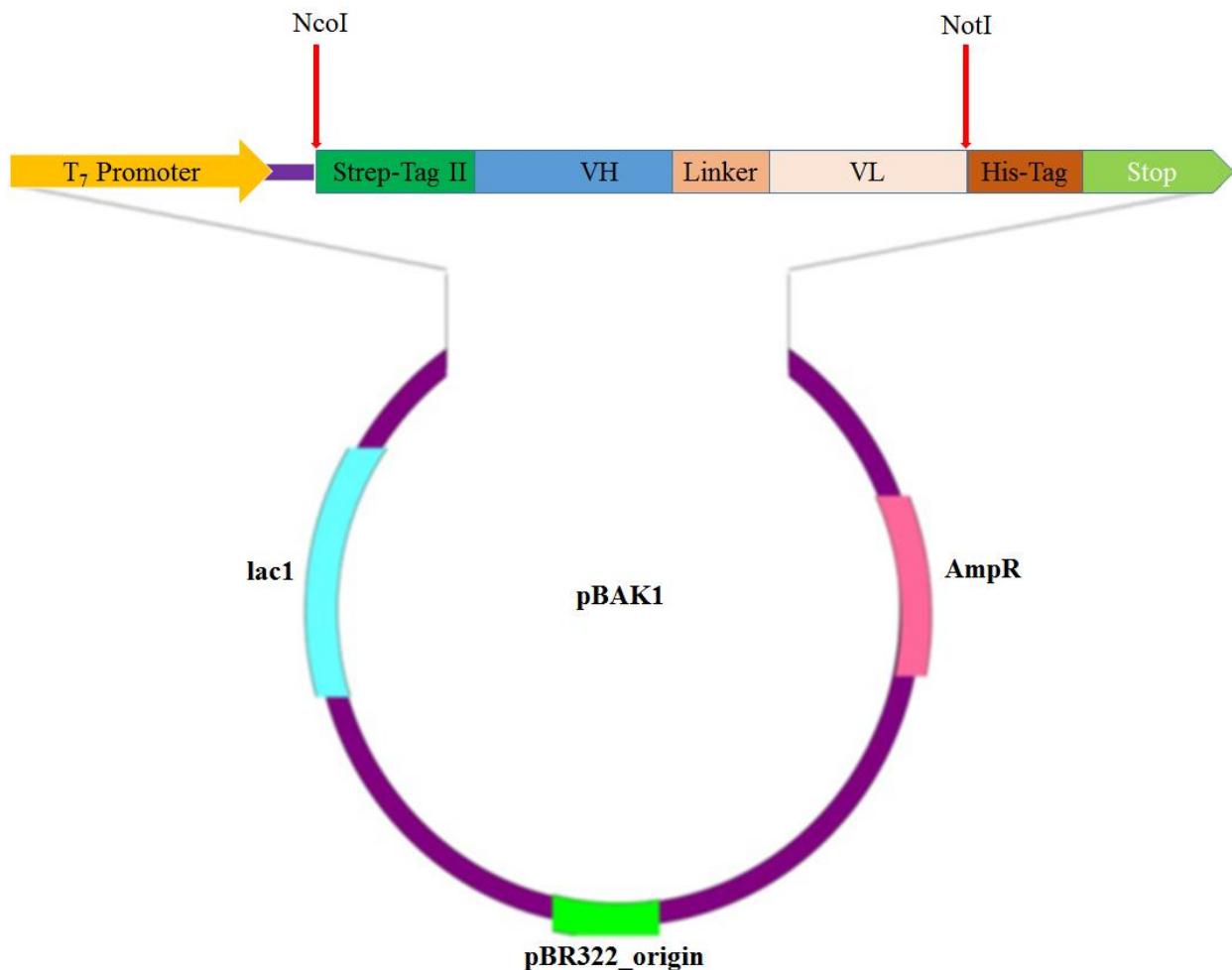
	CDR2 V _H	CDR3 V _H
EbovGPscFv18-1	IRLKSNNYAIHYVESVKGRFTISRDDSKSSVYLRMNNLKAEDTGIYYC	TRHYYG-----CMDY
EbovGPscFv35-2	ISYDG---SNYNPNSLKNRISITRDTSKNQFFLKLNSVTTEDTATYYCARESPYYG--SSHWYFGW	
EbovGPscFv10-3	IRNKANGYTTEYSASVVKGRFTISRDNQSILYLMQNALRAEDSATYYRARPYYS----NYVGFA	
EbovGPscFv4-2	IDPENG--DIEYASKFQGKATITADTSSNTAYLQLSSLTSEDTAVYYCTT-YYY----GSAWFAY	
EbovGPscFv27-5	IDPEDG--ETKYAPKFQGKATITADISPNTAYLQPSSLTSEDTAVYYCTRGGYY----GPWYFDW	
EbovGPscFv13-1	FYPGSG--SIIKYDEKFKDCKATLTADKSSSTVYMELSLRTSEDSAVYFCARHEPYYYGSSYEGPDFY	
EbovGPscFv39-1	ILPGSG--STNYNEKFKGKATFTADTSSNTAYMQLSSPTTEDSAIYYCARDY----YDWYFDW	
EbovGPscFv16-1	IDPENG--DIEYASKLQGKATITADTSSNTAYLQLSSLTSEDTAVYYCARPIYYYG--SSHWYFDW	
EbovGPscFv3-2	IDPNSG--GTKYNEKFKSATLTVDKPSSTAYMQLSSLTSEDSAVYYCARTGYY----GSSWYFDW	
EbovGPscFv22-1	TNPNSNG--GTNYNEKFKSATLTVDKSSNTAYMQLSSLTSEDSAVYYCARSV-----SGNFAY	

	CDR2 V _L	CDR3 V _L
EbovGPscFv18-1	QRPQQT PQLLI YRVPNRFSGVLD RFSGTGTG DFTLK I S RVEAEDVG Y F C L Q V THVPP --	F G G G
EbovGPscFv35-2	QKQGKSPQLLV NAKT LAEGVPSR FS GSGSGT Q FSL K I NSL Q P EDF G SYYC Q H H Y G I P P - W	F G G G
EbovGPscFv10-3	QKQG I SPQLLV NAKT LAEGVPSR FS GSGSGT Q FSL K I NSL Q P EDF G NYYC Q H H Y T T P - F	F G G G
EbovGPscFv4-2	QKQGKSPQLLV NAKT LA DG VPSR FS GSGSGT Q Y S L K I N N L Q P EDF G SYYC Q H F W S T P - Y	F G G G
EbovGPscFv27-5	QKQGKSPQLLV NAKT LAEGVPSR FS GSGSGT Q FSL K I NSL Q P EDF G SYYC Q R H Y D T P - Y	F G G G
EbovGPscFv13-1	QKPGQPPPKLLI Y G A S T R E S G V P D R F T G S G S G T D F T L T I S S V Q A E D L A V Y Y C Q N D H S Y P - L	F G A G
EbovGPscFv39-1	QKPGQPPPKLLI Y G A S T R E S G V P D R L T G S G S G T D F T L T I S S V Q A E D L A V Y Y C Q N D H S Y P - W	F G G G
EbovGPscFv16-1	QKPGQSPKLLI Y K V S N R F S G V P D R F S G S G S G T D F T L K I S R V E A E D L G V Y F C S Q S T H V P - W	F G G G
EbovGPscFv3-2	QKPGQSPKLLI Y K V S N R F S G V P D R F S G S G S G T D F T L K I S R V E A E D L G V Y Y C F Q G S H V P P L Y	F G G G
EbovGPscFv22-1	QKPGQSPKLLI Y K V S N R F S G V P D R F S G S G S G T D F T L K I S R V E A E D L G V Y F C S Q S T H V P - W	F G G G

EbovGPscFv18-1	TKLELRRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFTHCQELQQDECS
EbovGPscFv35-2	TKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFTHCQELQQDECS
EbovGPscFv10-3	TKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFTHCQELQQDECS
EbovGPscFv4-2	TKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFTHCQELQQDECS
EbovGPscFv27-5	TKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFTHCQELQQDECS
EbovGPscFv13-1	TKLELKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFTHCQELQQDECS
EbovGPscFv39-1	TKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFTHCQELQQDECS
EbovGPscFv16-1	TKLEVKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFTHCQELQQDECS
EbovGPscFv3-2	TKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFTHCQELQQDECS
EbovGPscFv22-1	TKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFTHCQELQQDECS

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	CGAGAAGACCGGCAGCGGTGGGCAGAGCTTGTGAAGCCA
MVH_F2	CGAGAAGACCGGCAGCGGTGGAGGAGGCTTGATGCAACCT
MVH_F3	CGAGAAGACCGGCAGCGGTGGACCTGAGCTGGAGATGCCT
MVH_F4	CGAGAAGACCGGCAGCGGTGGACCTGGCCTGGTGAGACCT
MVH_F5	CGAGAAGACCGGCAGCGGTGGGGAGGCTTAGTGAAGCCT
MVH_F6	CGAGAAGACCGGCAGCGGTGGGCAGAGCTTGTGAAGCCA
MVH_F7	CGAGAAGACCGGCAGCGGTGGAGGGGGCTTGGTACAGCCT
MVH_F8	CGAGAAGACCGGCAGCGGTGGGCAGAGCTTGTGAGGTCA
MVH_R1	GGAGCCGCCGCCGCCAGAACCAACCAC <u>CCGGATCC</u> ACCACCCGAG GAAACGGTGACCGTGGT
MVH_R2	GGAGCCGCCGCCGCCAGAACCAACCAC <u>CCGGATCC</u> ACCACCCGAG GAGACTGTGAGAGTGGT
MVH_R3	GGAGCCGCCGCCGCCAGAACCAACCAC <u>CCGGATCC</u> ACCACCCGCA GAGACAGTGACCAAGAGT
MVH_R4	GGAGCCGCCGCCGCCAGAACCAACCAC <u>CCGGATCC</u> ACCACCCGAG GAGACGGTGACTGAGGT
VL primers	
MVL_F1	GGCGGGCGGGCTCCGGTGGTGG <u>GGATCC</u> GCAATCATGTCTGCATCTCC
MVL_F2	GGCGGGCGGGCTCCGGTGGTGG <u>GGATCC</u> GCCTCCCTATCTGTATCTGTG
MVL_F3	GGCGGGCGGGCTCCGGTGGTGG <u>GGATCC</u> GCCTCCCTATCTGCATCTGTG
MVL_F4	GGCGGGCGGGCTCCGGTGGTGG <u>GGATCC</u> CTACTTTGTCGGTTACCTT
MVL_F5	GGCGGGCGGGCTCCGGTGGTGG <u>GGATCC</u> CTCAGCCTTTCTCCCTGGGA
MVL_F6	GGCGGGCGGGCTCCGGTGGTGG <u>GGATCC</u> CTCCCTGAGTGTGTCAGCA
MVL_F7	GGCGGGCGGGCTCCGGTGGTGG <u>GGATCC</u> CTCCCTGCCGTGTCAGTCTT
MVL_F8	GGCGGGCGGGCTCCGGTGGTGG <u>GGATCC</u> CTCCCTGCCGTGTCAGTCTT
MKR	AGAACACTCATTCTGTTGAAGCTTTGACAATGGGTGAAGTTG
MKR_Not I	AGT <u>GGCGGCCG</u> CAGAACACTCATCCTGTTGAAGCTTTGACAATGGGTGAAGT TG
Strep Tag II	
KzSTREP II	CGAATTCCACCATGGCC TGG AGC CAT CCG CAG TTC GAG AAG ACC GGC AGC GG
T7 promoter	
RDT7	CTATAGAAGG GTAATACGACTCACTATA <u>GGCGAATTCCACCATGGCC</u>

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.