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Supplemental Information

Antibody-Dependent Enhancement of Ebola Virus Infection by Human Antibodies Isolated from Survivors

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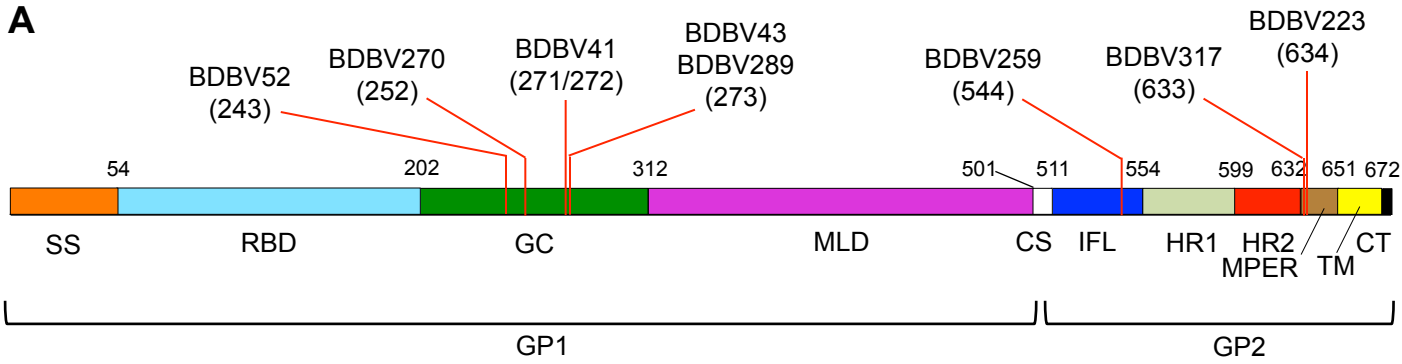
A



B

Antibody	Subclass	Neutralization IC_{50}, ng/mL			ELISA binding, EC_{50}, ng/mL		
		BDBV-GP	EBOV-GP	SUDV-GP	BDBV-GP	EBOV-GP	SUDV-GP
BDBV52	IgG1	>	>	>	27	>	>
BDBV270	IgG1	182	3365	>	154	173	159
BDBV41	IgG1	93	>	>	25	>	>
BDBV43	IgG1	18	506	308	29	22	21
BDBV289	IgG1	32	588	>	20	29	103
BDBV259	IgG1	59	>	>	26	>	>
BDBV317	IgG1	6	2083	>	9	191	208
BDBV223	IgG3	<0.1	70	>	22	24	106

Fig. S1 (related to Fig. 1)**. A.** Amino acid numbers (in parentheses) corresponding to escape mutations for the indicated mAb, shown on the map of BDBV GP1 and GP2 [adapted from Lee et al., 2008, *Nature* 454(7201):177-182 and Lennemann at al., 2014, *mBio*. 5(1):e00862-13]. The escape mutations were identified by serial passaging of EBOV/BDBV-GP or VSV/BDBV-GP viruses in presence of corresponding mAbs and sequencing of viral GP ORFs (see Materials and Methods). SS, signal sequence, RBD, receptor-binding domain, GC, glycan cap, MLD, mucin-like domain, CS, furin cleavage site (amino acids 501/502), IFL, internal fusion loop, HR1, heptad repeat 1, HR2, heptad repeat 2, MPER, membrane-proximal external region, TM, transmembrane domain, CT, cytoplasmic tail; positions of the first amino acid of each domain are indicated. **B.** Neutralizing and binding potencies of BDBV-specific mAbs against BDBV, EBOV and SUDV. IC_{50} and EC_{50} values greater than 10,000 ng/mL are indicated by a “>” symbol. The figure adapted from Ilinykh et al., 2018 (manuscript in submission).

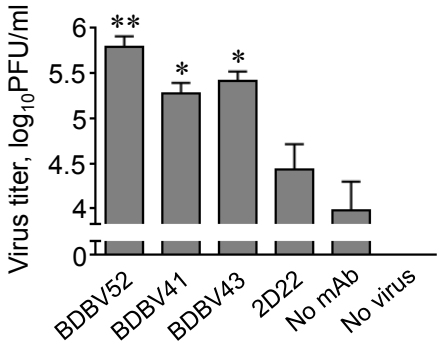


Fig. S2 (related to Fig. 1)**.** THP-1 monocytes infected with EBOV/BDBV-GP in presence of mAb release infectious viral particles to the media. Cells were incubated with mAbs at 100 μg/ml, infected at an MOI of 1 PFU/cell for 1 hour, washed, and supplemented with fresh medium with no mAbs added. In 48 hours, THP-1 supernatants were collected, and virus titer was determined by plaque assay. Mean values ±SD based on triplicate samples. Limit of detection: 20 PFU/ml. Differences to no antibody control: * $p<0.01$, ** $p<0.001$ (Unpaired t-test).

A

mAb	Neutralization IC_{50}, µg/mL	Binding (ELISA), EC_{50}, µg/mL
EBOV-68	-	0.036
EBOV-87	3	0.724
EBOV-91	-	0.011
EBOV-141	50	6.159
EBOV-520	5.738	0.012
EBOV-333	1	0.016
13c6	2	0.033
2G4	0.1	0.187

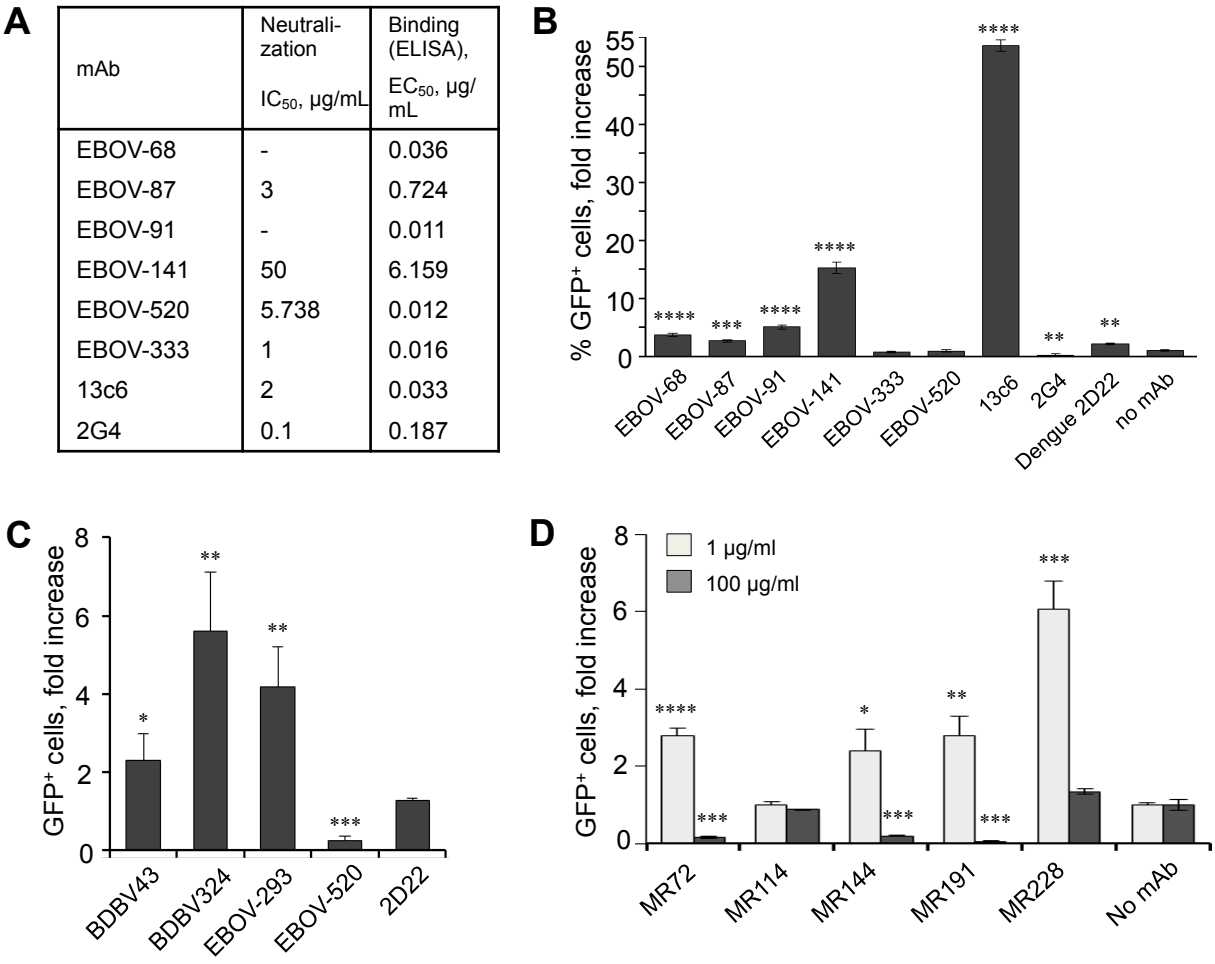


Fig. S3 (related to Fig. 1)**.** ADE of additional filovirus species infections. **A.** Neutralizing and binding properties of mAbs isolated from EBOV survivors or two humanized murine mAb components of zMapp. **B-D**, fold increases of infected (eGFP$^+$) THP-1 cells after inoculation with EBOV (B), EBOV/SUDV-GP (C) or EBOV/MARV-GP (D) in presence of 100 µg/mL (B) or 10 µg/mL (C) of the indicated mAb. Mean values ± SD based on triplicate samples. Differences to controls: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 (B, C, Unpaired t-test, D, Multiple t-test).

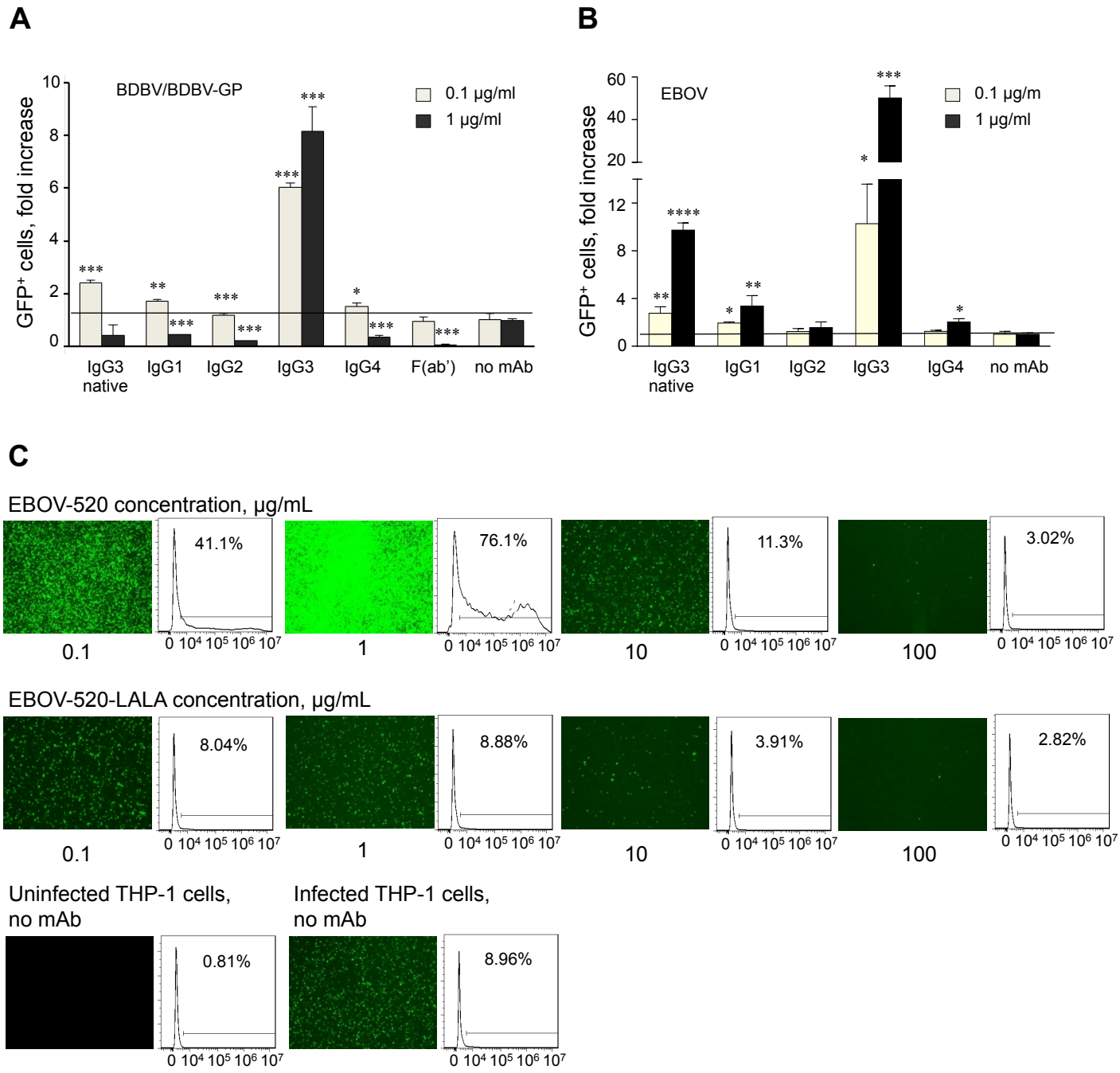


Fig. S4 (related to Fig. 3)**.** ADE depends on the Fc fragment of recombinant IgG. THP-1 cells inoculated with EBOV/BDBV-GP **(A)** or EBOV **(B)** in the presence of hybridoma-produced BDBV223, which is IgG3 (IgG3 native), or its recombinant forms of various subclasses at the indicated concentrations. Mean values ± SD based on triplicate samples. Differences to no antibody control: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 (Multiple t-test). **C.** The LALA mutation of EBOV-520 IgG1 abrogates ADE *in vitro*. Fluorescent microscopy and flow cytometry analysis of eGFP+ THP-1 cells inoculated with EBOV-eGFP at MOI of 1.0 PFU/cell and treated with the indicated concentrations of EBOV-520 IgG1, EBOV-520 IgG1/LALA, or incubated with no mAb.

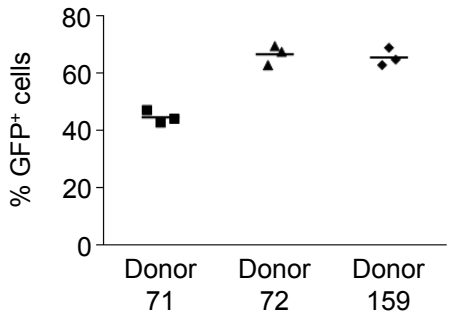


Fig. S5 (related to Fig. 4)**.** The percentages of infected Vero E6 cells after co-cultivation with infected PBMCs. Isolated PBMCs were infected with EBOV/BDBV-GP at an MOI of 1 PFU/cell. At 24 hours after infection, they were harvested and placed atop of the Vero E6 cell monolayers. After 48 hours of co-cultivation, Vero E6 cells were analyzed by flow cytometry. Values for individual donors are indicated by symbols, and mean values are indicated by horizontal bars.

Table S1. Neutralization of EBOV or EBOV/BDBV-GP by Various Forms of BDBV223

Form of BDBV223	**Subclass**	**Neutralization activity against indicated virus (IC_{50}, µg/mL)**	
		EBOV	**EBOV/BDBV-GP**
Hybridoma-produced	IgG3	0.07	0.0000177
Recombinant	IgG1	14.3	0.0004047
	IgG2	0.53	0.0071740
	IgG3	0.00069	0.0006622
	IgG4	122.6	0.0001383
	F(ab′)	0.09	0.0000199