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Development of a human antibody cocktail that deploys multiple functions to confer pan-ebolavirus protection

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SUMMARY

Passive administration of monoclonal antibodies (mAbs) is a promising therapeutic approach for Ebola virus disease (EVD). However, all mAbs and mAb cocktails that have entered clinical development are specific for a single member of the *Ebolavirus* genus, Ebola virus (EBOV), and

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AUTHOR CONTRIBUTIONS

Conceptualization, A.Z.W., Z.A.B., A.S.H., L.M.W., J.M.D., L.Z., X.Q. and K.C. Methodology, A.Z.W., A.S.H., G.A., L.M.W., J.M.D., X.Q., and K.C. Investigation, A.Z.W., Z.A.B., S.H., A.S.H., E.G., A.S.W., B.M.G., Z.Z., W.Z., G.L., D.M.A., C.L.M., R.K.J., R.M.J., R.R.B., N.B., O.B., D.H.K., J.V. and R.H.B. III. Writing – Original Draft, A.Z.W. and K. C. Writing – Review & Editing, A.Z.W., Z.A.B., A.S.H., A.S.W., B.M.G., R.K.J., T.G., S.J.A., L.M.W., L.Z., X.Q. and K.C. Visualization, A.Z.W., Z.A.B., A.S.H. and K.C. Funding acquisition, J.M.D., L.Z., X.Q. and K.C. Resources, T.G. and S.J.A. Supervision, Z.A.B., A.S.H., K.J.W., G.A., L.M.W., J.M.D., L.Z., X.Q. and K.C.

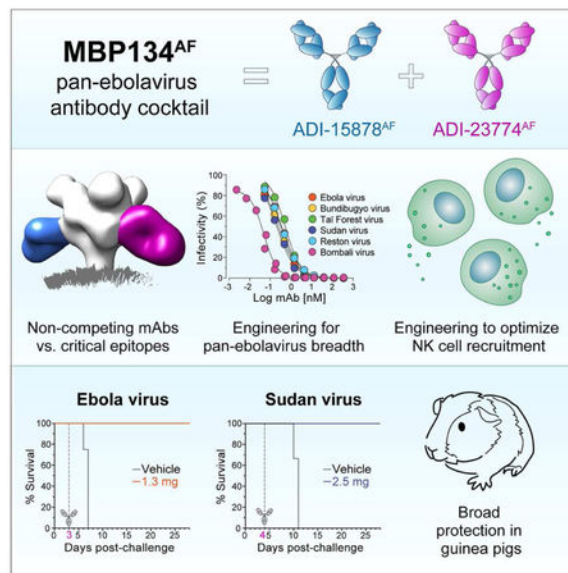
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DECLARATION OF INTERESTS

A.Z.W., Z.A.B., L.Z., L.M.W., and K.C. are co-inventors on a provisional patent application (No. 62/460,200) assigned to Mapp Biopharmaceutical Inc., Adimab LLC, and Albert Einstein College of Medicine Inc.) describing the development and use of MBP134 and variants thereof for anti-ebolavirus immunotherapy. A.Z.W., E.G., and L.M.W. are employees and shareholders in Adimab LLC. Z.A.B., D.M.A., C.L.M., N.B., O.B., D.H.K., M.H.P., J.V., K.J., and L.Z. are employees and shareholders in Mapp Biopharmaceutical Inc. K.J. and L.Z. are CEO and President of Mapp Biopharmaceutical Inc., respectively.

ineffective against outbreak-causing Bundibugyo virus (BDBV) and Sudan virus (SUDV). Here, we advance MBP134, a cocktail of two broadly neutralizing human mAbs, ADI-15878 from an EVD survivor and ADI-23774 from the same survivor but specificity-matured for SUDV GP binding affinity, as a candidate pan-ebolavirus therapeutic. MBP134 potently neutralized all ebolaviruses and demonstrated greater protective efficacy than ADI-15878 alone in EBOV-challenged guinea pigs. A second-generation cocktail, MBP134^{AF}, engineered to effectively harness natural killer (NK) cells afforded additional improvement relative to its precursor in protective efficacy against EBOV and SUDV in guinea pigs. MBP134^{AF} is an optimized mAb cocktail suitable for evaluation as a pan-ebolavirus therapeutic in nonhuman primates.

Graphical abstract



eTOC blurb:

Broadly protective therapies against filoviruses are urgently needed. Wec et al. advance a cocktail of two human mAbs as a candidate pan-ebolavirus therapeutic. The mAbs were selected for antiviral potency and breadth, engineered to enhance Fc effector functions and tested against multiple ebolaviruses in guinea pig models of lethal challenge.

INTRODUCTION

Viruses of the family *Filoviridae* (filoviruses) cause outbreaks of a lethal disease for which no FDA-approved treatments or vaccines are available. During the unprecedented 2013–2016 Ebola virus disease (EVD) epidemic in Western Africa and in its aftermath, the passive administration of monoclonal antibodies (mAbs) emerged as a promising treatment approach (Corti et al., 2016; Mire et al., 2017; Olinger et al., 2012; Pascal et al., 2018; Qiu et al., 2013, 2014, 2016). To date, three mAbs and mAb cocktails—ZMapp, REGN-EB3, and mAb114/VRC 608—have entered clinical development (National Institutes of Health Clinical Center, 2018; PREVAIL II Writing Group et al., 2016; Sivapalasingam et al., 2018). However, all of these investigational treatments suffer a key liability—they are specific for a

single member of the *Ebolavirus* genus, Ebola virus (EBOV), and ineffective against the divergent outbreak-causing ebolaviruses Bundibugyo virus (BDBV) and Sudan virus (SUDV) (Corti et al., 2016; Murin et al., 2014; Pascal et al., 2018; Saphire et al., 2018), which accounted for $\approx 40\%$ of all ebolavirus infections prior to 2013 (Burk et al., 2016). New broadly active immunotherapeutics are thus needed to combat the urgent public health threat posed by BDBV, SUDV, and novel ebolaviruses yet to emerge into human populations, such as the recently described Bombali virus (BOMV) (Goldstein et al., 2018).

To discover broadly protective human antibodies, we previously isolated and characterized 349 GP-specific mAbs from a survivor of the West African EVD epidemic (Bornholdt et al., 2016). A systematic analysis of this library for breadth of the neutralizing mAb response against ebolaviruses identified ADI-15878 as a promising candidate therapeutic (Wec et al., 2017). ADI-15878 possesses potent pan-ebolavirus neutralizing activity through its recognition of a highly conserved conformational fusion-loop epitope in GP with subnanomolar affinity and enhanced targeting of a cleaved GP intermediate generated in late endosomes. *In vivo*, ADI-15878 fully protected mice challenged with EBOV and SUDV (Bornholdt et al., 2016; Wec et al., 2017). However, monotherapy with ADI-15878 was only partially protective in ferrets (Wec et al., 2017) and guinea pigs (Figure 1A, this study), supporting prior observations that mAb cocktails targeting multiple GP epitopes may be necessary for complete protection (Flyak et al., 2016; Oswald et al., 2007; Qiu et al., 2013, 2014; Wec et al., 2017; Zhao et al., 2017).

Here, we screened existing human and nonhuman primate mAbs for a suitable partner to ADI-15878 and identified a previously described human neutralizing mAb, ADI-15946, which recognizes a broadly conserved but non-overlapping epitope in the base subdomain of ebolavirus GP (Bornholdt et al., 2016; Wec et al., 2017). Complementing data-driven mAb selection with the optimization of mAb combining sites and Fc effector functions afforded a two-mAb cocktail, MBP134^{AF}, which could fully protect guinea pigs against EBOV and SUDV challenge with a single ≈ 10 mg/kg dose administered as late as 3–5 days post-virus exposure. Our findings set the stage for evaluation of MBP134^{AF} as a pan-ebolavirus therapeutic in nonhuman primates (see companion manuscript, Bornholdt *et al.*).

RESULTS AND DISCUSSION

Identification of Candidate Partner mAbs for ADI-15878

Because previous work has demonstrated the predictive value of the guinea pig models of ebolavirus challenge for therapeutic antibody efficacy in nonhuman primates (Cross et al., 2015; Marzi, 2017; Wong et al., 2012), we exposed guinea pigs to a uniformly lethal dose of guinea pig-adapted EBOV (EBOV-GPA) (Connolly et al., 1999) and then treated them with equivalent doses of ADI-15878 or the ZMapp cocktail (Qiu et al., 2014) at 3 days post-challenge. Monotherapy with ADI-15878 afforded only 33–50% survival and was less effective than ZMapp (100% survival) (Figure 1A–B), suggesting the need to target an additional GP epitope to achieve complete protection.

Potential ADI-15878 partner mAbs were filtered on the basis of: (i) their host origin (human or nonhuman primate); (ii) neutralization potency (50% inhibition of EBOV infectivity

[IC₅₀] <10 nM); (iii) anti-ebolavirus binding and neutralization breadth (recognition and neutralization of EBOV, BDBV, and SUDV); (iv) neutralization of both uncleaved and endosomally cleaved forms of ebolavirus GP, a feature previously associated with antiviral potency and breadth (Wec et al., 2017; Zhao et al., 2017); (v) lack of cross-reactivity with the secreted glycoprotein sGP present at high levels in EVD patients (to prevent sGP from "soaking up" circulating mAbs) (Mohan et al., 2012; Sanchez et al., 1996; Volchkov et al., 1995); and (vi) demonstrated post-exposure protection against EBOV in mice. This selection process yielded two candidates, ADI-15946 (Bornholdt et al., 2016; Wec et al., 2017) and CA45 (Zhao et al., 2017). Like ADI-15878, these broadly neutralizing mAbs recognize conserved conformational epitopes in the "base" subdomain of the trimeric GP spike (Figure 1C), raising the possibility that they can compete with ADI-15878 for GP binding and limit cocktail efficiency. Indeed, preincubation of EBOV GP with saturating amounts of ADI-15878 abolished binding by CA45 in a biolayer interferometry (BLI) assay (Figure 1D) but did not interfere with ADI-15946 binding (Figure 1E), indicating that ADI-15878 and CA45 compete for GP recognition, whereas ADI-15878 and ADI-15946 do not. Therefore, we selected ADI-15946 for further evaluation as a cocktail partner for ADI-15878.

Specificity Maturation of ADI-15946 to SUDV GP

We previously showed that ADI-15946 neutralizes EBOV, BDBV, and SUDV; however, it is less potent against SUDV (Wec et al., 2017) (see Figure 3A–C). This neutralization deficit arises from the reduced binding affinity of ADI-15946 for SUDV GP relative to EBOV GP (Figures 2A and S2A), consistent with the sequence divergence between these glycoproteins at the proposed sites of mAb contact (Wec et al., 2017). To ensure that our mAb cocktail effectively targets all known virulent ebolaviruses at two distinct sites, we sought to specificity-mature ADI-15946 to SUDV GP using yeast-display technology (Xu et al., 2013). Libraries were generated by introducing diversity into the ADI-15946 heavy and light chains (HC and LC, respectively) through oligonucleotide-based mutagenesis and transformation into *Saccharomyces cerevisiae* by homologous recombination. Improved variants were identified after 2 (LC) or 3 (HC) rounds of selection with a recombinant SUDV GP protein (Figure S1) and cross-screening for retention of EBOV and BDBV binding was performed on the best SUDV GP binder, ADI-23774 (Figure S2A–C). Combining beneficial LC and HC mutations yielded a variant, ADI-23774, with 5–10 \times enhanced binding affinity to SUDV GP and slightly improved binding to EBOV and BDBV GP relative to its ADI-15946 parent (Figures 2 and S2A–C). These gains in GP:mAb affinity effected by specificity maturation were primarily driven by reductions in the dissociation rate constant (k_{off}) (Figures 2C and S2D). Next, because *in vitro* affinity maturation can increase antibody polyspecificity with potential risks of off-target binding and reduced serum half-life *in vivo* (Hötzel et al., 2012), we assessed the polyspecificity of ADI-15946 and ADI-23774 as described (Jain et al., 2017; Xu et al., 2013). Fortuitously, specificity maturation also reduced ADI-23774's nonspecific binding relative to that of ADI-15946 (Figure 2D). Thus, both ADI-15878 and ADI-23774 display a low level of polyspecificity, a highly desirable property for early-stage therapeutic candidates.

Antiviral Neutralization Potency and Breadth of ADI-23774 In Vitro

To evaluate the breadth and potency of neutralization by ADI-23774, we performed dose-response neutralization assays with recombinant vesicular stomatitis viruses (rVSVs) bearing GPs from SUDV, EBOV, and BDBV (Figure 3A–B) and with authentic SUDV, EBOV, and BDBV (Figure 3C). Concordant with ADI-23774's improved binding to both recombinant GP proteins (Figures 2 and S2B–C) and rVSVs bearing full-length transmembrane GP (Figure S2A), ADI-23774 neutralized both rVSV-SUDV GP (Figure 3A) and authentic SUDV (Figure 3C) more potently than did ADI-15946 [IC_{50} ($\pm 95\%$ confidence intervals) = 1 ± 1 nM for ADI-23774 vs. 35 ± 1 nM for ADI-15946 against rVSV-SUDV GP (Figure 3A)]. Further, ADI-23774 could neutralize rVSV-SUDV GP and authentic SUDV completely without leaving an un-neutralized fraction, whereas ADI-15946 could not (Figure 3A,C). These trends were also apparent against rVSV-SUDV GP_{CL}, bearing a proteolytically cleaved form of GP (GP_{CL}) resembling an endosomal entry intermediate (Figure 3B). ADI-23774's increased effectiveness against SUDV did not compromise its capacity to neutralize rVSVs bearing EBOV and BDBV GP or the authentic agents (Figure 3A, C).

Protective Efficacy and Breadth of ADI-23774 in Mice

To assess the effects of these gains in neutralization potency on mAb protective efficacy, we evaluated ADI-23774 in murine models of EBOV (Bray et al., 1998) and SUDV (Brannan et al., 2015) challenge. ADI-23774 resembled ADI-15946 in its capacity to protect animals from challenge with mouse-adapted EBOV (EBOV-MA) when administered three days post-exposure (Figure 4A–B). However, unlike ADI-15946, which conferred no benefit against SUDV (Wec et al., 2017), ADI-23774 stemmed weight loss in SUDV-challenged interferon α/β receptor-knockout (IFN α/β R^{-/-}) mice and fully protected them when administered one and four days post-exposure (Figure 4C–D). ADI-23774 is an optimized candidate to serve as a partner mAb for ADI-15878, with enhancements over its precursor in biophysical properties, neutralization breadth, and anti-ebolavirus protective breadth in mice.

In Vitro Neutralization Properties of a Cocktail Comprising ADI-15878 and ADI-23774 and its Protective Efficacy in Guinea Pigs

We combined ADI-15878 and ADI-23774 into a cocktail, MBP134, and examined its functional properties in vitro. MBP134 potently neutralized rVSVs bearing GP proteins from ebolaviruses belonging to all six species, including BOMV, recently discovered in free-tailed bats in Sierra Leone (Figure 5). Cultivation of rVSV-EBOV GP in the presence of MBP134 yielded a single escape mutant genotype after seven serial passages; however, this mutant was only partially resistant to MBP134 neutralization (data not shown). MBP134's capacity to broadly and effectively neutralize ebolaviruses suggests that its utility could extend beyond the currently recognized agents to unknown viruses and viral variants that may pose a spillover risk in the future.

To evaluate the protective potential of MBP134, we exposed guinea pigs to a uniformly lethal dose of EBOV-GPA and then treated them with increasing doses of MBP134 at three days post-challenge (Figure 6A–B). Treatment with 2.5–3.3 mg of MBP134 (total mAb dose) per animal afforded near complete protection (Figure 6A), whereas treatment with

ADI-15878 alone at 2.5–5 mg per animal afforded 50% protection (Figure 1A), providing evidence for the superior efficacy of the cocktail over the monotherapy (see Table S1 for statistical comparisons of survival curves in Figure 6A).

Optimization of MBP134-mediated immune effector functions *in vitro* through Fc glycan engineering

To further enhance the *in vivo* potency of MBP134, we set out to optimize an orthogonal property of its component mAbs—their capacity to harness the host antiviral innate immune response through antigen-dependent engagement of activating Fc receptors on immune cells. Because cytotoxicity by NK cells has been demonstrated to play a critical role in protection against EBOV infection (Gunn et al., 2018; Warfield et al., 2004), we measured the EBOV GP-dependent activation of human NK cells derived from four seronegative human donors by ADI-15878 and ADI-23774. Both mAbs were poorly active, as judged by their limited capacity to induce three markers of NK cell activation—degranulation (Figure 7A), and production of IFN- γ (Figure 7B) and MIP-1 β (Figure 7C)—relative to the ZMapp component mAb c13C6 (Qiu et al., 2014) (also see Figure S3). Thus, viral neutralization is likely the primary mode of MBP134's antiviral activity. We postulated that NK cell recruitment, activation, and infected-cell killing by MBP134 could be improved by enhancing its capacity to engage the activating Fc receptor Fc γ RIIIa on NK cells. Indeed, previous work has demonstrated that mAbs bearing uniformly afucosylated glycan structures display precisely this property (Ferrara et al., 2011; Shields et al., 2002), which has been linked to their enhanced protective efficacy against several viral pathogens, including EBOV (Forthal et al., 2010; Gunn et al., 2018; Hiatt et al., 2014; Saphire et al., 2018; Zeitlin et al., 2011). Accordingly, we generated and evaluated afucosylated variants of the MBP134 mAbs (ADI-15878^{AF} and ADI-23774^{AF}, respectively). Both variant mAbs significantly outperformed their fucosylated precursors in all three NK cell activation assays (Figure 7A–C), confirming their enhanced capacity to engage the innate immune system.

Protective Efficacy and Breadth of the MBP134^{AF} cocktail in Guinea Pigs

Finally, we assessed the protective potential of the second-generation MBP134^{AF} cocktail in guinea pigs, which possess an Fc receptor (gpFc γ RIV) homologous to human Fc γ RIIIA with enhanced binding affinity for afucosylated human IgGs relative to their fucosylated counterparts (Mao et al., 2017). Only 1.3 mg (total mAb dose) of MBP134^{AF} administered at three days post-challenge was required to fully protect animals from EBOV-GPA (Figure 7D–E)—an approximately-twofold reduction in dosage relative to the parent MBP134 cocktail ($P=0.0064$, 1.3 mg MBP134 vs. 1.3 mg MBP134^{AF}) (Table S2). Moreover, a single MBP134^{AF} dose as low as 0.8 mg administered at four days post-challenge was sufficient to protect >80% of guinea pigs challenged with guinea pig-adapted SUDV (SUDV-GPA) (Wong et al., 2016) (Figure 7F). Remarkably, MBP134^{AF} afforded 70–100% protection from SUDV-GPA challenge (at 2.5–5.0 mg total mAb dose) even when treatment was delayed to five days post-challenge (Figure 7G). (see Table S2 for statistical comparisons of survival curves in Figure 7D,F). These are, to our knowledge, the lowest single doses (0.8–1.3 mg total mAb) and longest post-exposure treatment windows (4–5 days) demonstrated to protect guinea pigs from lethal ebolavirus challenge. All previous studies with both therapeutic mAbs and mAb cocktails used total doses of 5–10 mg and did not report initial

mAb treatments past 3 days post-exposure (Flyak et al., 2016, 2018; Gilchuk et al., 2018; Howell et al., 2016; Qiu et al., 2012; Zhao et al., 2017).

Summary and Concluding Remarks

Herein, we have used a rigorous mAb selection strategy coupled to yeast-based specificity maturation and Fc glycan engineering to develop a next-generation human mAb cocktail, MBP134^{AF}, which can protect against EBOV and SUDV in small and large rodents. MBP134^{AF} targets all known ebolavirus GP proteins [including that from a recently discovered "pre-emergent" agent (Goldstein et al., 2018)]; recognizes GPs from EBOV, BDBV, TAFV, SUDV, and BOMV at two independent antigenic sites; and neutralizes both the extracellular and endosomal intermediate forms of GP, thereby conferring potency as well as robustness to viral neutralization escape. Further, it is optimized to leverage both mechanical neutralization and Fc-linked innate immune functions to block viral infection and spread. By virtue of these advanced features, MBP134^{AF} affords unparalleled improvements in therapeutic potency against broad ebolavirus challenge in the stringent guinea pig challenge model relative to any mAbs or mAb cocktails that have been described previously. Our findings set the stage for evaluation of MBP134^{AF} as a pan-ebolavirus therapeutic in nonhuman primates (see companion manuscript, Bornholdt *et al.*).

STAR METHODS

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by Lead Contact Dr. Kartik Chandran (kartik.chandran@einstein.yu.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell lines.—Vero female African grivet monkey kidney cells and HEK293T female human embryonic kidney fibroblast cells obtained from American Type Culture Collection (ATCC) and maintained in high-glucose Dulbecco's modified Eagle medium (DMEM; ThermoFisher, Carlsbad, CA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Flowery Branch, GA), 1% GlutaMAX (ThermoFisher), and 1% penicillin-streptomycin (ThermoFisher). Cells were maintained in a humidified 37°C, 5% CO₂ incubator. Suspension adapted HEK-293F (ThermoFisher) were maintained in serum free Freestyle HEK-293F expression medium (ThermoFisher). Cells were maintained in a humidified 37°C, 8% CO₂, 125 rpm shaking incubator. *Drosophila melanogaster* S2 cells (ThermoFisher) were cultured in complete Schneider's medium at 27°C. Cell lines were not authenticated following purchase.

Culture of primary human innate immune cells.—Primary human NK cells from deidentified donors were cultured and maintained in RPMI1640 supplemented with 10% fetal bovine serum, L-Glutamine, and penicillin/streptomycin at 37C in a humidified incubator at 5% CO₂ for the duration of the assays.

Ethics statement for primary human innate immune cells.—Innate immune effector cells were isolated from fresh peripheral blood samples collected by the Ragon

Institute or the MGH Blood bank from healthy human volunteers. All subjects signed informed consent and the study was approved by the MGH Institutional Review Board. Samples were collected from human adults older than 18 years of age, and were completely deidentified prior to use and thus, researchers were blinded to gender and age of donors.

Mice.—Female BALB/c mice (Jackson Labs, Bar Harbor, ME), aged 10-12 weeks old and female Type 1 IFN α/β receptor knockout mice (Type 1 IFN α/β R $^{-/-}$) (Jackson Labs) aged 6-8 weeks old were used for *in vivo* protection studies. Animals were provided food and water *ad libitum* and housed in individual ventilated cages.

Guinea pigs.—Outbred Hartley guinea pigs (250-300 g), aged 4-6 weeks old, were purchased from Charles River Laboratories (Wilmington, MA). Animals were provided food and water *ad libitum* and given environmental enrichment according to the guidelines for the species. Cleaning of the animals was completed three times per week which included a complete cage and bedding material change. Animals were kept 2–3 per cage in the large shoe box cages from IVC Alternative Design. Each unit is ventilated with a HEPA blower system.

Animal welfare statement.—Murine challenge studies were conducted under IACUC-approved protocols in compliance with the Animal Welfare Act, PHS Policy, and other applicable federal statutes and regulations relating to animals and experiments involving animals. The facility where these studies was conducted (USAMRIID) is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC), and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011. Guinea pig challenge studies were approved by the Animal Care Committee (ACC) of the Canadian Science Centre for Human and Animal Health (CSCHAH) in Winnipeg, Canada, in accordance with guidelines from the Canadian Council on Animal Care (CCAC).

Recombinant vesicular stomatitis viruses (rVSVs).—Recombinant vesicular stomatitis Indiana viruses (rVSV) expressing eGFP in the first position, and encoding representative GP proteins from EBOV/Mayinga (EBOV/H.sap-tc/COD/76/Yambuku-Mayinga), BDBV (BDBV/H.sap/UGA/07/But-811250), SUDV/Boneface (SUDV/C.por-lab/SSD/76/Boneface), BOMV (BOMV/M.con/SLE/16/MF319185), TAFV Muc (TAFV/H.sap-tc/CIV/94/CDC807212), RESTV (RESTV/M.fas-tc/USA/89/Phi89-AZ-1435) in place of VSV G have been generated using a plasmid based rescue system in HEK293T and described previously (Goldstein et al., 2018; Ng et al., 2014; Wec et al., 2016; Whelan et al., 1995; Wong et al., 2010).

Authentic filoviruses.—The authentic filoviruses EBOV/“Zaire 1995” (EBOV/H.sap-tc/COD/95/Kik-9510621) (Jahrling et al., 1999), mouse-adapted EBOV/Mayinga (EBOV-MA)(Bray et al., 1998), SUDV/Boneface-USAMRIID111808, and BDBV/200706291 (Towner et al., 2008), Guinea pig-adapted EBOV/Mayinga (EBOV-GPA) (Ebola virus VECTOR/C.porcellus-lab/COD/76/Mayinga-GPA) (Connolly et al., 1999), Guinea pig-adapted SUDV/Boneface (SUDV-GPA) (Sudan virus/NML/C.porcelluslab/SSD/76/Nzara-Boneface-GP) (Wong et al., 2016) were used in this study.

Animal welfare statement.—Murine challenge studies were conducted under IACUC-approved protocols in compliance with the Animal Welfare Act, PHS Policy, and other applicable federal statutes and regulations relating to animals and experiments involving animals. The facility where these studies was conducted (USAMRIID) is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC), and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011. Guinea pig challenge studies were approved by the Animal Care Committee (ACC) of the Canadian Science Centre for Human and Animal Health (CSCHAH) in Winnipeg, Canada, in accordance with guidelines from the Canadian Council on Animal Care (CCAC).

METHOD DETAILS

Expression and purification of mAbs.—Recombinant mAbs ADI-15946, ADI-23774 and ADI-15878 were produced in HEK-293F cells (ThermoFisher) by transient transfection, and purified by protein A affinity chromatography, as described previously (Wec et al., 2016). MBP134^{AF} (afucosylated) component mAbs were produced in *XTFT N. benthamiana* tobacco plants via transient expression and purification as previously described (Olinger et al., 2012).

Expression and purification of recombinant GP ectodomains.—Ebola virus GPs lacking the mucin domain and transmembrane domain (EBOV: aa32-632, 311-463), (SUDV: aa1-637, 270-473), (BDBV: aa1-640, 312-470) were produced from stable *Drosophila melanogaster* S2 cell lines. Briefly, Effectene (Qiagen) was used to transfect S2 cells with a modified pMT-puro vector plasmid containing the GP gene of interest, followed by stable selection in the presence of puromycin (6 µg/ml). Cells were cultured at 27° in complete Schneider's medium and then adapted to HyClone SFM4insect media (GE Healthcare) for large-scale expression in 2-liter Erlenmeyer flasks. Expression was induced with 1 mM CuSO₄, and supernatant harvested when cell viability dropped below 80%, as determined by a trypan blue exclusion assay. All GPs were engineered with a C-terminal double Strep-tag to facilitate purification using a StrepTrap HP 5 mL prepacked column (GE Healthcare) using an AKTA pure 25M3 (GE Healthcare) and then further purified over a Superdex Increase 200 in PBS.

VSV infectivity measurements and neutralization assays.—Viral infectivity was determined by automated counting of eGFP⁺ cells (infectious units; IU) using a CellInsight CX5 imager (ThermoFisher) at 12–14 h post-infection. For mAb neutralization experiments, pre-titrated amounts of recombinant vesicular stomatitis viruses (rVSVs) expressing eGFP and GP from EBOV, BDBV, TAFV, SUDV, RESTV and BOMV particles (MOI ≈ 1 IU per cell) were incubated with increasing concentrations of test mAb at room temp for 1 h, and then added to confluent Vero cell monolayers in 96-well plates (Goldstein et al., 2018; Wec et al., 2016; Wong et al., 2010). Viral neutralization data were subjected to nonlinear regression analysis to derive IC₅₀ values (4-parameter, variable slope sigmoidal dose-response equation; GraphPad Prism, La Jolla, CA).

Authentic filovirus microneutralization assays.—The authentic filoviruses EBOV/“Zaire 1995” (EBOV/H.sap-tc/COD/95/Kik-9510621) (Jahrling et al., 1999), SUDV/Boneface-USAMRIID111808 (Brannan et al., 2015), and BDBV/200706291 (Towner et al., 2008) were used in this study. Antibodies were diluted to indicated concentrations in culture media and incubated with virus for 1 h. Vero E6 cells were exposed to antibody/virus inoculum at an MOI of 0.2 (EBOV, BDBV) or 0.5 (SUDV) plaque-forming unit (PFU) per cell for 1 h. Antibody/virus inoculum was then removed, and fresh culture media was added. At 48 h post-infection, cells were fixed, and infected cells were immunostained and quantitated by automated fluorescence microscopy, as described (Wec et al., 2016).

ADI-15946 affinity maturation.—Affinity maturation libraries were generated by introducing diversity into the ADI-15946 heavy and light chains via site-saturation mutagenesis. Degenerate oligonucleotides sampling all 20 amino acids at each position within each CDR were incorporated into ADI-15946 via DNA shuffling, as described previously (Stemmer, 1994). The resulting libraries were then transformed into *Saccharomyces cerevisiae* using homologous recombination, as described (Xu et al., 2013). To identify improved variants, selections were carried out using flow cytometry as generally described (Chao et al., 2006). Briefly, libraries were incubated with titrating amounts of a recombinant SUDV monomer and sorted by FACS. Variants with improved binding affinities were identified from the HC and LC libraries by the second and third rounds of selection, respectively (see Figure S1). Selection outputs were plated, and colonies were picked for sequence analysis and IgG production. Beneficial mutations identified from the HC and LC library selections were then rationally combined and the resulting variants were screened for further improvements in binding activity via biolayer interferometry assays (ForteBio, Pall LLC).

GP:mAb binding ELISA.—To compare binding of ADI-15946 and ADI-23774 to SUDV, EBOV, and BDBV GP displayed on rVSVs (see Figure S2), normalized amounts of sucrose gradient purified rVSVs bearing SUDV, EBOV, and BDBV GPs were coated overnight onto plates at 4°C. Plates were then blocked with PBS containing 3% bovine serum albumin (PBSA) and incubated with dilutions of test antibody. Bound Abs were detected with anti-human IgG conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Dallas, TX) and Ultra-TMB colorimetric substrate (ThermoFisher). All incubations were performed for 1 h at 37°C.

GP:mAb kinetic binding analysis by biolayer interferometry (BLI).—The OctetRed™ system (ForteBio) was used to measure binding competition to a trimeric EBOV GP ectodomain between ADI-15946, ADI-15878 and CA45. Ni-NTA (NTA, ForteBio) sensors were used to capture hexahistidine-tagged EBOV GP trimers (40 µg/mL) in 1× Kinetics Buffer (PBS supplemented with 0.002% Tween-20 and 1 mg/mL BSA). Binding of test mAbs to GP was performed in two separate association stages. The sensors were dipped in the first test mAb at 50 µg/mL and then transferred to the next test mAb solution at 50 µg/mL also containing 25 µg/mL of the first test mAb. The baseline and dissociation steps were carried out in 1 × Kinetics Buffer according to the manufacturer’s recommendations.

GP:mAb kinetic binding analysis by surface plasmon resonance (SPR).—SPR biosensor analysis was conducted at 25°C in a HBS-EP+ buffer system (10 mM HEPES [pH 7.4], 150 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20) with 0.1% BSA using a Biacore 8K optical biosensor (GE Healthcare, Marlboro, MA) docked with a C1 sensor chip (GE Healthcare). The sample compartment was maintained at 10°C. Goat anti-human IgG capture antibody (Jackson ImmunoResearch, West Grove, PA) was immobilized (990 ± 23 RU) to both flow cells of the sensor chip using standard amine coupling chemistry. This surface provided a format for reproducible capture of fresh analysis antibody after each regeneration step. Flow cell 2 was used to analyze captured antibody (137 ± 8 RU) while flow cell 1 was used as a reference flow cell. Antigen concentrations ranging from 81 to 0.3 nM (3-fold dilutions) were prepared in running buffer. Each of the antigen concentrations were run as a single replicate. Three blank (buffer) injections were run and used to assess and subtract system artifacts. The association (420 s) and dissociation (1800 s) phases for all antigen concentrations were monitored at a flow rate of 25 µL per min. The surface was regenerated with two injections of 10 mM glycine [pH 1.5] for 30 s, at a flow rate of 30 µL per min. The data were aligned, double referenced, and fit using the 1:1 binding model from the Biacore 8K Evaluation Software, version 1.0. Sensorgram images were generated in GraphPad Prism.

Antibody-mediated activation of human NK (natural killer) cells.—To evaluate the ability of *N. benthamiana*- and HEK293-produced mAbs (afucosylated and fucosylated, respectively) to activate NK function, human NK cells were enriched from the peripheral blood of four different human donors by negative selection using the RosetteSep kit (STEMCELL Technologies, Cambridge, MA) followed by Ficoll separation. NK cells were rested overnight in the presence of 1 ng/ml recombinant IL-15 (PeproTech, Rocky Hill, NJ). 3 ng per well of EBOV GP was coated on a Nunc Maxisorp ELISA plate (ThermoFisher) at 4° overnight, and plates were blocked with 5% BSA prior to addition of dilutions of antibodies (10 µg/ml) in PBS for 2 hours at 37°. Unbound antibodies were removed by washing wells 3× with PBS prior to addition of NK cells. The NK cells were added at 5×10⁴ cells/well in the presence of brefeldin A (Sigma Aldrich), GolgiStop (BD Biosciences, Franklin Lake, NJ), and α-CD107a-PE-Cy5 antibody, and incubated for 5 h at 37°C. NK cells were stained with flow cytometry antibodies for the following surface markers: α-CD3-AlexaFluor700, α-CD56-PE-Cy7, and α-CD16-APC-Cy7, followed by intracellular staining for IFN. (FITC) and MIP-1β (PE). All antibody conjugates were from BD Biosciences. Cells were analyzed by flow cytometry on a BD LSR2 flow cytometer and data was processed using FlowJo software (Ashland, OR).

EBOV and SUDV challenge studies in mice.—10–12-week old female BALB/c mice (Jackson Labs, Bar Harbor, ME) were challenged via the intraperitoneal (i.p.) route with mouse-adapted EBOV/Mayinga (EBOV-MA) (Bray et al., 1998) (100 PFU; ~3,000 LD₅₀). Mice were treated i.p. 3 days post-challenge with PBS vehicle or 300 µg of each mAb (0.3 mL volume, ≈15 mg mAb/kg). Animals were observed daily for clinical signs of disease and lethality for 28 days. Daily observations were increased to a minimum of twice daily while mice were exhibiting signs of disease. Moribund mice were humanely euthanized on the basis of IACUC-approved criteria. 6–8-week old female Type 1 IFN α/β receptor knockout

mice (Type 1 IFN α / β R $^{-/-}$) (Jackson Labs) were challenged with WT SUDV (1000 PFU i.p.). Animals were treated i.p. 1 and 4 days post-challenge with PBS vehicle or 300 μ g (0.3 mL volume, \approx 15 mg mAb/kg) per dose and monitored and euthanized as above.

EBOV and SUDV challenge studies in guinea pigs.—4-6-week old female guinea pigs (250-300 g) were randomly assigned to experimental groups with 6 guinea pigs per group (n=6). All animals were challenged via the i.p. route with a 1000 LD₅₀ of EBOV-GPA (Connolly et al., 1999) or SUDV-GPA (Wong et al., 2016) in 1 mL of DMEM. ADI-15878, ZMapp, MBP134, and MBP134^{AF} were administered i.p. at 3 days post-challenge (EBOV-GPA), or 4 or 5 days post-challenge (SUDV-GPA). Control-group animals received DPBS. Animals were observed for clinical signs of disease, survival and weight change for up to 20 days, and survival was monitored for an additional 12 days.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis.—Dose-response neutralization curves were fit to a logistic equation by nonlinear regression analysis. 95% confidence intervals (95% CI) for the extracted IC₅₀ parameter were estimated under the assumption of normality. Analysis of survival curves was performed with the Mantel-Cox (log-rank) test. Statistical comparisons of NK cell activity were carried out by one-way ANOVA with Tukey's correction for multiple comparisons testing. Testing level (alpha) was 0.05 for all statistical tests. Detailed statistical comparisons on groups in Figures 6–7 are shown in Figure S3. Technical and biological replicates are indicated in the Figure Legends. All analyses were carried out in GraphPad Prism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- Bornholdt ZA, Turner HL, Murin CD, Li W, Sok D, Souders CA, Piper AE, Goff A, Shamblin JD, Wollen SE, et al. (2016). Isolation of potent neutralizing antibodies from a survivor of the 2014 Ebola virus outbreak. *Science* 351, 1078–1083. [PubMed: 26912366]
- Brannan JM, Froude JW, Prugar LI, Bakken RR, Zak SE, Daye SP, Wilhelmsen CE, and Dye JM (2015). Interferon α / β Receptor-Deficient Mice as a Model for Ebola Virus Disease. *J. Infect. Dis* 212 Suppl 2, S282–94. [PubMed: 25943199]
- Bray M, Davis K, Geisbert T, Schmaljohn C, and Huggins J (1998). A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. *J. Infect. Dis* 178, 651–661. [PubMed: 9728532]

- Burk R, Bollinger L, Johnson JC, Wada J, Radoshitzky SR, Palacios G, Bavari S, Jahrling PB, and Kuhn JH (2016). Neglected filoviruses. *FEMS Microbiol. Rev* 40, 494–519. [PubMed: 27268907]
- Chao G, Lau WL, Hackel BJ, Sazinsky SL, Lippow SM, and Wittrup KD (2006). Isolating and engineering human antibodies using yeast surface display. *Nat. Protoc* 1, 755–768. [PubMed: 17406305]
- Connolly BM, Steele KE, Davis KJ, Geisbert TW, Kell WM, Jaax NK, and Jahrling PB (1999). Pathogenesis of experimental Ebola virus infection in guinea pigs. *J. Infect. Dis* 179 Suppl 1, S203–17. [PubMed: 9988186]
- Corti D, Misasi J, Mulangu S, Stanley DA, Kanekiyo M, Wollen S, Ploquin A, Doria-Rose NA, Staupé RP, Bailey M, et al. (2016). Protective monotherapy against lethal Ebola virus infection by a potentially neutralizing antibody. *Science* 351, 1339–1342. [PubMed: 26917593]
- Cross RW, Fenton KA, Geisbert JB, Mire CE, and Geisbert TW (2015). Modeling the Disease Course of Zaire ebolavirus Infection in the Outbred Guinea Pig. *J. Infect. Dis* 212 Suppl2, S305–15. [PubMed: 26038397]
- Ferrara C, Grau S, Jager C, Sondermann P, BrCinker P, Waldhauer I, Hennig M, Ruf A, Rufer AC, Stihle M, et al. (2011). Unique carbohydrate-carbohydrate interactions are required for high affinity binding between FcγRIIIb and antibodies lacking core fucose. *Proc Natl Acad Sci USA* 108, 12669–12674. [PubMed: 21768335]
- Flyak AI, Shen X, Murin CD, Turner HL, David JA, Fusco ML, Lamplé R, Kose N, Ilinykh PA, Kuzmina N, et al. (2016). Cross-Reactive and Potent Neutralizing Antibody Responses in Human Survivors of Natural Ebolavirus Infection. *Cell* 164, 392–405. [PubMed: 26806128]
- Flyak AI, Kuzmina N, Murin CD, Bryan C, Davidson E, Gilchuk P, Gulka CP, Ilinykh PA, Shen X, Huang K, et al. (2018). Broadly neutralizing antibodies from human survivors target a conserved site in the Ebola virus glycoprotein HR2-MPER region. *Nat. Microbiol* 3, 670–677. [PubMed: 29736037]
- Forthal DN, Gach JS, Landucci G, Jez J, Strasser R, Kunert R, and Steinkellner H (2010). Fc-glycosylation influences Fcγ receptor binding and cell-mediated anti-HIV activity of monoclonal antibody 2G12. *J. Immunol* 185, 6876–6882. [PubMed: 21041724]
- Gilchuk P, Kuzmina N, Ilinykh PA, Huang K, Gunn BM, Bryan A, Davidson E, Doranz BJ, Turner HL, Fusco ML, et al. (2018). Multifunctional Pan-ebolavirus Antibody Recognizes a Site of Broad Vulnerability on the Ebolavirus Glycoprotein. *Immunity* 49, 363–374.e10. [PubMed: 30029854]
- Goldstein T, Anthony SJ, Gbakima A, Bird BH, Bangura J, Tremeau-Bravard A, Belaganahalli MN, Wells HL, Dhanota JK, Liang E, et al. (2018). The discovery of Bombali virus adds further support for bats as hosts of ebolaviruses. *Nat. Microbiol* 3, 1084–1089. [PubMed: 30150734]
- Gunn BM, Yu W-H, Karim MM, Brannan JM, Herbert AS, Wee AZ, Halfmann PJ, Fusco ML, Schendel SL, Gangavarapu K, et al. (2018). A Role for Fc Function in Therapeutic Monoclonal Antibody-Mediated Protection against Ebola Virus. *Cell Host Microbe* 24, 221–233.e5. [PubMed: 30092199]
- Hiatt A, Bohorova N, Bohorov O, Goodman C, Kim D, Pauly MH, Velasco J, Whaley KJ, Piedra PA, Gilbert BE, et al. (2014). Glycan variants of a respiratory syncytial virus antibody with enhanced effector function and in vivo efficacy. *Proc Natl Acad Sci USA* 111, 5992–5997. [PubMed: 24711420]
- Hötzel I, Theil F-P, Bernstein LJ, Prabhu S, Deng R, Quintana L, Lutman J, Sibia R, Chan P, Bumbaca D, et al. (2012). A strategy for risk mitigation of antibodies with fast clearance. *MAbs* 4, 753–760. [PubMed: 23778268]
- Howell KA, Qiu X, Brannan JM, Bryan C, Davidson E, Holtsberg FW, Wee AZ, Shulenin S, Biggins JE, Douglas R, et al. (2016). Antibody Treatment of Ebola and Sudan Virus Infection via a Uniquely Exposed Epitope within the Glycoprotein Receptor-Binding Site. *Cell Rep.* 15, 1514–1526. [PubMed: 27160900]
- Jahrling PB, Geisbert TW, Geisbert JB, Swearingen JR, Bray M, Jaax NK, Huggins JW, LeDuc JW, and Peters CJ. (1999). Evaluation of immune globulin and recombinant interferon-alpha2b for treatment of experimental Ebola virus infections. *J. Infect. Dis* 179 Suppl 1, S224–34. [PubMed: 9988188]

- Jain T, Sun T, Durand S, Hall A, Houston NR, Nett JH, Sharkey B, Bobrowicz B, Caffry I, Yu Y, et al. (2017). Biophysical properties of the clinical-stage antibody landscape. *Proc Natl Acad Sci USA* 114, 944–949. [PubMed: 28096333]
- Mao C, Near R, and Gao W (2017). Identification of a Guinea Pig Fey Receptor that Exhibits Enhanced Binding to Afucosylated Human and Mouse IgG. *J. Infect. Dis. Med* 01.
- Marzi A (2017). Evaluation of ebola virus countermeasures in guinea pigs. *Methods Mol. Biol* 1628, 283–291. [PubMed: 28573629]
- Mire CE, Geisbert JB, Borisevich V, Fenton KA, Agans KN, Flyak AI, Deer DJ, Steinkellner H, Bohorov O, Bohorova N, et al. (2017). Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody. *Sci. Transl. Med* 9.
- Mohan GS, Li W, Ye L, Compans RW, and Yang C (2012). Antigenic subversion: a novel mechanism of host immune evasion by Ebola virus. *PLoS Pathog.* 8, e1003065.
- Murin CD, Fusco ML, Bornholdt ZA, Qiu X, Olinger GG, Zeitlin L, Kobinger GP, Ward AB, and Saphire EO (2014). Structures of protective antibodies reveal sites of vulnerability on Ebola virus. *Proc Natl Acad Sci USA* 111, 17182–17187. [PubMed: 25404321]
- National Institutes of Health Clinical Center (2018). Safety and Pharmacokinetics of a Human Monoclonal Antibody, VRC-EBOMAB092-00-AB (MAb14), Administered Intravenously to Healthy Adults.
- Ng M, Ndungo E, Jangra RK, Cai Y, Postnikova E, Radoshitzky SR, Dye JM, Ramírez de Arellano E, Negredo A, Palacios G, et al. (2014). Cell entry by a novel European filovirus requires host endosomal cysteine proteases and Niemann-Pick C1. *Virology* 468–470, 637–646.
- Olinger GG, Pettitt J, Kim D, Working C, Bohorov O, Bratcher B, Hiatt E, Hume SD, Johnson AK, Morton J, et al. (2012). Delayed treatment of Ebola virus infection with plant-derived monoclonal antibodies provides protection in rhesus macaques. *Proc Natl Acad Sci USA* 109, 18030–18035. [PubMed: 23071322]
- Oswald WB, Geisbert TW, Davis KJ, Geisbert JB, Sullivan NJ, Jahrling PB, Parren PW, and Burton DR (2007). Neutralizing antibody fails to impact the course of Ebola virus infection in monkeys. *PLoS Pathog.* 3, e9. [PubMed: 17238286]
- Pascal KE, Dudgeon D, Trefry JC, Anantpadma M, Sakurai Y, Murin CD, Turner HL, Fairhurst J, Torres M, Rafique A, et al. (2018). Development of clinical-stage human monoclonal antibodies that treat advanced Ebola virus disease in non-human primates. *J. Infect. Dis*
- PREVAIL II Writing Group, Multi-National PREVAIL II Study Team, Davey RT, Dodd L, Proschan MA, Neaton J, Neuhaus Nordwall J, Koopmeiners JS, Beigel J, Tierney J, et al. (2016). A randomized, controlled trial of zmapp for ebola virus infection. *N. Engl. J. Med* 375, 1448–1456. [PubMed: 27732819]
- Qiu X, Fernando L, Melito PL, Audet J, Feldmann H, Kobinger G, Alimonti JB, and Jones SM (2012). Ebola GP-specific monoclonal antibodies protect mice and guinea pigs from lethal Ebola virus infection. *PLoS Negl. Trop. Dis* 6, e1575.
- Qiu X, Audet J, Wong G, Fernando L, Bello A, Pillet S, Alimonti JB, and Kobinger GP (2013). Sustained protection against Ebola virus infection following treatment of infected nonhuman primates with ZMAb. *Sci. Rep* 3, 3365. [PubMed: 24284388]
- Qiu X, Wong G, Audet J, Bello A, Fernando L, Alimonti JB, Fausther-Bovendo H, Wei H, Aviles J, Hiatt E, et al. (2014). Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature* 514, 47–53. [PubMed: 25171469]
- Qiu X, Audet J, Lv M, He S, Wong G, Wei H, Luo L, Fernando L, Kroeker A, Fausther Bovendo H, et al. (2016). Two-mAb cocktail protects macaques against the Makona variant of Ebola virus. *Sci. Transl. Med* 8, 329ra33.
- Sanchez A, Trappier SG, Mahy BW, Peters CJ, and Nichol ST (1996). The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. *Proc Natl Acad Sci USA* 93, 3602–3607. [PubMed: 8622982]
- Saphire EO, Schendel SL, Fusco ML, Gangavarapu K, Gunn BM, Wee AZ, Halfmann PJ, Brannan JM, Herbert AS, Qiu X, et al. (2018). Systematic Analysis of Monoclonal Antibodies against Ebola Virus GP Defines Features that Contribute to Protection. *Cell* 174, 938–952.e13. [PubMed: 30096313]

- Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, Weikert SHA, and Presta LG (2002). Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcγ3 and antibody-dependent cellular toxicity. *J. Biol. Chem.* 277, 26733–26740. [PubMed: 11986321]
- Sivapalasingam S, Kamal M, Slim R, Hosain R, Shao W, Stoltz R, Yen J, Pologe LG, Cao Y, Partridge M, et al. (2018). Safety, pharmacokinetics, and immunogenicity of a co-formulated cocktail of three human monoclonal antibodies targeting Ebola virus glycoprotein in healthy adults: a randomised, first-in-human phase 1 study. *Lancet Infect. Dis* 18, 884–893. [PubMed: 29929783]
- Stemmer WP (1994). DNA shuffling by random fragmentation and reassembly: in vitro recombination for molecular evolution. *Proc Natl Acad Sci USA* 91, 10747–10751. [PubMed: 7938023]
- Towner JS, Sealy TK, Khristova ML, Albariño CG, Conlan S, Reeder SA, Quan P-L, Lipkin WI, Downing R, Tappero JW, et al. (2008). Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathog.* 4, e1000212.
- Volchkov VE, Becker S, Volchkova VA, Ternovoj VA, Kotov AN, Netesov SV, and Klenk HD (1995). GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. *Virology* 214, 421–430. [PubMed: 8553543]
- Warfield KL, Perkins JG, Swenson DL, Deal EM, Bosio CM, Aman MJ, Yokoyama WM, Young HA, and Bavari S (2004). Role of natural killer cells in innate protection against lethal ebola virus infection. *J. Exp. Med* 200, 169–179. [PubMed: 15249592]
- Wee AZ, Nyakatura EK, Herbert AS, Howell KA, Holtsberg FW, Bakken RR, Mittler E, Christin JR, Shulenin S, Jangra RK, et al. (2016). A “Trojan horse” bispecific-antibody strategy for broad protection against ebolaviruses. *Science* 354, 350–354. [PubMed: 27608667]
- Wee AZ, Herbert AS, Murin CD, Nyakatura EK, Abelson DM, Fels JM, He S, James RM, de La Vega M-A, Zhu W, et al. (2017). Antibodies from a Human Survivor Define Sites of Vulnerability for Broad Protection against Ebolaviruses. *Cell* 169, 878–890.e15. [PubMed: 28525755]
- Whelan SP, Ball LA, Barr JN, and Wertz GT (1995). Efficient recovery of infectious vesicular stomatitis virus entirely from cDNA clones. *Proc Natl Acad Sci USA* 92, 8388–8392. [PubMed: 7667300]
- Wong AC, Sandesara RG, Mulherkar N, Whelan SP, and Chandran K (2010). A forward genetic strategy reveals destabilizing mutations in the Ebolavirus glycoprotein that alter its protease dependence during cell entry. *J. Virol* 84, 163–175. [PubMed: 19846533]
- Wong G, Richardson JS, Pillet S, Patel A, Qiu X, Alimonti J, Hogan J, Zhang Y, Takada A, Feldmann H, et al. (2012). Immune parameters correlate with protection against ebola virus infection in rodents and nonhuman primates. *Sci. Transl. Med* 4, 158ral46.
- Wong G, He S, Wei H, Kroeker A, Audet J, Leung A, Cutts T, Graham J, Kobasa D, Embury-Hyatt C, et al. (2016). Development and Characterization of a Guinea Pig-Adapted Sudan Virus. *J. Virol* 90, 392–399. [PubMed: 26491156]
- Xu Y, Roach W, Sun T, Jain T, Prinz B, Yu T-Y, Torrey J, Thomas J, Bobrowicz P, Vásquez M, et al. (2013). Addressing polyspecificity of antibodies selected from an in vitro yeast presentation system: a FACS-based, high-throughput selection and analytical tool. *Protein Eng. Des. Sel* 26, 663–670. [PubMed: 24046438]
- Zeitlin L, Pettitt J, Scully C, Bohorova N, Kim D, Pauly M, Hiatt A, Ngo L, Steinkellner H, Whaley KJ, et al. (2011). Enhanced potency of a fucose-free monoclonal antibody being developed as an Ebola virus immunoprotectant. *Proc Natl Acad Sci USA* 108, 20690–20694. [PubMed: 22143789]
- Zhao X, Howell KA, He S, Brannan JM, Wee AZ, Davidson E, Turner HL, Chiang C-L, Lei L, Fels JM, et al. (2017). Immunization-Elicited Broadly Protective Antibody Reveals Ebolavirus Fusion Loop as a Site of Vulnerability. *Cell* 169, 891–904.e15. [PubMed: 28525756]

Highlights:

- Cocktail of two human mAbs affording pan-ebolavirus neutralization was selected
- Cocktail outperforms single antibody in protecting against EBOV infection in guinea pigs
- Second-generation cocktail with optimized Fc effector functions enhances protection
- Single low dose of optimized cocktail protects guinea pigs against two ebolaviruses

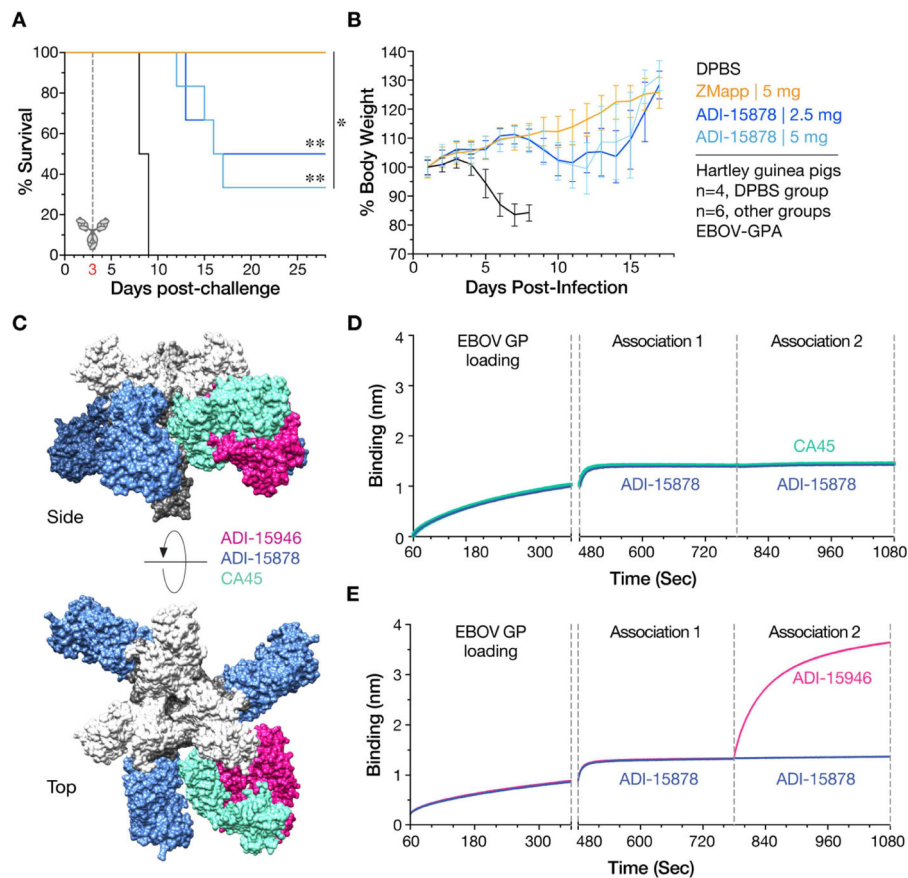


Figure 1. Selection of ADI-15946 as a candidate cocktail partner for ADI-15878

(A) Hartley guinea pigs were challenged with guinea-pig adapted EBOV (EBOV-GPA) and then treated with single doses of ZMapp, ADI-15878, or vehicle (Dulbecco's phosphate-buffered saline, DPBS) at three days post-exposure. Survival curves (vehicle vs. test mAb and ZMapp (5 mg) vs. ADI-15878 (5 mg)) were compared by Mantex-Cox test. **, $P < 0.01$. *, $P < 0.05$. (B) Body weights of surviving animals in each treatment group in panel A. Averages \pm SD ($n=6$ for ZMapp, $n=3-6$ for ADI-15878) are shown. Data are from single cohorts. (C) In silico models of the ADI-15878, ADI-15946, and CA45 Fabs were fitted into negative-stain EM reconstructions of GP:Fab complexes (Wec et al., 2017; Zhao et al., 2017) and are superimposed onto a single EBOV GP structure [PDB ID: 5JQ337] to illustrate the approximate binding footprint and angle of approach of ADI-15946 and CA45 relative to ADI-15878. Light gray and dark gray, GP1 and GP2 subunits, respectively. (D–E) Analysis of competitive binding of candidate IgGs to EBOV GP by biolayer interferometry (BLI). Each GP-bearing probe was sequentially dipped in analyte solutions containing ADI-15878 and then ADI-15878 (D–E, control), CA45 (D), or ADI-15946 (E). Results from a representative experiment are shown.

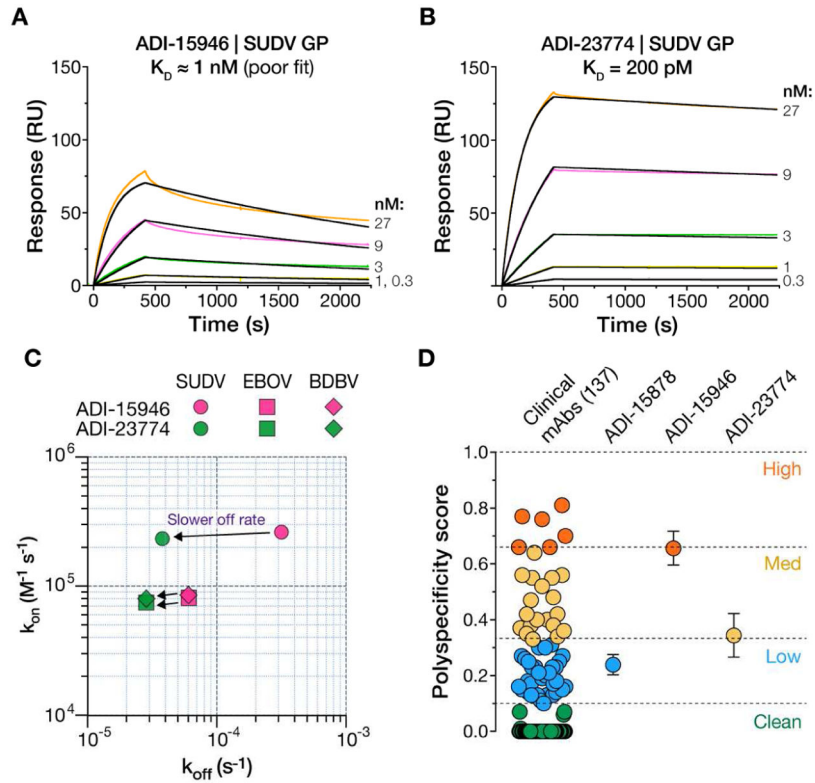


Figure 2. Binding and polyspecificity properties of ADI-15946 and its specificity-matured variant ADI-23774

(A–B) BLI sensorgrams for IgG-SUDV GP interactions with ADI-15946 (A) and ADI-23774 (B). Experimental curves (colored traces) were fit using a 1:1 binding model (black traces). The corresponding flow analyte (GP) concentration is indicated at the right of each curve. (C) Comparison of association (k_{on}) and dissociation (k_{off}) rate constants for IgG interactions with EBOV, BDBV, and SUDV GP. Arrows indicate changes in the values of these constants following ADI-15946 specificity maturation. (D) Polyspecificity scores for candidate mAbs were determined as described previously (Xu et al., 2013). The scores for 137 mAbs in commercial clinical development (Jain et al., 2017) are shown for comparison. Averages \pm SD ($n=3$ for ADI-15878 and ADI-15946, $n=2$ for ADI-23774) from 2–3 independent experiments. See also Figure SI for specificity maturation of ADI-15946 to ADI-23774 and Figure S2 for GP:mAb kinetic binding constants derived from BLI.

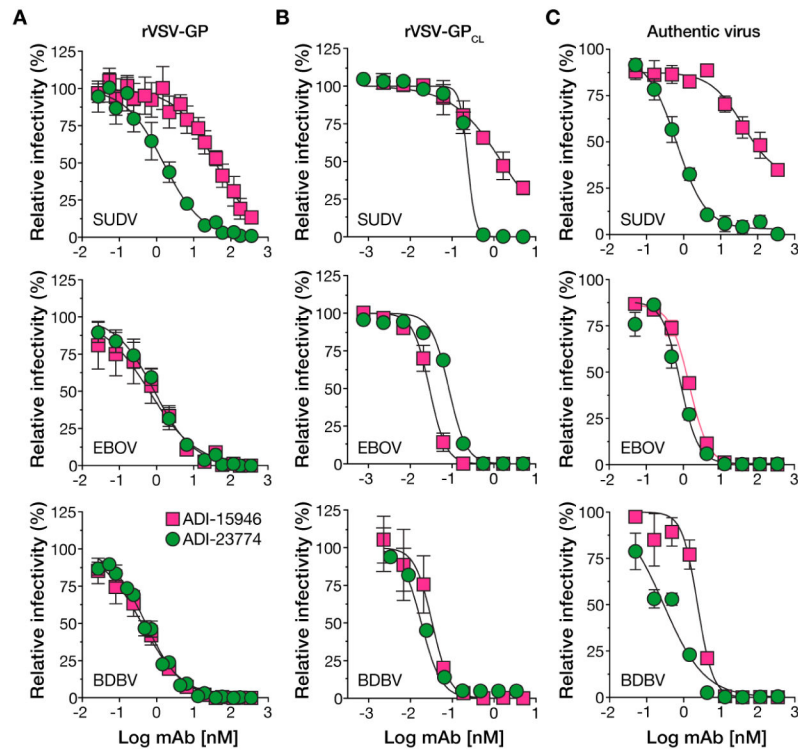


Figure 3. Neutralizing activity of ADI-23774

Neutralization of rVSVs encoding enhanced green fluorescent protein (eGFP) and bearing uncleaved (A) or cleaved (B) ebolavirus GP proteins (rVSV-GP and rVSV-GP_{CL}, respectively). Virions were preincubated with increasing concentrations of each mAb and then exposed to cells for 12 to 14 hours at 37°C. Infection was measured by automated counting of eGFP+ cells and normalized to infection obtained in the absence of antibody. Averages±SD (n=6–9 in panel A, n=3 in panel B) from 3 independent experiments. (C) Neutralization of authentic filoviruses measured in a microneutralization assay. Virions were preincubated with increasing concentrations of each mAb and then exposed to cells for 48 h at 37°C. Infected cells were immunostained for viral antigen and enumerated by automated fluorescence microscopy. Averages±SD (n=2–4) from two independent experiments.

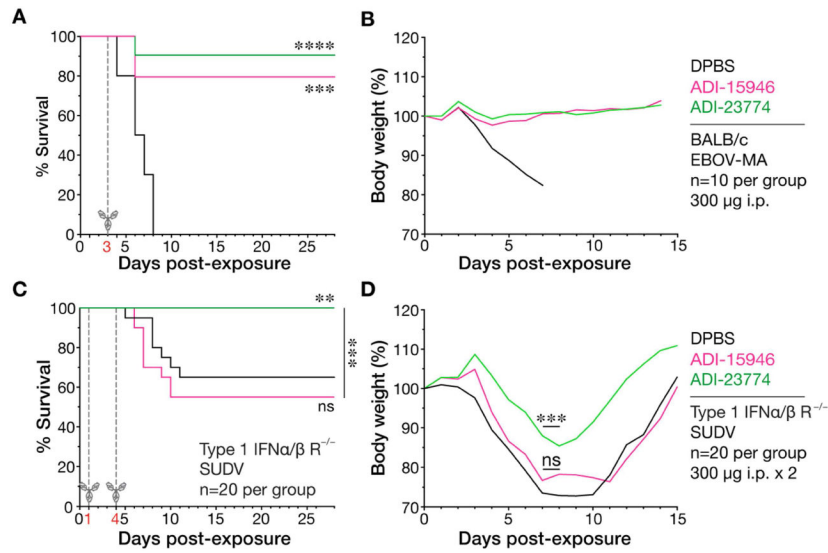


Figure 4. Protective efficacy of ADI-23774 against ebolavirus challenge in mice

(A) BALB/c mice were challenged with mouse-adapted EBOV (EBOV-MA) and then treated with single doses of the indicated mAbs or vehicle (DPBS) at 3 days post-exposure. Survival curves (vehicle vs. test mAb) were compared by Mantex-Cox test. (B) Combined body weights of surviving animals in each treatment group in panel A. Data in panels A–B are from single cohorts. (C) Type 1 IFN α / β R^{-/-} mice were challenged with wild-type SUDV and then treated with two doses of the indicated mAbs or vehicle (DPBS) at 1 and 4 days post-exposure. Survival curves (vehicle vs. test mAb and ADI-15946 vs ADI-23774) were compared by Mantex-Cox test. (D) Combined body weights of surviving animals in each treatment group in panel C. Groups (vehicle vs. test mAb) were compared by two-way ANOVA with repeated measures and Dunnett’s test. Significance values for comparison of body weights on days 6–7 are shown. Data in panels C–D are pooled from two cohorts. ****, $P < 0.0001$. ***, $P < 0.001$. **, $P < 0.01$. *, $P < 0.05$. ns, $P > 0.05$.

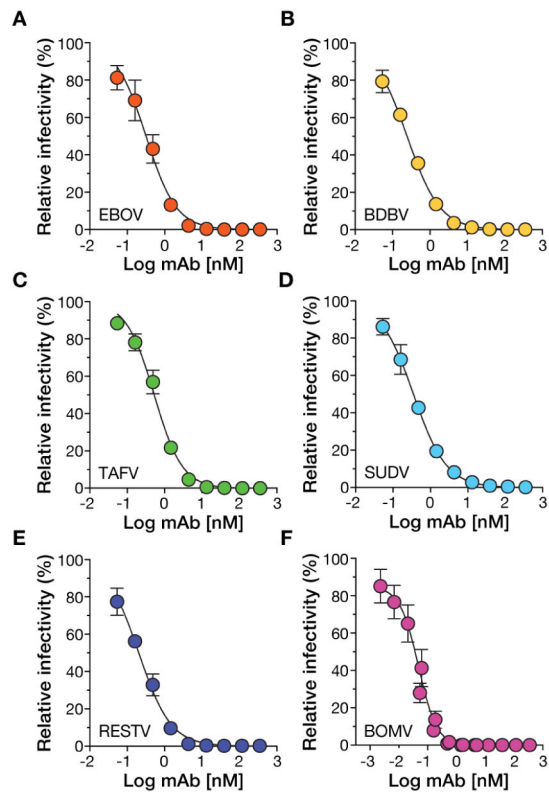


Figure 5. Neutralizing activity of MBP134 cocktail

(A–F) Neutralization of rVSVs encoding eGFP and bearing GP proteins from EBOV (A), BDBV (B), Tai Forest virus (TAFV) (C), SUDV (D), Reston virus (RESTV) (E), and BOMV (F) was determined as in Figure 3. Averages \pm SD (n=6) from three independent experiments are shown.

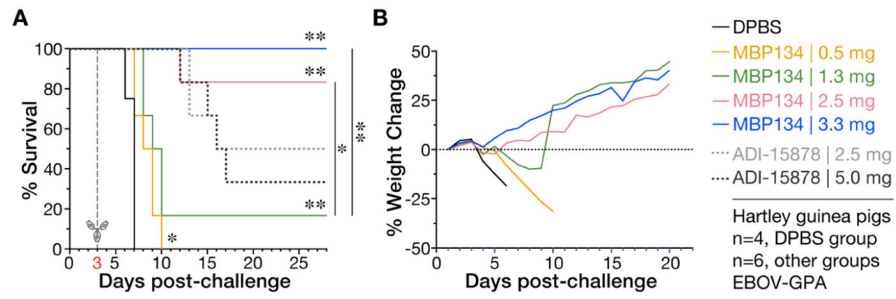


Figure 6. Protective efficacy of MBP134 in guinea pigs

(A) Hartley guinea pigs were challenged with EBOV-GPA and then treated with single doses of ADI-15878, MBP134 (1:1 mixture of ADI-15878 and ADI-23774), or vehicle (DPBS) at three days post-exposure. Survival curves were compared by Mantex-Cox test. (B) Body weights of surviving animals in each treatment group in panel A. Data are from single cohorts. **, $P < 0.01$. *, $P < 0.05$. ns, $P > 0.05$. See also Table S1 for statistical details on the groups compared in panel A.

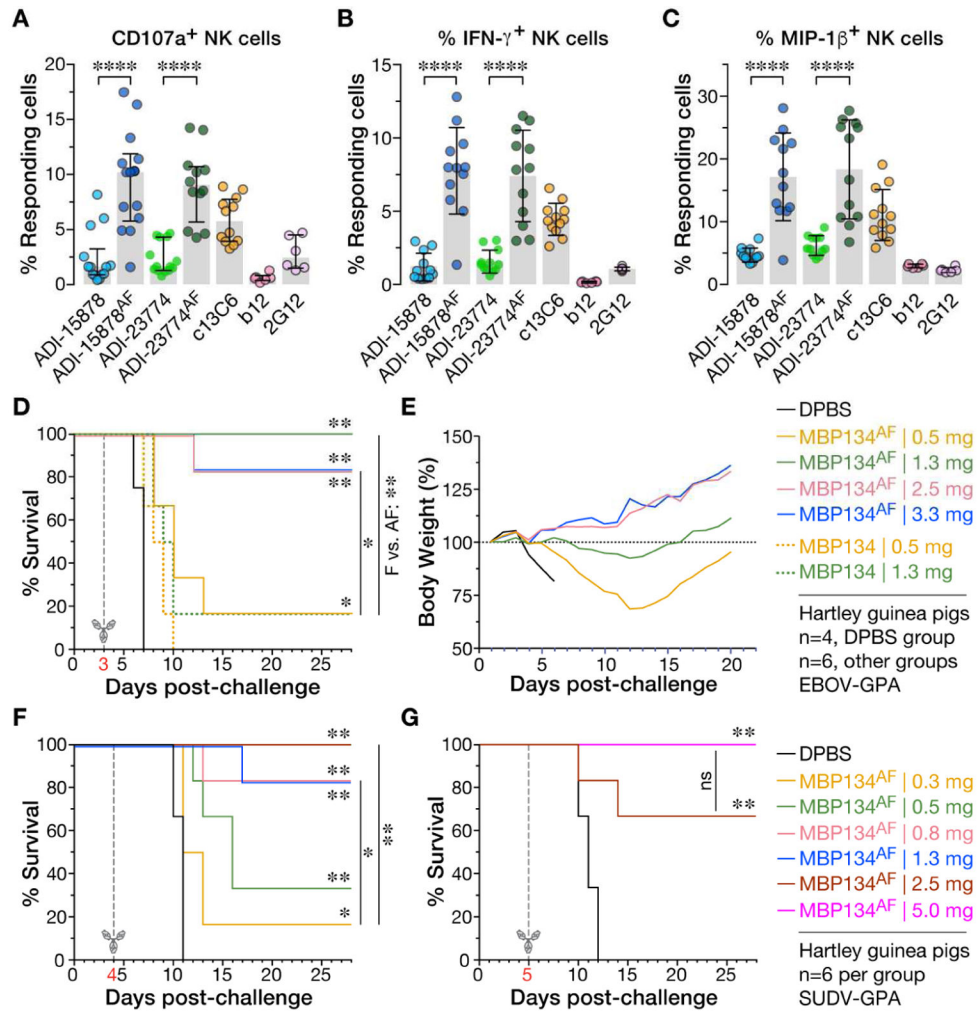


Figure 7. Development and evaluation of the second-generation MBP134^{AF} cocktail
 (A–C) Activation of human natural killer (NK) cells by EBOV GP:IgG complexes. NK cells enriched from the peripheral blood of four different human donors were incubated with complexes between EBOV GP and the indicated IgGs (10 μ g/mL) for 5 h at 37°C, and then stained with antibody-fluorophore conjugates specific for the cell-surface markers CD3, CD56, and CD16, followed by intracellular staining for markers of NK cell activation, CD107a (degranulation) (A), IFN- γ (B), and MIP-1 β (C). CD3⁻/CD56^{dim}/CD16⁺ NK cells were analyzed by flow cytometry. Data with cells from all four donors are pooled. HIV-1 glycoprotein-specific mAbs b12 and 2G12 are included as (negative) controls for antigen specificity. EBOV GP-specific mAb c13C6 produced in transgenic *Nicotiana benthamiana* tobacco plants to bear a highly functional afucosylated/agalactosylated bisected glycan is included as a positive control. Averages \pm SD (n = 12–14 for all mAbs except b12 and 2G12 (n=6) from 4 independent experiments). The indicated groups were compared by one-way ANOVA and Tukey’s test. (D) Hartley guinea pigs were challenged with EBOV-GPA and then treated with single doses of MBP134, MBP134^{AF} (1:1 mixture of ADI-15878^{AF} and ADI-23774^{AF}), or vehicle (DPBS) at three days post-exposure. (E) Body weights of surviving animals in each treatment group in panel F. Data in panels D–E are from single

cohorts. (F–G) Guinea pigs were challenged with SUDV-GPA and then treated with single doses of MBP134^{AF} or vehicle (DPBS) at four (F) or five (G) days post-exposure. Data in panels F–G are from single cohorts. Survival curves in panels D, F were compared by Mantex-Cox test. ****, $P < 0.0001$. **, $P < 0.01$. *, $P < 0.05$. ns, $P > 0.05$. See also Figure S3 for flow-cytometric gating strategies in panels A–C and Table S2 for statistical details on the groups compared in panels D, F.

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