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Ervebo Vaccine Provides Cross-Protection Against Bundibugyo Virus Infection in Macaques

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ERVEBO Cross-Protects Against Bundibugyo Virus Infection in Macaques

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The 2026 Bundibugyo virus (BDBV) disease outbreak in the Democratic Republic of the Congo (DRC) was declared a public health emergency (PHEIC) in May 2026 [1]. Regional security concerns, debilitated medical infrastructure, diagnostic limitations, and lack of approved BDBV–specific medical countermeasures have contributed to rapid escalation of the outbreak [2]. Currently, there are no licensed medical countermeasures for BDBV [3]. Several BDBV-specific vaccines are in development [4], including a vesicular stomatitis virus (VSV)–based vaccine expressing the BDBV glycoprotein which has demonstrated strong preclinical efficacy [5-7]; however, these candidates have not yet completed clinical evaluation. A licensed VSV-based vaccine expressing the Ebola virus glycoprotein (VSV-EBOV) [8] is available for human use and clinical (ERVEBO) and research grade (VSV-EBOV) material has been shown to elicit cross-reactive, cross-neutralizing and inconclusive cross-protective immune responses against the related BDBV [5,9-14]. ERVEBO's protective efficacy against BDBV, however, has not been formally established in the standard macaque model of BDBV infection. Macaques received a single human dose of ERVEBO or an unrelated VSV-based control vaccine and were challenged with BDBV 14 days after vaccination. ERVEBO vaccinated macaques exhibited significantly reduced clinical disease and strikingly increased survival, as well as significantly reduced viremia, mucosal viral shedding and disease-associated biochemistry markers consistent with protection against BDBV-mediated hepatorenal disease. These findings indicate that ERVEBO can rapidly mediate cross-protective immunity against BDBV.

Bundibugyo virus (*Orthoebolavirus bundibugyoense*) (BDBV) is a human pathogenic zoonotic orthoebolavirus in the family *Filoviridae* capable of causing Bundibugyo virus disease (BVD) [15]. BVD ranges from 32%- 55% in case fatality and is characterized by a 2–21-day incubation period followed by fever, weakness, gastrointestinal symptoms, hemorrhaging in some cases, shock, and multi-organ failure [16,17]. Initial transmission occurs through direct contact with the unknown animal reservoir; sustained transmission is through direct contact with the blood or body fluids of infected people or contaminated materials often involving healthcare settings and close household contacts [3]. BDBV emerged in 2007-2008 during an outbreak in the Bundibugyo District in western Uganda, hence its name [18,19]. The virus resurfaced in 2012 causing an outbreak in the Orientale Province of the Democratic Republic of Congo (DRC) [20] before triggering the 2026 unprecedented and uncontrolled epidemic in and around the Ituri and North Kivu provinces of the DRC [21]. The latest outbreak, which was declared a public health emergency of international concern (PHEIC), rapidly escalated into the largest BDBV and second-largest orthoebolavirus outbreak in history. It has now surpassed 10,000 confirmed cases and over 5,00 deaths with cases spread beyond DRC into Uganda, Kenya and Nigeria [1,21]. Because of a lack of BDBV-specific vaccines and treatment options, current BDBV response activities rely heavily on rapid diagnostic detection, patient isolation, early aggressive supportive care, and strict infection prevention and control protocols.

Vaccination is widely established as one of the most effective preventive public health countermeasures for controlling and interrupting community transmission during an infectious disease outbreak [22]. Regarding orthoebolaviruses, current licensed vaccines and

65 treatments are mainly designed for Ebola virus (EBOV). Therefore, other human pathogenic
66 orthoebolaviruses, such as BDBV and Sudan virus (SUDV), remain largely unaddressed
67 significant public health concerns. Several vaccine candidates are under evaluation for use
68 against BDBV, but all are at different developmental stages and must undergo or finish
69 clinical trials before they are widely available for human use [4]. One of these platforms is the
70 VSV-based vaccine that contains the BDBV glycoprotein (VSV-BDBV), which has shown
71 great success in preclinical laboratory studies [6,7]. ERVEBO, the FDA-approved vaccine for
72 EBOV, is based on the same VSV platform containing the EBOV glycoprotein (VSV-EBOV) [8]
73 and was successfully used in clinical trials during the West African Ebola outbreak in 2015
74 and the DRC during the 2018-2020 Ebola outbreak under investigational new drug
75 applications [23,24]. Previous preclinical studies have not conclusively shown VSV-
76 EBOV/ERVEBO mediated cross-protection against BDBV challenge in macaques; one
77 reason for not initially supporting ERVEBO deployment in the current BDBV outbreak [5,9].
78 More recent studies, however, have demonstrated BDBV cross-reactive and cross-
79 neutralizing antibodies in VSV-EBOV and ERVEBO vaccinated ferrets and humans,
80 respectively, again raising the question of potential cross-protection against BDBV
81 infection [10-14].

82 In this study, we assessed the potential for ERVEBO cross-protection against BDBV in the
83 cynomolgus macaque model, the ultimate pre-clinical model for filoviruses. Animals
84 received either a control VSV-Andes virus (VSV-ANDV) vaccine (n=5) or the ERVEBO (VSV-
85 EBOV) vaccine (n=6) (Fig. 1a). Macaques were vaccinated intramuscularly (IM) 14-days
86 prior to IM challenge with BDBV and monitored daily for 35 days post challenge (DPC) using

87 an approved clinical score sheet (Extended Data Table 1). Clinical scores were elevated on
88 6 -7 DPC in one ERVEBO vaccinated and three VSV-ANDV vaccinated macaques (Fig. 1b
89 and Extended Data Fig. 1a). These four macaques met endpoint criteria between 8 and 9
90 DPC (Fig. 1c). A fourth VSV-ANDV vaccinated macaque began developing clinical signs of
91 disease on 9 DPC and met endpoint criteria on 12 DPC (Fig. 1b,c and Extended Data Fig.
92 1a). The fifth macaque in the VSV-ANDV group did develop moderate clinical signs and
93 recovered. The five remaining ERVEBO vaccinated macaques developed minor and
94 transient signs of disease between 6-10 DPC and quickly recovered (Fig. 1b,c and Extended
95 Data Fig. 1a). Most of the macaques, regardless of vaccination group, had slightly
96 decreased body weight following 3 DPC (Fig. 1d and Extended Data Fig. 1b) and slightly
97 elevated temperatures peaking between 6-9 DPC (Fig. 1e and Extended Data Fig. 1c)
98 compared to normalization at 0 DPC. However, the averaged values over time were the
99 same between groups for both body weight and temperature (Fig. 1d,e). Overall, compared
100 to the VSV-ANDV vaccinated macaques (control group), the ERVEBO vaccinated group
101 showed significantly improved clinical disease (Fig. 1b and Extended Data Fig. 1a) and
102 demonstrated strikingly increased survival (83% vs 20%) (Fig. 1c).

103 Blood samples collected during clinical exams were analyzed for biochemical and
104 hematological indices. Overall, biochemical markers remained largely unchanged from -14
105 DPC to 3 DPC. Thereafter, levels of aspartate aminotransferase (AST), alanine
106 aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TBIL), blood urea
107 nitrogen (BUN), and creatinine (CRE) levels increased for both vaccine groups with ERVEBO
108 vaccinated animals displaying significantly lower values (Fig. 2a-f and Extended Data Fig.

1d-i). Levels of these markers in non-survivors of both groups continued to increase until endpoint criteria were met while levels in most survivors quickly returned to normal values. Additional metrics, such as decreased albumin (ALB) and total carbon dioxide (tCo2) levels, were also observed (Fig. 2g,h and Extended Data Fig. 1j,k). Altogether, the data suggests that ERVEBO protected animals from BDBV-mediated impaired hepatorenal function. Hematological parameters with measurable responses to infection were not observed until about 3 DPC. Thereafter, a decline in platelet (PLT) levels in both groups was observed (Fig. 2i and Extended Data Fig. 1l). PLT levels, however, quickly rebounded to baseline levels in the five surviving ERVEBO vaccinated macaques. Signs of a leukogram stress response were observed which included a decrease in lymphocyte (LYM) counts at 6 DPC (Fig. 2j and Extended Data Fig. 1m) before quickly rebounding in the surviving macaques, while the monocyte (MON) counts rapidly increased between 9-14 DPC (Fig. 2k and Extended Data Fig. 1n). Additional biochemical (Extended Data Fig. 2) and hematological indices (Extended Data Fig. 3) were assessed but showed minor differences. Overall, the observed biochemical and hematological changes were more pronounced in macaques that met euthanasia criteria.

Viral RNA quantified from blood samples was first detectable, and at similar levels in both vaccination groups, at 3 DPC (Fig. 3a). Viral RNA levels peaked in both groups at 6 DPC before decreasing at 9 DPC. Importantly, the levels of viral RNA in the ERVEBO vaccinated animals were significantly lower at 6 and 9 DPC compared to the VSV-ANDV vaccinated macaques (Fig. 3a). At 6 DPC, the ERVEBO macaque that met euthanasia criteria had viral RNA levels equivalent to the VSV-ANDV macaques that also met euthanasia criteria.

Conversely, the survivor in the VSV-ANDV group had viral RNA levels equivalent to the surviving ERVEBO macaques (Fig. 3a). In conjunction with the viremia data, macaques that succumbed to disease had high viral loads in the tissues collected at euthanasia (Fig. 3b). While, as expected, surviving macaques had reduced viral loads in the tissues collected during euthanasia at study endpoint (35 DPC) (Fig. 3b). Even though viral loads cannot be directly compared between the two groups, the data suggests a strong correlation between peak viremia, organ viral load, and clinical outcome.

Viral shedding was measured from oral and rectal swabs collected during the clinical exams. Viral RNA was undetectable in the oral swabs at 3 DPC but was present in the one non-surviving ERVEBO vaccinated macaque and three of the five VSV-ANDV macaques at 6 DPC (Fig. 3c). The five surviving ERVEBO vaccinated macaques had significantly lower viral RNA in oral swabs at 9 DPC compared to VSV-ANDV vaccinated macaques (Fig. 3c), which peaked at the humane endpoint (Fig. 3e). Rectal swabs showed a similar pattern, but viral RNA was not detected until 6 DPC (Fig. 3d). Rectal viral shedding was significantly higher in the VSV-ANDV vaccinated macaques compared to the ERVEBO vaccinated macaques at 6 and 9 DPC which was driven by the macaques that met humane endpoint criteria (Fig. 3d,e). Interestingly, the recipient of VSV-ANDV that survived had sustained viral RNA shedding in the rectal swab out to 21 DPC (Fig. 3d). Collectively this data indicates that ERVEBO significantly reduces viral shedding kinetics.

A known protective mechanism of VSV-based vaccines is the humoral immune response [25,26]. Therefore, we assessed binding and neutralizing antibody responses in serum samples on day of vaccination (-14 DPC; baseline), challenge (0 DPC), established disease (6 DPC),

and convalescence (35 DPC). ERVEBO vaccinated macaques had significantly higher levels of EBOV GP-reactive IgG compared to VSV-ANDV vaccinated animals at 0 DPC and 6 DPC (Fig. 3f), and these levels were also significantly elevated over time when compared to -14 DPC (Fig. 3f). ERVEBO vaccinated macaques also had significantly higher levels of BDBV GP-cross-reactive IgG compared to VSV-ANDV vaccinated animals at 0 DPC and 6 DPC (Fig. 3g). Interestingly, the VSV-ANDV vaccinated macaque that survived challenge had no difference in BDBV GP cross-reactive IgG titer, but reduced EBOV GP specific IgG titer compared to the ERVEBO vaccinated macaques at study endpoint (35 DPC) (Fig. 3f,g). BDBV cross-neutralizing antibody titers were assessed with data shown as 50% fluorescence reduction ($FRNT_{50}$) of GFP-positive cells. The ERVEBO vaccinated macaques had significantly increased cross-neutralizing antibodies at 35 DPC when compared to -14, 0 and 6 DPC (Fig. 3h). Interestingly, two of the VSV-ANDV vaccinated macaques that succumbed to disease had an increase in neutralizing titer at 6 DPC while the VSV-ANDV vaccinated macaque that survived showed a modest increase at 35 DPC (Fig. 3h). This data highlights the rapid homologous and cross-reactive humoral response generated by ERVEBO.

The current macaque challenge study provides strong evidence that a single dose of ERVEBO conferred rapid cross-protection against BDBV challenge, although breakthrough infection was observed in a single animal. This finding is highly relevant to current outbreak response activities in the DRC because there are currently no approved vaccines or therapeutics available to specifically target BDBV infections since all available commercial medical countermeasures target EBOV.

Since the PHEIC declaration for the current BDBV outbreak in the DRC, public health discussions have focused on accelerating the development of BDBV-specific vaccine candidates. Organizations such as the Coalition of Epidemic Preparedness Innovations (CEPI), in collaboration with academic and commercial partners, have fast-tracked development of vaccines specifically designed to target BDBV. Examples include chimpanzee adenovirus-vectored vaccines [27], mRNA vaccines [28], and VSV-vectored vaccines [29]. Although several candidates have advanced through preclinical development and limited clinical studies have started, none are currently licensed or routinely stockpiled for outbreak response [4]. Once an investigational BDBV-specific vaccine becomes available, deployment would likely occur under a clinical-trial protocol.

Early in the current BDBV outbreak, use of ERVEBO or other EBOV-targeting vaccines were dismissed because evidence for cross-protection against BDBV was considered inconclusive and efficacy unlikely [30]. This view may have also been influenced by the lack of cross-protective efficacy of VSV-EBOV against SUDV in the macaque model [31] and the decision not to deploy ERVEBO during the 2022 SUDV outbreak in Uganda, despite initial requests by the Ugandan government [32]. The situation for the current BDBV outbreak, however, is different from that of the 2022 SUDV outbreak. In 2011, VSV-EBOV was reported to confer partial protection against BDBV challenge [9]. In a subsequent study, simultaneous co-administration of monovalent VSV-EBOV and VSV-SUDV provided limited protection, whereas heterologous prime-boost vaccination resulted in full protection against BDBV challenge [5]. Nevertheless, the evidence for cross-protection against BDBV was considered limited and difficult to interpret largely because of the small group sizes in these studies.

This uncertainty prompted renewed investigation into the cross-protective potential of ERVEBO against BDBV infection. Initial evidence of BDBV-cross-reactive IgG was detected in sera derived from VSV-EBOV vaccinated ferrets [10]. Subsequently, clinical data from individuals previously vaccinated with ERVEBO or Zabdeno/Mvabea demonstrated detectable cross-reactive antibody responses to BDBV. More recently, cross-neutralizing antibodies were identified in sera from human ERVEBO vaccinees [11-14]. Based on the earlier animal studies and the published serology data, our macaque study was designed to assess cross-protection of ERVEBO vaccination in the most relevant preclinical model, the cynomolgus macaque. Our results reinforced the indirect data on cross-reactive antibody responses in ERVEBO or Zabdeno/Mvabea vaccinees and provided proof of cross-protection against BDBV challenge. While finalizing our manuscript, a parallel study reported cross-protection of ferrets against BDBV challenge mediated by ERVEBO vaccination [33]. Another parallel study reported partial cross-protection against BDBV challenge in cynomolgus macaques following ERVEBO vaccination three months before (ATL, AM, unpublished). Together with our study results, there is now increasing preclinical evidence of cross-protective efficacy through ERVEBO vaccination against BDBV challenge.

Assessment of medical countermeasures against BDBV in macaques is more challenging because the model is not uniformly lethal with mortality ranging from about 40% -75% [5-7,9]. In contrast to the variable and asymmetric survival distribution observed in macaques, BDBV challenge in ferrets is lethal and therefore the ferret provides a stringent screening model for evaluating candidate countermeasures [34,35]. At present, small-rodent models

using wild-type BDBV strains are not established. Since the initial inconclusive evidence of cross-protective efficacy had already been generated in macaques [5,9,33], we selected the cynomolgus macaque model as the most relevant model for ERVEBO-mediated cross-protection against BDBV challenge. Compared with control vaccinated macaques, ERVEBO vaccinated animals showed markedly higher survival (83% vs 20%) and displayed significantly reduced clinical disease, BDBV viremia, and BDBV shedding from mucosal membranes. Consistent with responses typically elicited by VSV-based vaccines, ERVEBO vaccination generated a robust specific humoral response against the homologous EBOV GP vaccine antigen, along with IgG cross-reactive antibodies against the unmatched BDBV GP. Homologous humoral vaccine responses were to similar levels as previous preclinical studies, suggesting that the increased clinical dosage of $7.2x10^7$ PFU (clinical dose) used in our study does not impact the magnitude of the vaccine response [36]. Unsurprisingly the magnitude of the BDBV cross-reactive humoral vaccine response was blunted in comparison to a homologous vaccine response [7]. Antibody neutralization testing demonstrated the development of a BDBV cross-reactive, functional antibody response. ERVEBO-vaccinated macaques that survived to the study endpoint showed a significant increase in neutralizing antibody titers over time. Although the surviving macaque in the control cohort also developed neutralizing antibodies as a response to BDBV challenge, the increase in titers from baseline was smaller than that observed in the ERVEBO-vaccinated macaques. These findings suggest a robust functional humoral response in the ERVEBO-vaccinated macaques. However, at the critical timepoint (6 DPC) there was no difference in neutralization titers between groups indicating that antibody-mediated neutralization is not

241 the primary protective mechanism. Significantly higher levels of EBOV GP–specific and
242 BDBV GP cross-reactive IgG at 0 and 6 DPC in ERVEBO vaccinated macaques compared
243 with control VSV-ANDV vaccinated animals further support a role for vaccine-induced
244 antibody responses in protection. These findings are consistent with previous reports
245 associating total antigen-specific IgG levels as correlates of protection in other preclinical
246 filovirus vaccine models [7,37-39]. In humans, a post hoc analysis of the PREVAIL, STRIVE, and
247 FLW trials similarly suggested that GP-specific IgG response could serve as a potential
248 correlate of protection [40].

249 Rapid vaccine-mediated protection is important for filovirus outbreak response, which has
250 been achieved in the past through ERVEBO ring vaccination as shown during EBOV
251 outbreaks in West Africa as well as DRC [23,24]. A hallmark preclinical study in support of the
252 ring vaccination strategy showed that VSV-EBOV completely or partially protected
253 cynomolgus macaques with a single dose given as late as 7 and 3 days before homologous
254 EBOV challenge, respectively [36]. Here we chose a slightly prolonged (14 days) single-dose
255 ERVEBO vaccination scheme to somewhat compensate for the heterologous BDBV
256 challenge situation with the outcome supporting the rapid development of cross-protective
257 immunity against BDBV. Interestingly, a single-dose VSV-EBOV vaccination did not provide
258 protection against SUDV challenge in another cynomolgus macaque study [31]. This result
259 may be explained by the closer phylogenetic relationship of BDBV to EBOV than to SUDV
260 and Reston virus [18]. Thus, our data supports the evaluation of ERVEBO in a ring vaccination
261 trial among contacts of BDBV cases in the current DRC outbreak.

Together, our findings strongly indicate ERVEBO-mediated cross-protection against BDBV. This cross-protection, however, is not complete as evidenced by the one ERVEBO vaccinated macaque that developed fulminant BDBV disease with progression similarly observed in most control macaques that succumbed to disease. This drawback may be a risk for public health intervention but also is a vast improvement in a scenario of no available vaccine options as currently in the DRC outbreak. A not fully protective vaccine can still play a vital part in aiding in the control of an outbreak. Epidemiological modeling studies have suggested that a vaccine with a 45% cross-protective efficacy rate can reduce mortality by an additional 24.8% when implemented in a reactive 2 ring vaccine program [40].

Our study has some limitations. First, as in previous macaque studies, group sizes remain relatively small, although our group sizes were larger than those in earlier reports, making it difficult to reach significance. Larger group sizes would be preferable for statistical evaluation to improve precision, but availability of cynomolgus macaques is limited, ethical considerations of the use of macaques must be balanced, and maximum biocontainment space is restricted for larger animal species. Our study design was a consequence of those issues. Second, the BDBV macaque model is not uniformly lethal, which complicates assessment of medical-countermeasure efficacy. Nevertheless, we believe that the stringent infection and partially lethal BDBV cynomolgus macaque model provides convincing and sufficient clinical, pathological, virological, and serological readout parameters to evaluate vaccine-mediated protection or cross-protection. Third, we did not perform an in-depth immunological characterization to fully assess differences in

humoral and cellular responses between the groups. This analysis will take time and is planned for a follow-up study.

The World Health Organization recently issued emergency guidance and allocated ERVEBO doses to be used for frontline workers and clinical studies in the ongoing, uncontrolled BDBV outbreak in the DRC [41]. Because use of ERVEBO against BDBV remains investigational, recipients are fully informed of the potential risks, benefits, and limitations, particularly regarding cross-species efficacy. Priority groups for preventive vaccination include health-care workers, burial-team members, and other frontline responders. ERVEBO is also used for contacts of confirmed BDBV cases as part of a ring-vaccination strategy. At the time of this article, the Tumaini Phase 2/3 ring vaccination trial (NCT07842107) was currently poised to implement ERVEBO as a medical countermeasure. Therefore, our findings are extremely timely and highly relevant as they strengthen the strategic change in public health response for this outbreak. Until a BDBV-specific vaccine becomes available, ERVEBO will likely serve as an important interim tool alongside established outbreak-control measures, including rapid case detection, isolation, optimized supportive care, contact tracing, infection prevention and control, and safe burials.

METHODS

Biosafety and ethics. The study was performed in the maximum containment laboratory (BSL4) at Rocky Mountain Laboratories, National Institute of Allergy and Infectious

Diseases (NIAID), National Institutes of Health (NIH). All work, including sample processing and inactivation was approved by the Institutional Biosafety Committee (IBC) and followed IBC-approved Standard Operating Protocols. This project was also reviewed and approved by the NIH Dual Use Research of Concern-Institutional Review Entity (DURC-IRE). The animal work was performed in accordance with the approved IACUC and following recommendations from the Guide for the Care and Use of Laboratory Animals of the Office of Animal Welfare, National Institutes of Health and the Animal Welfare Act of the US Department of Agriculture, in an AAALAC-accredited facility. Cynomolgus macaques (*Macaca fascicularis*) were individually housed in cages placed adjacent to one another to encourage social interaction. Each macaque was also given manipulanda and commercial toys with videos and music in the room for entertainment. Treats and fruit were given twice daily with commercial monkey chow; water was provided *ad libitum*. Macaques were maintained in a climate-controlled room with a fixed 12-hour light–dark cycle. Human interaction occurred daily throughout the study period.

Study design. Twelve Cynomolgus macaques (6 males and 6 females; age 6-18 years; weight 2.5-11 kg, Indonesian origin) were randomly divided into two groups. Unfortunately, one macaque, which had to be euthanized for animal welfare reasons prior to study start, could not be replaced. Therefore, one group (n=5) received $1.5x10^7$ PFU of VSV-ANDV that served as the control group. A second group (n=6) received $7.2x10^7$ PFU of ERVEBO, which is the approved human clinical dose. Macaques were vaccinated IM with equal amounts in each bicep at 14 days prior to challenge (-14 DPC). On 0 DPC, $1x10^4$ $TCID_{50}$ of BDBV ($5.62x10^3$ $TCID_{50}$/mL back-titration) was administered via IM injection into the caudal thigh.

Clinical scores were taken daily in accordance with the IACUC-approved scoresheet (Extended Data Table 1). Scoring was based on the following criteria: general appearance and activity level, appearance of skin and coat, bodily fluid discharge, respiration frequency, feces and urine output, and appetite. The predetermined end point was a score of 35 or higher. Clinical examinations were physically conducted during the vaccination period (-14, -13, -11, -7 DPC) and following challenge (0, 1, 3, 6, 9, 14, 21, 28 and 35 DPC). Swabs (oral and rectal) and blood samples were collected for the purpose of determining viral load, as well as hematology, serum biochemistry and antibody levels. Tissue samples were collected for the purpose of determining viral load.

Viruses and cells. Vero E6 cells (Mycoplasma negative, RRID: CVCL_0059) were grown at 37 °C and 5% CO2 in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, St. Louis, MO) containing 10% fetal bovine serum (FBS) (Wisent Inc., St. Bruno, Canada), 2 mM L-glutamine, 50 U/mL 300 penicillin, and 50 mg/mL streptomycin (all supplements from Thermo Fisher Scientific, 301 Waltham, MA). BDBV strain 200706291 (GenBank accession No. MK028856.1), was propagated and titered on Vero E6 cells, and stored in liquid nitrogen until challenge. Viral stocks were confirmed by sequencing and confirmed to be mycoplasma negative.

Vaccine candidates. Generation, growth and titration of the VSV-ANDV has been described previously [42]. The ERVEBO vaccine used was a current clinical lot.

Viral load determination. Inactivated whole blood, swab medium (DMEM supplemented with 2% Penicillin and Streptomycin) or clarified tissue homogenate were used for RNA

extraction with the QIAamp Viral RNA Kit (Qiagen) per the manufacturer's instructions. RNA was detected using a BDBV RT-qPCR assay for the detection of the BDBV L gene. Isolated RNA was run using Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs) with 5µl sample RNA and 20 µl reaction mix containing 10 µM forward primer 5'-GAAGGGTCAGGTGCCTTATTAC-3', 10 µM reverse primer 5'-GTGTGGTTGTCCCAGAAACT-3', and 10 µM probe 5'-FAM-ACAGAGCATAGCATCGAGGCAGAA-3'-3IABkFQ. Primers and probes were acquired from Integrated DNA Technologies (Coralville, Iowa, USA). RT-qPCR reactions were performed on QuantStudio 5 thermocycler. BDBV standards with known infectious titers were used to calculate the standard curve in infectious equivalents.

Hematology and blood chemistry. Blood samples were collected in tubes containing EDTA and hematology data was determined via Zoetis Vetscan HM5 (Zoetis, Parsippany, NJ, USA). Serum biochemistry was analyzed on a Vetscan VS2 Chemistry Analyzer (Zoetis, Parsippany, NJ, USA).

Serology-ELISA. Nunc MaxiSorp flat bottom 96-well plates (Thermo Fisher Scientific) were coated with 100 ng in 100 µl/well of recombinant BDBV-GP minus the transmembrane region; BDBV GPΔTM (IBT Bioservices, 0505-015) and recombinant EBOV-GP minus the transmembrane domain; EBOV GPΔTM (IBT Bioservices, 0501-001). Plates were refrigerated overnight, blocked with 150 µL of 5% casein in 1x PBS (ThermoFisher), and incubated with four-fold serially diluted macaque sera (beginning at 1:100) in duplicate. Immunoglobulin G (IgG) antibodies were detected by peroxidase-labelled Goat anti-Monkey IgG (H+L) Secondary Antibody (ThermoFisher, PA1-84631) at a dilution of 1:2000 in 5% casein followed by 3,3',5,5'-Tetramethylbenzidine 2-component peroxidase substrate

(TMB) (Seracare, 5120–0047) and stop solution (Seracare, 5150-0021). The optical density was measured at 450 nm (OD450) immediately following the addition of the stop solution. Wells were washed three times with 1x PBS containing 0.1% Tween in between each step, with a final six washes prior to the addition of TMB.

Neutralization assay. Neutralization assay with VSV-BDBV GP-GFP was performed as previously described [43]. The VSV-BDBV GP-GFP assay was optimized for incubation of the serum/virus mix on the Vero E6 cells lasted for 18 hours at 37°C. Samples were run on the FACSymphony A5 Cell Analyzer (BD Biosciences, Mississauga, ON, Canada) and the GFP-positive cell count was analyzed using FlowJo v. 10 software.

Statistical analysis and software. Data distribution was assumed to be non-parametric in nature, but this was not formally tested. Due to species constraints, low numbers of macaques and subsequent samples could be collected. Data collection and analysis were performed blinded. Significance tests were performed as indicated where appropriate for the data using GraphPad Prism 10-11 and Claude Haiku 4.5. Unless stated otherwise, statistical significance levels were determined as follows: ns = $p > 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** = $p \leq 0.0001$. The exact nature of tests is stated where appropriate.

Data availability. The datasets that support the findings of this study are available in figshare with the identifier DOI: 10.6084/m9.figshare.34888785.

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AUTHOR CONTRIBUTION

All authors contributed to the study and manuscript as follows: Conceptualization: Kyle O'Donnell, Paige Fletcher, Andrea Marzi, Kyle Rosenke & Heinz Feldmann; Methodology: all authors; Investigation: all authors; Funding acquisition: Heinz Feldmann; Project administration: Kyle Rosenke & Heinz Feldmann; Supervision: Kyle Rosenke & Heinz Feldmann; Writing – original draft: Kyle Rosenke, Kyle O'Donnell, Paige Fletcher & Heinz Feldmann; Writing – review & editing: all authors.

COMPETING INTERESTS:

Heinz Feldmann claims intellectual property for VSV-based vaccines against viral hemorrhagic fevers. All other authors declare any competing interests.

FIGURES

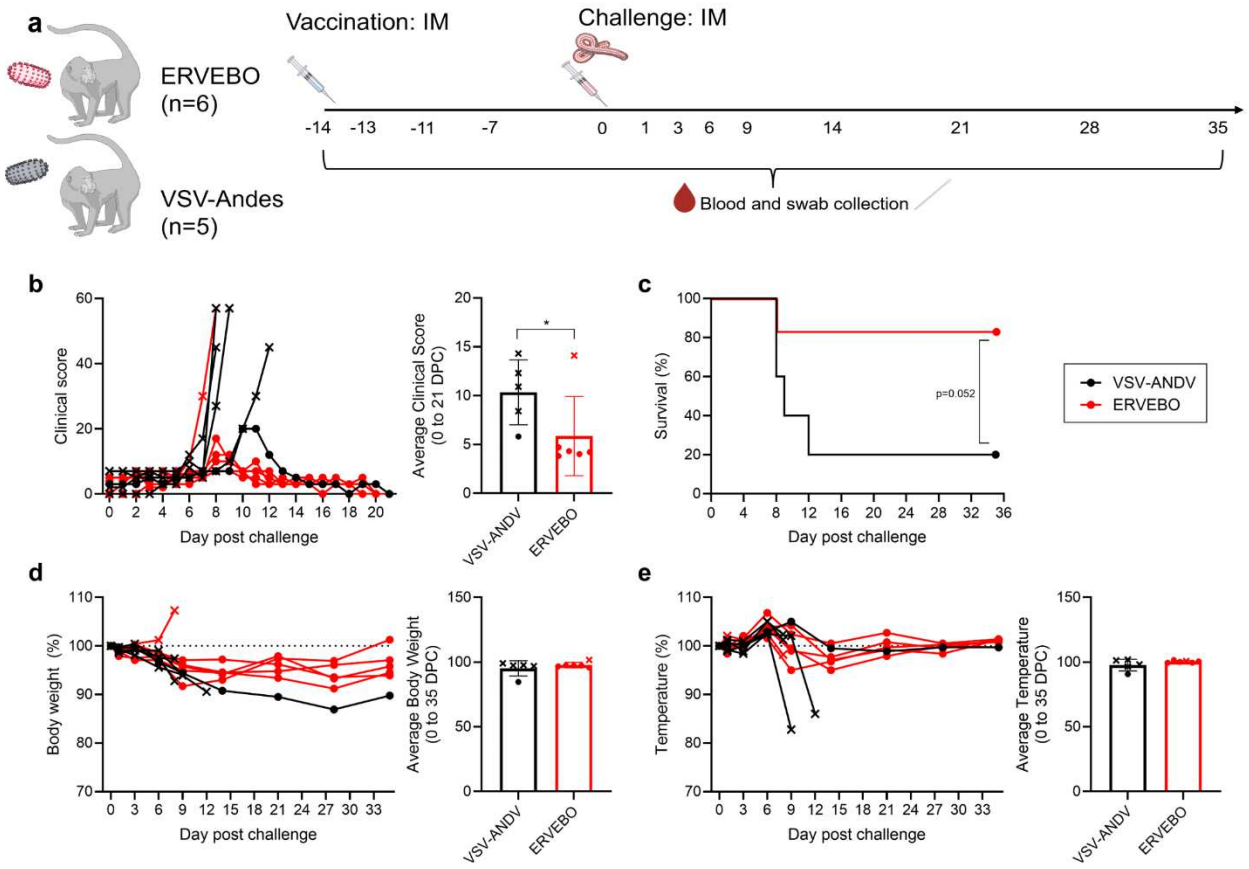
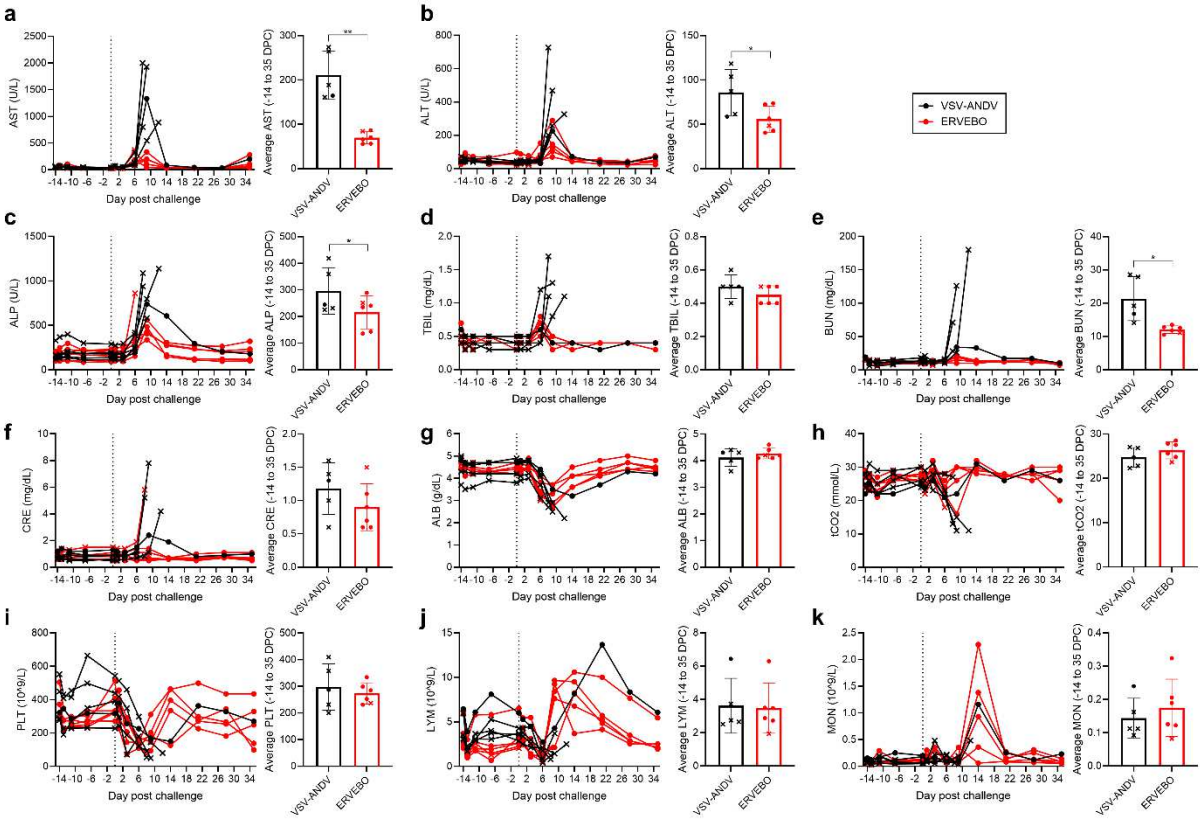


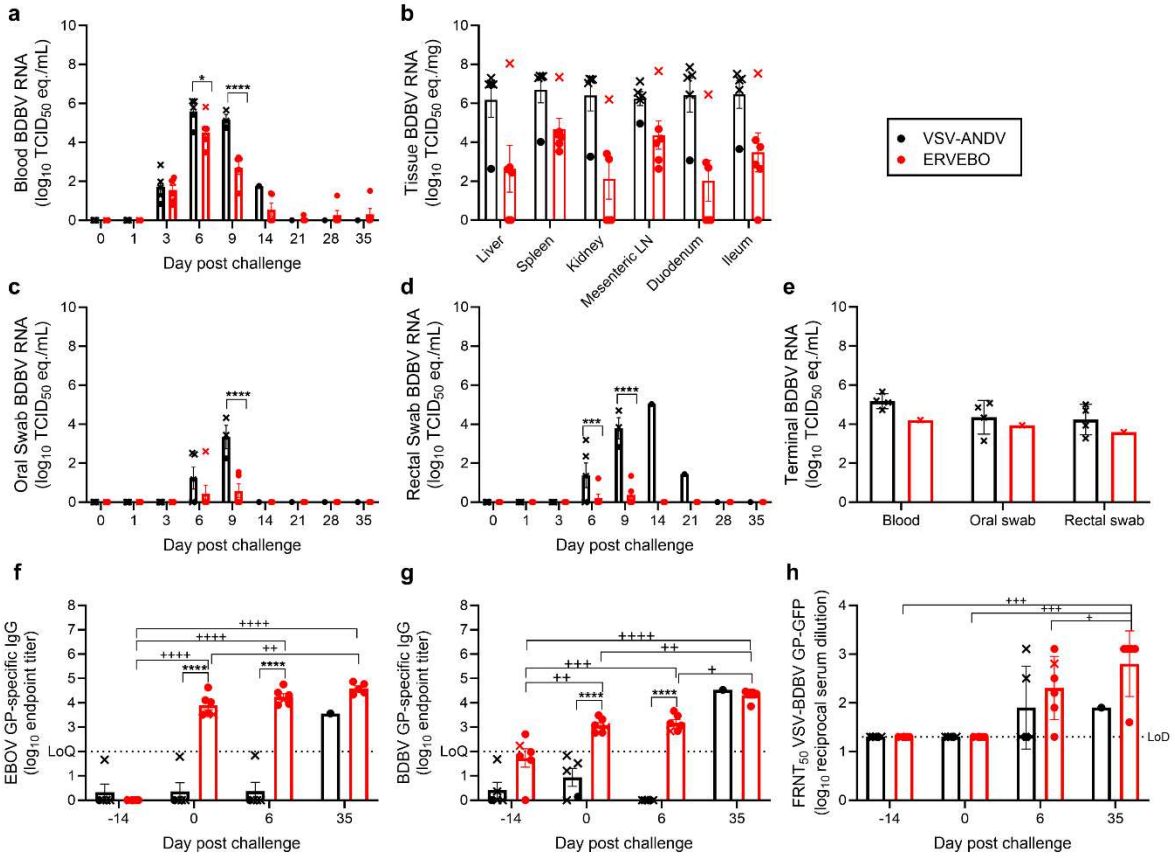
Fig 1: Study design and clinical changes in macaques after BDBV challenge. a, Study design. **b**, Clinical score. **c**, Survival curve with comparison determined by Mantel-Cox test. **d**, Body weight percentage normalized to the day of challenge (0 DPC). **e**, Body temperature percentage normalized to the day of challenge (0 DPC). Dotted line denotes normalization at 100% (**d,e**). Data is shown as individual macaques. Macaques that succumbed are depicted with an “X”. Statistical significance by unpaired t-test with Welch’s correction (one-tailed p-value) of averaged values over time shown as mean +/- SD (**b**).



574

575 **Fig 2: Biochemistry and hematology analysis after BDBV challenge.** Biochemical
576 marker levels measured in the serum and hematology analysis measured in the blood.
577 **a**, Aspartate aminotransferase (AST). **b**, Alanine aminotransferase (ALT). **c**, Alkaline
578 phosphatase (ALP). **d**, Total bilirubin (TBIL). **e**, Blood urea nitrogen (BUN). **f**, Creatinine
579 (CRE). **g**, Albumin (ALB). **h**, Total carbon dioxide (tCO2). **i**, Platelets (PLT). **j**,
580 Lymphocytes (LYM). **k**, Monocytes (MON). Dotted line denotes the day of challenge (0
581 DPC). Data is shown as individual macaques. Macaques that succumbed are depicted
582 with an “X”. Statistical significance by unpaired t-test with Welch’s correction (one-tailed
583 p-value) of averaged values over time shown as mean +/- SD.

584



585

586 **Fig 3: BDBV viral load & serology after challenge.** BDBV RNA levels in the blood (**a**),
587 tissue samples (**b**), oral swabs (**c**), rectal swabs (**d**), and terminal endpoint samples (**e**).
588 Serum levels were assessed at day of vaccination (-14 DPC), challenge (0 DPC),
589 established disease (6 DPC), and convalescence (35 DPC) for EBOV GP-specific IgG
590 titers (**f**), BDBV GP-specific IgG titers (**g**), and VSV-BDBV neutralizing antibody titers
591 presented as $FRNT_{50}$ of GFP-positive cell count (**h**). Data is shown as mean +/- SEM or
592 geometric mean +/- geometric SD. Macaques that succumbed are depicted with an "X".
593 Statistical significance by ordinary one-way ANOVA with Tukey's multiple comparisons
594 test compared within groups (+) (**f,g,h**) or by two-way ANOVA with Sidak's multiple

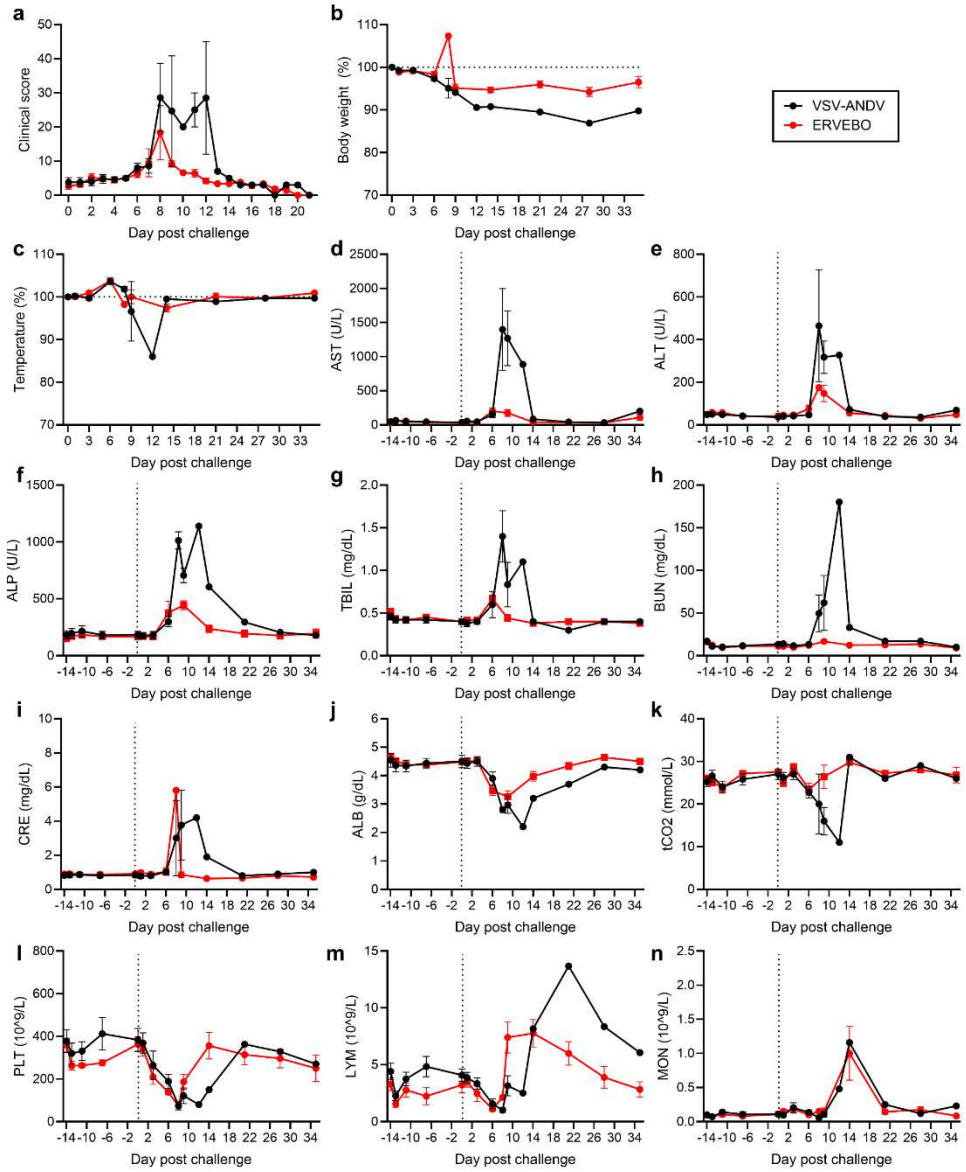
comparisons test compared between groups (*) (**a,c,d,f,g**). Dotted line denotes the limit of quantification (LoQ) (**f,g**) or the limit of detection (LoD) (**h**).

EXTENDED DATA – FIGURE / TABLE LEGENDS

Extended Data Table 1: Clinical score sheet parameters

Parameter	Clinical Signs	Score*
General appearance	Normal, alert	0
	Abnormal or hunched posture, dull appearance to eyes	5
	Dehydration, notable weight loss, obvious swelling, tissue mass	10
	Bleeding from any orifice (not related to routine procedures or menstruation)	15
Skin and fur	Normal	0
	Ruffled fur, unkept appearance	5
	Rash, pallor, redness, icterus, petechiae, ecchymoses, wound, abscess, ulcer	10
Nose, mouth, eyes, head	Normal	0
	Nasal discharge, excessive salivation, ocular discharge, lacrimation, reddened eyes, ptosis	5
Respiration	Normal	0
	Increased or decreased respirations, cough, sneezing	5
	Dyspnea, open mouth breathing, cyanosis	15
Feces and urine	Normal consistency and volume	0
	Decreased feces, dry, wet and pasty, watery feces, discoloration, decreased urine, discoloration	2
	Feces absent	5
	Liquid feces (if debilitating), urine absent, blood in feces or urine	10
Food intake	Normal (eating more than half the biscuits)	0
	Decreased (eating less than half the biscuits)	3
	Eating fruit but no biscuits	5
	Severely decreased (not eating biscuits or fruit)	10
Locomotor	Normal	0
	Hyperactivity (circling, increased aggression), hypoactivity (hunched, inactive on camera, active with people in room)	5
	Ataxia, neurological signs (tremors, head tilt), loss of interest in treats	10
	Reluctant to move, uses cage for support, difficulty getting to food or water (decreased response to human presence), seizures	15
	Down with no or minimal response to human approach, coma	35

*Macaques who reached a score ≥35 met the humane endpoint criteria



616

617 **Extended Data Fig 1: Clinical changes with biochemical and hematological**

618 **analysis in macaques after BDBV challenge. a**, Clinical score. **b,** Body weight

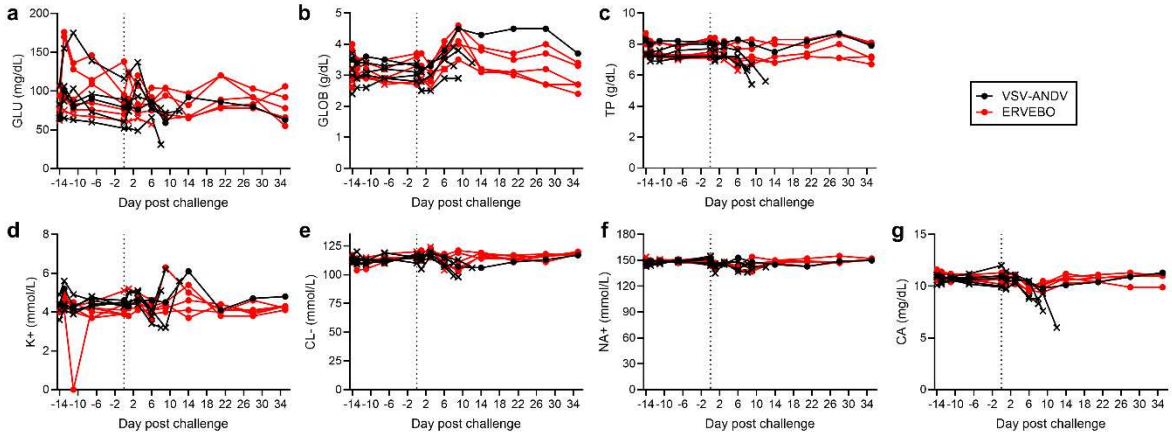
619 percentage normalized to the day of challenge (0 DPC). **c**, Body temperature

620 percentage normalized to the day of challenge (0 DPC). Biochemical analysis in the

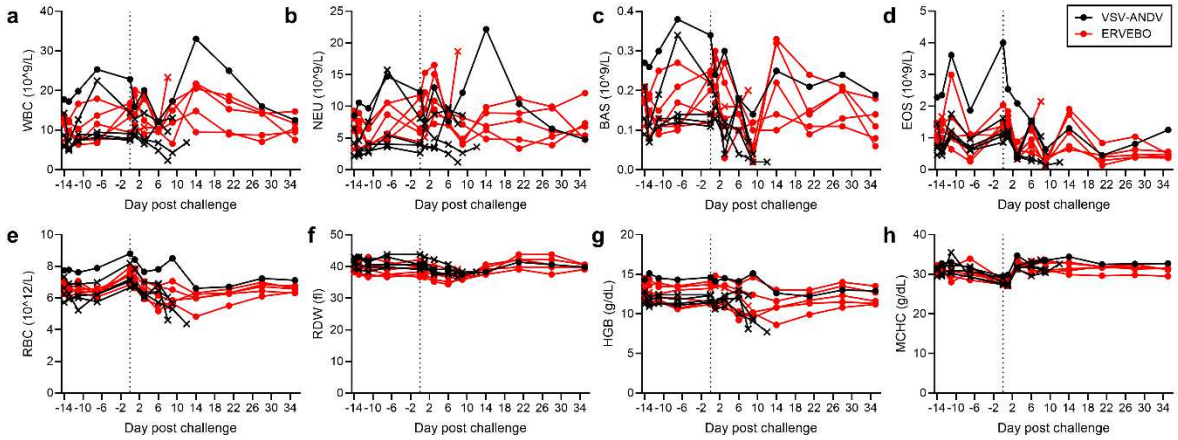
621 serum for aspartate aminotransferase (AST) (**d**), alanine aminotransferase (ALT) (**e**),

622 alkaline phosphatase (ALP) (**f**), total bilirubin (TBIL) (**g**), blood urea nitrogen (BUN) (**h**),

creatinine (CRE) (**i**), albumin (ALB) (**j**), and total carbon dioxide (tCO2) (**k**). Hematological analysis in the blood for platelets (PLT) (**l**), lymphocytes (LYM) (**m**), and monocytes (MON) (**n**). Data is shown as mean +/- SEM. Dotted line denotes either normalization at 100% (**b,c**) or the day of challenge (0 DPC) (**d-n**).



Extended Data Fig 2: Biochemical markers in serum of macaques at vaccination and after BDBV challenge. a, Glucose (GLU). **b**, Globulin (GLOB). **c**, Total protein (TP). **d**, Potassium (K+). **e**, Chloride (CL-). **f**, Sodium (NA+). **g**, Calcium (CA). Dotted line denotes the day of challenge (0 DPC). Data is shown as individual macaques. Macaques that succumbed are depicted with an “X”.



638

639 **Extended Data Fig 3: Hematology analysis in blood of macaques at vaccination**

640 **and after BDBV challenge. a,** White blood cells (WBC). **b**, Neutrophils (NEU). **c**,

641 Basophils (BAS). **d**, Eosinophils (EOS). **e**, Red blood cells (RBC). **f**, Red cell distribution

642 width (RDW). **g**, Hemoglobin (HGB). **h**, Mean corpuscular hemoglobin concentration

643 (MCHC). Dotted line denotes the day of challenge (0 DPC). Data is shown as individual

644 macaques. Macaques that succumbed are depicted with an “X”.

645

Supplementary Files

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- RosenkeetalExtendedData.docx