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Supplementary Materials for

Single-dose VSV-based vaccine protects against Kyasanur Forest disease in nonhuman primates

Bharti Bhatia *et al.*

Corresponding author: Heinz Feldmann, feldmannh@niaid.nih.gov

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Figure S1: Study layout. Pigtailed macaques were vaccinated intramuscularly with $1x10^7$ PFU of either VSV-KFDV (study group; n=6) or VSV-EBOV (control group; n=4) at D-28, challenged subcutaneously and intravenously with $1x10^5$ $TCID_{50}$ of KFDV each at D0, and euthanized for necropsy at D9. Animals were monitored throughout the study at least twice daily for clinical signs and examinations, including blood draw, swabs and body weight determination, were performed at D-28, D-27, D-25, D-18, D0, D1, D3, D6, and D9. Samples were used for virology, immunology and pathology.

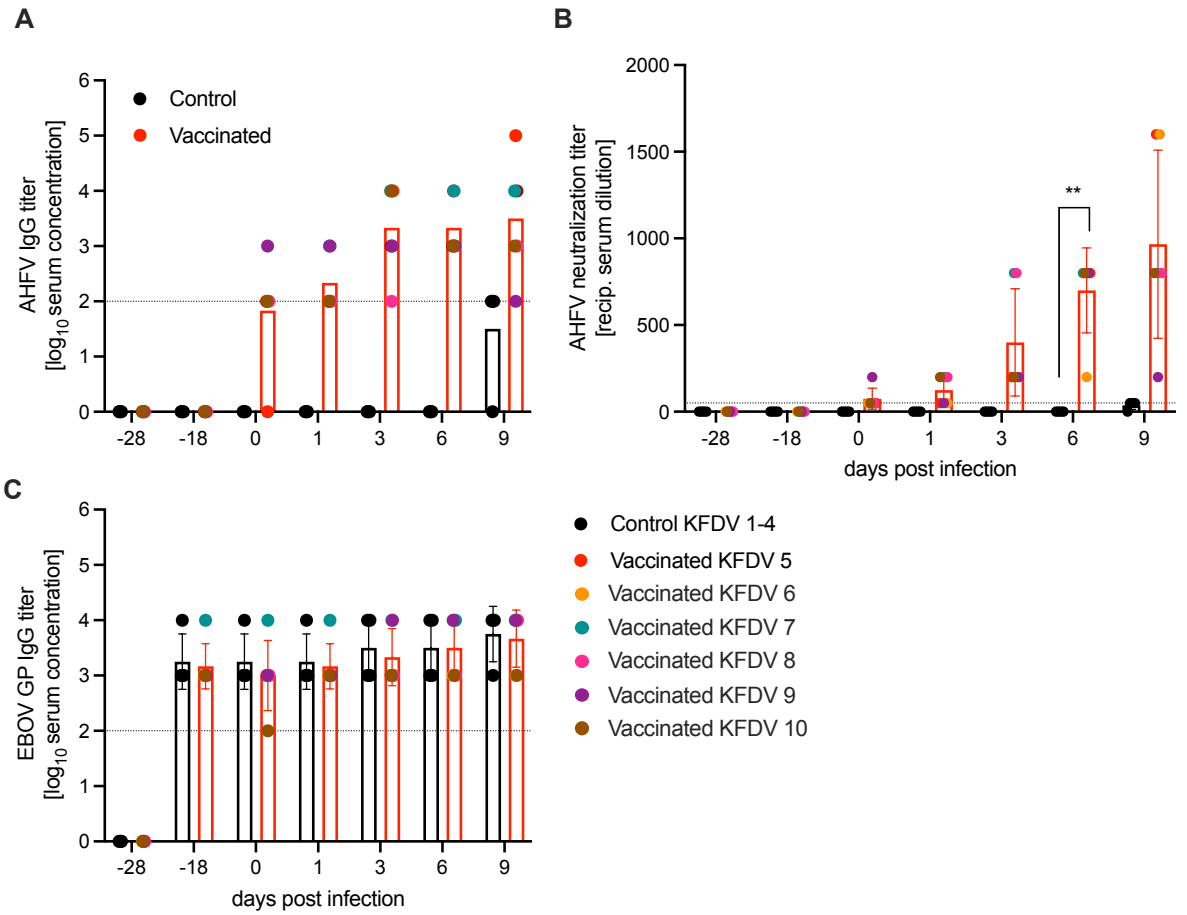

Figure S2: Cross-reactive humoral immune responses after VSV-KFDV vaccination and KFDV challenge. (A) Total IgG-specific antibodies to AHFV were determined by ELISA on serum samples collected from animals immunized with VSV-KFDV (n=6), and VSV-EBOV (n=4). (B) The same serum samples were tested for their neutralizing activity against AHFV. The highest titer that completely neutralized 100 $TCID_{50}$ of AHFV are shown. (C) Total IgG-specific antibodies to EBOV GP were determined by ELISA on the same serum samples. Each dot represents a single animal. The dotted lines represent assay limits. Statistical significance was analyzed using two-way Anova and Tukey's multiple comparisons; significant results are indicated as $^{**}p < 0.01$.

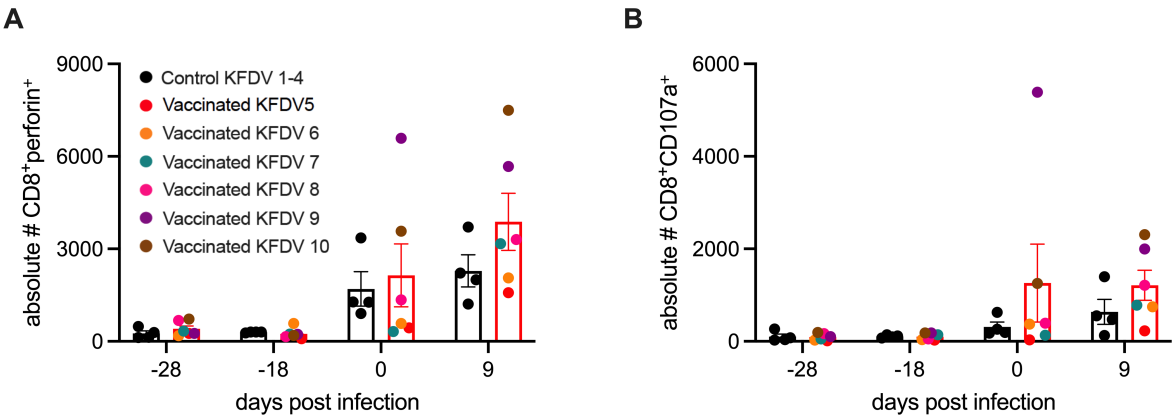

Figure S3: Adaptive immune responses after VSV-KFDV vaccination and KFDV challenge. (A-B) VSV-KFDV-specific T-cell responses. Cryopreserved PBMCs were stimulated with pooled overlapping peptides derived from the KFDV E protein and analyzed by flow cytometry. All measurements were performed in duplicate for each animal. (A) $CD8^{+}$perforin, and (B) $CD8^{+}$ CD107a. Statistical significance between control (black, VSV-EBOV) or study (red, VSV-KFDV) macaques was analyzed by two-way Anova with Sidak's multiple comparisons.

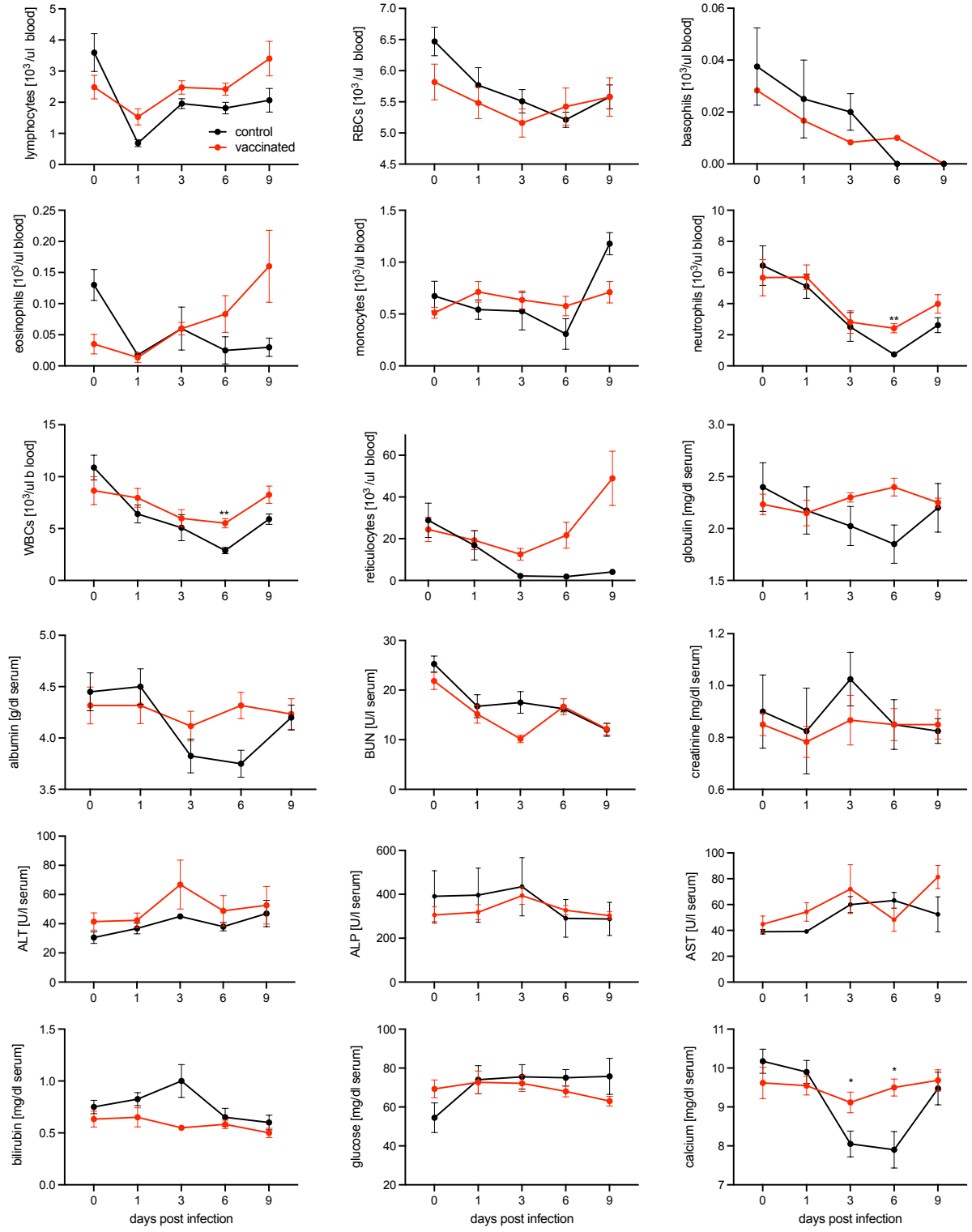

Figure S4: Hematologic and blood chemistry parameters post KFDV challenge.
Hematology was performed on EDTA blood and blood chemistry on serum samples collected

from animals immunized with VSV-KFDV (n=6), and VSV-EBOV (n=4). Lymphocytes, red blood cells (RBCs), basophils, eosinophils, monocytes, neutrophils, white blood cells (WBC), reticulocytes, globulin, albumin, blood urea nitrogen (BUN), creatinine, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin, glucose, and calcium. Data are shown as mean plus standard error of the mean. Statistical significance was analyzed using two-way Anova with Sidak's multiple comparisons; significant results are indicated as $*p < 0.05$, $**p < 0.01$.

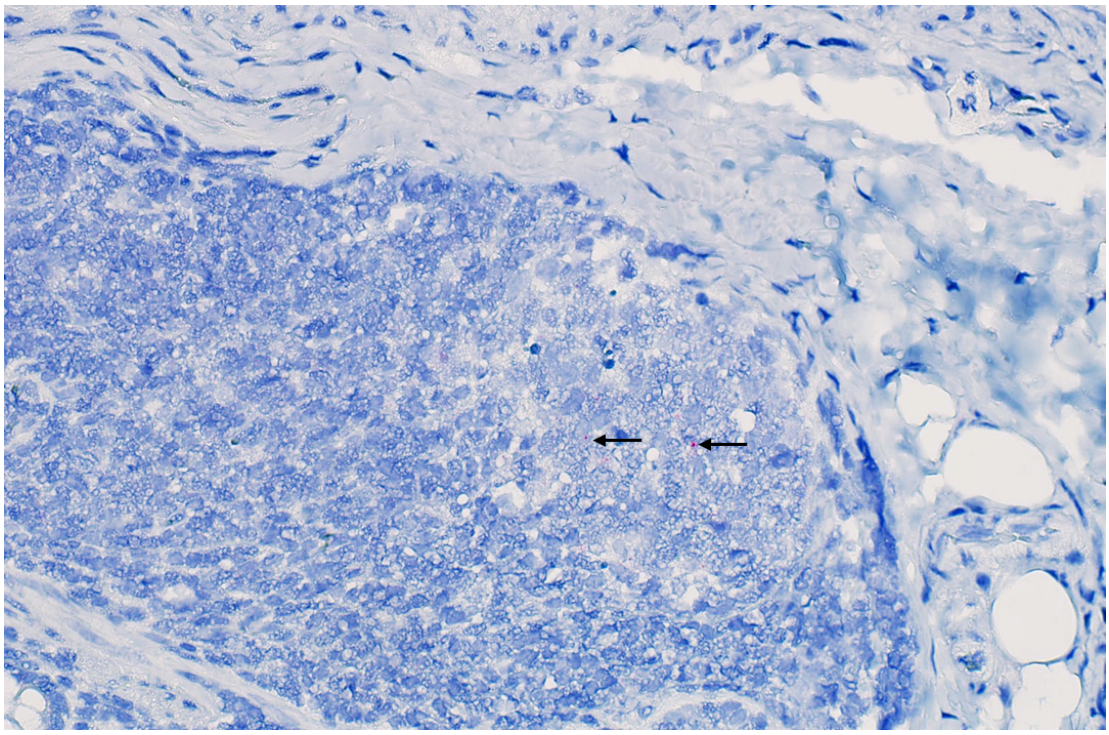

Figure S5: Detection of KFDV genomic RNA in colon section. Animals were euthanized and necropsied at D9 following KFDV challenge. Formalin-fixed tissue section were processed for i*n-situ* hybridization targeting KFDV genome RNA. The image shows a section from a lymphoid follicle in the colon of a representative vaccinated animal with only minimal KFDV genomic signal (magnification at 400x).

Monkey	Signs
KFDV 1	Severely decreased appetite, piloerection, clear nasal discharge, hunched posture, tired, slow movements, mild facial edema, irregular respirations, muddy mucus membranes day 6&9
KFDV 2	Severely decreased appetite, hunched posture, slow& careful movements, abdominal respirations, rubbing his nose, mildly dehydrated (5-8%), elevated temperature on day 1
KFDV 3	Decreased appetite, nasal discharge, irregular abdominal breathing, hunched posture, slow& tired, shivering
KFDV 4	Severely decreased appetite, bloody nasal discharge, hunched posture, increased irregular respirations
KFDV 5	normal
KFDV 6	Decreased appetite
KFDV 7	Decreased appetite
KFDV 8	Decreased appetite
KFDV 9	Decreased appetite
KFDV 10	Decreased appetite

Table S1: Clinical observations after KFDV challenge. Animals KFDV 1-4 were VSV-EBOV vaccinated (control group) and animals KFDV 5-10 were VSV-KFDV vaccinated (study group). All animals were challenged with $2x10^5$ $TCID_{50}$ of KFDV 28 days after vaccination. Animals were monitored twice daily for clinical signs form D0 (challenge) to D9 (necropsy).

Monkey	Signs
KFDV 1	Liver: chronic congested, enlarged, reticulated, rounded edges, hemorrhage Spleen: enlarged, firm, turgid Heart: flabby ventricle, rounded, large R ventricle plaque in pulmonary; thickened valves Lungs: tan aspects, possible HF Brain& meninges: injected
KFDV 2	Spleen: enlarged, firm, turgid Mesenteric LN: enlarged Lungs: hemorrhagic, ecchymosis, L7R dorsal & ventral Brain& meninges: injected
KFDV 3	Brain & meninges: injected
KFDV 4	Bilateral epistaxis
KFDV 5	Crusted skin lesion on back, site of subcutaneous inoculation
KFDV 6	Small intestine: ileus
KFDV 7	Small intestine: ileus
KFDV 8	normal
KFDV 9	Liver: pale
KFDV 10	Liver: pale

Table S2: Gross pathology after KFDV challenge. Animals KFDV 1-4 were VSV-EBOV vaccinated (control group) and animals KFDV 5-10 were VSV-KFDV vaccinated (study group). All animals were challenged with $2x10^5$ $TCID_{50}$ of KFDV 28 days (D0) after vaccination and euthanized for necropsy on D9.