Supplementary Information for

A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against lethal Nipah virus disease

Stephanie L. Foster¹²³, Courtney Woolsey¹²³, Viktoriya Borisevich¹², Krystle N. Agans¹², Abhishek N. Prasad¹², Daniel J. Deer¹², Joan B. Geisbert¹², Natalie S. Dobias¹², Karla A. Fenton¹², Robert W. Cross¹², Thomas W. Geisbert¹²*.

¹Galveston National Laboratory, University of Texas Medical Branch, Galveston, TX, USA, 77555-0610.

²Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA, 77555-0610.

³These authors contributed equally to the work.

*Corresponding author: Thomas W. Geisbert, 301 University Blvd. Rte. 0610, Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX, USA, 77555-0610, (409) 266-6906, twgeisbe@utmb.edu

Email: twgeisbe@utmb.edu

This PDF file includes:

- Supplementary Materials and Methods
- Figures S1 to S4
- Tables S1 to S2
**Supplementary Materials and Methods**

**Cloning of pVSV-ΔG-NiV<sub>B</sub>G.** The pVSV-ΔG-NiV<sub>B</sub>G plasmid was constructed by Gibson assembly of fragments encoding the NiV<sub>B</sub>G gene and the pVSV-ΔG backbone. To generate vector material for Gibson assembly, a previously constructed pVSV-ΔG plasmid was digested with MluI-HF and AvrII restriction enzymes (New England Biolabs) and purified by SDS-PAGE electrophoresis. Oligonucleotide PCR primers designed with large overhangs for Gibson assembly were manufactured at IDT. Inserts were generated by using the primers to amplify the NiV<sub>B</sub>G gene out of the previous pVSV-ΔG-NiV<sub>B</sub>G-GFP construct by PCR using Platinum<sup>TM</sup> SuperFi<sup>TM</sup> DNA polymerase according to the manufacturer's instructions. The full-length pVSV-ΔG-NiV<sub>B</sub>G construct, encoding rVSV-ΔG with NiV<sub>B</sub>G in place of VSV G in the pBluescript (pBS) plasmid backbone, was assembled using the NEBuilder<sup>®</sup> HiFi DNA Assembly Cloning Kit according to the manufacturer's instructions. Presence of the NiV<sub>B</sub>G gene was confirmed, and cloning borders were checked by Sanger sequencing and diagnostic restriction digest. A large culture of *E. coli* transformed with the construct was grown, and plasmid was purified by alkaline lysis cesium chloride plasmid preparation.

**Recovery of rVSV-ΔG-NiV<sub>B</sub>G.** BHK-21 clone WI-2 cells (from Dr. Michael A. Whitt, The University of Tennessee Health Science Center, Memphis, TN) were maintained in high-glucose DMEM supplemented with 5% heat-inactivated FBS, 1% penicillin/streptomycin solution (10,000 units/ml penicillin and 10,000 µg/ml streptomycin), and 1% GlutaMAX Supplement. The VSV G-complemented rVSV-ΔG-NiV<sub>B</sub>G G vaccine construct was recovered using a protocol modified from Dr. Michael A. Whitt (77). Cells were seeded in 6-well plates to be 70 to 80% confluent for infection and transfection the next day. First, the cells were infected with modified vaccinia virus expressing bacteriophage T7 polymerase (vTF7-3; ATCC; Cat. No. VR-2153) at an MOI of 5. Immediately afterward, cells were transfected with pBS-VSV G, pBS-VSV N, pBS-VSV P, pBS-VSV L, and the full-length pVSV-ΔG-NiV<sub>B</sub>G G plasmid (all under the control of the T7 promoter) at a ratio of 8:3:5:1:5 using TransfectACE reagent at a ratio of 3.5 µL per µg of plasmid DNA. Plates were incubated at 37°C and 5% CO2 for 4-5 hours, at which point the growth medium was changed. Plates were returned to the incubator for 48 hours to allow production of infectious virions.

Recovered virus supernatants were subsequently filtered through a 0.22-µm Millex-GS syringe filter to remove contaminating vTF7-3 and passaged on VSV G-complemented BHK cells for plaque purification. Positive p1 supernatants were collected when VSV-specific cytopathic effect was observed (~24 hours), clarified to remove cell debris, and used to infect 10-centimeter cell culture dishes of VSV G-complemented BHK cells at an MOI of 1 for p2. After VSV-specific cytopathic effect was observed, the supernatant was collected, clarified, and aliquoted into 2-mL screwcap tubes for storage of p2 rVSV-ΔG-NiV<sub>B</sub>G seed stocks at -80°C.

**Characterization of rVSV-ΔG-NiV<sub>B</sub>G Vaccine.** Viral RNA was isolated from a p2 seed stock in TRizol LS Reagent using the Direct-zol RNA miniprep kit (Zymo Research) according to the manufacturer's instructions. The complete viral RNA genome was sequenced with the NextSeq 550 system with a depth of 130 million reads. DNA was extracted from p2 seed stock in TRizol LS according to the manufacturer's instructions for Mycoplasma testing with the e-Mycoplus Mycoplasma PCR Detection Kit (LilIF Diagnostics). The p2 seed stock was subjected to endotoxin testing using Endosafe<sup>®</sup>-PTS Limulus Amebocyte Lysate cartridges (Charles River Laboratories).

The vaccine was tested and found to be negative for mycoplasma and endotoxin contamination, and cloning borders and the NiV<sub>B</sub>G gene were sequenced using Sanger sequencing. The complete sequences of viral RNA and full-length plasmid were obtained through next-generation sequencing using the NextSeq 550 system and were found to match the expected sequences.

**Immunofluorescence Assay.** To check for expression of NiV<sub>B</sub>G in rVSV-ΔG-NiV<sub>B</sub>G G-infected cells, BHK-21 clone WI-2 cells and Vero 76 cells were seeded in 6-well plates at a density of 2 × 10<sup>6</sup> cells per well to be 50- to 70% confluent for infection the next day. Cells were infected with rVSV-ΔG-NiV<sub>B</sub>G at an MOI of 3 or mock-infected with growth media only. At eight hours post...
infection, cells were fixed with a solution of 4% paraformaldehyde in water and then quenched overnight in PBS with 100 mM glycine. Half of the wells for each cell line were permeabilized with 0.5% TritonX-100 in PBS-glycine, while the remaining wells were processed without permeabilization. All wells were blocked for one hour in 3% sterile BSA in PBS (hereafter known as blocking buffer). Conditions receiving m102.4 primary antibody for the detection of the NiVΔG attachment glycoprotein (kindly provided by Dr. Christopher Broder, Uniformed Services University of the Health Sciences, Bethesda, MD) were incubated with 50 µg/mL antibody in blocking buffer at 4°C overnight, while secondary-only infected wells were incubated in blocking buffer only at 4°C overnight. All wells were washed with blocking buffer and then incubated with secondary antibody solution comprising goat anti-human IgG conjugated to Alexa Fluor™ 488 (dilution 1:5,000) in blocking buffer for one hour, protected from light. Wells were then washed with blocking buffer and stored under PBS-glycine for imaging on a Nikon Eclipse Ti inverted fluorescent microscope. Images were obtained using the fluorescein isothiocyanate filter and were exposed for 300 milliseconds at 20% power. Magnification from eyepiece and objectives totaled 100X.

**Challenge virus.** The isolate of NiVΔG used in the study (200401066) was obtained from a fatal human case during the outbreak in Rajbari, Bangladesh in 2004. The challenge material was passaged on Vero E6 cells twice. Four notable mutations of sufficient frequency were found between the P2 stock of NiVΔG and the reference sequence GenBank Accession number AY988601.1. Of these, one was non-coding, and the other three led to single amino acid changes: one in the M protein and two in the F protein. The cell supernatants were stored at -80°C as ~ 1 ml aliquots. No detectable mycoplasma or endotoxin levels were measured (< 0.5 EU/ml).

**NHP Vaccination and Challenge.** For the first study, nine healthy, adult AGMs from St. Kitts (*Chlorocebus aethiops*; Worldwide Primates) weighing 3.0-5.7 kg were randomized into a group of six experimental animals and a group of three control animals. The six experimental animals were specifically vaccinated by intramuscular (i.m.) injection of 1 × 10⁷ PFU of rVSVΔG-NiVΔG, and control animals were vaccinated by i.m. injection of 1 × 10⁷ PFU of a nonspecific (78). All nine AGMs were exposed 7 days after vaccination to 5 × 10⁵ PFU of NiVΔG, with the dose being equally divided between i.t. and the i.n. routes. For the second study, nine healthy, adult AGMs from St. Kitts (*Chlorocebus aethiops*; PreLabs) weighing 3.6-8.2 kg were randomized into a group of six experimental animals and a group of three control animals. The six experimental animals were specifically vaccinated by i.m. injection of 1 × 10⁷ PFU of rVSVΔG-NiVΔG, and control animals were vaccinated by i.m. injection of 1 × 10⁷ PFU of a nonspecific rVSVΔG-EBOV-GP vaccine. All AGMs were exposed 3 days after vaccination to 5 × 10⁵ PFU of NiVΔG with the dose being equally divided between the i.t. and the i.n. routes.

All animals for both studies were given physical examinations, and blood was collected before vaccination (day 0); and on days 4, 7, 10, 14/15, 21, 28, and 35 after virus challenge. The AGMs were monitored daily and scored for disease progression with an internal NiV humane endpoint scoring sheet approved by the UTMB IACUC. UTMB facilities used in this work are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and adhere to principles specified in the eighth edition of the Guide for the Care and Use of Laboratory Animals, National Research Council. The scoring changes measured from baseline included posture and activity level, attitude and behavior, food intake, respiration, and central nervous system abnormalities. A score of ≥ 9 out of 22 indicated that an animal met the criteria for euthanasia.

**Hematology and Serum Biochemistry.** Total white blood cell counts, white blood cell differentials, red blood cell counts, platelet counts, hematocrit values, total hemoglobin concentrations, mean cell volumes, mean corpuscular volumes, and mean corpuscular hemoglobin concentrations were analyzed from blood collected in tubes containing EDTA using a Vetscan HM5 laser based hematologic analyzer (Zoetis). Serum samples were tested for concentrations of albumin, amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), calcium, creatinine (CRE), C-
reactive protein (CRP), gamma-glutamyltransferase (GGT), glucose, total protein, and uric acid by using a Piccolo point-of-care analyzer and Biochemistry Panel Plus analyzer discs (Abaxis).

**RNA Isolation from NiV<sub>b</sub>-infected AGMs.** On specified procedure days, 100 μl of blood was added to 600 μl of AVL viral lysis buffer (Qiagen) for RNA extraction. For tissues, approximately 100 mg was stored in 1 ml RNAlater (Qiagen) for 7 days for stabilization. RNAlater was completely removed, and tissues were homogenized in 600 μl RLT buffer (Qiagen) in a 2 mL cryovial using a Tissue Lyser (Qiagen) and ceramic beads. The tissues sampled included axillary, inguinal, mandibular, and mesenteric lymph nodes; upper, middle, and lower lobes of both left and right lungs; liver; spleen; kidney; adrenal gland; frontal cortex of brain; brainstem; cervical spinal cord; submandibular salivary gland; tonsil; heart; duodenum; pancreas; ileocecal junction; transverse colon; urinary bladder; ovary or testis; uterus or prostate; nasal mucosa; conjunctiva; and eye. Full sets of tissues were not obtained from all animals in this study. All blood samples were inactivated in AVL viral lysis buffer, and tissue samples were homogenized and inactivated in RLT buffer prior to removal from the BSL-4 laboratory. Subsequently, RNA was isolated from blood using the QiAamp viral RNA kit (Qiagen), and from tissues using the RNeasy minikit (Qiagen), according to the manufacturer’s instructions supplied with each kit.

**Quantification of Viral Load.** Viral loads of RNA from blood or tissues were quantitatively assessed using reverse transcriptase quantitative PCR (RT-qPCR) and primers/probe targeting the N gene and intergenic region between N and P of NiV<sub>b</sub>. Probe sequences were 6FAM-5’CGTCACACATCAGCTCTGACAA 3’-TAMRA for NiV<sub>b</sub> (Life Technologies, Carlsbad, CA), as described previously (76). Threshold cycle (CT) values representing viral genomes were analyzed with CFX Manager software, and the data are shown as genome equivalents (GEq). To create the GEq standard, RNA from viral stocks was extracted, and the number of strain-specific genomes was calculated using Avogadro’s number and the molecular weight of each viral genome.

Virus titration was performed by plaque assay using Vero 76 cells (ATCC CRL-1587) from all plasma samples as previously described (22). Briefly, increasing 10-fold dilutions of the samples were adsorbed to Vero 76 cell monolayers in duplicate wells (200 μl/well) and overlaid with 0.8% agarose in 1X Minimum Essentials Medium (MEM) with 5% FBS and 1% penicillin/streptomycin. After 2-3 days incubation at 37°C/5% CO<sub>2</sub>, neutral red stain was added, and plaques were counted after 24-hour incubation. The limit of detection for this assay was 25 PFU/mL.

**Transcriptomics.** NHPV2_Immunology reporter and capture probesets (Nanostring Technologies) were hybridized with ~5 μl of blood RNA at 65 °C for ~24 hours as previously described (79). The RNA:probeset complexes were loaded into an nCounter® microfluidics cartridge and assayed on a NanoString nCounter® SPRINT Profiler. To estimate abundance of each of the 769 unique mRNA immune-related targets included in the NHPV2_Immunology panel, fluorescent reporter barcodes were imaged and counted in each sample lane. The nCounter® RCC files were imported into NanoString nSolver® 4.0 software. All samples met the established quality control criteria. To compensate for varying RNA inputs, housekeeping genes and spiked-in positive and negative controls were used to normalize raw counts. The data were analyzed using the NanoString nSolver® Advanced Analysis 2.0 package to generate principal-component analysis figures and volcano plots, as well as to determine differential expression of transcripts (a full list of probes detected for each sample group along with log fold changes and P values is featured in Supplementary Data). Normalized data (log fold change values and BH-adjusted P values) were exported as a.CSV file (Microsoft Excel Office for Mac v.14.1.0) for Ingenuity Pathway Analysis (IPA)-based (QIAGEN) functional enrichment of DE RNAs. Z scores were imported into GraphPad Prism v.9 to produce DE mRNAs and canonical signaling heat maps. Human annotations were added for each respective gene to perform immune cell profiling and generate cell-type plots within nSolver®. To validate our nSolver®-derived cell type predictions, we used CIBERSORT web-based deconvolution software (57).

**Histology.** Tissue sections were deparaffinized and rehydrated through xylene and graded ethanolys. Slides went through heat antigen retrieval in a steamer at 95°C for 20 minutes in Sigma.
Citrate Buffer, pH6.0, 10x (Sigma Aldrich, St. Louis, MO). To block endogenous peroxidase activity, slides were treated with a 3% hydrogen peroxide and rinsed in distilled water. The tissue sections were processed for IHC using the Thermo Autostainer 360 (ThermoFisher, Kalamazoo, MI). Sequential 15-minute incubations with avidin D and biotin solutions (Vector Laboratories, Burlingame, CA #SP-2001) were performed to block endogenous biotin reactivity. Specific anti-NiV immunoreactivity was detected using an Anti-NiV primary antibody at a 1:4000 dilution for 60 min. Secondary antibody used was biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA #BA-1000) at 1:200 for 30 min followed by Vector Horseradish Peroxidase Streptavidin, R.T.U (Vector Laboratories #SA-5704) for 30 min. Slides were developed with Dako DAB chromogen (Dako, Carpenteria, CA #K3468) for 5 min and counterstained with hematoxylin for 45 seconds.
**Fig. S1. Vector design for rVSV-ΔG-NiV<sub>B</sub> G.**

Schematic of the RNA genomes of (A) rVSV-ΔG-NiV<sub>B</sub>G-GFP and (B) rVSV-ΔG-NiV<sub>B</sub>G. The GFP gene was excised from the vaccine vector for immunization of NHPs. Each gene is indicated (VSV N, VSV P, VSV M, NiV<sub>B</sub> G, GFP, and VSV L). For both panels, the genes are shown as boxes, while the intergenic regions and 3' and 5' untranslated regions are shown as black lines. VSV genes are shown in blue; the NiV<sub>B</sub> G gene is shown in orange; and the GFP gene is shown in green. The 3' and 5' trailers of each negative-sense virus are indicated. Abbreviations: GFP, green fluorescent protein; NiV<sub>B</sub>, Nipah virus Bangladesh strain; RNA, ribonucleic acid; rVSV, recombinant vesicular stomatitis virus; VSV, vesicular stomatitis virus.
Fig. S2. rVSV-ΔG-NiV<sub>B</sub> G expresses NiV<sub>B</sub> G protein.

IFA of BHK-21 clone WI-2 (panels A through E) and Vero 76 cells (panels F through K) infected with G*-rVSV-ΔG-NiV<sub>B</sub> G (panels A, B, D, E, G, H, J, and K) or mock-infected with growth medium only (panels C, F, and I). Cells were either permeabilized (panels B and C and F through H) or not permeabilized (panels C through E and I through K) and incubated with human m102.4 primary antibody (panels B, C, E, F, H, I, and K) followed by a secondary antibody against human IgG conjugated to AlexaFluor 488 (all panels). Note: some background fluorescence was prominent due to the high concentration of m102.4 primary antibody used.

Abbreviations: BHK, baby hamster kidney; IFA, immunofluorescence assay; IgG, immunoglobulin G; NiV<sub>B</sub>, Nipah virus Bangladesh strain; rVSV, recombinant vesicular stomatitis virus.
Fig. S3. Respiratory rates following challenge of AGMs with NiV<sub>B</sub>.

Line graph of respiratory rates in breaths per minute tracked daily throughout Study 1 for animals vaccinated with rVSV-ΔG-NiV<sub>B</sub>G (blue symbols) or nonspecific control rVSV-ΔG-EBOV 76 (red symbols) seven days prior to challenge (A) or three days prior to challenge (B) with NiV<sub>B</sub>. Animal IDs are shown in the legend to the right of the graph. AGM, African green monkey; EBOV, Zaire ebolavirus; NiV<sub>B</sub>, Nipah virus Bangladesh strain; rVSV, recombinant vesicular stomatitis virus.
**Fig. S4. CIBERSORT predicted cell subset frequencies in AGMs challenged with NiV.**

Heatmap of Study 2 CIBERSORT-derived cell-type quantities in vaccinated (n=6) versus vector control (n=3) datasets filtered by DPI (4, 7, 10 (survivors) or the terminal time point in fatal cases). Abbreviations: 3S, vaccinated survivor group (n=4) for Study 2 (-3-day immunization); 3F, vaccinated fatal group (n=2) for Study 2 (-3 immunization); DPI, days post infection.
Table S1. Clinical description and outcome of cynomolgus macaques vaccinated 7 days prior to NiV challenge

| Subject No. | Sex  | Clinical illness* | Clinical pathology* |
|-------------|------|-------------------|---------------------|
| C7-1        | Female | Tachypnea (d7); dyspnea (d7); anorexia (d7); depression (d7). Subject euthanized (d7). | Leukopenia (d0); lymphopenia (d0, 7); monocytopenia (d0, 4, 7); eosinopenia (d0, 4); basopenia (d0, 4, 7); thrombocytopenia (d7); neutrophilia (d7); ↑ CRP (d7). |
| C7-2        | Female | Dyspnea (d7); shallow breathing (d8); severe depression (d8); fever (d7). Subject euthanized (d8). | Leukopenia (d8); lymphopenia (d0, 7, 8); eosinopenia (d0, 4, 7, 8); basopenia (d0, 4, 7, 8); thrombocytopenia (d7, 8); hyperglycemia (d8); ↑ BUN (d8); ↓ CRE (d0); ↑ ALT (d7, 8); ↑ AST (d8); ↑ CRP (d7); ↑↑ CRP (d8). |
| C7-3        | Male | Tachypnea (d9); shallow breathing (d9); dyspnea (d9); decreased appetite (d8, 9); depression (d9). Subject euthanized (d9). | Lymphopenia (d9); eosinopenia (d0, 4, 7); basopenia (d0, 4, 7, 9); thrombocytopenia (d9); monocytosis (d9); neutrophilia (d0, 9); ↓ CRE (d4); ↑ CRP (d9). |
| V7-1        | Female | Decreased appetite (d1, 5-11, 20, 22). Subject survived to study endpoint (d35). | Leukopenia (d35); lymphopenia (d35); monocytopenia (d35); neutropenia (d4); eosinopenia (d0, 7); basopenia (d0, 7); thrombocytopenia (d4); monocytosis (d4); eosinophilia (d4); basophilia (d4, 15); ↓ CRE (d15, 35). |
| V7-2        | Female | Decreased appetite (d16); fever (d4). Subject survived to study endpoint (d35). | Leukopenia (d28, 35); monocytopenia (d0, 4, 7, 10, 15, 21, 28, 35); neutropenia (d28, 35); eosinopenia (d0, 4, 7, 10, 15, 21, 28, 35); basopenia (d28, 35); neutrophilia (d4); thrombocytosis (d10); ↓ CRE (d35); ↑ CRP (d4). |
| V7-3        | Female | Decreased appetite (d1, 5, 8, 11, 16, 18-24, 27-32, 34, 35). Subject survived to study endpoint (d35). | Leukocytosis (d7, 10); monocytosis (d4, 7, 10, 15, 21, 35); neutrophilia (d4, 7, 10); eosinophilia (d4, 7, 10, 15); basophilia (d4, 7, 10, 15); ↓ CRE (d35). |
| V7-4        | Male | None. Subject survived to study endpoint (d35). | Leukocytosis (d4, 7, 10, 15); lymphocytosis (d0, 4, 7, 10, 15); neutrophilia (d0, 4, 7, 10, 15, 21, 28, 35); basophilia (d15); monocytopenia (d0, 4, 7, 10, 15, 21, 28, 35); eosinopenia (d28); basopenia (d21, 28). |
| V7-5        | Male | None. Subject survived to study endpoint (d35). | Monocytopenia (d0, 4, 7, 10, 15, 28, 35); neutrophilia (d0, 4, 7, 10, 15, 21, 28, 35); ↓ CRE (d44). |
| V7-6        | Male | None. Subject survived to study endpoint (d35). | Monocytopenia (d0, 4, 7, 10, 15, 21, 28, 35); eosinopenia (d7); neutrophilia (d0, 4, 7, 10, 15, 21, 28, 35); basophilia (d0, 21, 35); ↓ CRE (d0, 10, 15, 21); ↑ CRP (d4). |

*Days after NiV<sub>5</sub> challenge are in parentheses. All reported findings are in comparison to baseline (day of vaccination, i.e. d-7) values. Decreased appetite is defined as some food but not all food consumed from the previous day. Anorexia is defined as no food consumed from the previous day. Fever is defined as a temperature more than 2.5 °F over baseline, or at least 1.5 °F over baseline and ≥ 103.5 °F. Hypothermia is defined as a temperature ≤3.5 °F below baseline. Lymphocytopenia, monocytopenia, erythrocytopenia, thrombocytopenia, neutropenia, eosinopenia, and basopenia are defined by a ≥35% drop in numbers of lymphocytes, monocytes, erythrocytes, platelets, neutrophils, eosinophils, or basophils, respectively. Lymphocytosis, monocytosis, neutrophilia, eosinophilia, and basophilia are defined by a 100% or greater increase in numbers of lymphocytes, monocytes, neutrophils, eosinophils, and basophils, respectively. Hyperglycemia is defined as a 100% or greater increase in levels of glucose. Hypoglycemia is defined by a ≥25% decrease in levels of glucose. Anemia is defined as a concurrent ≥25% decrease in erythrocyte count, Hct, and Hgb. Hypoalbuminemia is defined by a ≥25% decrease in levels of albumin. Hypoproteinemia is defined by a ≥25% decrease in levels of total protein. Hypoamylasemia is defined by a ≥25% decrease in levels of serum amylase. Hypocalcemia is defined by a ≥25% decrease in levels of serum calcium. Increases in ALT, AST, ALP, CRE, CRP, BUN, and Hgb were graded on the following scale: ↑ = 1-5-fold, ↑↑ = >5-10 fold, ↑↑↑ = >10-20 fold, ↑↑↑↑ = >20-fold, ↓ = ≥50% decrease. (BUN) blood urea nitrogen, (ALT) alanine aminotransferase, (AST) aspartate aminotransferase, (ALP) alkaline phosphatase, (CRE) Creatinine, (CRP) C-reactive protein, (Hct) hematocrit, (Hgb) hemoglobin.
Table S2. Clinical description and outcome of cynomolgus macaques vaccinated 3 days prior to NiV challenge

| Subject No. | Sex | Clinical illness* | Clinical pathology* |
|-------------|-----|-------------------|---------------------|
| C3-1        | Female | Dyspnea (d7, 8); anorexia (d8); depression (d8); fever (d7). Subject euthanized (d8). | Leukocytosis (d8); monocytes (d0, 4, 8); neutrophilia (d7, 8); lymphocytopenia (d7, 8); neutropenia (d4); eosinopenia (d0, 4, 7); basopenia (d4, 7); thrombocytopenia (d8); ↑ CRE (d8); ↑ ALT (d8); ↑ CRP (d7); ↑↑ CRP (d8). |
| C3-2        | Male | Tachypnea (d8); dyspnea (d8); decreased appetite (d5-7); anorexia (d8); depression (d8). Subject euthanized (d8). | Lymphopenia (d8); monocytes (d7); eosinopenia (d0, 4, 7, 8); basopenia (d0, 8); neutrophilia (d8); hypoalbuminemia (d8); ↑ ALT (d0, 4, 7); ↑↑ AST (d8); hypoamylasemia (d8); ↑ CRE (d8). |
| C3-3        | Male | Tachypnea (d7); dyspnea (d7); decreased appetite (d5-7); depression (d7). Subject euthanized (d7). | Lymphopenia (d7); eosinopenia (d0, 4, 7); basopenia (d0, 4, 7); thrombocytopenia (d7); monocytes (d7); ↑ CRE (d7). |
| V3-1        | Female | Tachypnea (d5, 6); dyspnea (d6); anorexia (d5, 6); depression (d6); fever (d4). Subject euthanized (d6). | Leukocytosis (d6); monocytes (d0, 4, 6); neutrophilia (d6); eosinopenia (d6); lymphocytopenia (d4); eosinopenia (d4); basopenia (d4); thrombocytopenia (d4, 6); hyperglycemia (d6); ↑ CRE (d4); ↑ CRE (d6); hypoalbuminemia (d6); hypoproteinemia (d6); ↑ CRP (d4, 6). |
| V3-2        | Female | Tachypnea (d6); dyspnea (d6); decreased appetite (d5, 6); depression (d6); fever (d4); seizures (d6); epistaxis (d6). Subject euthanized (d6). | Leukocytosis (d6); monocytes (d0, 6); neutrophilia (d6); eosinopenia (d6); lymphocytopenia (d4); eosinopenia (d4); basopenia (d4); thrombocytopenia (d4, 6); hyperglycemia (d6); ↑ CRE (d4); ↑ CRE (d6); hypoalbuminemia (d6); hypoproteinemia (d6); ↑ CRP (d4, 6). |
| V3-3        | Female | Tachypnea (d5, 6, 7); decreased appetite (d5-9, 11, 12). Subject survived to study endpoint (d35). | Leukopenia (d0); lymphocytopenia (d4, 35); monocytes (d0, 21, 35); neutropenia (d0, 21); eosinopenia (d0, 7, 14, 21, 28); basopenia (d7, 28); monocytosis (d14); neutrophilia (d4); eosinopenia (d10); basophilia (d10); hypoglycemia (d35); hypoalbuminemia (d7); ↑ ALT (d35); ↑ AST (d7); hypoamylasemia (d4); ↑ CRP (d4, 7). |
| V3-4        | Male | Tachypnea (d8); dyspnea (d7); decreased appetite (d5-11); fever (d7). Subject survived to study endpoint (d35). | Leukopenia (d10, 14); lymphocytopenia (d14); neutrophilia (d4, 7, 10, 14, 21); basophilia (d14); eosinopenia (d9, 4, 10, 35); basopenia (d4, 7, 10); thrombocytopenia (d7); ↑ AST (d10); hypoamylasemia (d4, 7, 14, 21, 28, 35); ↑↑ CRP (d7). |
| V3-5        | Male | Decreased appetite (d5-8; 11, 15, 22, 29, 30, 33, 35); fever (d4, 7). Subject survived to study endpoint (d35). | Monocytosis (d7, 10, 14); neutrophilia (d10); eosinopenia (d35); eosinopenia (d0, 4, 10, 21); basopenia (d0, 4, 7, 10, 21); thrombocytopenia (d7, 35); ↑ ALT (d0, 35); ↑↑ AST (d35); hypoamylasemia (d4); ↑ CRP (d7). |
| V3-6        | Male | Tachypnea (d6, 7); dyspnea (d8); decreased appetite (d5-7, 9-11, 15, 22, 24, 25, 29, 30, 32-34); anorexia (d8); hypothermia (d10). Subject survived to study endpoint (d35). | Leukopenia (d10, 14); lymphocytopenia (d14); monocytes (d10); neutrophilia (d7; 10, 14); lymphocytopenia (d7); monocytes (d0, 4, 35); eosinopenia (d7); basopenia (d4, 7); thrombocytopenia (d4, 7); ↑ CRP (d7). |

*Days after NiVs challenge are in parentheses. All reported findings are in comparison to baseline (day of vaccination, i.e. d - 3) values. Decreased appetite is defined as some food but not all food consumed from the previous day. Anorexia is defined as no food consumed from the previous day. Fever is defined as a temperature more than 2.5 °F over baseline, or at least 1.5 °F over baseline and ≤ 103.5 °F. Hypothermia is defined as a temperature ≤3.5°F below baseline. Lymphocytopenia, monocytes, erythrocytopenia, thrombocytopenia, neutropenia, eosinopenia, and basopenia are defined by a ≥35% drop in numbers of lymphocytes, monocytes, erythrocytes, platelets, neutrophils, eosinophils, or basophils, respectively. Lymphocytosis, monocytosis, neutrophilia, eosinophilia, and basophilia are defined by a 100% or greater increase in numbers of lymphocytes, monocytes, neutrophils, eosinophils, and basophils, respectively. Hyperglycemia is defined as a 100% or greater increase in levels of glucose. Hypoglycemia is defined by a ≥25% decrease in levels of glucose. Anemia is defined as a concurrent ≥25% decrease in erythrocyte count, Hct, and Hgb. Hypoalbuminemia is defined by a ≥25% decrease in levels of albumin. Hypoproteinemia is defined by a ≥25% decrease in levels of total protein. Hypoamylasemia is defined by a ≥25% decrease in levels of serum amylase. Hypocalcemia is defined by a ≥25% decrease in levels of serum calcium. Increases in ALT, AST, ALP, CRE, CRP, Hct, and Hgb were graded on the following scale: ↑ = 1-5-fold, ↑↑ = >5-10 fold, ↑↑↑ = >10-20 fold, ↑↑↑↑ = >20-fold, ↓ ≤ 50% decrease. (BUN) blood urea nitrogen, (ALT) alanine aminotransferase, (AST) aspartate aminotransferase, (ALP) alkaline phosphatase, (CRE) Creatinine, (CRP) C-reactive protein, (Hct) hematocrit, (Hgb) hemoglobin.