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Supplementary Information for

Development of an antibody cocktail for treatment of Sudan virus infection

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This PDF file includes:

Supplementary text
Figures S1 to S5
Tables S1 to S2

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Supplementary Information Text

Materials and Methods

Antibody production in mammalian expression system. A total of six chimeric anti-SUDV GP full-length heavy chain and light chain sequences were designed using the variable domain amino acid sequences of 16F6, 17F6, X10B1, X10B6, X10F3 and X10H2. All antibody sequences were constructed utilizing murine light and heavy chain signal peptides, human IgG_1 heavy constant sequences and human kappa constant sequences. All genes were codon and sequence optimized to ensure high level expression in murine cells and synthetized (GeneArt/ThermoFisher Scientific). Stable NS0 cell lines were developed at BioFactura, Inc. (Frederick, MD) using the StableFast platform and the single cholesterol selection strategy as described by Sampey, *et. al.* (39). Briefly, bicistronic expression plasmids were constructed coding for both heavy and light chains of each mAb. Each chain coding sequence was flanked upstream by cytomegalovirus (CMV)-derived promoters and downstream by bovine growth hormone (BGH) polyadenylation sequences (poly-A) comprising independent heavy and light chain expression cassettes. The cholesterol selection marker 17β-hydroxysteroid dehydrogenase type 7 or Hsd17β7 was regulated by an SV40 promoter and SV40 poly-A. The serum-free medium adapted cholesterol auxotrophic NS0 host cell line (NS0-SF, ECACC, Cat No. 03061601) was transfected by electroporation with expression plasmids and stable cell lines were selected by withdrawal of exogenous cholesterol. Best performing pools for each mAb were scaled to shaker or spinner flasks and stirred-tank single-use bioreactors (BioBLU, Eppendorf) and operated in fed-batch mode for 7-9 days. Cultures were subsequently clarified by centrifugation and filtration and mAbs were purified by a single Protein A capture step. Briefly, clarified supernatants were loaded onto Amsphere Protein A columns (JSR Life Sciences). Columns were washed with equilibration buffer (20 mM sodium phosphate, 150 mM sodium chloride) followed by a high salt wash with 500 mM sodium chloride. The mAbs were eluted with 0.2 M glycine at pH 3.0 directly into a container with 1 M Tris-HCl pH 8.0 to neutralize the eluate. Final purified mAbs were dialyzed into 1x PBS, pH 7.4 and sterile filtered. Final purified mAbs were characterized for identity, purity and endotoxin levels.

Antibody production in plant expression system. Anti-SUDV GP IgG_1 full light chain and heavy chain sequences were designed using variable domain amino acid sequences. Signal peptide derived from *Nicotiana tabacum* pathogenesis-related 1a (PR1a) protein (X06930) was fused with variable heavy and light chain regions to target expression of antibodies to the apoplast. The light chain variable domains of 16F6, 17F6, X10B1, X10B6, X10F3 and X10H2 were fused to a human kappa constant region to produce full length chimeric light chains, while the heavy chain variable domains were fused to a gamma constant human IgG_1 region to produce chimeric IgG_1 heavy chains. All genes were codon optimized to ensure high level expression in *Nicotiana benthamiana* plants and synthetized (ThermoFisher Scientific). All genes were then cloned into the binary expression vector pGR-D35S, where expression of heavy and light chains is under control of the double 35S *Cauliflower mosaic virus* (CaMV) promoter and *Tobacco etch virus* (TEV) enhancer. *Agrobacterium tumefaciens* strain AGL1 was transformed with the plasmids. *N. benthamiana* plants were vacuum infiltrated using agrobacterium cultures and aerial tissue was harvested after four to six days.

Antibodies used in the mouse studies were purified with Protein A. Biomass containing Sudan specific mAbs were homogenized after harvest in a Tris based buffer followed by detergent extraction for 20 minutes. The extract was clarified by centrifugation and filtration prior to loading onto on a MabSelect prepacked column (GE Healthcare).

Elution was achieved with a citrate buffer, pH 3.0 directly into a container with 2 M Tris pH 9.0 to neutralize the eluate. The recovered mAbs were dialyzed overnight into 1xPBS, pH 7.4, concentrated in a centrifugal device to > than 1 mg/mL and terminally filtered with a 0.22 µm PVDF membrane prior to storage at ≤ -60°C. Plant expressed mAbs for use in the NHP studies were captured on Protein A and held overnight in the neutralized eluate. The following day, the eluate was diluted to a pH of approximately 5.6 and loaded onto an SP column and eluted with 150 mM salt. The SP eluate was concentrated and buffer exchanged with ultrafiltration and diafiltration prior to loading on to Capto-Q and collected in the unbound fraction. The final mAb was augmented with polysorbate-80 to 0.005%, aseptically filtered (0.22 µm), aliquoted and held frozen at ≤ -60°C. All chromatography media was obtained from GE Healthcare. Final, purified mAbs for NHP studies were released based on attributes of identity, purity (≥ 90%) and endotoxin ≤ 0.1 EU/mg among other attributes.

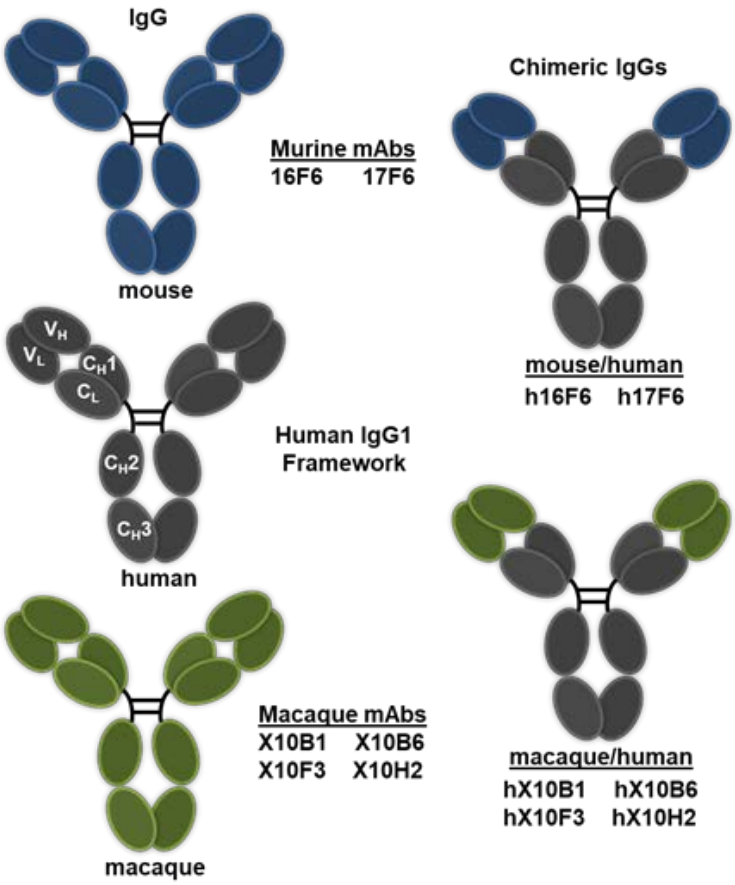

Fig. S1. Chimerization strategy for humanized SUDV-specific mAbs. Variable heavy and variable light chains from SUDV-specific murine or macaque mAbs were cloned into human IgG1 constant heavy and constant light chain expression vectors, respectively. These expression vectors were then co-expressed in either mammalian or plant production systems to generate human IgG1 chimeric mAbs with SUDV specificity.

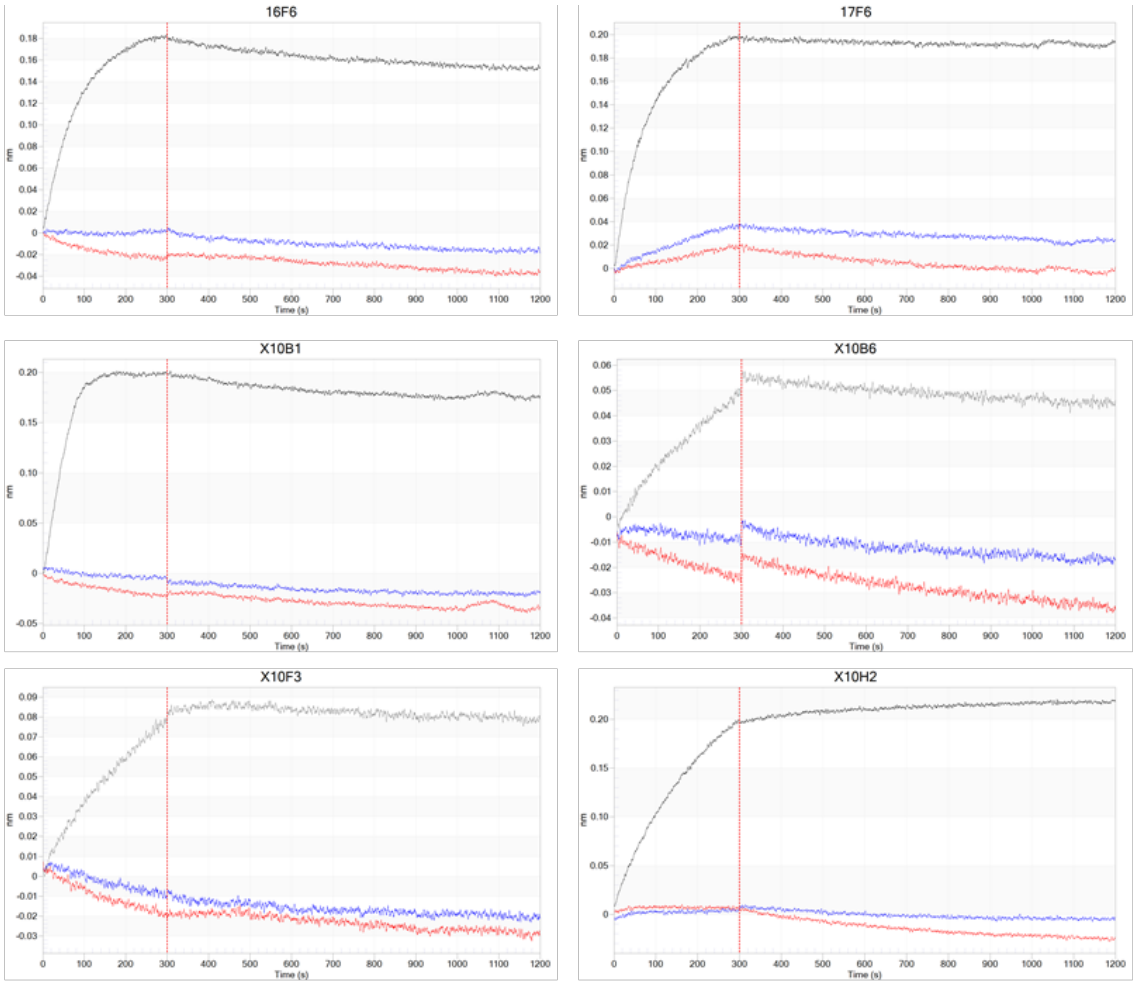

Fig. S2. Cross-reactive specificity of humanized SUDV-specific mAbs by BLI. AHC biosensors coated with indicated mAbs were dipped into assay buffer containing recombinant SUDV GP (grey), EBOV GP (blue), or BDBV GP (red). Antibody association (left of red line) and dissociation (right of red line) was assessed.

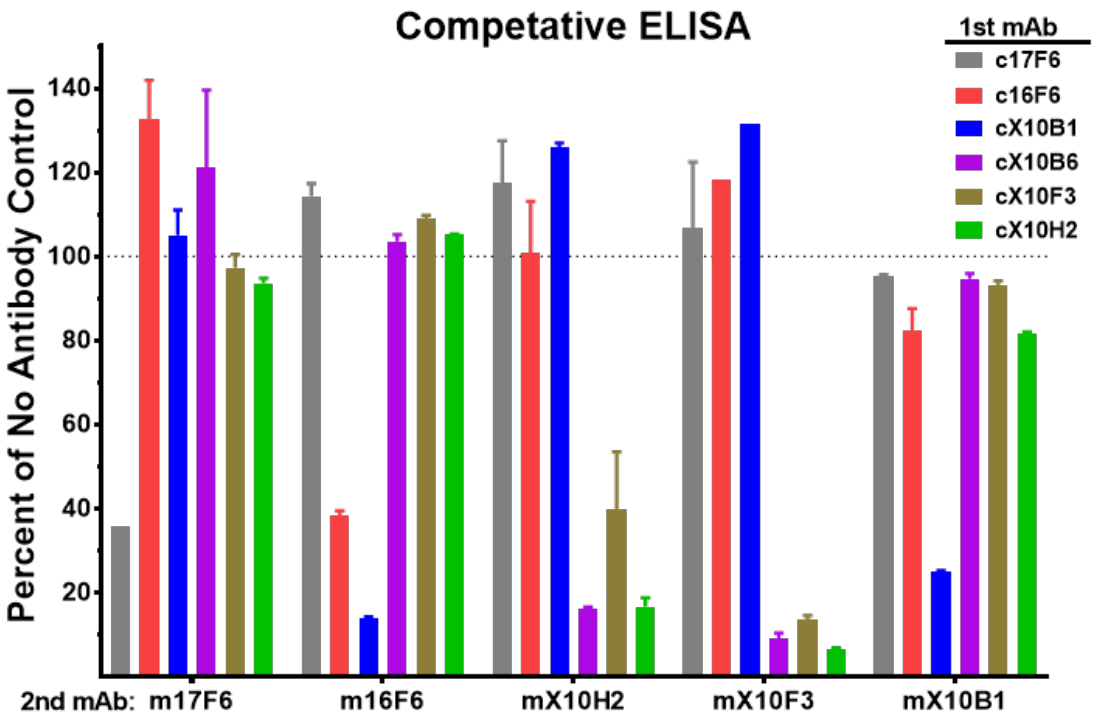

Fig. S3. Competitive binding of humanized SUDV-specific mAbs. ELISAs were completed to identify mAbs that compete for SUDV GP binding. Chimeric (c) human version of indicated mAbs were added to antigen coated plates prior to the addition of murine (m) version of indicated mAbs. Murine antibody binding was detected using anti-mouse IgG-HRP secondary antibody. Data is presented as the percent of murine antibody alone for each mAb.

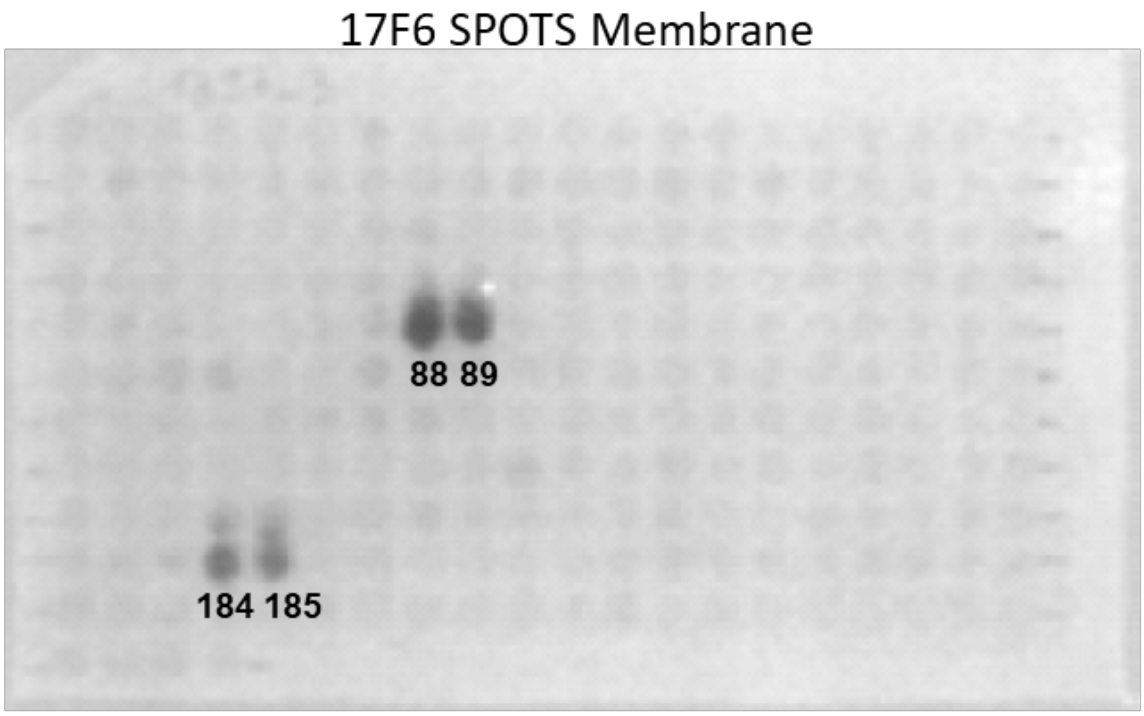

Fig. S4. Linear epitope specificity of SUDV GP-specific mAb 17F6. SPOTS membrane, coated with 13mer peptides of SUDV GP from Boniface and Gulu isolates, was sequentially incubated with 17F6, HRP conjugated secondary antibody, and substrate to identify epitope specificity of 17F6. Amino acid sequence: 88 = GMVSLHVPEGETT (Boniface); 89 = LHVPEGETTLPSQ (Boniface); 184 = GMVPLHIPEGETT (Gulu); 185 = LHIPEGETTLPSQ (Gulu)

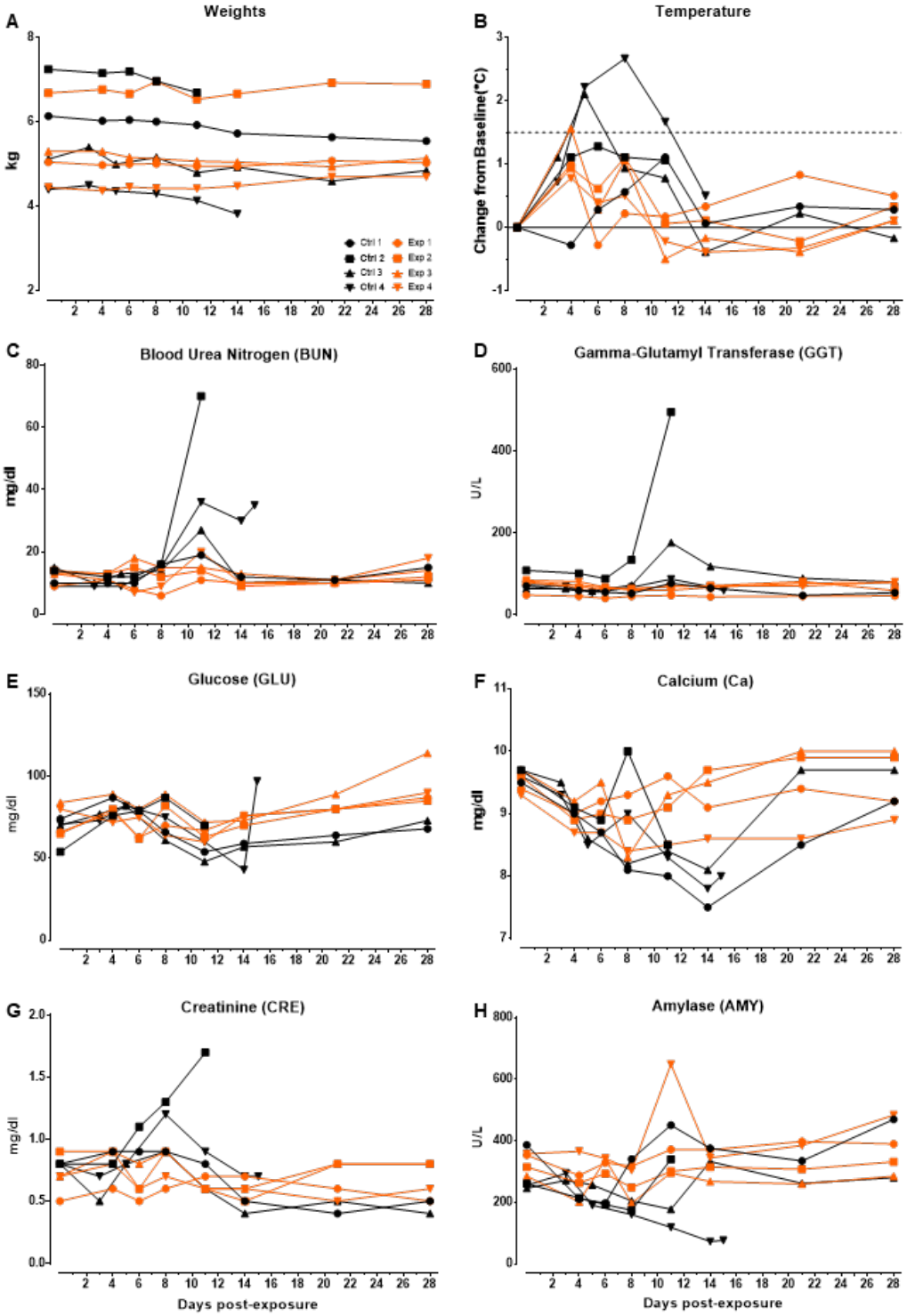

Fig. S5. Weights, temperatures, and serum chemistries of rhesus macaques following SUDV exposure. Rhesus macaques were exposed intramuscularly to 1750 plaque forming units of SUDV. Groups (N=4) were treated intravenously with either 50 mg/kg of SUDV-specific mAb cocktail 16F6/X10H2 or and equal volume to weight ratio of vehicle (0.9% NaCl) on days 4 and 6 post-exposure (Ctrl 3 and Ctrl 4 treated on day 5 only). (**A**) Weights and (**B**) temperatures were collected on indicated days post-exposure. Serum was collected on indicated days post-exposure to evaluate serum enzymes (**C**) BUN, (**D**) GGT, (**E**) GLU, (**F**) Ca, (**G**) CRE, and (**H**) AMY by Piccolo.

Table S1. SUDV-Gulu GP Affinity

mAb	Source	K_D (nM)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})
16F6	Mammalian	0.16	3.22E+05	5.60E-05
17F6	Mammalian	0.80	2.40E+05	2.32E-04
X10B1	Plant	0.001	6.07E+04	1.00E-07
X10H2	Plant	8.82	3.00E+03	2.55E-05
X10B6	Plant	1743	1.93E+02	3.10E-04
X10F3	Plant	227.5	7.86E+02	1.85E-04

Table S2. Clinical signs of disease following SUDV I.M. exposure

NHP	Clinical Observations	Status
Ctrl 1	Responsiveness score 2-3 (days 8-14), fever (day 11), dehydration (days 6-14), edema (day 11), 2-3 fold increase in ALT, >5 fold increase in AST, ALP, >5 fold decrease in platelets	Survived
Ctrl 2	Responsiveness score 2-3 (days 8-11), moderate rash (day 8), bloody nasal discharge (day 11), anorexia (days 8-11), severe dehydration (day 11), severe testicular edema (day 11), 3-4 fold increase in ALT, >5 fold increase in AST, ALP, 3-4 fold decrease in platelets	Euthanized day 11 (secondary criteria)
Ctrl 3	Responsiveness score 1 (days 9-11), mild rash (days 5-11), fever (day 5), diarrhea (days 10-11), vomiting (day 9), severe testicular edema (days 11-14), 2-3 fold increase in ALT, >5 fold increase in AST, ALP, 1.5 fold decrease in platelets	Survived
Ctrl 4	Responsiveness score 2-3 (days 8-15), fever (days 5-11), 15% weight loss, diarrhea (days 10-11), vomiting (day 11), bloody nasal discharge (day 14), agonal breathing (days 14-15), severe dehydration (day 11-15), moderate edema (days 8-15), >5 fold increase in AST, 2-3 fold increase in ALT, ALP, 2-3 fold decrease in platelets	Euthanized day 15 (respiratory distress)
Exp 1	Responsiveness score 1 (days 4-6)	Survived
Exp 2	Edema (day 14)	Survived
Exp 3	Fever (day 4)	Survived
Exp 4	2 fold increase in AMY	Survived

ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, AMY: amylase