

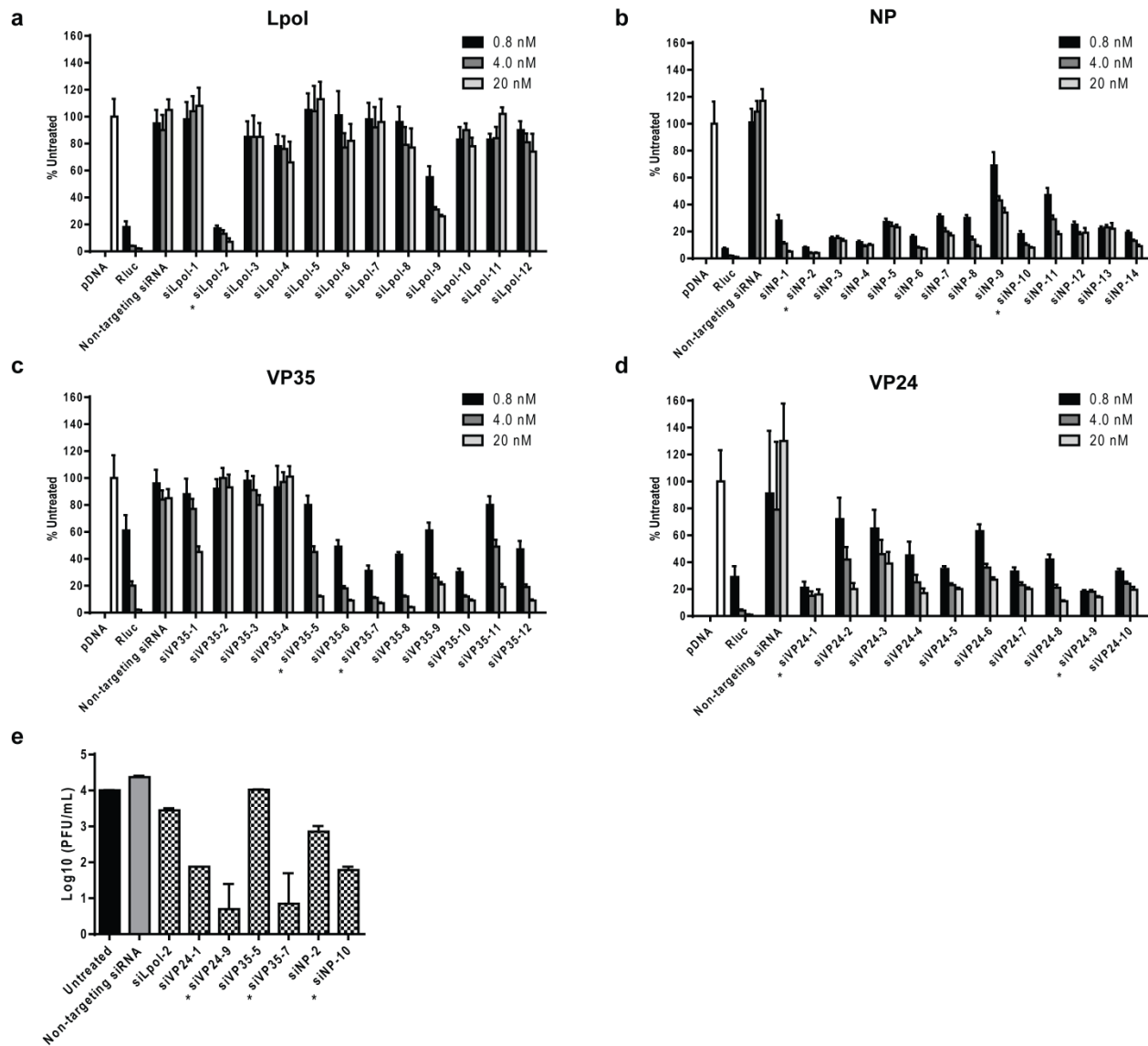
Rescue of non-human primates from advanced *Sudan ebolavirus* infection with lipid encapsulated siRNA

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Subject No.	Sex	siRNA	Treatment time (h)	Outcome	Clinical illness	Clinical and gross pathology
C-1	F	None	None	Non-survivor	Depression (d5-10); lethargy (d7-10); loss of appetite (d5-10); mild rash (d7-9); ecchymotic rash (d10); animal euthanized in pm of d10	Leukocytosis (d10); granulocytosis (d3,6,10); lymphopenia (d6,10); thrombocytopenia (d6,10); hypoalbuminemia (d10); ALT > 3-fold↑ (d10); AST > 10-fold↑ (d10); ALP > 2-fold↑ (d6); ALP > 7-fold↑ (d10); BUN > 6-fold↑ (d10); CRE > 3-fold↑ (d10); GGT > 5-fold↑ (d10); CRP > 10-fold ↑ (d6,10)
C-2	M	None	None	Non-survivor	Depression (d5-8); lethargy (d6-8); loss of appetite (d4-8); mild rash (d6); moderate rash (d7,8); ecchymotic rash (d8); rectorrhagia (d8); animal euthanized in pm of d8	Leukocytosis (d3,6,8); granulocytosis (d3,6,8); lymphopenia (d6); thrombocytopenia (d6,8); hypoalbuminemia (d6,8); ALT > 2-fold↑ (d6); ALT > 6-fold↑ (d8); AST > 9-fold↑ (d6); AST > 10-fold↑ (d8); ALP > 2-fold↑ (d6); BUN > 2-fold↑ (d6); BUN > 10-fold↑ (d8); CRE > 3-fold↑ (d6); CRE > 6-fold↑ (d8); GGT > 2-fold↑ (d8); CRP > 2-fold ↑ (d3); CRP > 10-fold ↑ (d6,8)
C-3	M	None	None	Non-survivor	Depression (d8); loss of appetite (d5-8); mild rash (d8); moderate rash (d9); animal expired in am on d9	Granulocytosis (d6); lymphopenia (d6); AST > 2-fold↑ (d6); CRP > 10-fold ↑ (d6)
C-4	M	Luc LNP	96	Non-survivor	Fever (d4-6); depression (d6-9); loss of appetite (d5-9); mild rash (d6); moderate rash (d7-9); epistaxis (d9); rectorrhagia (d9); animal euthanized in am of d9	Leukocytosis (d4); granulocytosis (d4,9); lymphopenia (d9); thrombocytopenia (d7,9); hypoalbuminemia (d9); ALT > 9-fold↑ (d7); ALT > 10-fold↑ (d9); AST > 10-fold↑ (d7); AST > 10-fold↑ (d9); BUN > 4-fold↑ (d7); BUN > 2-fold↑ (d9); CRE > 2-fold↑ (d7); GGT > 2-fold↑ (d9); CRP > 7-fold ↑ (d4); CRP > 10-fold ↑(d7); CRP > 3-fold ↑(d9)
C-5	F	None	None	Non-survivor	Fever (d5); depression (d6-9); lethargy (d7-9); loss of appetite (d5-9); mild rash (d8); ecchymotic rash (d9); epistaxis (d9); rectorrhagia (d9); animal euthanized in pm of d9	Leukocytosis (d5); granulocytosis (d5,9); lymphopenia (d5); thrombocytopenia (d8,9); hypoalbuminemia (d8,9); ALT > 3-fold↑ (d8); ALT > 2-fold↑ (d9); AST > 3-fold↑ (d8); AST > 10-fold↑ (d8); AST > 7-fold↑ (d8); GGT > 2-fold↑ (d8,9); CRP > 10-fold ↑ (d5); CRP > 5-fold ↑ (d8); CRP > 4-fold ↑ (d9)
VP35-1	F	Anti-VP35	24	Survivor	Loss of appetite (d6-11)	Leukocytosis (d3,10); granulocytosis (d3,10); lymphopenia (d3,6); CRP > 10-fold ↑ (d3,6,10)
VP35-2	M	Anti-VP35	24	Survivor	Loss of appetite (d8-9)	Granulocytosis (d6,10); lymphopenia (d6); CRP > 10-fold ↑ (d3); CRP > 2-fold ↑ (d6); CRP > 4-fold ↑(d10)
VP35-3	M	Anti-VP35	48	Survivor	None	Granulocytosis (d3); thrombocytopenia (d6,10); CRP > 10-fold ↑ (d3); CRP > 2-fold ↑(d6,10)
VP35-4	F	Anti-VP35	48	Survivor	Fever (d7); depression (d10,11); loss of appetite (d4-15); mild rash (d10-12)	Leukocytosis (d10); granulocytosis (d3,6,10); lymphopenia (d3); thrombocytopenia (d10,14); ALT > 3-fold↑ (d10); ALP > 2-fold↑ (d6,14); ALP > 5-fold↑ (d10); CRP > 3-fold ↑ (d3); CRP > 10-fold ↑(d6,10)
VP35-5	M	Anti-VP35	72	Survivor	Fever (d10); depression (d13); loss of appetite (d11-16)	Leukocytosis (d3); granulocytosis (d3,6, 10,14); lymphopenia (d10); hypoalbuminemia (d14); ALP > 2-fold↑ (d14); CRP > 7-fold ↑ (d6); CRP > 3-fold ↑(d10); CRP > 10-fold ↑(d14)
VP35-6	F	Anti-VP35	72	Survivor	Fever (d3); loss of appetite (d4-10); mild rash (d9-12)	Granulocytosis (d3,6); thrombocytopenia (d14); AST > 4-fold↑ (d6); AST > 5-fold↑ (d10); CRP > 3-fold ↑ (d3); CRP > 6-fold ↑ (d6); CRP > 4-fold ↑ (d10)
VP35-7	F	Anti-VP35	96	Survivor	Fever (d8,9); loss of appetite (d5-12)	Leukocytosis (d4,7); granulocytosis (d4,7,10,14); thrombocytopenia (d10); hypoalbuminemia (d14); ALT > 3-fold↑ (d10); AST > 2-fold↑ (d7); AST > 10-fold↑ (d10); CRP > 10-fold ↑ (d4); CRP > 10-fold ↑(d7); CRP > 3-fold ↑(d10)
VP35-8	F	Anti-VP35	96	Survivor	Loss of appetite (d6,8,9)	Leukocytosis (d4); granulocytosis (d4,7); lymphopenia (d3); AST > 2-fold↑ (d7); AST > 3-fold↑ (d10); CRP > 3-fold ↑ (d4); CRP > 7-fold ↑(d7); CRP > 5-fold ↑(d10)
VP35-9	M	Anti-VP35	96	Survivor	Fever (d4,5,7-9); depression (d9-12); loss of appetite (d5-7,10-18); mild rash (5-7,10-14)	Leukocytosis (d4,10,21); granulocytosis (d4,7,10,21); thrombocytopenia (d7,10); ALT > 7-fold↑ (d7); ALT > 6-fold↑ (d10); ALT > 2-fold↑ (d14,21); AST > 7-fold↑ (d7); AST > 10-fold↑ (d10); ALP > 3-fold↑ (d7,14,21); ALP > 4-fold↑; BUN > 10-fold↑ (d8,10); CRE > 7-fold↑ (d8); CRE > 10-fold↑ (d10); GGT > 2-fold↑ (d21); CRP > 10-fold ↑ (d4); CRP > 4-fold ↑ (d7)
VP35-10	M	Anti-VP35	96	Survivor	Fever (d4,8,9); loss of appetite (d5-8,10-12)	Leukocytosis (d4,10,28); granulocytosis (d4,7,10,28); thrombocytopenia (d7,14); hypoalbuminemia (d10,14); AST > 10-fold↑ (d10); CRP > 5-fold ↑ (d4); CRP > 3-fold ↑(d7); CRP > 6-fold ↑(d10)
VP35-11	M	Anti-VP35	120	Non-survivor	Fever (d5); depression (d5-8); lethargy (d7,8); loss of appetite (d5-8); mild rash (d5-7); ecchymotic rash (d8); rectorrhagia (d8); animal euthanized in am of d8	Granulocytosis (d5); lymphopenia (d5); thrombocytopenia (d5,8); hypoalbuminemia (d8); ALT > 10-fold↑ (d8); AST > 10-fold↑ (d8); ALP > 2-fold↑ (d8); BUN > 10-fold↑ (d8); CRE > 4-fold↑ (d8); GGT > 2-fold↑ (d8); CRP > 10-fold ↑ (d5,8)
VP35-12	F	Anti-VP35	120	Survivor	Fever (d5); depression (d9-12); mild rash (d6,10-13); loss of appetite (d5-14,18,19)	Leukocytosis (d5,28); granulocytosis (d5,8); lymphopenia (d8,11); thrombocytopenia (d5,8,11,15); hypoalbuminemia (d8,11,15,21); AST > 4-fold↑ (d8); AST > 8-fold↑ (d11); ALP > 2-fold↑ (d11,15,21); CRP > 10-fold ↑ (d5,8); CRP > 6-fold ↑(d11); CRP > 3-fold ↑(d21)
VP35-13	M	Anti-VP35	120	Non-survivor	Fever (d5,6); depression (d6-10); lethargy (d7-10); loss of appetite (d5-9); mild rash (d5,6); moderate rash (d7); ecchymotic rash (d8-10); mild rectorrhagia (d8,9); rectorrhagia (d10); animal euthanized in am of d10	Leukocytosis (d5); granulocytosis (d5,8,10); lymphopenia (d5); thrombocytopenia (d8,10); hypoalbuminemia (d8,10); ALT > 8-fold↑ (d8); ALT > 10-fold↑ (d10); AST > 2-fold↑ (d5); AST > 10-fold↑ (d8,10); AST > 7-fold↑ (d8); CRP > 10-fold ↑ (d5,8,10)
VP35-14	F	Anti-VP35	120	Survivor	Fever (d5,9,10); depression (d9-12); lethargy (d10,11); loss of appetite (d10-14); mild rash (d7-13)	Leukocytosis (d5); granulocytosis (d5,8,11,21,28); lymphopenia (d5,8); thrombocytopenia (d8,11); hypoalbuminemia (d11,15); AST > 2-fold↑ (d8); AST > 10-fold↑ (d11); BUN > 3-fold↑ (d11); CRP > 10-fold ↑(d5,11); CRP > 5-fold ↑(d8)

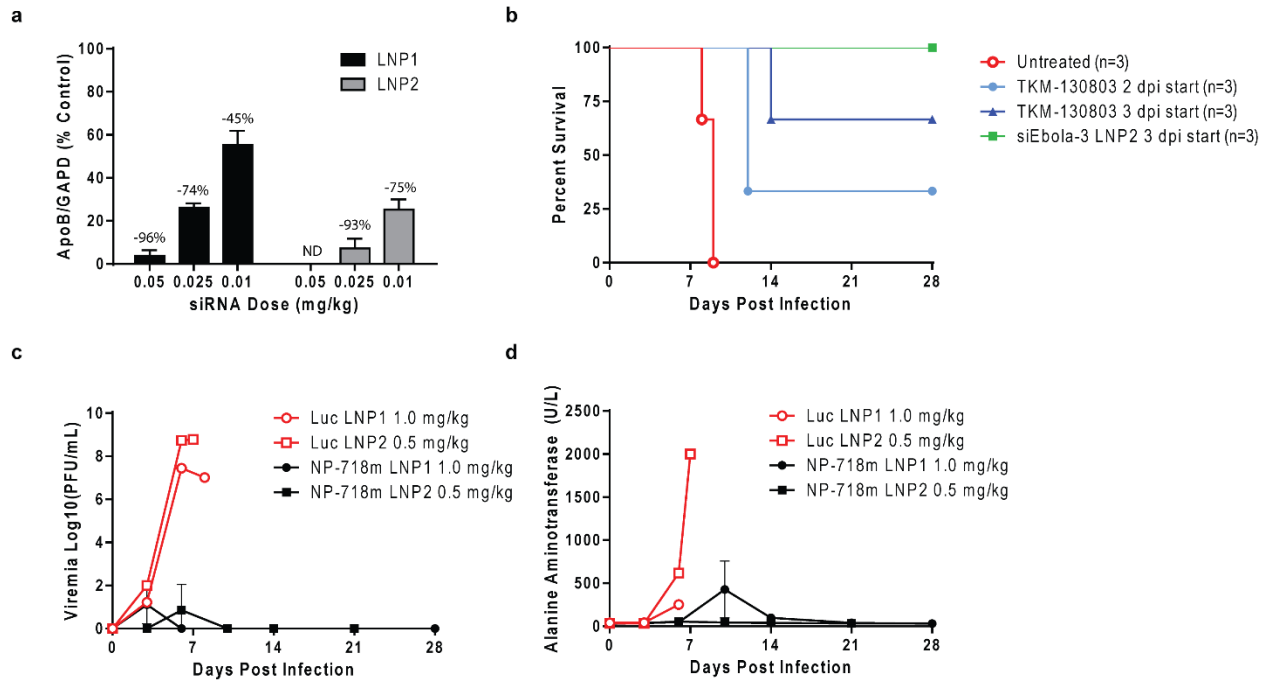
Days after SUDV challenge are in parentheses. Fever is defined as a temperature more than 2.5°F over baseline or at least 1.3°F over baseline and ≥ 103.5°F or 1.1°F over baseline and ≥ 104.0°F. Mild rash: focal areas of petechiae covering less than 10% of the skin; Moderate rash: areas of petechiae covering between 10% and 40% of the skin. Lymphopenia and thrombocytopenia are defined by a ≥35% drop in numbers of lymphocytes and platelets, respectively. Leukocytosis and granulocytosis are defined by a two-fold or greater increase in numbers of white blood cells over base line. Hypoalbuminemia is defined by a ≥35% decrease in levels of albumin. (ALT) alanine aminotransferase, (ALP) alkaline phosphatase, (AST) aspartate aminotransferase, (BUN) blood urea nitrogen, (CRE) creatinine, (CRP) C-reactive protein, (GGT) gamma glutamyltransferase.

Supplementary Table 1. Clinical description and outcome of *Sudan ebolavirus* challenged NHPs, controls and siVP35-LNP treated.
Outcomes, clinical signs, and pathology of controls and animals treated with siVP35-LNP.

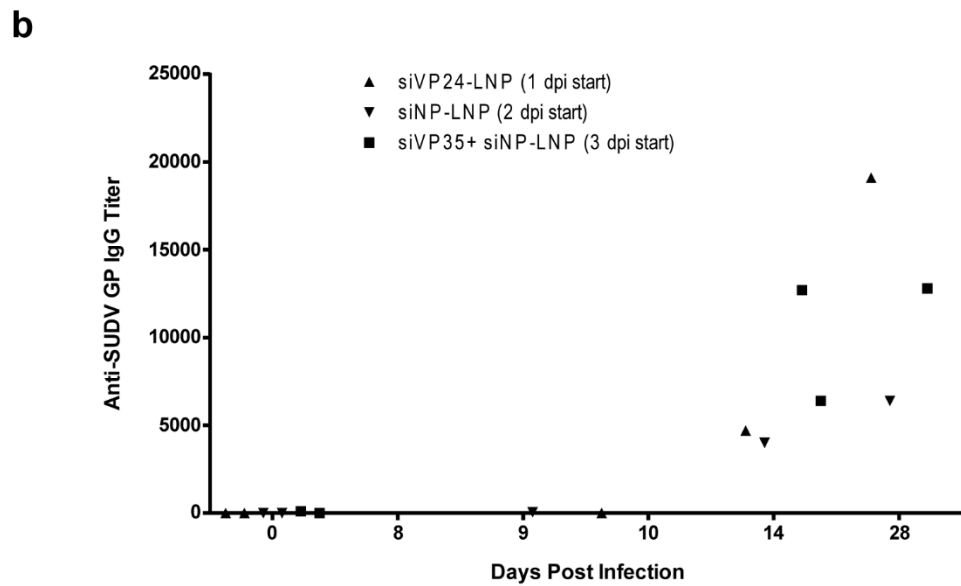
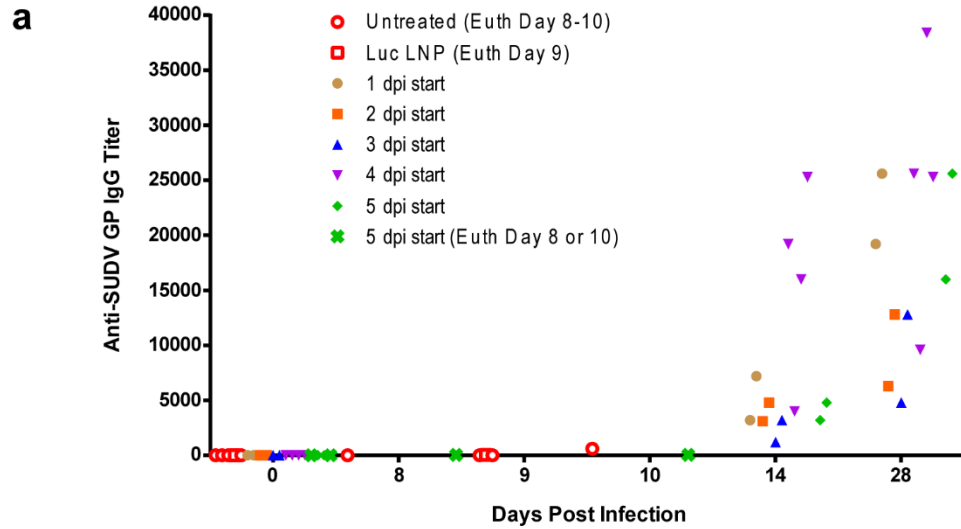


Supplementary Figure 1. Downselection of siRNAs for evaluation in NHP studies. a-d, 48 siRNAs were screened using the dual luciferase reporter assay described in Methods. siRNA-LNP targeting Renilla luciferase (Rluc) or non-targeting siRNA were positive and negative controls, respectively. Data are means \pm SD of one biological replicate normalized to untreated. *siRNAs with the most potent activity were evaluated in e) SUDV-infected HepG2 cells for antiviral activity. Data are means \pm SEM of one biological replicate. *siRNAs taken into NHP

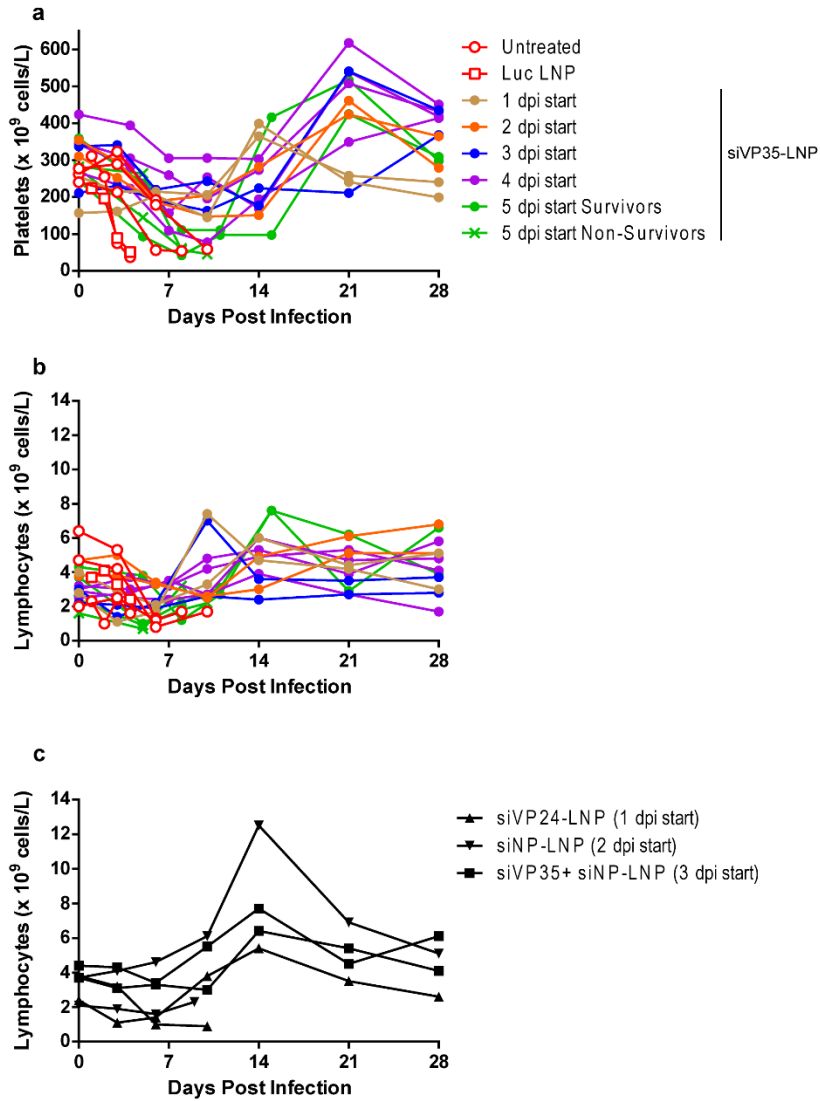
studies for efficacy assessment. Data for downselected siRNAs is in Figure 1. siNP-10 is referred to as siNP, siVP35-7 as siVP35 and siVP24-9 as siVP24 in the remainder of the manuscript.



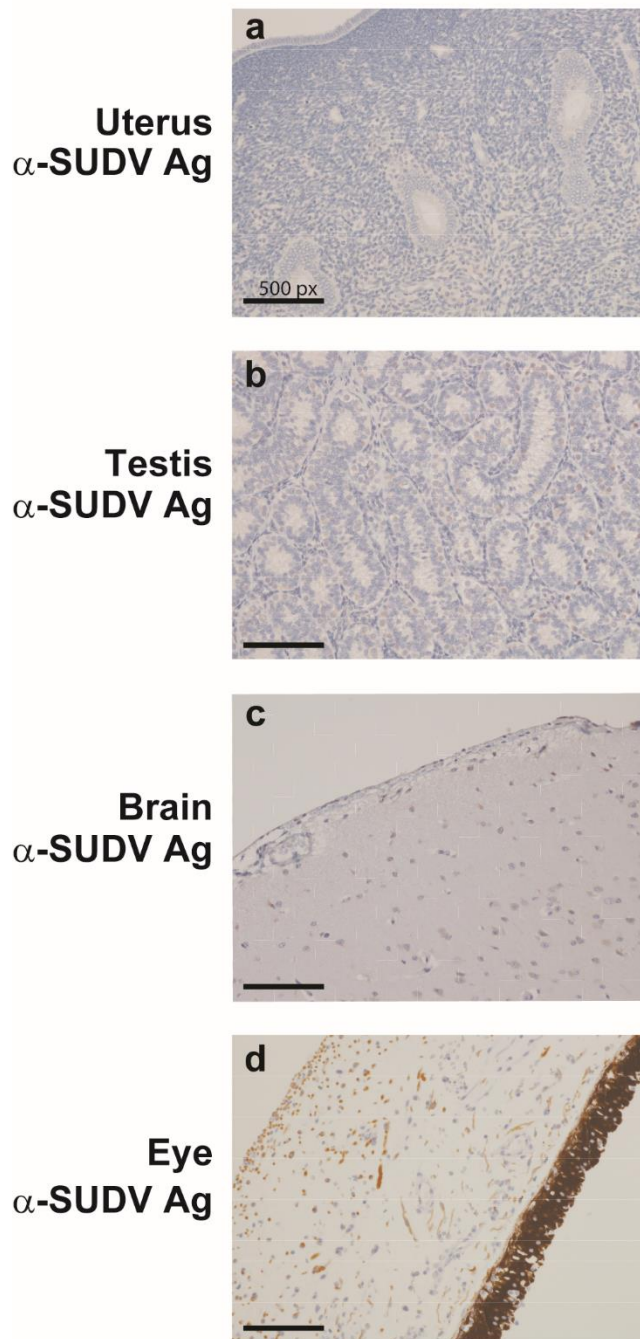
Supplementary Figure 2. Comparison of potencies between LNP formulations. **a**, BALB/c mice were administered siRNA targeting ApoB in LNP1 or LNP2 and liver mRNA reduction by branched DNA assay was assessed 48 h after dosing. Data are ApoB: GAPDH ratio means of $n = 4$ animals \pm SD, normalized to PBS control. **b**, siEbola-3 siRNA cocktail confers greater protection in LNP2 compared to LNP1 (aka, TKM-130803) in rhesus macaques infected with EBOV-Makona. Marburg virus (MARV) siRNA in LNP2 confers equivalent or better control of **c**, viremia and **d**, liver dysfunction at half the dosage of the same siRNA in LNP1. NP-718m LNP1 and siEbola-3 LNP2 data were published previously.



Supplementary Figure 3. siRNA-LNP treated animals have appreciable serological responses after reductions in viral load are observed post-treatment. a, siVP35-LNP treated animals develop robust IgG titers against SUDV GP by Day 14 and retain these responses by study endpoint. **b,** Surviving animals treated with siRNAs targeting VP24, NP or the cocktail combination of VP35- and NP- show a similar pattern of serological responses as siVP35-LNP treated animals.



Supplementary Figure 4. Hematology of control and siRNA-LNP treated animals confirm infection. a, thrombocytopenia and **b** and **c** lymphopenia were observed in all infected animals.



Supplementary Figure 5. siVP35-LNP treated animals that survive lethal challenge do not have observable SUDV antigen in the eyes, reproductive tissues, or neural tissues. Lack of SUDV antigen staining by immunohistochemistry in **a**, uterus, **b**, testis, **c**, brain and **d**, eye tissues from siVP35-LNP treated survivors. All images acquired at 20 \times magnification. Scale bar

= 500 microns. Representative images taken from animal VP35-12 (**a**, **c-d**), and animal VP35-9 (**b**).