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Supplemental Information

A Nanostructured Lipid Carrier for Delivery of a Replicating Viral RNA Provides Single, Low-Dose Protection against Zika

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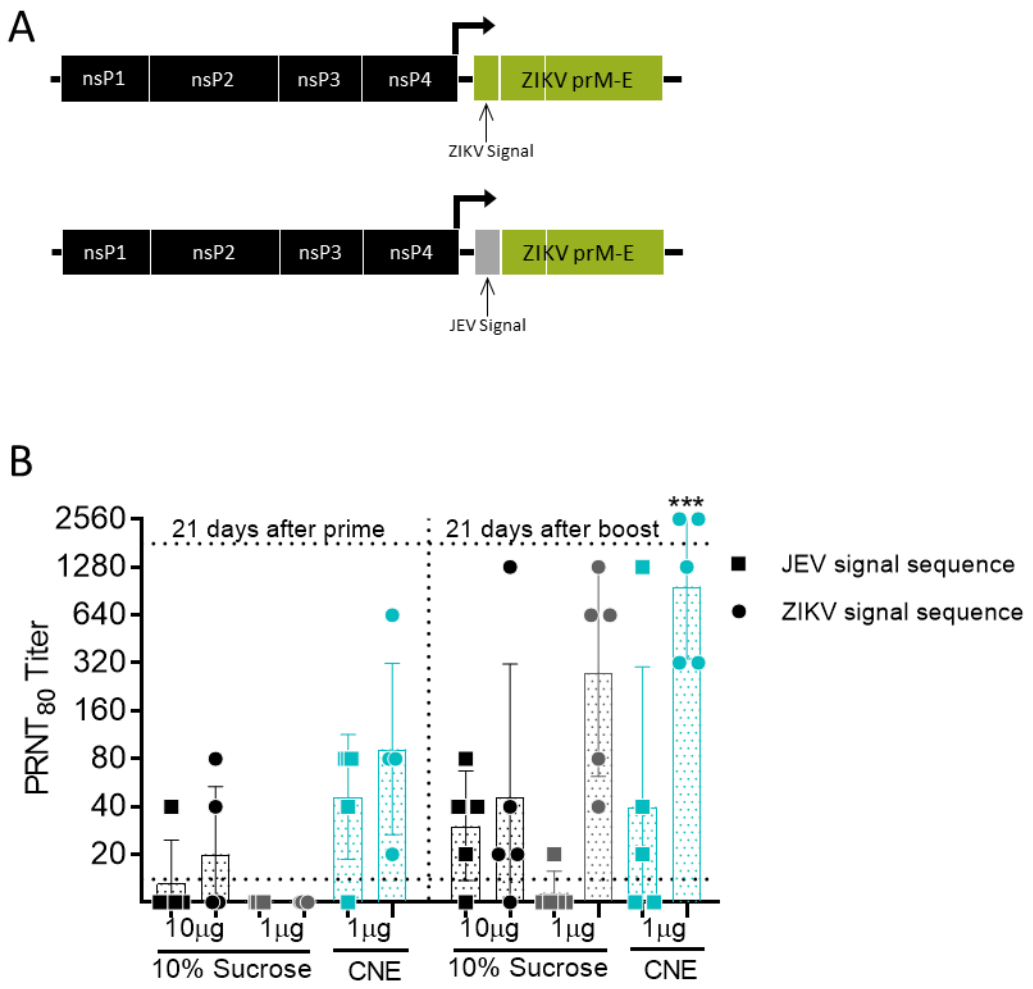
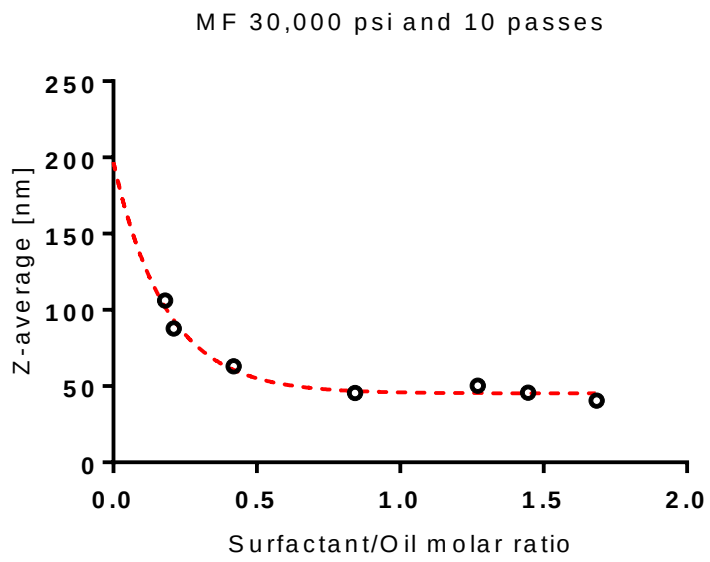
SUPPLEMENTAL FIGURES



Supplemental Figure 1. Comparison of signal sequences derived from Zika (ZIKV) or Japanese Encephalitis (JEV) Viruses. (**A**) Design of rvRNAs encoding ZIKV prM/E with a ZIKV or JEV signal sequence upstream of prM. (**B**) rvRNAs encoding ZIKV prM/E with ZIKV or JEV signal sequences were formulated in 10% sucrose (naked) or with cationic nanoemulsion (CNE) and 10 μg of the former or 1 μg of the latter were injected via IM route into C57BL/6 mice (n=5/group) and $PRNT_{80}$ titers were measured in the serum 21 days later. Mice were then boosted with a second immunization and $PRNT_{80}$ titers were measured an additional 21 days later.

SUPPLEMENTAL FIGURES



Supplemental Figure 2. Effect of surfactant to oil molar ratio on NLC particle size (Z-average). The surfactant to oil ratio was changed by varying the amount of total surfactant while holding the amount of oil constant. Experimental data was fit to a one-phase exponential decay equation (R-square = 0.972). All formulations were microfluidized under identical conditions (10 passes at 30,000 psi)

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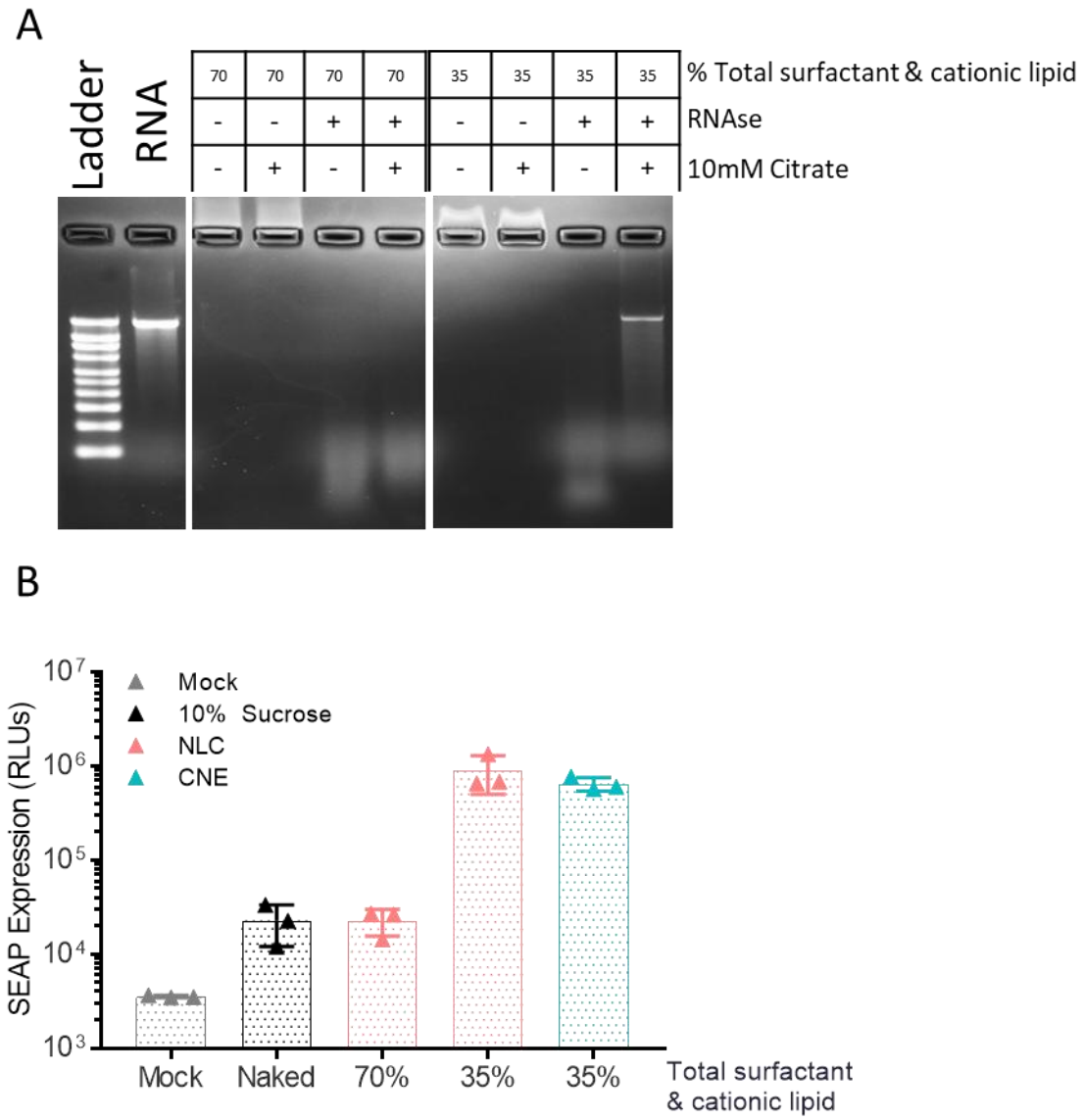

Supplemental Figure 3. Effect of hydrophilic surfactant on protection from RNase challenge and delivery of SEAP expressing rvRNA *in vivo*. rvRNA was complexed with variations of NLCs made with 70% or 35% total surfactant & cationic lipid manufactured with or without 10mM citrate buffer and assessed for their ability to protect rvRNA from RNase challenge (**A**). C57BL/6 mice (n=3/group) were administered rvRNA encoding SEAP complexed with 10% sucrose (naked) or with NLCs made with 70% or 35% total surfactant & cationic lipid, or with CNE, and serum SEAP activity was measured 3 days later (**B**).

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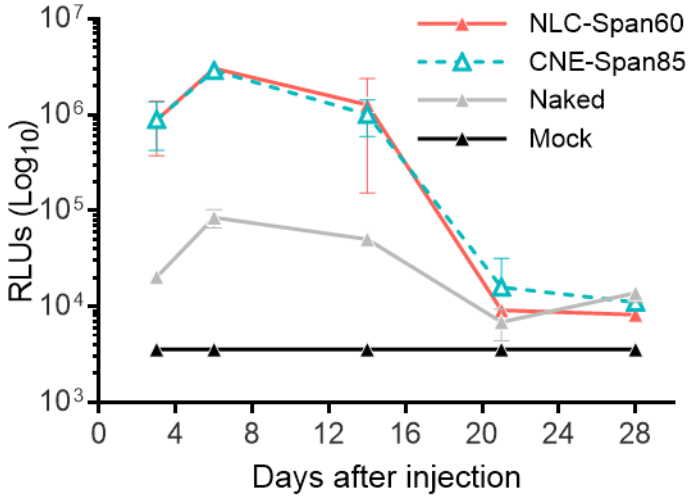
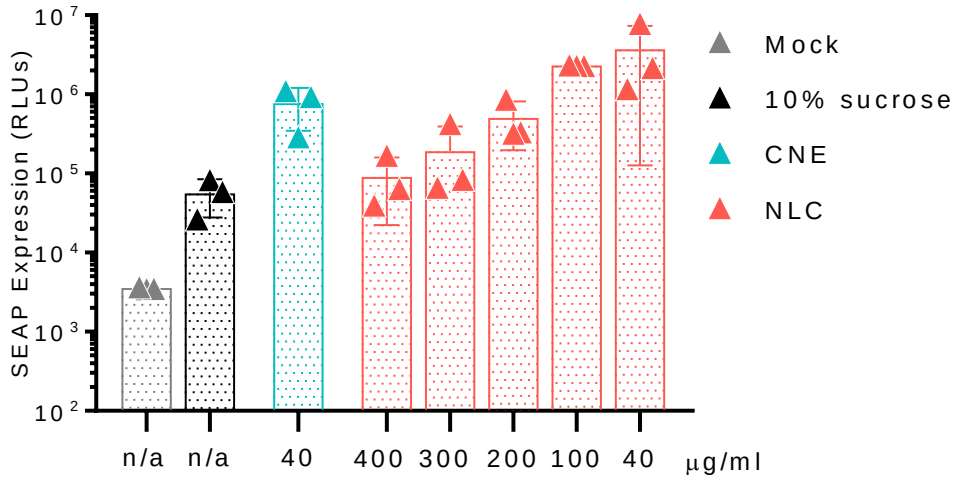
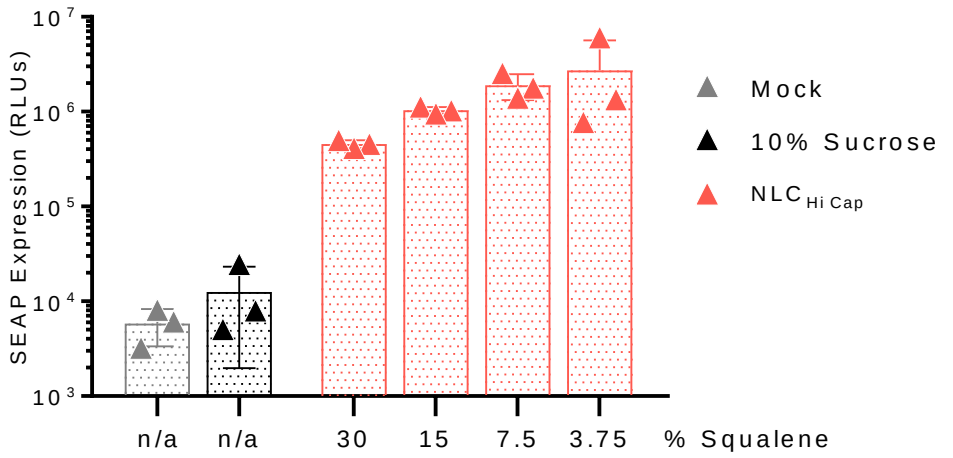


Supplemental Figure 4. Kinetics of protein expression following *in vivo* delivery of rvRNA formulated with NLC_{v1} or CNE. rvRNA encoding SEAP was formulated with either NLC_{v1}, CNE, or 10% sucrose (naked) and 100ng was administered via the IM route to C57BL/6 mice (n=3/group). Mice mock injected with 10% sucrose alone were used as a negative control. Mice were bled on days 3, 7, 14, 21, and 28 and serum SEAP activity was measured by SEAP assay.



Supplemental Figure 5. Optimization of rvRNA loading capacity of NLC_{v1}. rvRNA encoding SEAP was diluted in 10% sucrose to final concentrations of 400, 300, 200, 100, and 40 µg/ml and complexed 1:1 with NLC_{v1} by gentle pipetting. CNE was complexed 1:1 with 40 µg/ml of rvRNA. Complexed or unformulated rvRNA was then diluted in 10% sucrose for dosing such that each 50 µl injection IM resulted in a 100ng dose. Mice were then bled 3 days later and serum SEAP activity was measured by SEAP assay and compared to mock-injected mice.

SUPPLEMENTAL FIGURES



Supplemental Figure 6. Effect of decreasing squalene concentration in high-capacity NLCs on serum SEAP activity. High-capacity NLCs (containing 3% w/v DOTAP) manufactured with 30, 15, 7.5, and 3.75% w/v squalene were complexed 1:1 with rvRNA encoding SEAP at an N:P of 37 and 100ng was administered via IM route in C57BL/6 mice (n=3/group) and serum SEAP activity was measured 3 days later and compared to unformulated rvRNA- or mock-injected mice.

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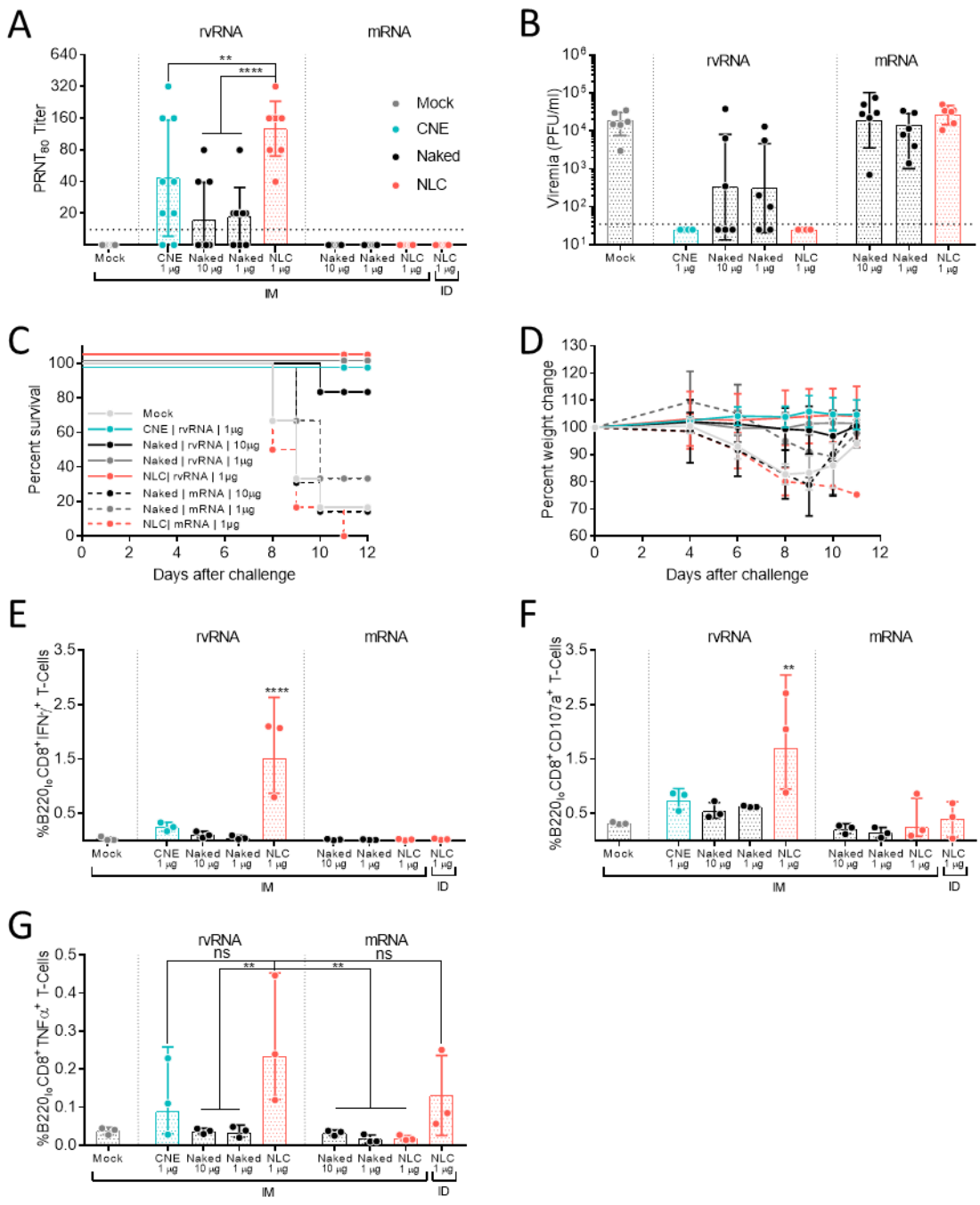
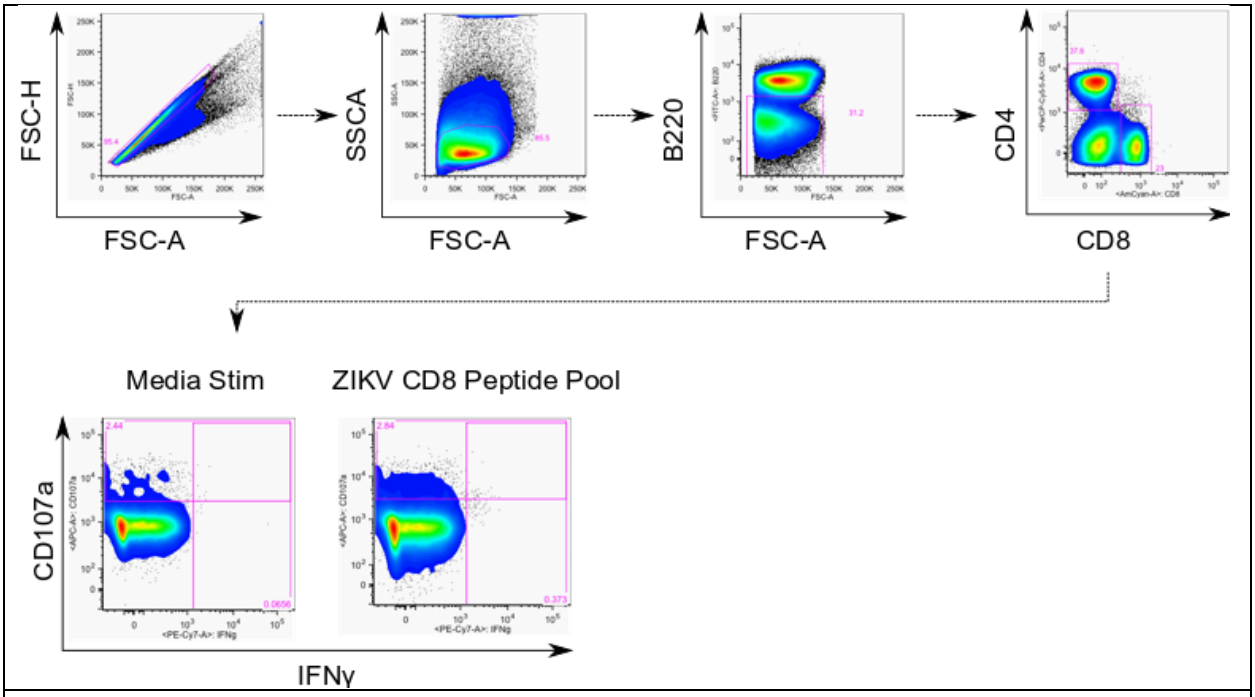
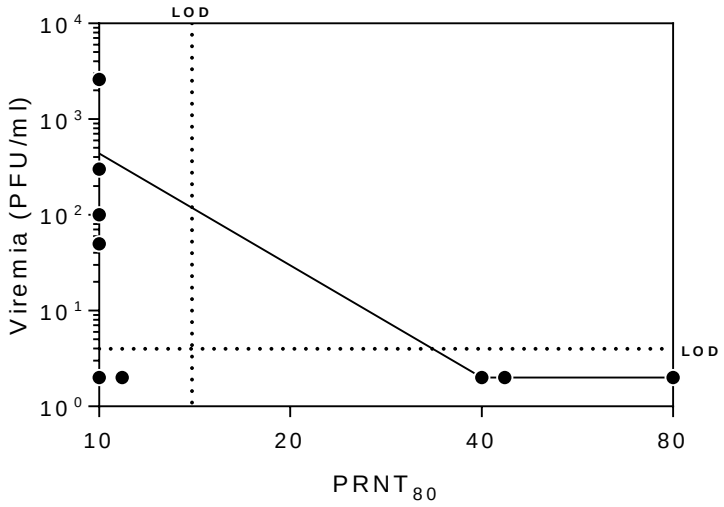


Supplemental Figure 7. Immunogenicity and efficacy of NLC_{v1}/rvRNA complexes in C57BL/6 mice. NLC_{v1} was complexed with rvRNA or mRNA encoding ZIKV prM/E at an N:P of 50 and a single 1 µg dose was administered to C57BL/6 mice via IM injection (n=9/group) and 14 days later, bled to assess neutralizing antibody titers by $PRNT_{80}$ (**A**) compared to mock vaccinated mice or 10 or 1 µg naked rvRNA or mRNA (n=9/group), or 1 µg rvRNA formulated with CNE at an N:P of 50. Data are presented as individual values as well as mean ± S.D. Thirty days after immunization, 6 mice per group were challenged with 5 $\log_{10}$ PFU of ZIKV Dakar strain 41525

following antibody blockade of type I interferon as described (49) and bled 4 days later to quantify viremia by plaque assay (**B**). Mice were monitored daily for survival (**C**) and weight loss (**D**). The remaining 3 mice per group were administered a second immunization on day 30 to assess post-boost $CD8^+$ T-cell responses. Fourteen days later, mice were euthanized and splenocytes were isolated, stained, and $\%B220_{lo}CD8^+$ T cells that were $IFN\gamma^+$ (**E**), $CD107a^+$ (**F**), or $TNF\alpha^+$ (**G**) were quantified by flow cytometry.



Supplemental Figure 8. Flow cytometry gating strategy.



Supplemental Figure 9. Correlation between day 14 $PRNT_{80}$ titer and post-challenge viremia in mice vaccinated with 3ng of ZIKV rvRNA formulated in NLC_{v2}.