Supplemental Information

Intramuscular Delivery of Replicon RNA

Encoding ZIKV-117 Human Monoclonal Antibody Protects against Zika Virus Infection

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Supplemental Figure 1. Evaluation of IRES elements. (A) Schematic of constructs used to evaluate panel of IRESs using nanoluciferase (nLUC) as a readout for expression levels (B) IRES-mediated nLUC expression in BHK cells 16 hours after transfection of RNA replicons. (C) Total IgG ELISAs of supernatants harvested from BHK cells 24 hours after transfection with replicons encoding variations of ZIKV-117. EMCV min, Encephalomyocarditis virus minimal sequence; EMCV opt, Encephalomyocarditis virus full length sequence; EV71, Human enterovirus 71; FGF1, Fibroblast growth factor 1; Arc1, Activity-regulated cytoskeleton-associated protein; VCIP, Vascular endothelial growth factor (VEGF) and type 1 collagen inducible protein; C-myc, c-myc proto-oncogene; BIP, Immunoglobulin heavy chain binding protein; HCV374, Hepatitis C virus 374 nucleotides; HCV392, Hepatitis C virus 392 nucleotides; DHV, Duck hepatitis A virus; CrPv, Cricket paralysis virus
**Supplemental Figure 2. Evaluation of alternative signal peptides.** (A) Schematic of constructs used to evaluate alternative signal peptides of the heavy chain (B) Total IgG ELISAs of supernatants harvested from BHK cells 24 hours after transfection with replicons encoding variations of ZIKV-117. *p<0.05 as determined by one-way ANOVA with Tukey’s multiple comparison test. Data are representative of 3 independent experiments (C) ZIKV-117 ELISAs of serum harvested 5 days after intramuscular injection of each RNA in C57BL/6 mice (n=5/group). Data are representative of 2 independent experiments.

**Supplemental Figure 3. Evaluation of alternative signal peptides.** (A) Schematic of constructs used to evaluate alternative signal peptides of the heavy chain (B) Total IgG ELISAs of supernatants harvested from BHK cells 24 hours after transfection with replicons encoding variations of ZIKV-117. *p<0.05 as determined by one-way ANOVA with Tukey’s multiple comparison test. Data are representative of 3 independent experiments (C) ZIKV-117 ELISAs of serum harvested 5 days after intramuscular injection of each RNA in C57BL/6 mice (n=5/group). Data are representative of 2 independent experiments.
Supplemental Figure 4. Titration of dose concentration for optimal antibody expression following intramuscular administration of NLC-formulated replicon RNA. C57BL/6 mice (n=3/group) were injected intramuscularly with a range of RNA concentrations in a 50 μL volume such that each group received between 0 and 25 μg of RNA. ZIKV-117 concentration then was measured by ELISA of sera harvested 5 days after injection. *p<0.05 as determined by one-way ANOVA with Tukey’s multiple comparison test. A subset of groups were repeated at n=5/group and similar results were observed.

Supplemental Figure 5. Effect of IFNAR blockade on antibody expression from replicon RNA. C57BL/6 mice (n=5/group) were pre-treated with MAR1-5A3 antibody or mock treated with PBS. One day later, both groups were injected intramuscularly with 40 μg of ZIKV-117 RNA, and ZIKV-117 concentration was measured by ELISA of sera harvested 0, 3, 5, and 7 days after RNA injection. *p<0.0001 between groups as determined by two-way ANOVA with Bonferroni’s multiple comparison test.