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Supplementary Materials for

NS1 DNA vaccination protects against Zika infection through T cell–mediated immunity in immunocompetent mice

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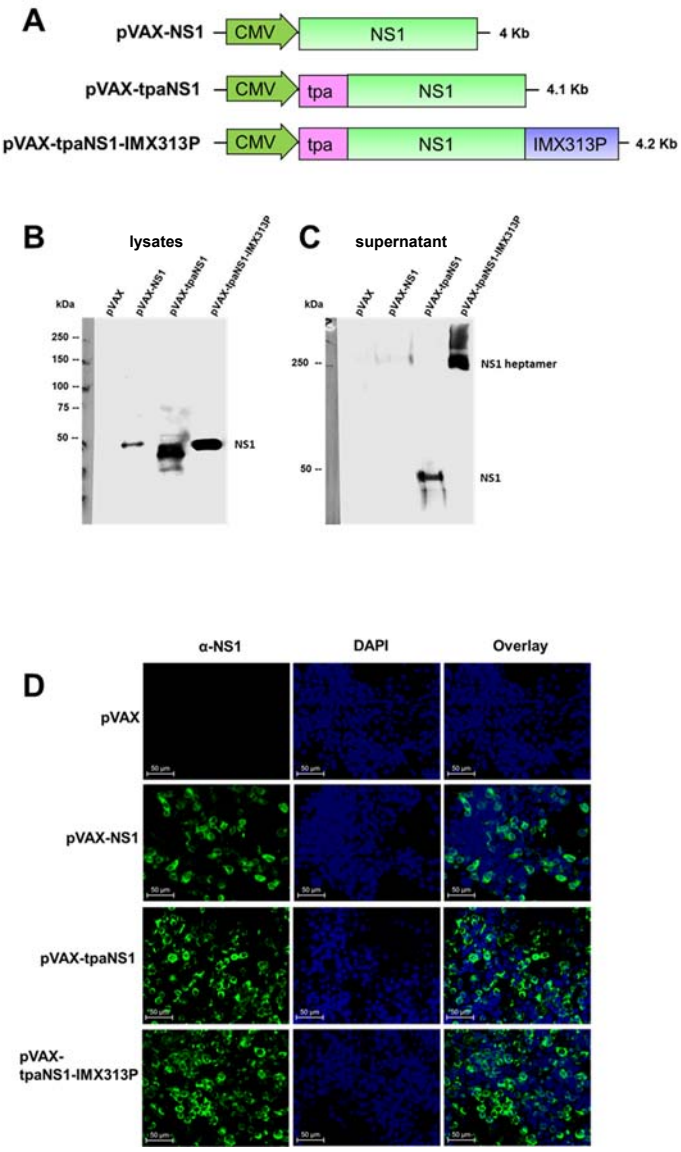

Fig. S1. DNA vaccine design and antigen expression. A) Schematic diagram of the NS1 DNA plasmid vaccines. **B)** Reducing immunoblot of cell lysates from HEK293T cells transfected with empty pVAX, pVAX-NS1, pVAX-tpaNS1 and pVAX-tpaNS1-IMX313P DNA using anti-ZIKV NS1 antibody. **C**) Non-reducing immunoblot of supernatant fluids from HEK293T cells transfected with pVAX-NS1, pVAX-tpaNS1 and pVAX-tpaNS1-IMX313P plasmid DNA. **D**) Immunofluorescence assay analysis for ZIKV NS1 protein expression in HEK293T cells. Photomicrographs of HEK293T cells transfected with the different DNA vaccines and pVAX control, stained with anti-ZIKV NS1 antibody. DAPI panels show control staining of cell nuclei. Overlay panels are combinations of anti-ZIKV NS1 and DAPI staining patterns.

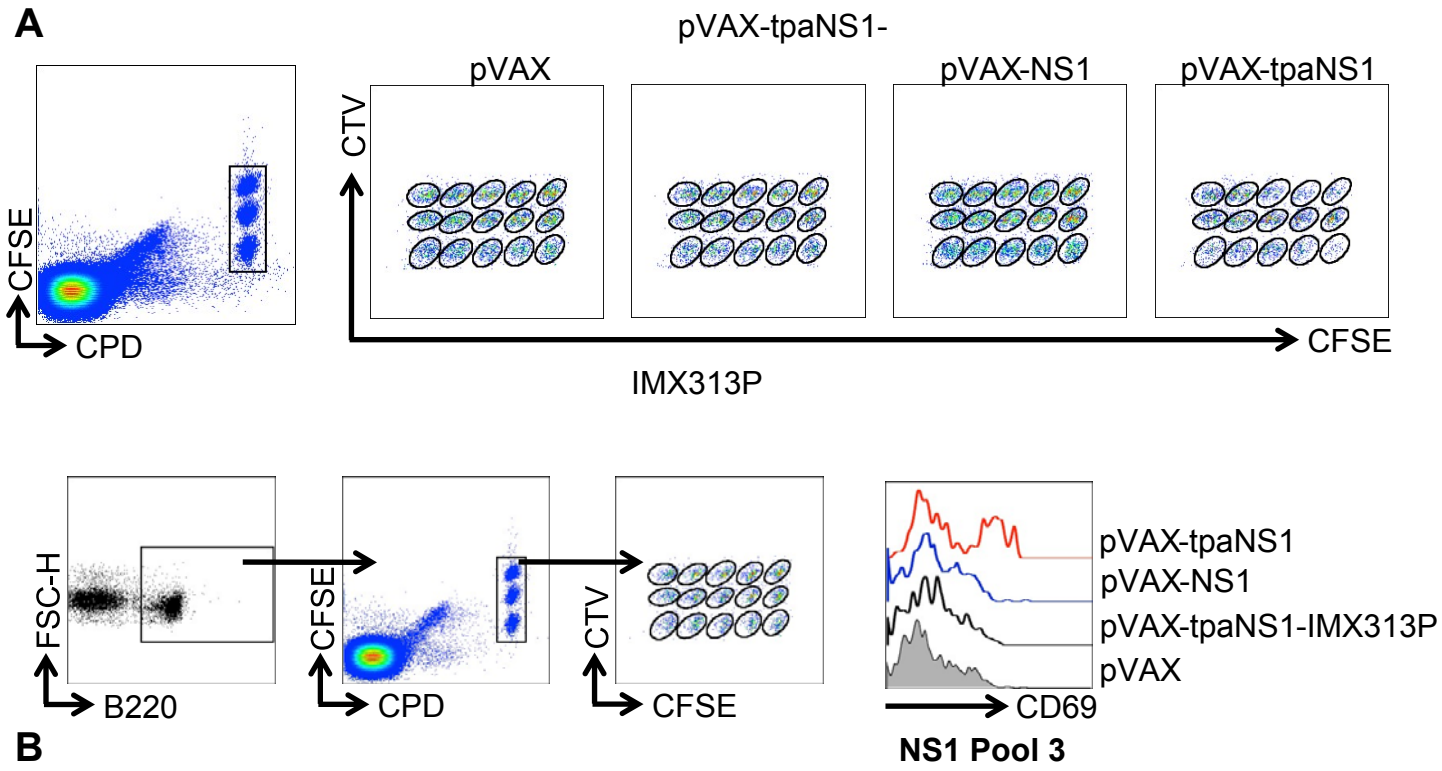

Fig. S2. FTA analysis. Flow diagram of the FTA analysis used in experiments described in figures 2, 3 and 4. Cells of interest were gated using FSC-A vs. SSC-A prior to excluding the doublets using FSC-H vs. FSC-W and SSC-H vs. SSC-W. **A**) The NS1 peptide pool targets were gated based on CFSE and CPD emission as shown. For simplicity, the plots show an example in which NS1 targets were gated from a representative mouse for each NS1 DNA vaccine group. The peptides-pulsed cell targets were gated and analysed for the percentage recovery relative to mock targets to determine the specific FTA cell loss using the equation: [(percent mock targets - percent peptides-pulsed targets)/percent mock targets] x 100. **B**) For Th cell responses analysis, cells of interest were gated based on B220+ expression. The NS1 peptide pool targets were gated based on CFSE and CPD emission as shown. The geometric mean fluorescent intensity (GMFI) of CD69 on peptide-pulsed targets was determined by flow cytometry. The values plotted in figures 3, 4 and 5 reflect: GMFI of CD69 on peptide-pulsed B220+ targets minus GMFI of CD69 on mock B220+ targets. The representative histogram plot shows CD69 expression on B220+ cell

targets pulsed with 10ug/ml of ZIKV NS1 Pool 3 in representative mice from groups vaccinated with pVAX, pVAX-NS1, pVAX-tpaNS1 and pVAXtpaNS1-IMX313P

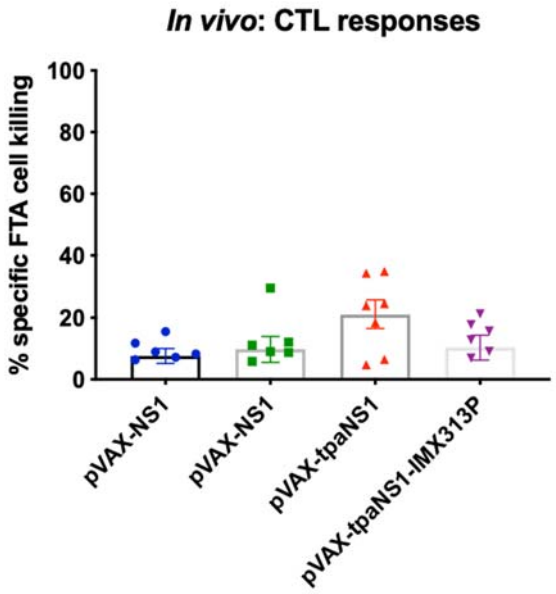

Fig. S3. Epitope confirmation. Groups of Balb/c mice (n = 6-7/ group) were vaccinated with NS1 DNA vaccine constructs three times at 2-week intervals and 13 days after the last vaccine dose FTA assay was performed. *In vivo* cell killing was measured against targets pulsed with ZIKV NS1 pool 4 peptides without CTL ID peptides (87 and 88). Peptide pulsed and mock target cells (1.5 x10^6 for each target cell cluster) were injected i.v. into vaccinated mice and splenocytes harvested 15h later and analysed by flow cytometry. % specific killing was calculated for the ZIKV NS1 CTL epitopes above mock. Data shows mean +/- SEM of NS1-specific CTL killing.

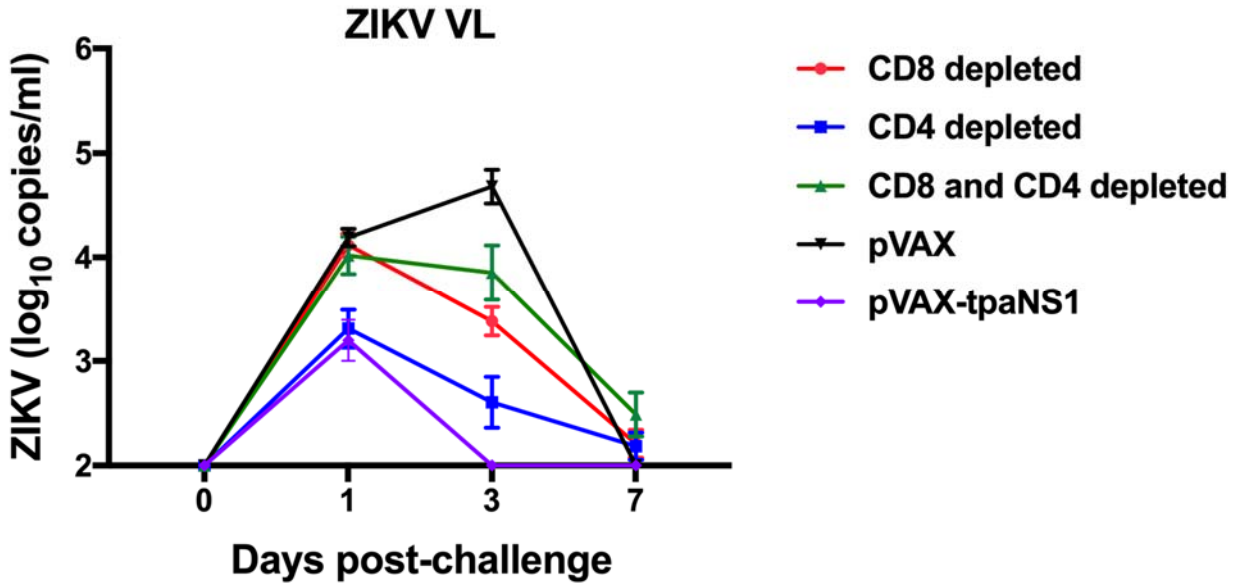

Fig. S4. ZIKV viral load over time in T cell–depleted mice. Serum viral loads in pVAX-tpaNS1 DNA vaccinated mice that were undepleted or depleted of $CD4^+$ and/or $CD8^+$ T lymphocytes prior to challenge with $ZIKV_{ZKV2015}$. Serum ZIKV viral loads (mean (n=10) ± SEM) on days 1, 3 and 7 post challenge are shown. pVAX immunised mice were used as controls.