Cell Reports, Volume *35*

Supplemental information

A vaccine inducing solely cytotoxic T lymphocytes fully prevents Zika virus infection and fetal damage

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Supplemental Figures, Titles, and Legends

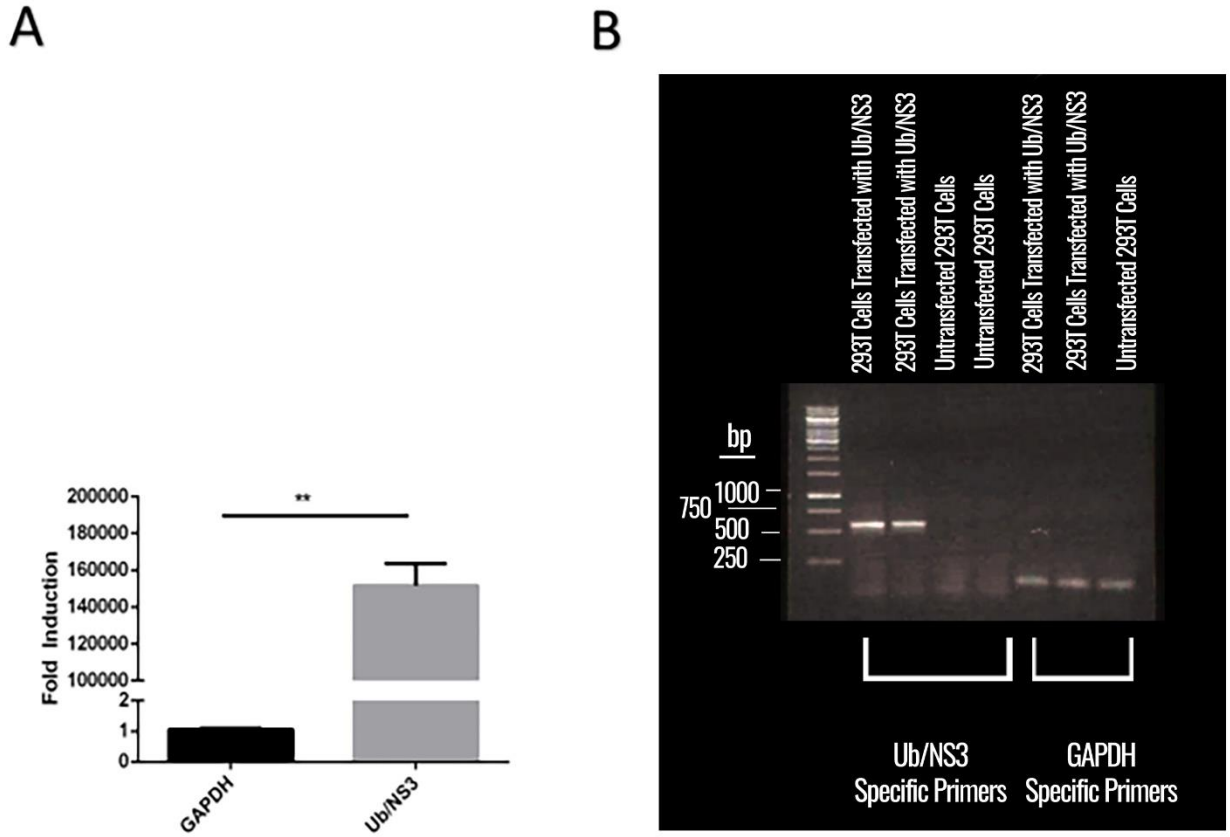

Fig. S1. mRNA levels of Ub/NS3 gene sequence in 293T cells. 293T cells were transfected with rearranged Ub/NS3 in PEI overnight. RNA was further isolated, cDNA was synthesized, and analysis of gene expression was conducted by qRT-PCR. (A) Relative gene expression of rearranged Ub/NS3 and GAPDH. (B) PCR products were run on a 1% agarose gel. Each treatment condition was replicated twice. Data was analyzed by student's *T* test. Error bars depict standard error of (s.e.m) the mean. ** indicates $P < 0.01$. Related to Fig. 1.

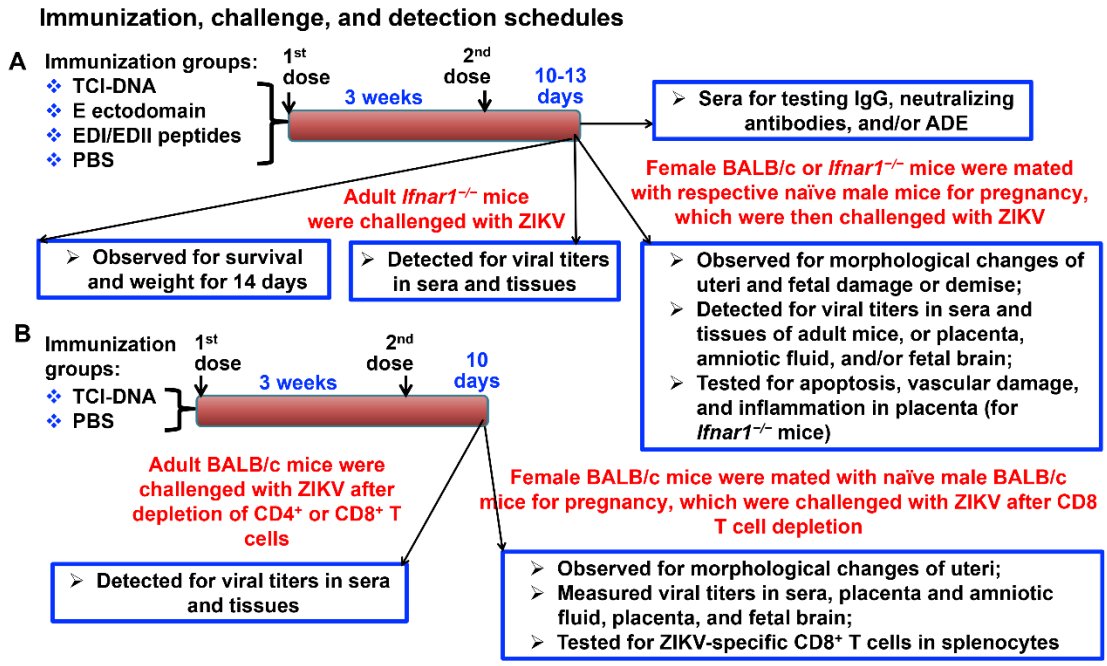

Fig. S2. Immunization, challenge, and detection schedules. (A) Schedule for BALB/c or *Ifnar1*$^{-/-}$ mice with TCI-DNA, E ectodomain, EDI/EDII peptides, or PBS. (B) Schedule for CD4/CD8 depletion experiments in BALB/c mice given either TCI-DNA or PBS. Related to STAR Methods sections titled "Immunization of mice with vaccines" and "ZIKV challenge studies and evaluation of vaccine efficacy in mice."

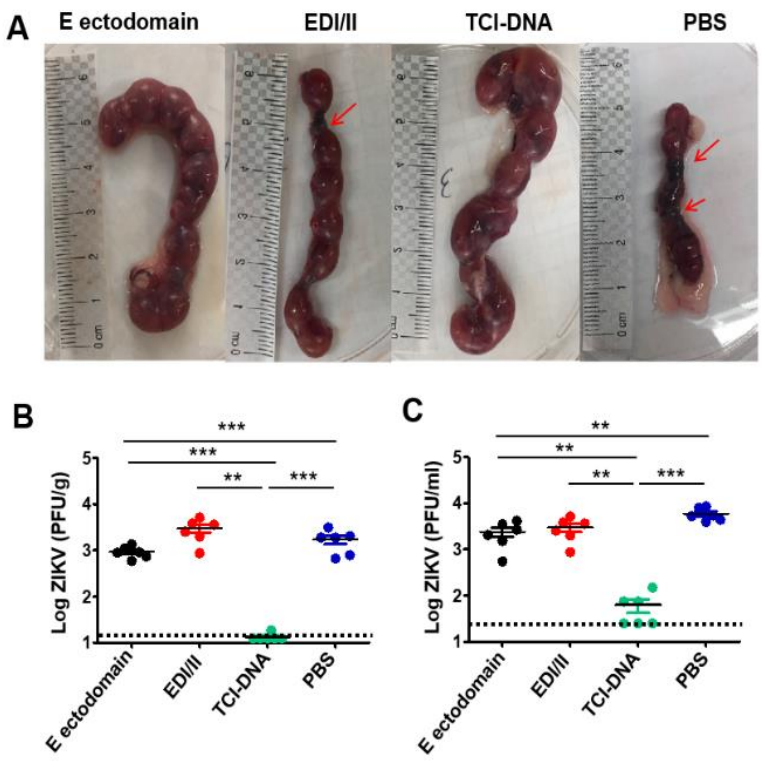

Fig. S3. ZIKV TCI-DNA vaccine protected female pregnant BALB/c mice and their fetuses against ZIKV challenge. Female BALB/c mice were immunized with ZIKV E ectodomain, EDI/II peptides, ZIKV TCI-DNA, or PBS control for two doses, sera collected, and mated with male BALB/c mice at 10 days post-2nd immunization. After receiving antibodies to IFNAR1, the pregnant mice (E5-E7) were intraperitoneally (I.P.) challenged with ZIKV (stain R103451, 2×10^5 plaque-forming unit (PFU)/mouse). Six days post-challenge, the mice were euthanized, examined for morphological changes of uteri, and for ZIKV titers using plaque-forming assay. (A) Morphology of representative images of uteri (E11-E13) of ZIKV-challenged pregnant mice. Arrows indicate fetal death. Viral titers in placenta (B) and amniotic fluid (C) of ZIKV-challenge pregnant mice. The detection limit was 12.5 PFU/g for placenta and 25 PFU/ml for amniotic fluid. The data in (B) and (C) are represented as mean ± s.e.m (n=6). ** and *** indicate $P < 0.01$ and $P < 0.001$, respectively. There are no significant differences between EDI/II and PBS groups. Related to Fig. 3.

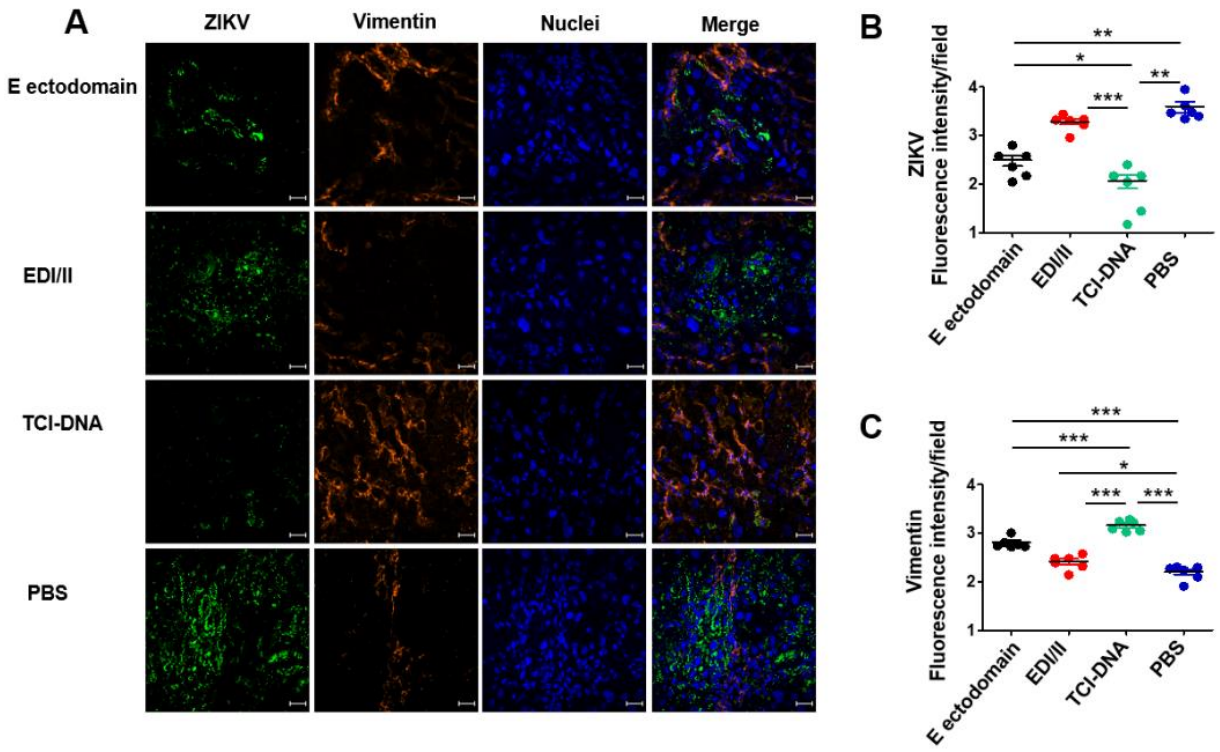

Fig. S4. ZIKV TCI-DNA vaccine prevented ZIKV-caused vascular damage in placenta of female pregnant *Ifnar1*$^{-/-}$ mice. Placenta collected from above ZIKV-challenged pregnant (E10-E12) *Ifnar1*$^{-/-}$ mice were stained for vimentin (a marker for fetal capillary endothelium and fetal blood vessels in placenta) by immunofluorescence assay. (A) Representative images of immunofluorescence staining of vimentin in placenta. ZIKV (green), vimentin (orange), and Nuclei (blue) were stained with anti-ZIKV antibody, anti-vimentin antibody, and DAPI, respectively. The images were magnified at 63X, with a scale bar of 20 µm. Quantification of ZIKV^{+} (B) and vimentin^{+} (C) staining in (A) by ImageJ software. The data are presented as mean ± s.e.m of fluorescence intensity for ZIKV^{+} or vimentin^{+} staining in each field (n = 6: "n" indicates numbers of image from different placentas). *, ** and *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Related to Fig. 4.

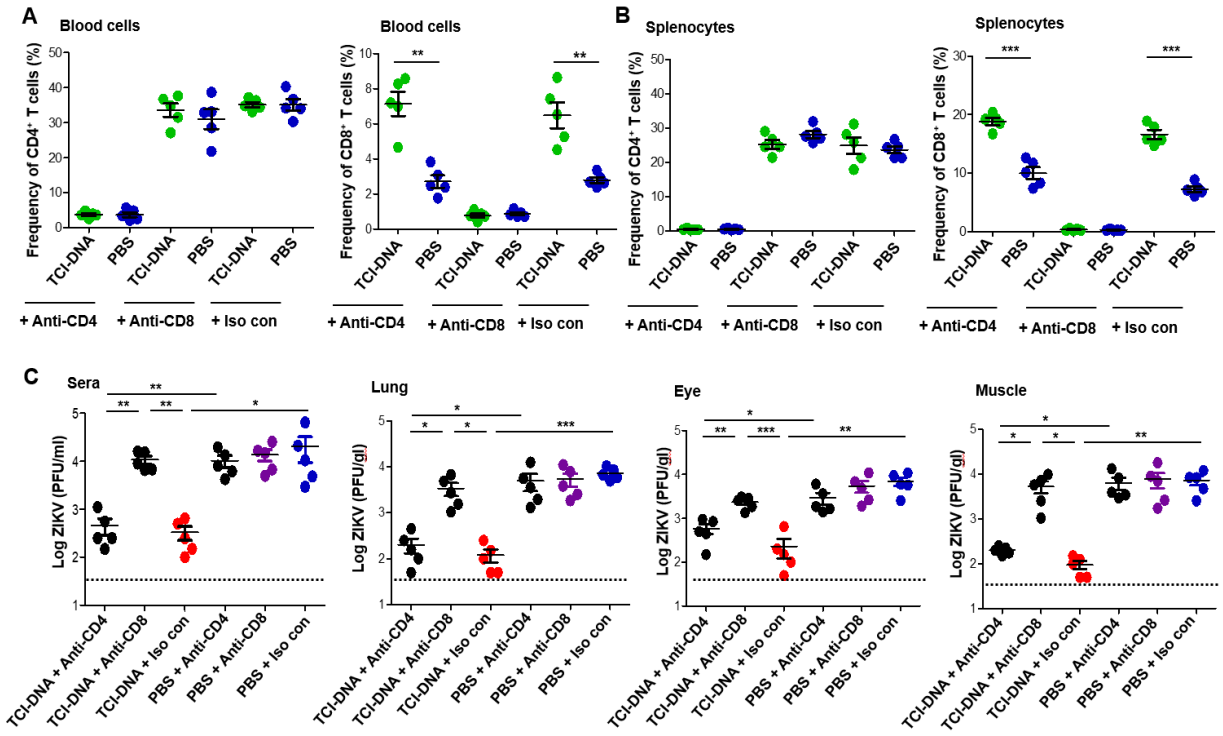

Fig. S5. CD8$^+$ T-cells induced by ZIKV TCI-DNA vaccine were essential in protecting adult mice against ZIKV infection. Male and female BALB/c mice were immunized with ZIKV TCI-DNA or PBS control for two doses; 10 days post-2nd dose, they were injected (I.P.) with anti-CD4 (for depleting CD4$^+$ T cells), anti-CD8a (for depleting CD8$^+$ T cells), or IgG2b isotype control (i.e., Iso con; without depleting CD4$^+$ and CD8$^+$ T cells) antibody (200 μg/mouse) for three times (-2, -1, and 1 day p.i.). One day before challenge, the mice were injected with anti-IFNAR1 blocking antibody (for depleting type I IFN; 2 mg/mouse), and then infected with ZIKV (strain R103451, 2.5×10^5 PFU/mouse). Three days post-challenge, the mice were sacrificed and the frequencies of CD4$^+$ or CD8$^+$ T cells in blood cells (A) and splenocytes (B) were quantified by flow cytometry analysis, as well as viral titers were determined by plaque-forming assay in sera and tissues (lung, eye, and muscle). The detection limit was 50 PFU/ml (for sera) or 50 PFU/g (for lung, eye, and muscle). The data are represented as mean ± s.e.m (n = 5). *, ** and *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Related to Fig. 7.