Supplementary Fig. 1, related to Figures 1-3. Fetal resorption in Ifnar1−/− dams with retro-orbital or intrafootpad infection with ZIKV.
Non-immune Ifnar1−/− dams were inoculated via retro-orbital (R.O.) or subcutaneous (via footpad) (S.C.) route at E7.5 with 10⁴ FFU of ZIKV FSS13025 or 10% FBS-PBS as Mock. (a) Representative pictures from uterus, fetus, and placenta with decidua at E14.5 are shown. Magenta arrows indicate the presence of fetal resorption. (b) Fetal body weight and (c) size were measured at E14.5. n = 38 fetuses from 5 separate mothers (Non-immune-Mock), n = 34 fetuses from 4 separate mothers (Non-immune-ZIKV-R.O.), and n = 11 fetuses from 2 separate mothers (Non-immune-ZIKV-S.C.). Weight and size were determined individually on the residual placenta if fetal resorption was observed. Data were pooled from two independent experiments. All data were expressed as a median with interquartile range. **p<0.01, ***p<0.001, ****p<0.0001. Kruskal-Wallis test was used for b and c.
Supplementary Fig. 2, related to Figure 1. The effect of CD4⁺, CD8⁺, or combined CD4⁺ and CD8⁺ T cell depletion on ZIKV viral burden in non-immune and DENV2-immune Ifnar⁻/- dams.

Non-immune and DENV2-immune Ifnar⁻/- dams were depleted of CD4⁺ and/or CD8⁺ T cells and challenged with ZIKV at E7.5 as described in Fig. 1. ZIKV RNA levels in maternal serum, brain, and spleen harvested at E14.5 were quantitated by qRT-PCR. ZIKV RNA levels were compared among 4 groups including 3 different combinations of T cell depletion: (a-c) Isotype versus Anti-CD8. (d-f) Isotype versus Anti-CD4. (g-i) Anti-CD4 versus Anti-CD8, and (j-l) Anti-CD8 vs Anti-CD4+CD8. n = 4 separate mothers (Non-immune-ZIKV+isotype), n = 5 separate mothers (Non-immune-ZIKV+Anti-CD8), n = 3 separate mothers (DENV2-immune-ZIKV+isotype), n = 3 separate mothers (DENV2-immune-ZIKV+Anti-CD8), n = 4 separate mothers (Non-immune-ZIKV+Anti-CD4), n = 4 separate mothers (DENV2-immune-ZIKV+Anti-CD4), n = 4 separate mothers (Non-immune-ZIKV+Anti-CD8+CD4), and n = 5 separate mothers (DENV2-immune-ZIKV+Anti-CD8+CD4). Data were pooled from two independent experiments and were expressed as a median. *p<0.05, **p<0.01. Kruskal-Wallis test was used for a-l.
Supplementary Fig. 3, related to Figures 2, 4 and 5. Phenotype of fetuses at E14.5 from non-immune or DENV2-immune WT dams treated with Ifnar1-blocking Ab with or without CD8+ T cell depletion.
Non-immune or DENV2-immune WT dams that were treated with Ifnar1-blocking Ab were challenged with ZIKV at E7.5 as described in Fig. 2. Tissues were harvested 7 days post-infection at E14.5. Representative images of fetuses and placentas from non-immune or DENV2-immune dams with or without anti-CD8 Ab administration are shown. n = 6 separate mothers (Non-immune-Mock), n = 5 separate mothers (Non-immune-ZIKV), n = 4 separate mothers (DENV2-immune-Mock), n = 5 separate mothers (DENV2-immune-ZIKV+isotype) and n = 4 separate mothers (DENV2-immune-ZIKV+Anti-CD8). Blue and magenta arrows indicate the presence of fetal growth restriction and resorption, respectively.
Supplementary Fig. 4, related to Figures 2-7. Efficiency of CD8+ T cell depletion in WT dams administered anti-CD8 Ab.
Pregnant non-immune or DENV2-immune WT mice were challenged with ZIKV at E7.5 as described in Fig 2. (a) The gating strategy used to analyze the presence of CD8+ T cells by flow cytometry in the spleen and decidua/placenta is illustrated. All plots represented were first gated on CD3+ cells. (b) The percentages of CD8+ T cell depletion in spleen and decidua/placenta are represented for isotype control or anti-CD8 Ab-treated dams in both non-immune and DENV-2 immune groups. Data are expressed as a median.
Supplementary Fig. 5, related to Figures 2 and 3. Phenotype of fetuses from DENV2-immune WT dams that were primed for 80 days prior to ZIKV challenge.

Non-immune and DENV2-immune WT dams that were primed for 80 days were treated with Ifnar1-blocking Ab and challenged with ZIKV at E7.5 as described in Fig. 2. Tissues were harvested 7 days after ZIKV infection at E14.5. (a) Representative images of fetuses and placentas are shown. n = 30 fetuses from 5 separate mothers (DENV2-immune-ZIKV) and n = 35 fetuses from 5 mothers (Non-immune-ZIKV). Blue arrows indicate fetal growth restriction. (b) Fetus weight and (c) size were recorded. (d-e) ZIKV RNA levels in the maternal spleen and placentas with decidua at E14.5 were measured by qRT-PCR. Data were pooled from two independent experiments. Data are expressed as a median. **p<0.01. Two-tailed Mann Whitney test was used.
Supplementary Fig. 6, related to Figures 2, 3 and 7. Maternal origin of cross-reactive CD8\(^+\) T cells in the decidua/placenta from WT mice treated with Ifnar1-blocking Ab.

CD45.1\(^+\) WT sires were bred with non-immune or DENV2-immune CD45.2\(^+\) WT dams, and cells from the placenta/decidua of ZIKV-infected dams were harvested at E14.5 as described in Fig. 2. Isolated cells were stimulated with a pool of 5 cross-reactive peptides for ICS and stained for cytokine production and CD45.1 and CD45.2 alleles. Representative plots of the gating strategy for IFN\(\gamma\), TNF\(\alpha\), and CD45.1/CD45.2-expressing antigen-specific CD8\(^+\) T cells were illustrated for non-immune (n=4, top panel) and DENV2-immune groups (n=4, bottom panel). Two independent experiments were performed.