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Supplemental information

A Zika virus mutation enhances transmission potential and confers escape from protective dengue virus immunity

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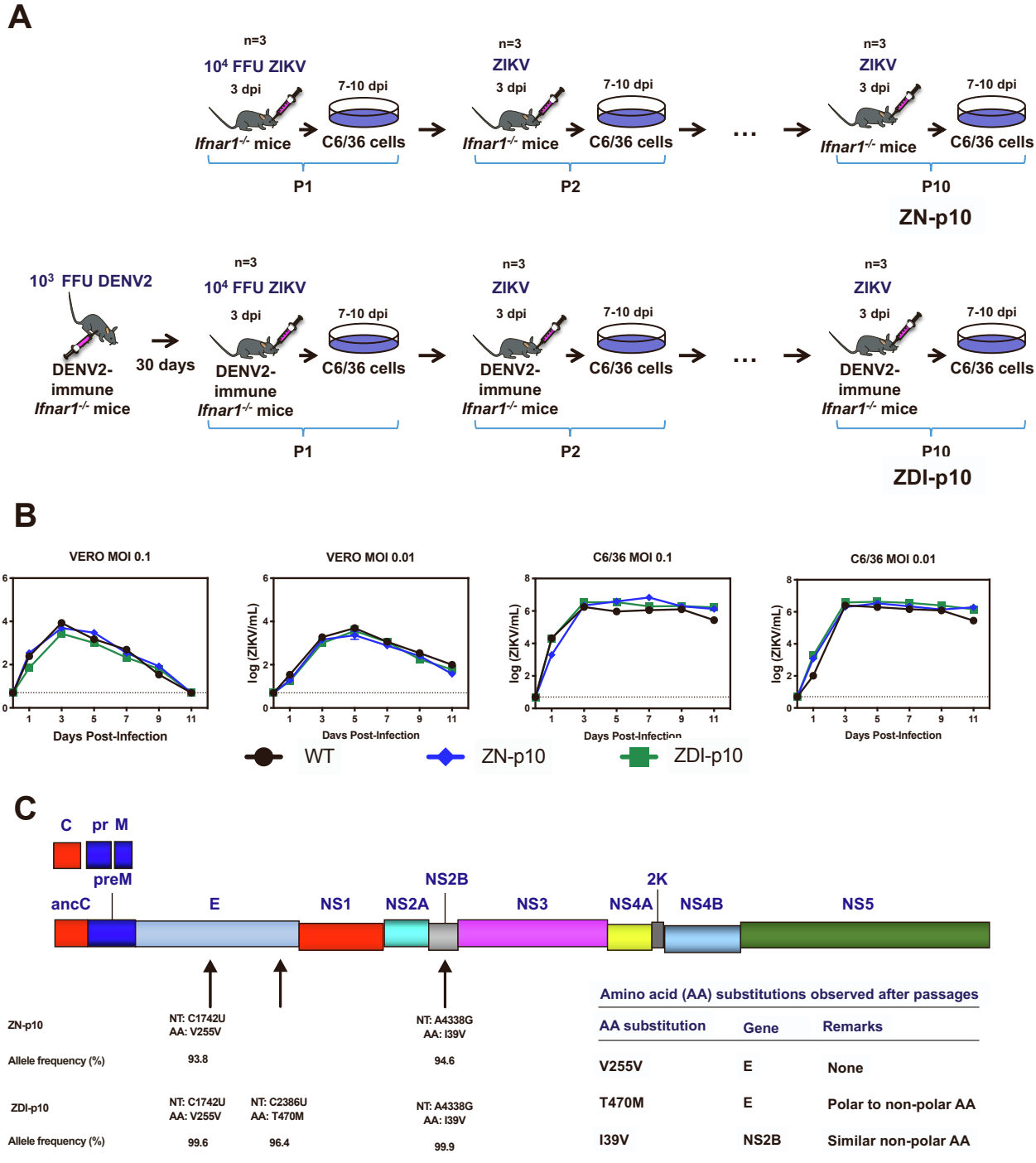
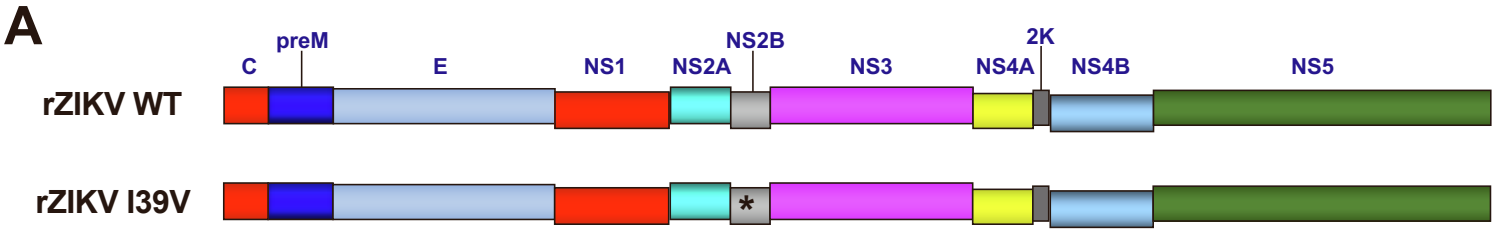
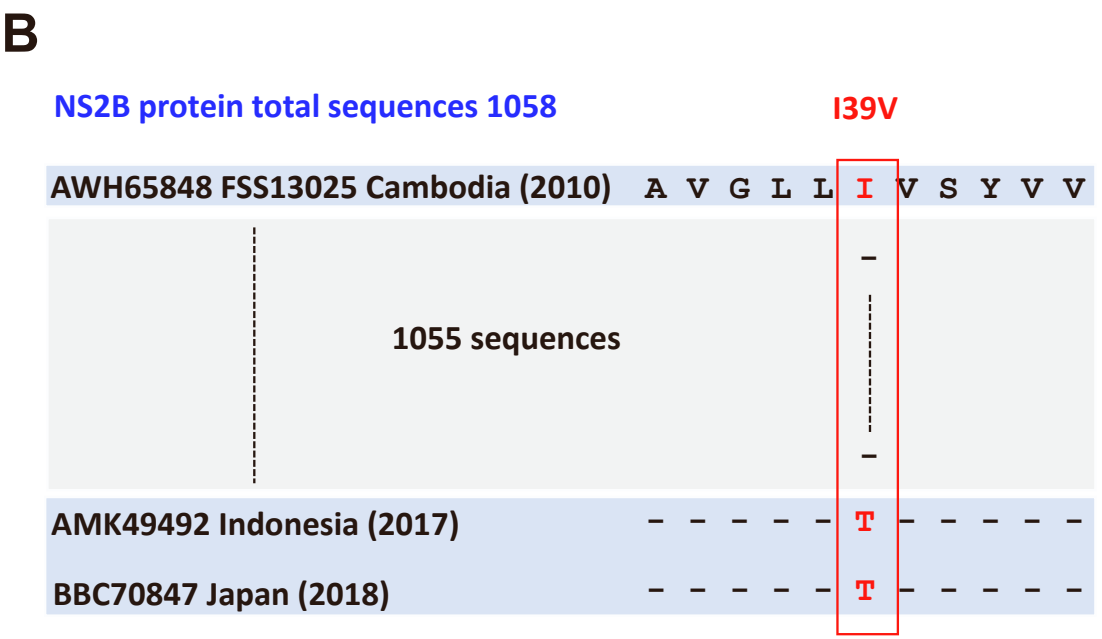


Supplementary Figure 1. Generation and characterization of two adapted ZIKV strains by passaging in mice and C6/36 mosquito cells (Related to Figure 2). (A) Schematic showing the protocols used to generate two new ZIKV strains by 10 cycles of alternate passaging in naïve *Ifnar1*$^{-/-}$ mice (strain ZN-p10) or DENV-immune *Ifnar1*$^{-/-}$ mice (strain ZDI-p10) and C6/36 cells. DENV2-immune *Ifnar1*$^{-/-}$ mice were generated by intraperitoneal injection with 10^3 FFU DENV2 strain S221 for 30 days. Group of 3 naïve (top) and 3 DENV2-immune (bottom) *Ifnar1*$^{-/-}$ mice were then inoculated intravenously with 10^4 FFU of ZIKV FSS13025, and the mice were bled at 3 dpi. Serum samples from the 3 mice per group were pooled and added to mosquito cells for 7–10 days. Virus-containing supernatants were harvested and 200 µL was injected IV into additional groups of naïve or DENV-immune *Ifnar1*$^{-/-}$ mice. After repeating this cycle a total of 10 times, we obtained ZIKV strains ZN-p10 and ZDI-p10. (B) Comparison of the growth kinetics of wild-type (WT) ZIKV, ZN-p10, or ZDI-p10 in Vero cells and C6/36 mosquito cells after infection at MOIs of 0.1 and 0.01. Viral titers were measured at the indicated time points using the FFA. Data are presented as the mean ± SEM of n=3 wells and are representative of three independent experiments. (C) Structure of the ZIKV genome, indicating the positions of key mutations in ZIKV ZN-p10 and ZDI-p10 strains. Viral RNA was analyzed by next-generation sequencing.





Supplementary Figure 2. Identification of naturally occurring I39 variants among clinical isolates (Related to Figure 4). (A) Structure of the ZIKV genome in the WT and I39V-mutated rZIKV strains. Asterisk denotes the position of the mutated I39V residue in the NS2B domain. (B) Sequence alignment of the NS2B protein from 1058 ZIKV clinical isolates, highlighting the substitutions at position 39.

Supplementary Table 1. Primers used for construction of recombinant ZIKV viruses (Related to STAR Methods).

Oligonucleotides, Sequence (5′—3′)	Source	Identifier
Env-T470M-F: CAAATTCTCATTGGAATGTTGCTGGTGTG	IDT	N/A
Env-T470M-R: CACACCAGCAACATTCCAATGAGAATTTG	IDT	N/A
NS2B-I39V-F: AGTGGGATGATCGTTCAGGATGTTGGACATGAA	IDT	N/A
NS2B-I39V-R: TTCATGTCCAACATCCTGAACGATCATCCCACT	IDT	N/A
NS2B-I39T-F: GTCGGTCTGCTAACTGTCAGTTACGTGGTC	IDT	N/A
NS2B-I39T-R: GACCACGTAACTGACAGTTAGCAGACCGAC	IDT	N/A

Supplementary Table 2. Primers and probes used for determination of ZIKV viral burden (Related to STAR Methods).

Oligonucleotides, Sequence (5′—3′)	Source	Identifier
18S forward primer: CGGCTACCACATCCAAGGAA	(Prestwood et al., 2008)	N/A
18S reverse primer: GCTGGAATTACCGCGGCT	(Prestwood et al., 2008)	N/A
18S probe – [Fam] – CTGTCTGGCA – [TamraQ]	Eurofins	N/A
ZIKV forward primer: TTGGTCATGATACTGCTGATTGC	IDT	N/A
ZIKV reverse primer: CCTTCCACAAAGTCCCTATTGC	IDT	N/A
ZIKV probe – [Fam] – CGGCATACAGCATCAGGTGCATAGGAG– [TamraQ]	Eurofins	N/A

Supplementary Table 3. Frequency of amino acids at residue 39 in flavivirus NS2B proteins (Related to Figure 4).

Virus	Most Frequent Amino Acid[a]	2nd Most Frequent Amino Acid[a]	3rd Most Frequent Amino Acid[a]
ZIKV	Ile (1056/1058)[a]	Thr (2/1058)	N/A
YFV	Met (452/452)	N/A	N/A
WNV	Phe (2627/2627)	N/A	N/A
JEV	Ala (387/387)	N/A	N/A
DENV-1	Ile (3642/3648)	Met (5/3648)	Val (1/3648)
DENV-2	Thr (3018/3023)	Ile (5/3023)	N/A
DENV-3	Ile (1682/1683)	Val (1/1683)	N/A
DENV-4	Leu (664/667)	Ile (3/667)	N/A

[a] (Number of clinical isolates with the indicated amino acid/total number of clinical isolates tested)
N/A=not applicable

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