Development, Characterization, and Application of Two Reporter-Expressing Recombinant Zika Viruses

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3551 3561 3571 3581 359 3601 3611 3621 CUGAGGAAGAGAUGGACGGCCAAGCUCA Majority **GGACCCUUUU**CCGCUU CUGGC JEV SA14 (KU323483) JEV SA14-14-2 (JN604986) UC.C. GAU U. G С G υ G С UC С GAU G U WNV NY99 (DQ211652) WNV B956 (AY532665) U U A A U G G C C C CG UC C C U ĊĞ υc A Ů MVEV MVE-1-51 (AF161266) A . A . A / Ū U А U GA υ MVEV V11-10 (JX123032) Ū Ū G c Ū ū Ū GA SLEV Hubbard (EU566860) G G Ú SLEV Imperial Valley (JF460774) С AΑ CU AGCA G AACGC U GG G A . A . A A AUGC ...GA DENV-1 16007 (AF180817) AAUG UG А GA AUCC GCAAA GC U DENV-2 16681 (U87411) DENV-3 BID-V4782 (JN183884) AG .G cu cu GG UG AA G. GA A G C U GUA СА UGCU CCU А AAC GC AGGA UUGG AAG AUG.U UA CAGG DENV-4 H780571 (JQ513344) υ G U UGCC υ UUUG GG A GA GUC AC AGC YFV Asibi (AY640589) YFV 17D (X03700) UG G G C C 00 Ü G GG CA GGAC AC AGC GGAC ZIKV MR-766 (KX377335) CU CAUGG U AG G GGU AU A CA ú AG G AUGG G GG AU AU GG AG CAUGG В JEV SA14 (expressing NS1' ZIKV MR-76 =× P6-740 UGGACGGCCAGAUUGA SL2 Ξ AAGAUCA JEV SA14-14-2 (not expressing NS1 ZIKV PRVA

Supplemental Figure S1

Figure S1. ZIKV lacks the ribosomal frameshift signal directing the expression of NS1'. (**A**) Nucleotide sequence alignment for seven major mosquito-borne flaviviruses (17 strains total). The consensus sequence is shown on top, and residues that match the consensus are hidden as dots to emphasize residues that differ from the consensus. (**B**) Predicted RNA folding involved in JEV NS1' frameshifting and its ZIKV counterpart. RNA secondary structures with pseudoknots were predicted using the IPknot program. Highlighted are the primary sequences and secondary structures important for the expression of JEV NS1': the heptanucleotide slippery sequence (blue), stem-loop 1 (SL1, orange), stem-loop 2 (SL2, magenta) and pseudoknot base-pairing (green). Also indicated is the silent point mutation G³⁵⁹⁹A (red circle) that is sufficient to abolish the synthesis of JEV SA14 NS1'.