

Table S1. Primers used in this study.

Description	Direction	Sequence
JEV Nakayama strain prM-E cloning into pcDNA3.1	Forward	5'-CCGGAATTCACCATGGAAGGCTCAATCATGTGG-3'
	Reverse	5'-GGCCTCGAGTTAAGCATGCACATTGGTCGC-3'
JEV Beijing-1 strain prM-E cloning into pcDNA3.1	Forward	5'-CCGGAATTCACCATGGAAGGCTCAATCATGTGG-3'
	Reverse	5'-GCTACCAATGTGCATGCTTA-ACTCGAGGCC-3'
JEV P3 strain prM-E cloning into pcDNA3.1	Forward	5'-CCGGAATTCACCATGGAAGGCTCAATCATGTGG-3'
	Reverse	5'-GCGACCAATGTGCATGCTTA-ACTCGAGGCC-3'
DENV-2 NGC strain prM-E cloning into pcDNA3.1	Forward	5'-CGGGATATCACCATGAACAGGAGACGCAGAACT-3'
	Reverse	5'-GGCCTCGAGTTAGGCCTGCACCATAACTCC-3'
Sequence primer designed on CMV promoter	Forward	5'-GGCGTGACGGTGGGAGGTC-3'
JEV prM-E sequence primer 1	Forward	5'-AGGCATTCCAAGACTAGTAGGAGATCCGTG-3'
JEV prM-E sequence primer 2	Forward	5'-GGGTCACAGGAAGGAGGC-3'
JEV prM-E sequence primer 3	Reverse	5'-AGTCGTTGAAAAGGCTTTGCC-3'
DENV-2 prM-E sequence primer 1	Forward	5'-GCATAGGAATATCAAATAGAG-3'
DENV-2 prM-E sequence primer 2	Forward	5'-GCTCTCCGAGAACGGGCCTCG-3'
DENV-2 prM-E sequence primer 3	Forward	5'-AACCTCCATTCTGGAGACAGC-3'
JEV Nakayama G106V introduction	Forward	5'-GTACTTTTCGGGAAGGGAAGCATTGAC-3'
	Reverse	5'-ACATCCGTTGCCCCACCCACGATCAG-3'
JEV Nakayama L107F introduction	Forward	5'-GGATTTTCGGGAAGGGAAGCATTGAC-3'
	Reverse	5'-ACATCCGTTGCCCCACCCACGATCAG-3'
JEV Nakayama G106V/L107F introduction	Forward	5'-GTATTTTCGGGAAGGGAAGCATTGAC-3'
	Reverse	5'-ACATCCGTTGCCCCACCCACGATCAG-3'
JEV Beijing-1 G106V introduction	Forward	5'-GTACTTTTCGGGAAGGGAAGTATTG-3'
	Reverse	5'-GATCGTGGGTGGGGCAACGGATGT-3'
JEV P3 G106V introduction	Forward	5'-GTACTTTTCGGGAAAGGAAGCATTG-3'
	Reverse	5'-GATCGTGGGTGGGGCAACGGATGT-3'
DENV-2 G106V introduction	Forward	5'-GTATTATTGAAAAGGAGGCATTGTG-3'
	Reverse	5'-ACATCCATTCCCCATCCTCTGTCC-3'

Underline: Restriction enzyme site.

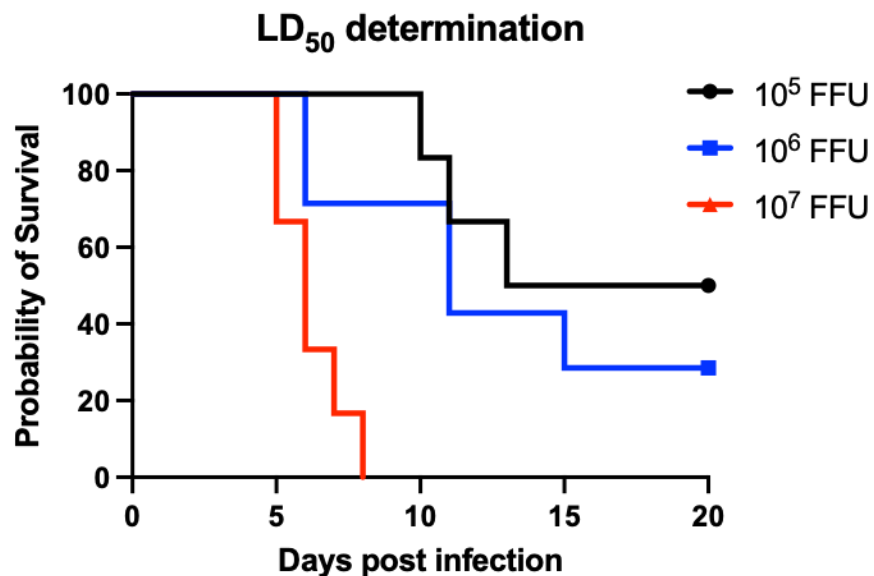


Figure S1. Determination of 50% lethal dose. A serially diluted JEV P3 strain was inoculated intraperitoneally into six or seven 6-week-old BALB/c mice to determine the 50% lethal dose (LD₅₀). The mice were monitored for 20 days. Mice were humanely euthanized if they showed apparent symptoms or a decrease of >20% in their initial body weight. The estimated LD₅₀ was 1.4×10^5 focus forming unit (FFU).