



**Supplemental Figure 1. Analysis of thermostability, Related to Figure 1.** (A) and temperature sensitivity (B) of WT and N154Q viruses in Vero cells. (A) Thermostability analysis. WT and N154Q virus were pre-incubated at 37°C or 40°C for 30 min or 60 min. After incubation, viral titers in each sample were determined by plaque assay. The viral activity was calculated by normalizing the viral titers of treatment groups to those of untreated groups. A one-way ANOVA test was performed to analyze the statistical differences between each treatment group and corresponding un-treated group. (B) Temperature sensitivity analysis. For each virus, viral titers determined using standard plaque assay on two sets of WT or N154Q virus-infected Vero cells. Sets were incubated for 4 days at 37°C or 40°C, respectively. The average results of three experiments with standard deviations are presented.

## **Supplemental Experimental Procedures**

**Virus thermostability assay.** Equal amounts ( $2 \times 10^5$  PFU/ml) of WT or N154Q diluted in 2% FBS DMEM were pre-incubating at 37°C or 40°C for 30 min or 60 min. After incubation, viral titers in each sample were determined by plaque assay. We calculated the relative infectivity by normalizing the viral titers of treatment groups to those of untreated groups (Xie et al. 2017).

**Virus thermo sensitivity assay.** Temperature sensitivity of each virus was determined using standard plaque assay on two sets of ZIKV WT or ZIKV E mutant virus-infected Vero cells. Sets were incubated for 4 days at 37°C or 40°C, respectively. The temperature sensitivity was calculated as followed:  $TS = \log_{10} \text{PFU at } 37^\circ\text{C} - \log_{10} \text{PFU at } 40^\circ\text{C}$  (Whiteman et al. 2011).

## Supplemental References

- Whiteman, M. C., J. A. Wicker, R. M. Kinney, C. Y. Huang, T. Solomon & A. D. Barrett (2011) Multiple amino acid changes at the first glycosylation motif in NS1 protein of West Nile virus are necessary for complete attenuation for mouse neuroinvasiveness. *Vaccine*, 29, 9702-10.
- Xie, X., Y. Yang, A. E. Muruato, J. Zou, C. Shan, B. T. Nunes, D. B. Medeiros, P. F. Vasconcelos, S. C. Weaver, S. L. Rossi & P. Y. Shi (2017) Understanding Zika Virus Stability and Developing a Chimeric Vaccine through Functional Analysis. *MBio*, 8.