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2 **Supplementary Figure 1. ZE4B-36 mutant is attenuated and induces a brief infection in mice.**

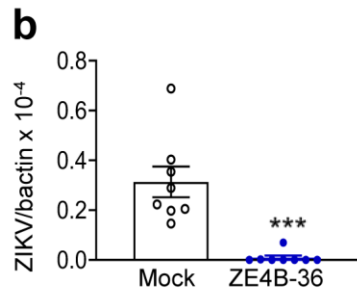
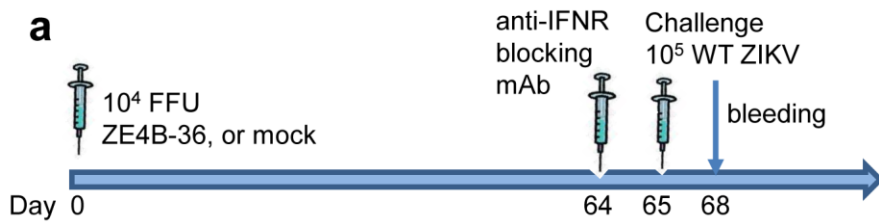
3 **a-b.** 4- to 6-week old AB6 were infected with 1×10^4 FFU WT FSS13025ic (WT, n=19), ZE4B-36
 4 (n=14) or PBS (mock, n=14). The immunized mice were monitored for weight loss (a), survival (b).

5 Weight loss is indicated by percentage, using the weight on the day before immunization to define
 6 100%. *** $P < 0.001$ compared to WT group (log rank test). **c-f.** 4 to 6-week old AB6 mice were

7 infected with 2.2×10^5 FFU ZE4B-36 or PBS (mock). Viral loads at day 6 in blood (c), brain (d),
 8 uterus (e) and testes (f) were determined by Q-PCR assays. n= 6. Data are presented as means \pm

9 standard error of the mean (s.e.m). *** $P < 0.001$ compared to mock group (Unpaired t test).

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12 **Supplementary Figure 2. Vaccination with ZE4B-36 protects WT B6 mice from ZIKV**

13 **infection.** 4- to 6-week old WT B6 mice were infected with ZE4B-36 or PBS (mock) subcutaneously.

14 On day 64 pi, mice were pretreated with mouse MAR1-5A3 followed by infection with 1x 10⁵ FFU

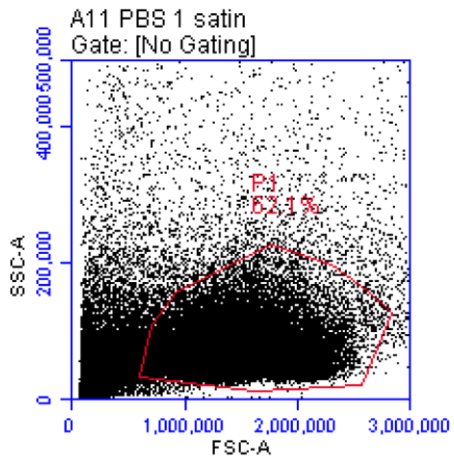
15 ZIKV-Dakar-MA strain one day later. **a.** Schematic of vaccination and challenge. **b.** Viremia was

16 determined by using Q-PCR at day 3 pi (n=8). Data are presented as means ± standard error of the

17 mean (s.e.m). *** P < 0.001 compared to mock group (Unpaired t test).

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21 **Gating Strategy:** Samples were processed with a C6 Flow Cytometer instrument. Total splenocytes
22 were gated. Dead cells were excluded on the basis of forward and side light scatter.

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