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**Supplementary Data**

**A single nonsynonymous mutation on ZIKV E protein-coding sequences leads to markedly increased neurovirulence *in vivo***

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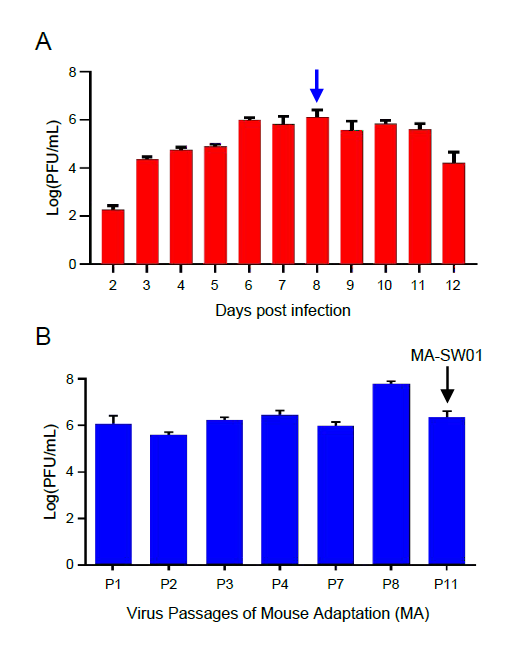
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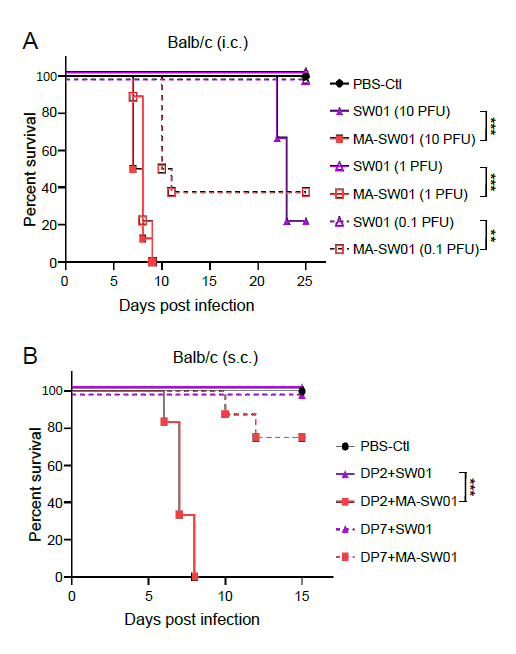
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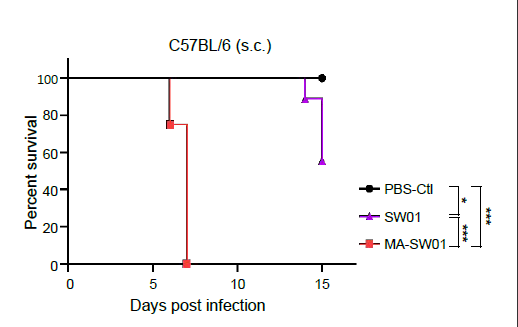
**Supplementary Figure S1.** **ZIKV clinical isolate SW01 can grow in the brain of neonatal mice.**

**(A)** DP2 (2 days postnatal) BALB/c mice were intracranially (i.c.) infected with 1,000 PFU ZIKV clinical isolate SW01 (n=3 for each group). Brains were collected from day 2 to day 12 after infection and homogenized. Virus titers of brains were tested by standard plaque assay. **(B)** Virus titers from MA-P1 to MA-P11 (MA-SW01) were determinated by standard plaque assay (n=3–5 for each group). The summary data were presented as mean ± standard deviation (SD). Data shown are representative of two independent experiments.



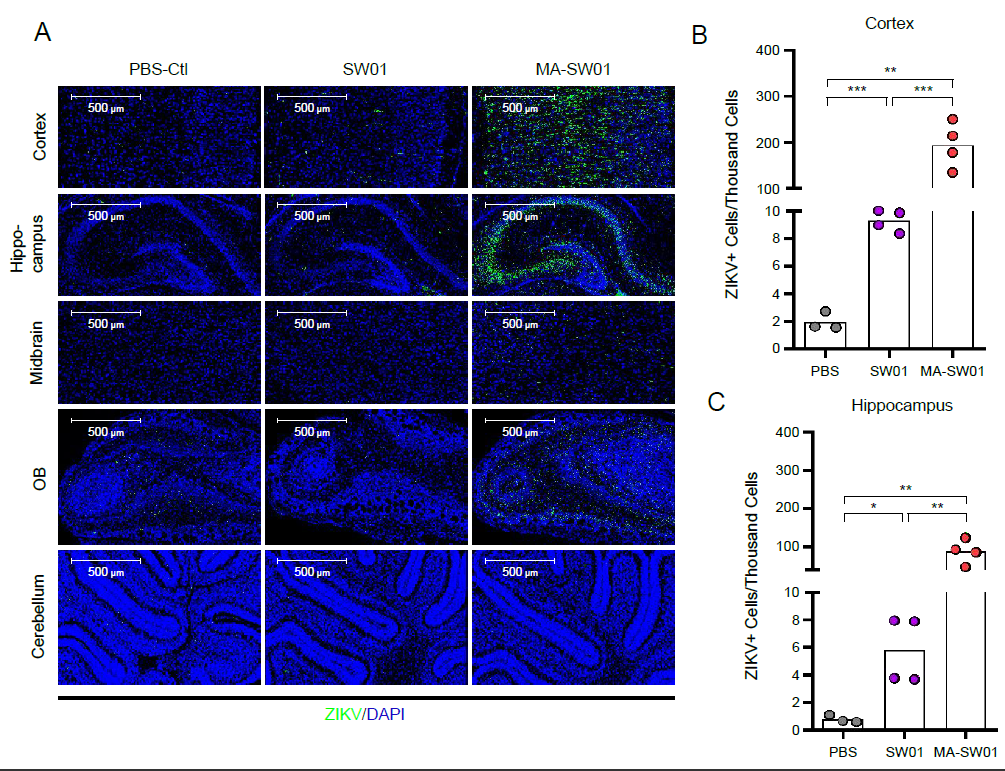
**Supplementary Figure S2. Increased virulence of the adapted MA-SW01 virus is dose and age dependent.**

**(A).** DP2 BALB/c mice were inoculated with 10, 1, and 0.1 PFU of SW01 virus or MA-SW01 virus, or the same volume of PBS control by intracranial (i.c.) route and monitored for survival rate from 0 to 25 days post infection (n=8-10 for each group). **(B).** DP2 and DP7 BALB/c mice were inoculated with either 100 PFU of SW01 or MA-SW01 virus, or the same volume of PBS control by subcutaneous (s.c.) route and monitored for survival rate from 0 to 15 days post infection (n=6-10 for each group). The difference between survival rate was analyzed by log rank test, P values were indicated by \*\* (*P*<0.01), or \*\*\* (*P*<0.001). Data shown are representative of two independent experiments.



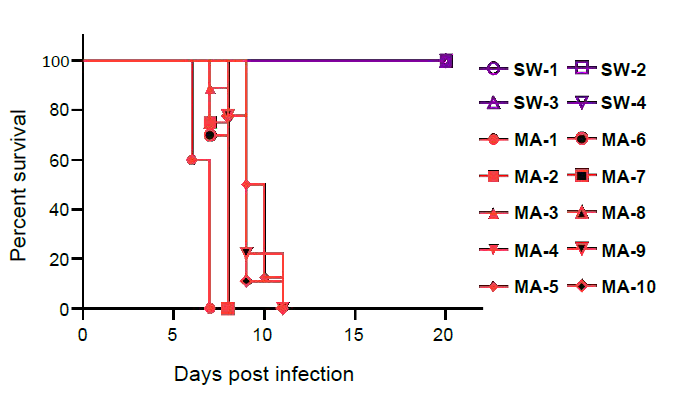
**Supplementary Figure S3. Increased virulence of the adapted MA-SW01 virus is reproducible in mice of a different genetic background.**

DP2 C57BL/6 mice were inoculated with 100 PFU SW01 or MA-SW01 virus, or PBS control by subcutaneous (s.c) route and monitored for survival rate from 0 to 15 days (n=8–9 for each group). The difference between survival rate was analyzed by log rank test, P values were indicated by \* (*P*<0.05), or \*\*\* (*P*<0.001). Data shown are representative of two independent experiments.

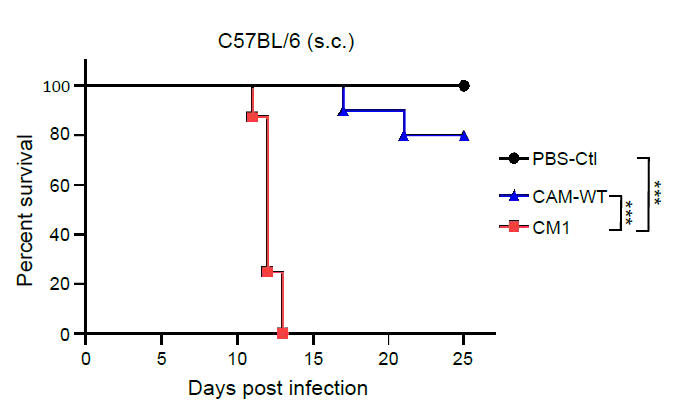


**Supplementary Figure S4. Cortex and hippocampus regions are the major target sites of MA-SW01 virus infection.**

**(A).** DP2 BALB/c mice were inoculated with 100 PFU SW01 virus, or MA-SW01 virus, or PBS control by s.c. route (n=3–4 for each group). Virus E protein expression in different brain regions (Cortex, hippocampus, midbrain, olfactory bulb (OB) and cerebellum) was shown by representative immunostaining; ZIKV positive cells in cortex **(B)** and hippocampus **(C)** were quantified and summarized as mean ± standard deviation (SD), with statistical analysis performed using student's t test, and *P* values were indicated by \* (*P*<0.05), or \*\* (*P*<0.01), or \*\*\* (*P*<0.001). Data shown are representative of two independent experiments.



**Supplementary Figure S5. Biological clones derived from the adapted MA-SW01 virus (MA) are more virulent than clones from the parental SW01 virus (SW).** DP2 BALB/c mice were i.c. injected with 10 PFU of cloned viruses isolated from either SW01 (SW) or MA-SW01 (MA), and then monitored for survival from 0 to 20 days (n=8-10 for each group). Purple symbols represent clones from SW01 (SW), and red symbols represent clones from MA-SW01 (MA). The difference between survival rate was analyzed by log rank test, *P* values were indicated by \* (*P*<0.05), or \*\* (*P*<0.01), or \*\*\* (*P*<0.001). Data shown are representative of two independent experiments.



**Supplementary Figure S6. Molecularly cloned ZIKV with E protein mutations (CM1) is more virulent than its parental virus (CAM-WT) in C57BL/6 mice.** DP2 C57BL/6 mice were injected with 100 PFU CAM-WT or CM1 virus, or PBS control by s.c. route and monitored for survival from 0 to 25 days post infection (n=8–10 for each group). Survival rate was analyzed by log rank test, and P values were indicated by \* (*P*<0.05), or \*\* (*P*<0.01), or \*\*\* (*P*<0.001). Data shown are representative of two independent experiments.

**Supplementary Table S1. Deduced E protein amino acid sequences on selected positions in SW01(SW) and MA-SW01(MA) derived viruses.**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Single Clone Viruses | Amino acid positions | | | | | | | | | |
| 6 | 67 | 68 | 69 | 154 | 296 | 335 | 370 | 401 | 440 |
| Consensus(WT) | V | D | M | A | N | D | T | E | H | S |
| 1-B-2(WT) | V | D | M | A | N | D | T | E | H | S |
| 1-C-3(WT) | V | D | M | A | N | D | T | E | H | S |
| 1-C-4(WT) | V | D | M | A | N | D | T | E | H | S |
| 1-D-4(WT) | V | D | M | A | N | D | T | E | H | S |
| 1-A-1(M) | V | N | I | A | D | D | R | A | H | S |
| 1-B-4(M) | V | N | I | A | D | D | R | E | H | S |
| 1-B-5(M) | V | N | I | A | D | D | R | E | H | S |
| 1-D-3(M) | V | N | I | A | D | D | R | E | H | P |
| 1-D-5(M) | V | N | M | V | N | D | T | E | Y | S |
| 2-B-3(M) | G | N | I | A | D | D | R | E | H | S |
| 2-C-3(M) | V | N | I | A | D | D | R | E | H | S |
| 2-C-5(M) | V | N | I | A | D | N | R | E | H | S |
| 2-D-5(M) | V | N | M | V | N | D | T | E | Y | S |
| 2-D-6(M) | V | N | M | V | N | D | T | E | Y | S |