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Dengue virus envelope protein domain III-elicited antibodies mediate cross-protection against Zika virus in a mouse model

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Highlights

- Dengue virus E protein domain III-induced antibodies provide cross-protection against Zika virus infection in wild-type mouse model;
- Maternally acquired anti-dengue virus E protein domain III antibodies increase the survival of 1-day-old mice born to mothers.

ABSTRACT

Dengue virus (DENV) and Zika virus (ZIKV) are antigenically related mosquito-transmitted viruses which represent a big public health problem. Although the antigenic cross-reactivity between two viruses were intensively investigated at the antibody and T cell levels, how DENV envelope protein domain III (EDIII)-elicited antibodies (Abs) impact the outcome of ZIKV infection is uncertain. Here, our results show that the sera isolated from DENV-EDIII-immunized wild-type mice recognized ZIKV-EDIII and cross-neutralized ZIKV in vitro. Passive transfer of DENV-EDIII-immune sera protected 1-day-old mice against lethal ZIKV challenge. Finally, maternally acquired anti-DENV-EDIII Abs significantly increased the survival of 1-day-old mice born to DENV-EDIII-immunized mothers post ZIKV challenge. These results reveal that DENV-EDIII-induced Abs provide cross-protection against ZIKV and may not mediate the Ab-dependent enhancement of ZIKV infection at the concentration used here. The present study would contribute to the development and application of DENV-EDIII-based vaccines.

1. Introduction

Family *flaviviridae*, genus flavivirus has more than 100 members including Yellow fever virus (YFV), Dengue virus (DENV), Japanese encephalitis virus (JEV), Zika virus (ZIKV), etc. According to the nucleotide variation, DENV is divided into four serotypes (DENV1-4) which generally cause dengue fever and occasionally dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)(Gintarong et al., 2018; Ko et al., 2018; Thomas et al., 2018). DHF and DSS usually occur in secondary heterotypic DENV infection and are supposed to be mediated by antibody (Ab)-dependent enhancement (ADE)(Dejnirattisai et al., 2010; Katzelnick et al., 2017). Currently, DENV spreads on a global scale and affects about 390 million people annually(Bhatt et al., 2013). Since the first isolation in Uganda in 1947, ZIKV did not attract attention until the circulation in Micronesia in 2007(Duffy et al., 2009; Posen et al., 2016) and big outbreaks in French Polynesia during 2013-2014 and South America during 2015-2016(Fauci and Morens, 2016; Musso et al., 2018). Like DENV, ZIKV infection usually gives rise to mild fever, rash but can cause the microcephaly of newborn when infecting pregnant women(Bautista, 2018; Hoen et al., 2018).

Tetravalent live attenuated DENV vaccine developed by Sanofi was licensed in 20 countries in Asia, Latin America, and Australia (Wilder-Smith et al., 2019). However, this vaccine is suggested to be applied only in people aged 9-45 years with DENV infection history and not to be used in individuals without DENV infection history because it may cause ADE of secondary heterologous DENV infection in vaccine-vaccinated population(Aguiar, 2018; Wilder-Smith et al., 2019). Therefore, it is urgent to develop a universal DENV vaccine which could be used in individuals without any limitation. The extracellular part of Flavivirus envelope (E) protein has three domains [I, II, and III (EDI, EDII, and EDII)] and EDIII is the major target for anti-flavivirus Ab response(Rey, 2013). Many studies demonstrated that DENV-EDIII-induced Ab mediates the neutralization of or protection against homologous or heterologous DENV infection in vitro or in vivo(Poggianella et al., 2015; Ramasamy et al., 2018; Zaneti et al., 2019). Therefore, EDIII is becoming a promising vaccine candidate for DENV. Blast searching results show that DENV-EDIII shares high level of amino acid homology with ZIKV-EDIII. But, whether and how DENV-EDIII-induced Abs affect the outcome of ZIKV infection is uncertain.

The present study is designed to evaluate the effect of DENV-EDIII-induced Abs on ZIKV infection in vitro and in vivo using wild-type (WT) mouse model. Our results show that DENV-EDIII-immune mouse sera cross-neutralized ZIKV in vitro and passive transfer of DENV-EDIII-immune sera protected 1-day-old WT mice against lethal ZIKV challenge. Furthermore, maternally acquired anti-DENV-EDIII Abs also protected 1-day-old mice born to DENV-EDIII-immunized mothers against ZIKV challenge. Overall, these results suggest that DENV-EDIII-induced Abs may not enhance the severity of ZIKV infection but mediate cross-protection against ZIKV challenge in mice.

2. Materials and methods

2.1. Cells, virus and mice

Vero cells and baby-hamster-kidney (BHK)-21 cells were purchased from the American Type Culture Collection (ATCC) and grown in RPMI-1640 medium (Gibco) and MEM-α medium (Gibco) supplemented with 5% heat-inactivated fetal bovine serum (FBS; Gibco), 100 U/ml Penicillin (Gibco), 100 μg/ml Streptomycin (Gibco), respectively. ZIKV strain Zhejiang04 (GenBank accession no: KX117076.1) was isolated from the serum of a Wenzhou Zika patient who traveled to the Republic of Suriname during 2016 Zika outbreak in South America. ZIKV was grown in Vero cells for two passages to produce the stock and tittered in BHK-21 cells using focus-forming assay (FFA) as described previously(Wen et al., 2017). The ZIKV titer was expressed as focus-forming unit (FFU)/ml. Six-week-old male and female WT (C57BL/6) mice were purchased from Nanjing Biomedical Research Institute of Nanjing University (NBRI) and bred in the animal center of Zhejiang Provincial Center for Disease Control and Prevention.

2.2. Recombinant expression of DENV-EDIIIs and ZIKV-EDIII

The soluble recombinant EDIII proteins of DENV1 (Hawaii strain, GenBank Accession No: ACF49259), DENV2 (NGC strain, GenBank Accession No: AAC59275), DENV3 (H87 strain, GenBank Accession No: AAA99437), DENV4 (H241 strain, GenBank Accession No: AAX48017) and ZIKV (FSS13025 strain, GenBank Accession No: JN860885) were expressed using prokaryotic expression vector. In brief, the codon-optimized gene sequence encoding DENV1-EDIII, DENV2-EDIII, DENV3-EDIII, DENV4-EDIII or ZIKV-EDIII, were synthesized by Genewiz (Suzhou, China) and cloned into prokaryotic plasmid pET21a, downstream of a 6×His-tag. The recombinant plasmids were transfected into *E. coli* BL21 strain and the recombinant EDIII proteins were purified by Ni-NTA column (Phygene, Fuzhou, China) according to the manufacturer's instructions. Purified EDIII proteins were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western Blot (WB).

2.3. Mouse immunization

DENV1-EDIII, DENV2-EDIII, DENV3-EDIII, DENV4-EDIII, DENV(1-4)-EDIII (the mixture of equal weight of DENV1-EDIII, DENV2-EDIII, DENV3-EDIII, DENV4-EDIII), and ZIKV-EDIII were emulsified in complete Freund's Adjuvant (CFA, Sigma), respectively, and injected subcutaneously into the back of 6-week-old female mice (50 μg/protein/mouse). Two weeks later, mice were boosted with the same protein emulsified in incomplete Freund's Adjuvant (IFA, Sigma). The mock-immunized mice received the adjuvants without antigen. Two weeks after the boosting, mice were euthanized by isoflurane and sera were collected. Both mock-immune and EDIII-immune sera were inactivated for 30 min at 56℃and used for ELISA, Micro-neutralization assay, and passive transfer experiments.

2.4. Enzyme-linked immunosorbent assay (ELISA)

Indirect capture ELISA was utilized to measure the DENV-EDIII-, ZIKV-EDIII-reactive IgG in mock-

immune and EDIII-immune sera. In brief, 96-well ELISA plate was coated with individual EDIII (DENV1-EDIII, DENV2-EDIII, DENV3-EDIII, DENV4-EDIII or ZIKV-EDIII; 100 ng/well) at 4℃overnight. Next day, ELISA plates were washed by PBST (0.1% Tween-20/PBS) and blocked by blocking buffer [1 % bovine serum albumin (BSA)/PBS] at 37 ℃for 1 h. Three-fold serially diluted mouse sera (starting from 1:10) was added to the well and incubated at 37℃for 1 h. The plates were washed using PBST and incubated with HRP-conjugated goat anti-mouse IgG Ab (Invitrogen, 1:5000) at 37℃ for 1 h. Finally, the plate was developed with 3,3 ',5,5'-tetramethyl benzidine (TMB) and the absorbance at 450 nm (OD450) was measured.

2.5. Micro-neutralization assay

Micro-neutralization (MN) assay was used to evaluate the neutralizing ability of mouse sera against ZIKV according to a recent publication with little modifications(Barba-Spaeth et al., 2016). In general, Vero cells were seeded to the well of 96-well culture plates (1×10^4/well). ZIKV (100 FFU/50 μl/well) and three-fold serial dilutions (starting from 1:3) of mouse sera (50 μl/well) were mixed and incubated for 1 h at 37℃. Blank well was given virus only. The mixture of virus and serum or virus alone was added to Vero cells and infected for 2 h at 37℃. The virus-serum mixture was discarded and 1% Methylcellulose/RPMI-1640 medium was used to cover Vero cells. Two days later, cells were fixed with 4 % paraformaldehyde/PBS and permeabilized with 1 % Triton X-100/PBS. After being blocked by 1 % BSA/PBS, the plate was incubated with mouse anti-ZIKV E protein mAb (1 μg/ml; ARG66286, Arigo Biolaboratories) for 1 h at room temperature (RT). Then, the plates were loaded with HRP-conjugated goat anti-mouse IgG Ab (Invitrogen, 1:5000) at RT for 1 h. Finally, the plate was developed with TMB and OD450 of each well was recorded. The percent neutralization was calculated based on the following formula: Percent neutralization=$[(OD450_{blank}-OD450_{serum})/OD450_{blank}]\times100\%$. 50% neutralization titer (NT50) was calculated as the average of mouse sera and presented as the reciprocal of serum dilution yielding 50% inhibition of the OD450 (compared with mock-immune sera).

2.6. ZIKV challenge of mice transferred with EDIII-immune sera

Different volumes [0.0001 μl (in 10 μl PBS), 0.1 μl (in 9.9 μl PBS), 2 μl (in 8 μl PBS), 10 μl] of pooled mock-immune or EDIII-immune (DENV1-EDIII, DENV2-EDIII, DENV3-EDIII, DENV4-EDIII, DENV(1-4)-EDIII or ZIKV-EDIII-immune) sera were injected subcutaneously into the back of 1-day-old WT mice. After 2 hours, 1×10^2 FFU (in 10 μl) of ZIKV was injected subcutaneously into the back of mice. Mouse weight and death were recorded daily for 28 days.

2.7. ZIKV challenge of 1-day-old mice born to DENV2-immunized mothers

Six-week-old female WT mice were immunized with DENV2-EDIII as above described. Four weeks later, DENV2-EDIII-immunized mice were mated with 10-week-old naive male WT mice. The 1-day-old mice born to naive or DENV2-EDIII-immunized mothers were sacrificed using isoflurane and sera were collected. The DENV-EDIII-, ZIKV-EDIII-reactive IgG levels in two-fold serially diluted sera (starting from 1:40) were detected using indirect capture ELISA as above described. In addition, the 1-day-old mice born to naive or DENV2-EDIII-immunized mothers were injected subcutaneously with 1×10^2 FFU (in 10 μl) of ZIKV. Mouse

weight and death were recorded daily for 28 days.

2.8. Statistical analysis

All data were analyzed using Prism 6 software (GraphPad Software, La Jolla, CA, USA) and expressed as the mean±SEM. Group means were compared using the two-tailed Mann-Whitney test. Survival data were analyzed using a log-rank test. $P<0.05$ was considered statistically significant.

3. Results

3.1. DENV-EDIII-immune serum cross-recognizes ZIKV-EDIII, vice versa

The amino acid sequences of DENV1-EDIII, DENV2-EDIII, DENV3-EDIII, DENV4-EDIII, and ZIKV-EDIII are aligned and shown in **Figure 1**. The percent identities between four DENV-EDIIIs and ZIKV-EDIII range from 44% to 49%. To determine whether DENV-EDIII-immune serum contains Ab cross-reactive with ZIKV-EDIII, we coated the ELISA plate with individual DENV-EDIII or ZIKV-EDIII and detected the level of DENV-EDIII- and ZIKV-EDIII-binding IgG in the sera of mice immunized with DENV1-EDIII, DENV2-EDIII, DENV3-EDIII, DENV4-EDIII, DENV(1-4)-EDIII or ZIKV-EDIII. As shown in **Figure 2**, the OD450 values measured by the naive (mock-immune) serum were significantly low as compared to EDIII-immune sera, indicating a negative result. In comparison, DENV-EDIII-immune sera not only bind to homologous DENV-EDIII but also heterologous DENV-EDIII and ZIKV-EDIII with the high OD450 values. Notably, the mixture of four DENV serotype EDIIIs immunization induced Ab response to DENV1 to DENV4-EDIIIs and ZIKV-EDIII, suggesting that four DENV serotype EDIIIs may not interfere with each other in inducing Ab response. Similarly, ZIKV-EDIII-immune sera recognized not only ZIKV-EDIII but also four DENV-EDIIIs (**Figure 2**). These results reveal the Ab cross-reactivity between DENV-EDIII and ZIKV-EDIII.

3.2. DENV-EDIII-induced Abs cross-neutralize ZIKV infection in vitro

We next assessed the neutralizing activity of DENV-EDIII-immune sera against ZIKV using MN assay on Vero cells. As shown in **Figure 3**, mock-immune sera did not obviously neutralize ZIKV. In contrast, both DENV-EDIII-immune and ZIKV-EDIII-immune sera efficiently neutralized ZIKV with NT50 of 1:10000-1:63095 and 1:36307, respectively. It is notable that DENV4-EDIII induced relatively weaker neutralizing Ab response than ZIKV-EDIII. NT50 titers for the other DENV-EDIII-immune sera (DENV1-EDIII, DENV2-EDIII, DENV3-EDIII, and DENV(1-4)-EDIII) are comparable to that of ZIKV-EDIII-immune sera. In general, these results suggest that each DENV serotype EDIII could induce appreciable cross-neutralizing Ab response to ZIKV.

3.3. DENV-EDIII-immune sera protect against lethal ZIKV challenge in mice

Since DENV-EDIII-immune sera could efficiently cross-neutralize ZIKV infection in vitro, we next evaluated whether passive transfer of DENV-EDIII-immune sera is sufficient to mediate cross-protection

against lethal ZIKV attack in vivo. In the present study, 1-day-old WT mice were chosen for use because recent studies revealed that ZIKV infection is lethal in 1-day-old WT mice but blocked by type I interferon system of adult WT mice(Li et al., 2018; Manangeeswaran et al., 2016). One-day-old mice were injected subcutaneously with pooled mock-immune, DENV1-EDIII-immune, DENV2-EDIII-immune, DENV3-EDIII-immune, DENV4-EDIII-immune, DENV(1-4)-EDIII-immune, or ZIKV-EDIII-immune sera, and then subcutaneously challenged with 1×10^2 FFU of ZIKV. As a result, passive transfer of mock-immune sera caused 30%-41% mouse death (**Fig. 4b, d, f, h**). High volume (10 μl) of EDIII-immune sera isolated from mice immunized with DENV1-EDIII, DENV2-EDIII, DENV3-EDIII, DENV4-EDIII, or ZIKV-EDIII significantly increased mouse survival relative to mock-immune sera (83%, 79%, 75%, 86%, 100% *vs* 30% survival, respectively; **Fig. 4h**). 0.1, 2 μl of DENV-EDIII-immune sera was neither to be protective as the high volume (10 μl) and nor to be pathogenic, suggesting low concentration of DENV-EDIII-induced Ab does not mediate the ADE of ZIKV infection. In comparison, both 0.1 μl and 2 μl of ZIKV-EDIII-immune sera significantly increased mouse survival as compared to mock-immune sera (86%, 100% *vs* 41% survival, respectively; **Fig. 4d, f**). To further determine whether DENV-EDIII-induced Abs have the potential capacity to enhance the severity of ZIKV infection when the serum is diluted enough to reach sub-neutralizing level, we then transferred a quite low volume (0.0001 μl) of DENV-EDIII-immune sera to recipient mice followed by ZIKV challenge. As shown in Fig. 4b, ZIKV-EDIII-immune sera did not protect against ZIKV again. Out of expectation, DENV-EDIII-immune sera did not significantly increase mouse death but just cause earlier death (**Fig. 4b**). Taken together, our results suggest that Abs induced by DENV1-EDIII, DENV2-EDIII, DENV3-EDIII or DENV4-EDIII could mediate cross-protection against ZIKV at the high concentration in vivo and may not cause ADE of ZIKV infection in vivo.

3.4. Maternally acquired anti-DENV2-EDIII Abs increase the survival of 1-day-old mice born to DENV2-EDIII-immunized mice post lethal ZIKV challenge

Since passive transfer of high volume of DENV2-EDIII-immune serum is able to decrease the severity of Zika disease in vivo, we further investigate whether maternally acquired anti-DENV2-EDIII Abs also protect against ZIKV infection in mice born to DENV2-EDIII-immunized mothers. We first detected high level of DENV2-EDIII-reactive IgG and relatively low level of ZIKV-EDIII-reactive IgG in 1-day-old mice born to DENV2-EDIII-immunized mothers (**Fig. 5**). One-day-old mice born to DENV2-EDIII-immunized mothers and challenged with ZIKV exhibited decreased weight loss and increased survival compared to naive mice (**Fig. 6**). 40% and 82% of the mice born to naive control group and DENV2-EDIII-immunized mothers survived, respectively (**Fig. 6b**). These results further confirmed the cross-protection of anti-DENV-EDIII Abs against ZIKV.

4. **Discussion**

Growing numbers of animal studies demonstrated that DENV-EDIII immunization could induce neutralizing Abs which neutralize DENV in vitro and reduce viremia in mice post DENV challenge(Chiang et al., 2014; Fahimi et al., 2018; Ramasamy et al., 2018). Since the advantage in reducing risk for developing

ADE of heterotypic DENV infection, EDIII is currently becoming a promising immunogen for constructing DENV vaccine(Fahimi et al., 2018). However, how DENV-EDIII-induced Abs affect the outcome of ZIKV infection is not determined. We show here that DENV-EDIII-immune mouse sera contain ZIKV-EDIII-reactive IgG which efficiently cross-neutralized ZIKV infection in vitro; Passive transfer experiments identified that DENV-EDIII-immune serum is able to significantly increase the mouse survival of ZIKV-challenged 1-day-old WT mice; Finally, maternally acquired anti-DENV2-EDIII Abs also significantly increased the survival of 1-day-old mice born to DENV2-EDIII-immunized mothers post ZIKV challenge.

Since DENV and ZIKV are co-circulating in many countries and share 47-52% homology at the protein level(Barba-Spaeth et al., 2016; Cerezo et al., 2019), the cross-reactive Ab and T cell immune responses between two viruses were intensively investigated(Wen et al., 2017). Cross-reactive Abs induced by DENV not only cross-protected against ZIKV infection but potentially enhanced the severity of ZIKV infection in vivo, vice versa. In contrast, cross-reactive T cells elicited by DENV or ZIKV protected against both DENV and ZIKV infection in animals(Wen and Shresta, 2019). Flavivirus protein E (exactly EDIII) is responsible for binding to susceptible cell receptors and infecting host cells, and then induces neutralizing Ab response. DENV-EDIII proteins have been used extensively for developing tetravalent dengue vaccine candidates and anti-EDIII monoclonal Abs have been confirmed to be able to efficiently block DENV entry into host cells(Crill and Roehrig, 2001). Although proteins expressed in prokaryotic and eukaryotic cells differ in antigenic properties caused by post-translational modification (like glycosylation, etc), they have similar structure and immunogenicity to native proteins. For instance, DENV-EDIII produced in E.*coli* blocked DENV infection in vitro and induced appreciable neutralizing antibody response in mice (Chiang et al., 2014; Chiang et al., 2013). Accordingly, ZIKV-EDIII expressed in insect cells inhibited ZIKV infection of cells in vitro and elicited ZIKV-neutralizing antibodies in mice (Qu et al., 2018). Our data show that DENV-EDIII expressed in E.*coli* induced high level of anti-DENV Ab responses, which is line with previous studies(Chiang et al., 2014; Chiang et al., 2013). The present study further shows that anti-DENV-EDIII mouse Abs recognized ZIKV-EDIII and efficiently cross-neutralized ZIKV in vitro, which is inconsistent with a recent mouse study. In that study, anti-DENV-EDIII Abs isolated from Balb/c mice immunized with plasmid encoding DENV-EDIII showed no neutralization [defined by focus reduction neutralization test (FRNT) on Vero cells] on ZIKV in vitro. Moreover, anti-DENV-EDIII Abs did not recognize ZIKV using ZIKV-infected Vero cells (defined by indirect fluorescence assay)(Slon Campos et al., 2017). The possible explanation for this discrepancy is: 1) different mouse background and immunization protocol: we immunized C57BL/6 mice with recombinant DENV-EDIII while Slon Campos et al., immunized Balb/c mice with DNA vector encoding DENV-EDIII; 2) different methods used for evaluating cross-reactivity and neutralization activity: we used ELISA and micro-neutralization assay, whereas Slon Campos et al., conducted indirect fluorescence assay and FRNT. In fact, DENV-EDIII and ZIKV-EDIII share 44% to 49% amino acid homology (Fig 1), which theoretically explains the Ab cross-reactivity between two viruses.

To investigate the immune response to ZIKV, researchers applied many animal models including *Ifnar1*$^{-/-}$ mice, *Ifngr1*$^{-/-}$ *Ifnar1*$^{-/-}$ mice, *Irf3*$^{-/-}$ *Irf5*$^{-/-}$ *Irf7*$^{-/-}$ mice, STAT2$^{-/-}$ mice, and anti-*Ifnar1* mAb-treated C57BL/6 mice(Li et al., 2018). Recently, two studies reported that 1-day-old WT mice were susceptible to ZIKV and

developed lethal disease post ZIKV challenge(Li et al., 2018; Manangeeswaran et al., 2016). In the present study, we evaluated whether anti-DENV-EDIII Abs have the protective activity against ZIKV in 1-day-old mice. Our data suggests that high volume of DENV-EDIII-immune sera significantly increased mouse survival while low, middle volumes of DENV-EDIII-immune sera mediated modest protection against ZIKV. Notably, we did not observe enhanced severity of ZIKV infection when transferring quite low volume (0.0001 μl) of DENV-EDIII-immune sera, which is in line with recent studies showing the Abs induced by ZIKV-EDIII produced in E. *coli* did not enhance DENV infection in vitro(Cabral-Miranda et al., 2019; Yang et al., 2017), and the sera from DENV2-EDIII-immunized mice have less enhancement capacities for heterotypic virus(Chiang et al., 2013). Our results then imply that anti-DENV-EDIII Abs may not mediate ADE of ZIKV in vivo at indicated concentration. We further asked whether maternally acquired anti-DENV-EDIII Abs also contribute to protection against ZIKV. We detected high level of DENV2-EDIII-reactive IgG and relatively low level of ZIKV-EDIII-reactive IgG in 1-day-old mice born to DENV2-EDIII-immunized mothers. This suggests that 1-day-old mice successfully acquired DENV2-EDIII-induced IgG from DENV2-EDIII-immunized mice. We challenged 1-day-old mice born to DENV2-EDIII-immunized mothers with ZIKV and observed that mice displayed reduced weight loss and increased percent survival relative to 1-day-old mice born to naive mothers. Unlike humans, mouse neonates could acquire maternal Abs through not only placental but also breast-feeding routes(Lee et al., 2016; Qi et al., 2012). Although the titer of ZIKV-EDIII-reactive IgG in neonates is not so high and low concentration of DENV-EDIII-induced Abs have negligible protection against ZIKV infection as identified above, mice could get Abs through breast-feeding route continuously, which may explain the maternal Abs-mediated protection against ZIKV. Thus, these data further confirm that anti-DENV-EDIII Abs could provide cross-protection against ZIKV in vivo.

Taken together, our results support the protective role of DENV-EDIII-induced Abs in ZIKV infection and suggest that DENV-EDIII is a safe vaccine candidate in terms of its cross-protection against ZIKV and reduced ADE.

Acknowledgements

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Figure legends

Figure 1. Amino acid sequence alignment between DENV-EDIII and ZIKV-EDIII.
Dots represent identical amino acids.

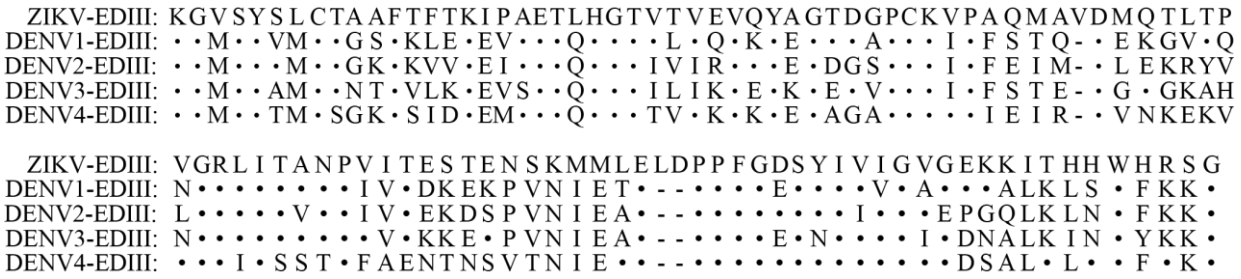


Figure 2. Sera from DENV-EDIII-immunized mice contain DENV-EDIII- and ZIKV-EDIII-reactive IgG. Mouse sera were collected from naive (mock-immune, n=6), DENV1-EDIII-immune (n=6), DENV2-EDIII-immune (n=6), DENV3-EDIII-immune (n=6), DENV4-EDIII-immune (n=6), DENV(1-4)-EDIII-immune (n=6), and ZIKV-EDIII-immune (n=6) mice. DENV1-EDIII-, DENV2-EDIII-, DENV3-EDIII-, DENV4-EDIII- and ZIKV-EDIII-reactive IgG levels were measured by indirect capture ELISA. Data are expressed as the mean±SEM.

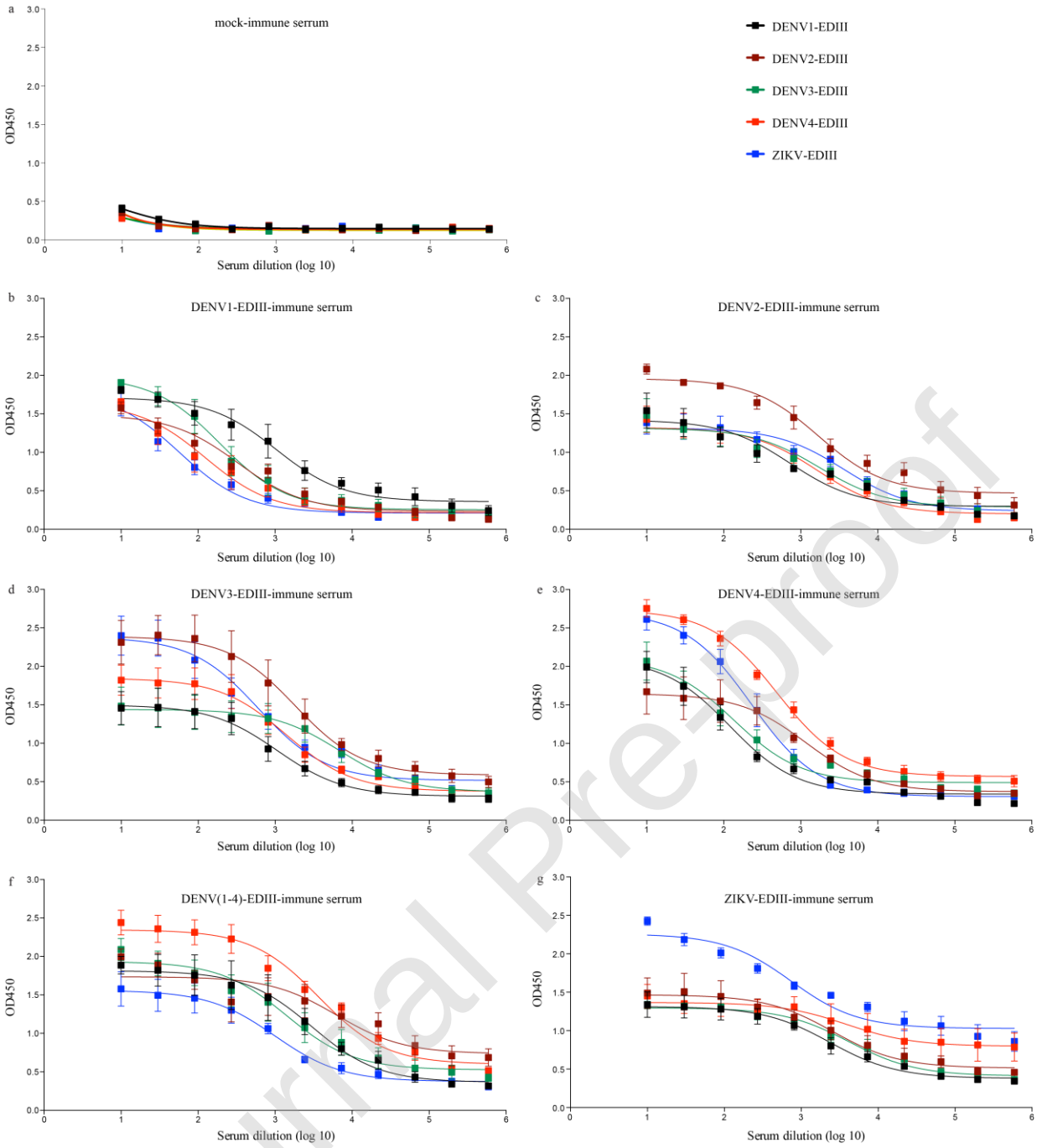


Figure 3. Sera from DENV-EDIIII-immunized mice neutralize ZIKV infection in vitro.

ZIKV samples (1×10^2 FFU/well) were incubated with mouse sera and then added to Vero cells for 2 h. Viral infection of cells was evaluated using ELISA analysis of ZIKV E protein expression. Neutralization is expressed as the percent reduction in infection compared with Vero cells incubated with ZIKV alone. Data are expressed as the mean±SEM of (**a**) naive (n=6), DENV1-EDIII-immune (n=6), DENV2-EDIII-immune (n=6), DENV3-EDIII-immune (n=6), DENV4-EDIII-immune (n=6), DENV(1-4)-EDIII-immune, and ZIKV-EDIII-immune sera. $^{**}P<0.01$ using a two-tailed Mann-Whitney test.

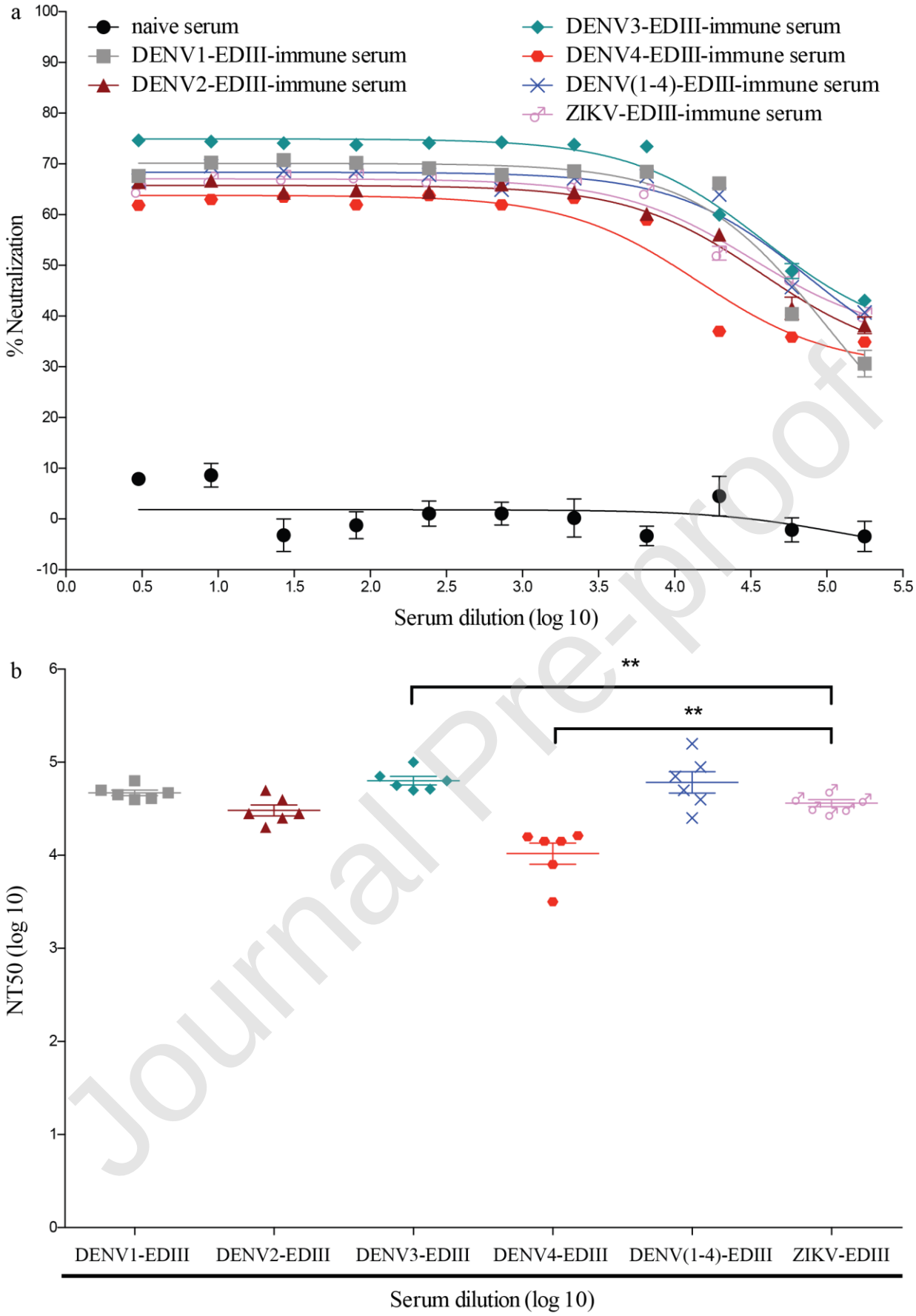


Figure 4. Passive transfer of DENV-EDIII-immune mouse sera increases the survival of ZIKV-infected mice.

One-day-old naive C57BL/6 mice were injected subcutaneously with 0.0001, 0.1, 2, 10 µl of naive, DENV1-EDIII-immune, DENV2-EDIII-immune, DENV3-EDIII-immune, DENV4-EDIII-immune, DENV(1-4)-EDIII-immune, ZIKV-EDIII-immune sera as described in the Materials and Methods. Two hours later, the mice were injected subcutaneously with 1×10^2 FFU of ZIKV. Mouse weight and death were recorded daily for 28 days. Data are expressed as the mean±SEM.*P<0.05, **P<0.01, ***P<0.001. **a**, **c**, and **e**: two-tailed Mann-Whitney test. **b**, **d**, and **f**: log-rank test.

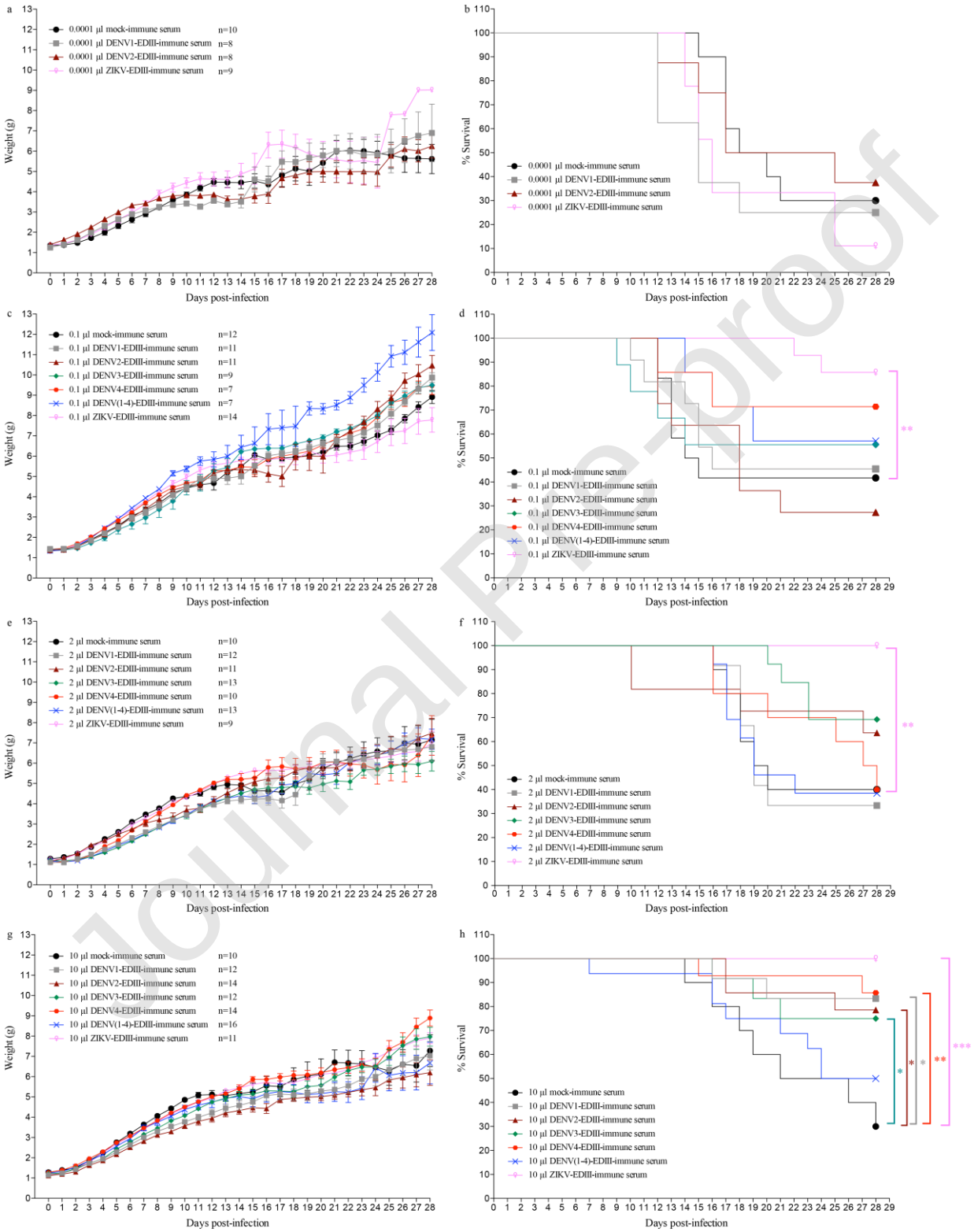


Figure 5. DENV2-EDIII- and ZIKV-EDIII-reactive IgG levels in 1-day-old mice born to naive or DENV2-EDIII-immune mothers.

Six-week-old female C57BL/6 mice were subcutaneously immunized with DENV2-EDIII twice as described in the Materials and Methods. Four weeks later, DENV2-EDIII-immunized female mice were mated with 10-week-old naive male mice. One-day-old mice born to naive or DENV2-EDIII-immunized females were sacrificed and sera were collected. DENV2-EDIII- and ZIKV-EDIII-reactive IgG levels were measured by indirect capture ELISA. Data are presented as the mean±SEM. *P<0.05. **b**: two-tailed Mann-Whitney test.

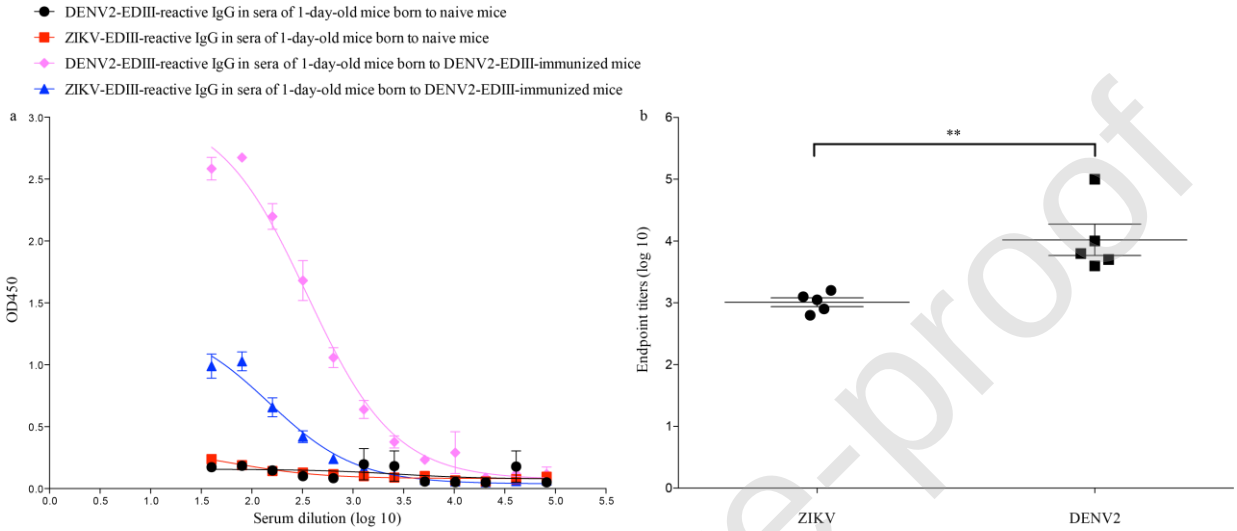
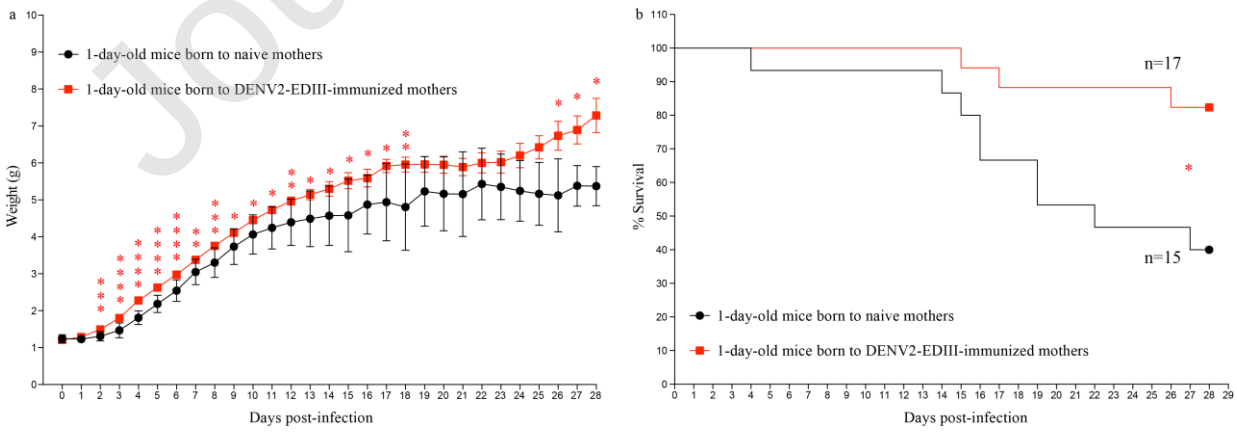


Figure 6. Maternally acquired DENV2-EDIII-induced antibodies increase the survival of ZIKV-infected 1-day-old mice born to DENV2-EDIII-immunized mothers.

Six-week-old female C57BL/6 mice were subcutaneously immunized with DENV2-EDIII twice as described in the Materials and Methods. Four weeks later, DENV2-EDIII-immunized females were mated with 10-week-old naive males. One-day-old mice born to naive or DENV2-EDIII-immunized mice were injected subcutaneously with 1×10^2 FFU of ZIKV. Mouse weight and death were recorded daily for 28 days. Data are presented as the mean±SEM. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. **a**: two-tailed Mann-Whitney test. b: log-rank test.



Authors' statement

YZ and JW designed the experiments. YZ, DC, LY, WZ, ZD performed the experiments. YZ, DC, LY, YZ and JW interpreted the data. YZ and JW wrote the paper. JW edited the paper

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