MD627 994 Seminar in Medical Microbiology 2 (2-0-4)

Rational Design of a Conserved Epitope-Driven Strategy for Developing Safe and Broadly Protective Flavivirus Vaccines

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Background

- Flavivirus
- Current gap in flavivirus therapeutics and vaccines
- Novel epitope for designing cross-reactive neutralizing flavivirus antibodies

First paper

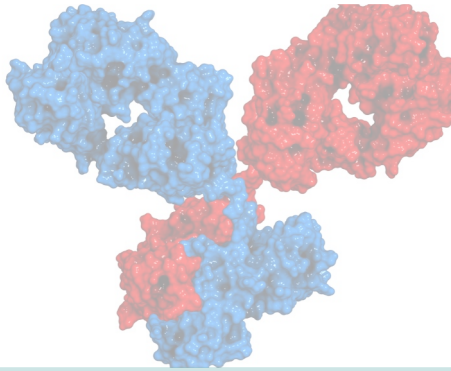
- Neutralizing antibodies targeting a novel epitope on the envelope protein exhibited broad protection against flaviviruses without risk of disease enhancement

Second paper

- A novel immunogen comprising a bc loop and mutant fusion loop epitopes generates potent neutralization and protective abilities against flaviviruses without risk of disease enhancement

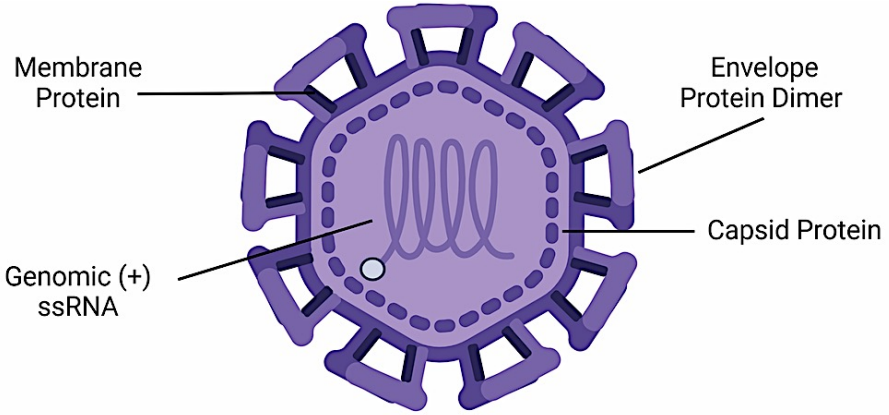
Criticisms

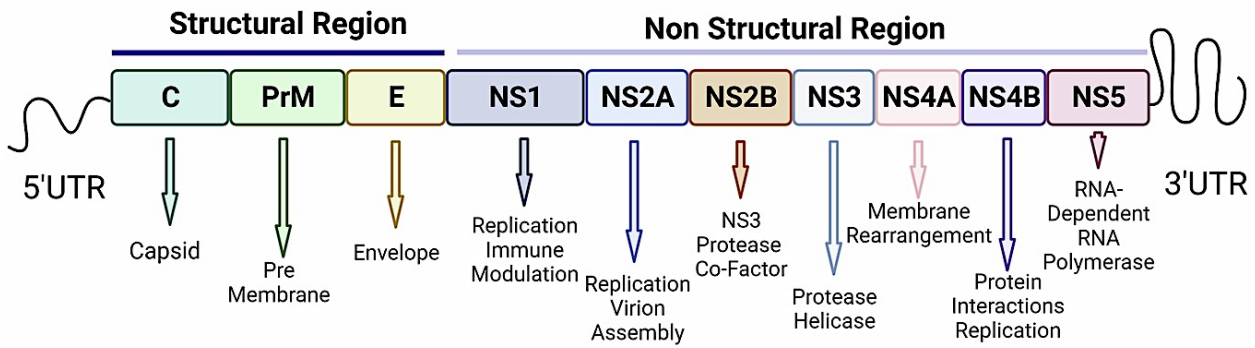
- Strong & weak points



The *Flavivirus* Genome

- **Family:** Flaviviridae
- **Genus:** *Flavivirus*
- **A single positive-stranded RNA virus**
- Dengue, Zika, Japanese Encephalitis, West Nile virus, and others.

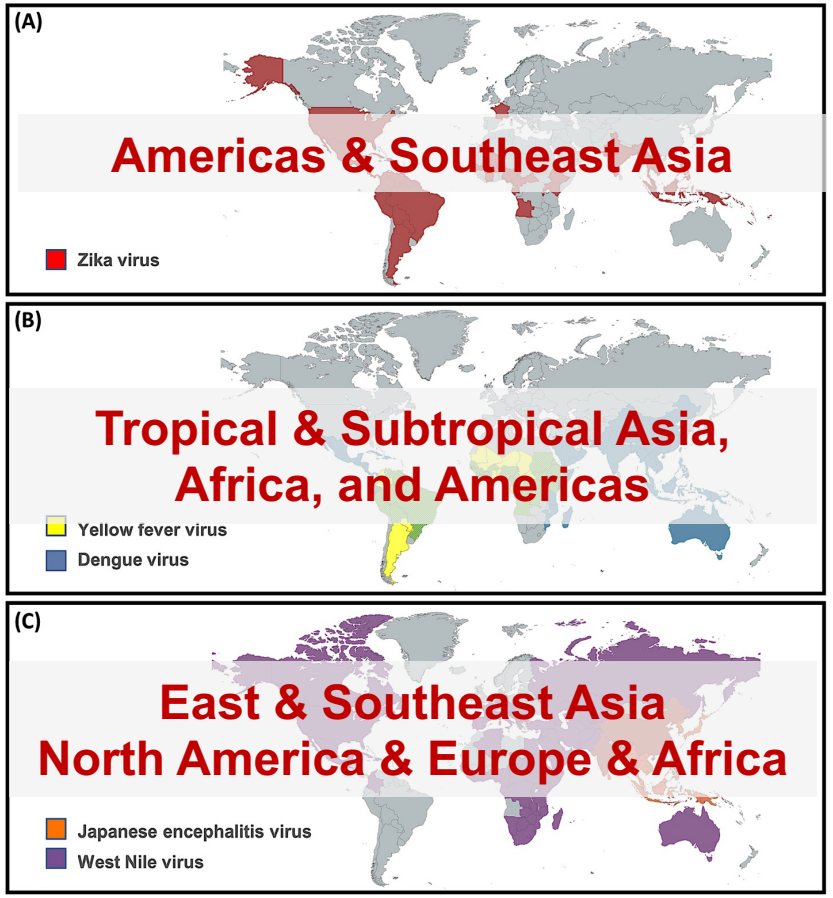




Tripathi et al., 2025

Major Global Public Health Concern.

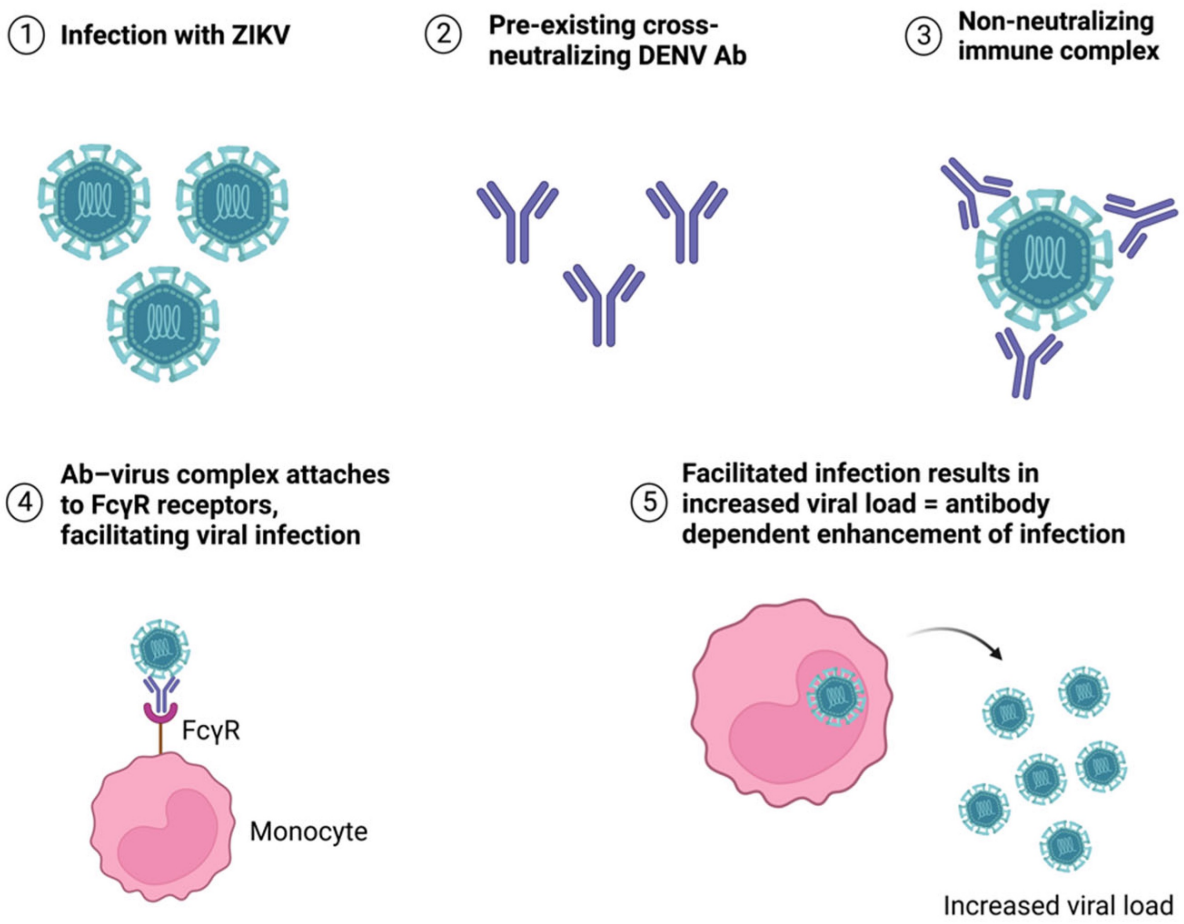
- High number of infections.
- Severe and potentially fatal diseases.
- Wide distribution and emerging threats.



(Annaleise et al., 2023)

Current gap in flavivirus therapeutics and vaccines

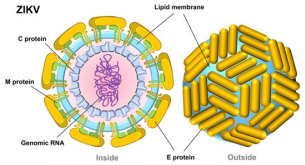
ADE of ZIKV infection caused by pre-existing cross-neutralizing DENV antibodies.



DENV

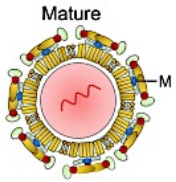
4 distinct serotypes & The licensed vaccine is incomplete and varies between serotypes.
(Jue et al.,2020)

ZIKV



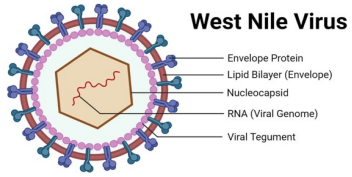
No licensed human vaccine & Strong cross-reactivity with DENV Abs
(Musso et al., 2019)

JEV



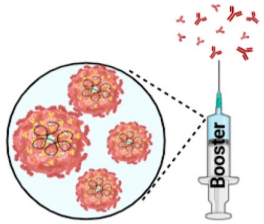
Vaccines are available, but coverage remains incomplete.
(Gielenny et al., 2024).

WNV



No licensed human vaccine & No specific antiviral treatment
(Ronca et al., 2021).

This highlights a critical need for a broad-spectrum vaccine that can provide cross-protection against multiple flaviviruses.



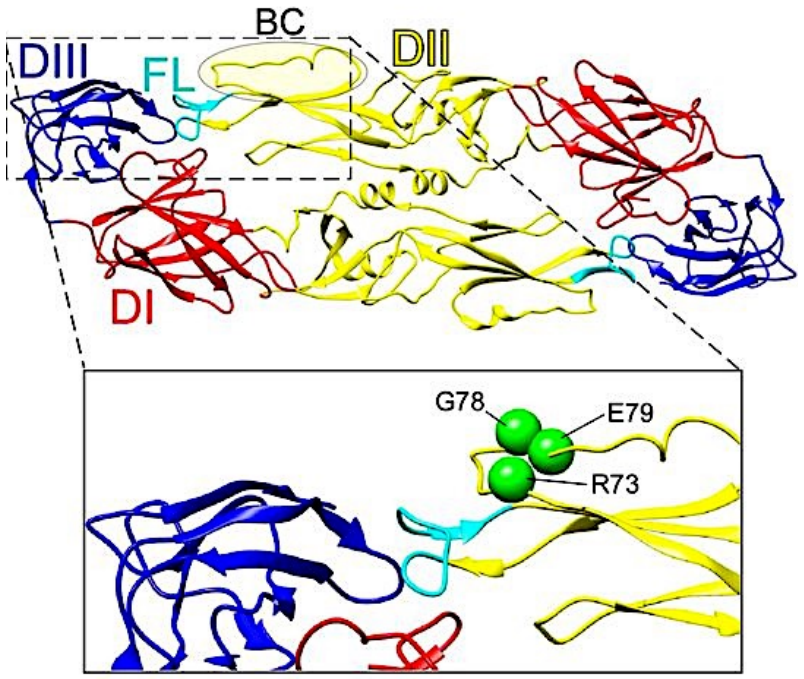
(Priscilla et al., 2022)

Novel epitope for designing cross-reactive neutralizing flavivirus antibodies

The Potent and Broadly Neutralizing Human Dengue Virus-Specific Monoclonal Antibody 1C19 Reveals a Unique Cross-Reactive Epitope on the bc Loop of Domain II of the Envelope Protein

Scott A. Smith,[a,b] A. Ruklanthi de Alwis,[c] Nurgun Kose,[b] Eva Harris,[d] Kristie D. Ibarra,[d] Kristen M. Kahle,[e] Jennifer M. Pfaff,[e] Xiaoxiao Xiang,[e] Benjamin J. Doranz,[e] Aravinda M. de Silva,[c] S. Kyle Austin,[f] Soila Sukupolvi-Petty,[f] Michael S. Diamond,[f] James E. Crowe, Jr.[b,g,h]

- ✓ Characterized 30 cross-neutralizing mAbs targeted the envelope (E) protein of DENVs.
- ✓ Identified one (1C19) that recognized a novel conserved site, which is the **bc loop on domain II (DII) (amino acids 73 to 79, RCPTQGE).**
- ✓ 1C19 conferred neutralization of all 4 DENV serotypes effectively.
- ✓ Able to compete for binding against the more common fusion loop Abs



This unique epitope suggests a focus for rational vaccine design, based on novel immunogens that present cross-reactive neutralizing determinants.

(Smith et al., 2013)

Yen *et al.*
Journal of Biomedical Science (2023) 30:41
https://doi.org/10.1186/s12929-023-00938-y

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Journal of Biomedical Science

RESEARCH Open Access

Neutralizing antibodies targeting a novel epitope on envelope protein exhibited broad protection against flavivirus without risk of disease enhancement

Li-Chen Yen[1], Hsin-Wei Chen[2], Chia-Lo Ho[3], Chang-Chi Lin[1,4], Yi-Ling Lin[1,5], Qiao-Wen Yang[1], Kuo-Chou Chiu[6,7], Shu-Pei Lien[2], Ren-Jye Lin[8] and Ching-Len Liao[1,2,8*]

Journal of Biomedical Science
Published in 2023, IF 12.1

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Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

A novel immunogen comprising a bc loop and mutant fusion loop epitopes generates potent neutralization and protective abilities against flaviviruses without risk of disease enhancement

Shiu-Bii Lien[a,1], Qiao-Wen Yang[b,1], Hong-Wei Huang[b,1], Kuo-Chou Chiu[c,d,e], Sui-Lung Su[f], Ching-Len Liao[b,g], Li-Chen Yen[b,*]

Vaccine
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Objective: To design a peptide-based vaccine targeting the conserved bc loop of the E protein, capable of eliciting broadly neutralizing antibodies that protect multiple flaviviruses without the risk of disease enhancement

RESULT 1 Identification of a potential cross-reactive immunogenic targeting E protein

Objective: To investigate the bc loop sequence in the E proteins of flaviviruses (DENV-1 to DENV- 4, ZIKV, and JEV)

Methods

- **DENV-1** (Hawaii)
- **DENV-2** (16681)
- **DENV-3** (H87)
- **DENV-4** (H214)
- **ZIKV** (PRVABC59)
- **JEV** (RP9)
- **WNV** (05002688)
- **YEF** (AAX4750.1)

↓

Amino acid alignments
by the MultAlin web server

↓

RCSB PDB PROTEIN DATA BANK

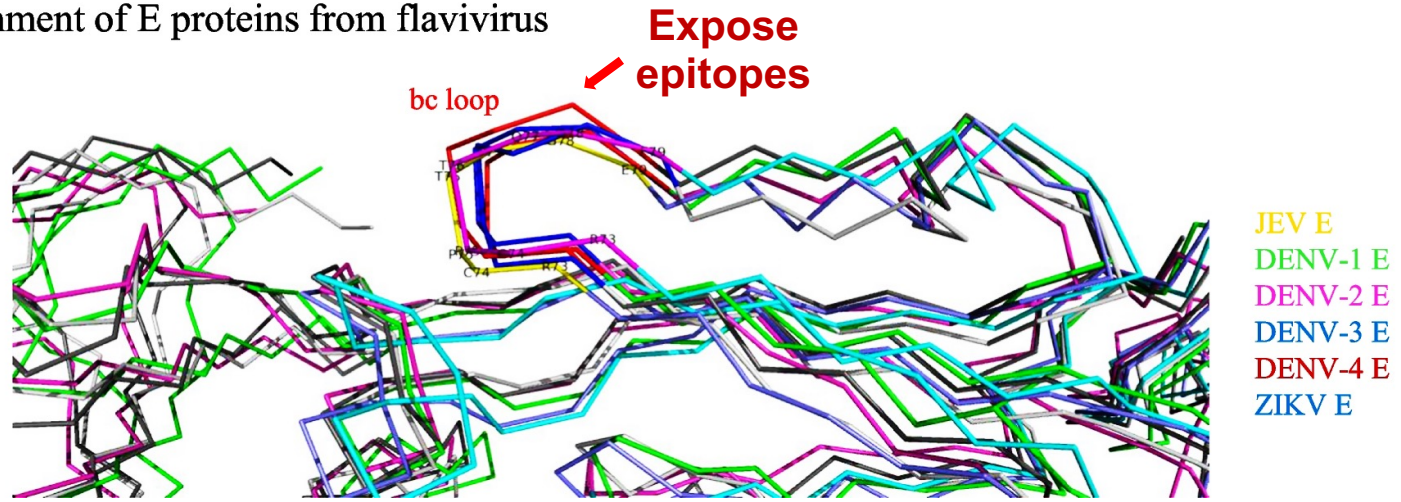
The alignment modeling
by the PyMol Program

A Alignment of the E protein

bc loop (amino acids 73 to 79)

```
                1        10        20        30        40        50        60        70        80        90       100       110
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+--
JEV             FNCLGMGNRDFIEGASGATWVDLVLEGDSCLTIMANDKPTLDVRMINIEASQLAEVRSYCYHASVTDISTVARCPTTGEAHNEKRADSSYVCKQGFTDRGWGNGCGLFGKGS
WNV             FNCLGMSNRDFLEGVSGATWVDLVLEGDSCVTIMSKDKPTIDVKMMNMEAANLAEVRSYCYLATVSDLSTKAACPTMGEAHNDKRADPAFVCRQGVVDRGWGNGCGLFGKGS
ZIKV            IRCIGVSNRDFVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGS
DENV-1          MRCVGIGNRDFVEGLSGATWVDVVLEHGSCVTTMAKNKPTLDIELLKTEVTNPAVLRKLCIEAKISNTTTDSRCPTQGEATLVEEQDANFVCRRTFVDRGWGNGCGLFGKGS
DENV-3          MRCVGVGNRDFVEGLSGATWVDVVLEHGGCVTTMAKNKPTLDIELQKTEATQLATLRKLCIEGKITNITTDSRCPTQGEAVLPEEQDQNYVCKHTYVDRGWGNGCGLFGKGS
DENV-2          MRCIGISNRDFVEGVSGGSWVDIVLEHGSCVTTMAKNKPTLDFELIKTEAKQLATLRKYCIEAKLTNTTTESRCPTQGEPSLNEEQDKRFVCKHSMVDRGWGNGCGLFGKGG
DENV-4          MRCVGVGNRDFVEGVSGGAWVDLVLEHGGCVTTMAQGKPTLDFELTKTTAKEVALLRTYCIEASISNITTATRCPTQGEPYLKEEQDQQYICRRDVVDRGWGNGCGLFGKGG
YFV             AHCIGITDRDFIEGVHGGTWVSATLEQGKCVTVMAPDKPSLDISLQTVAIDGPAEARKVCYSAVLTHVKINEKCPSTGEAHLAEENDGDNACKRTYSDRGWGNGCGLFGKGS
Consensus       .rC.G..#RDFvEGvsGgtWVd.vLEhg.CvT.Ma.dKPtlDie$..tea...Ae.RkyCyea..t...t..rCPtqGEa.l.ee.D...vCkrt.vDRGWGNGCGLFGKGs
```

B Alignment of E proteins from flavivirus



The bc loop sequence exhibits conservative characteristics.
: JEV epitope RCPTTGE and DENV/ZIKV epitope RCPTQGE.

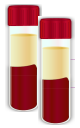
RESULT 2 Immunogenicity and neutralizing capability against flavivirus

Objective: To investigate the immunogenicity and neutralization capability induced by the synthesized peptides

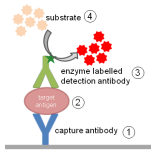
Methods

Synthesized peptides
- (JEV-NTE) RCPTTGE
- (DV/ZV-NTE) RCPTQGE

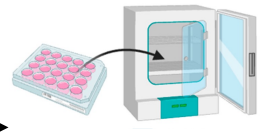
BALB/c mice

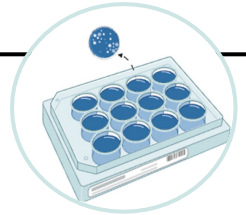


Immune sera



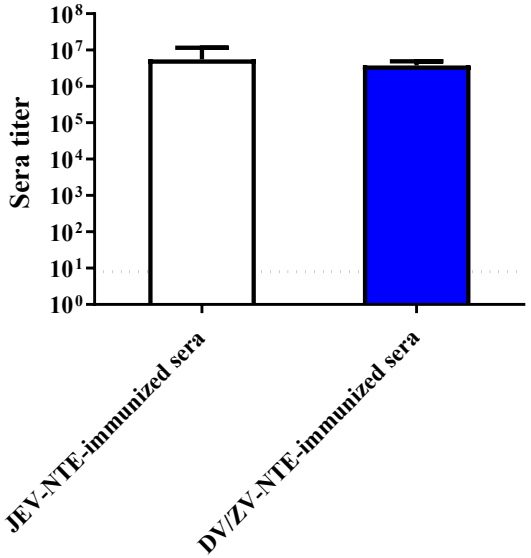
Antibody production using ELISA



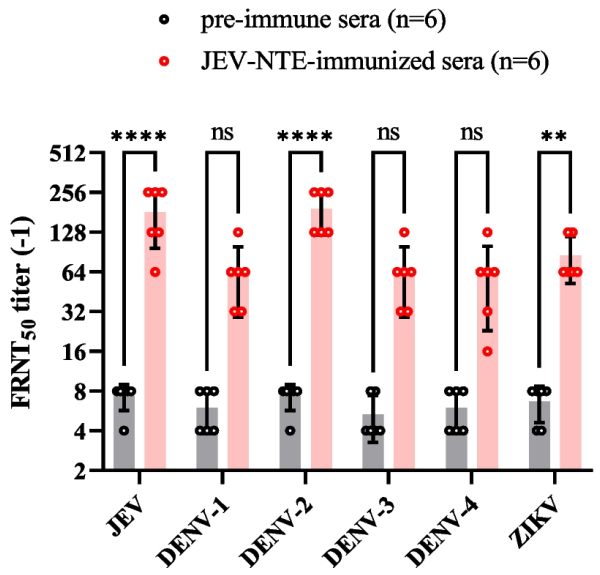


Focus reduction neutralization test ($FRNT_{50}$)

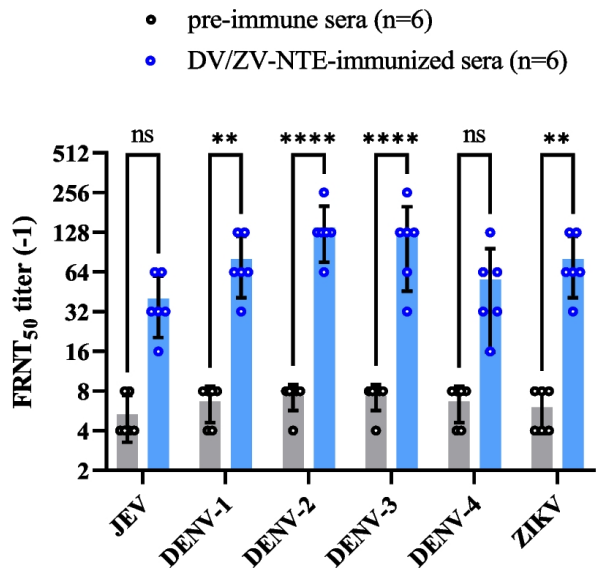
Immunogenicity induced by synthesized peptide sequences.



A Neutralization capability of JEV-NTE



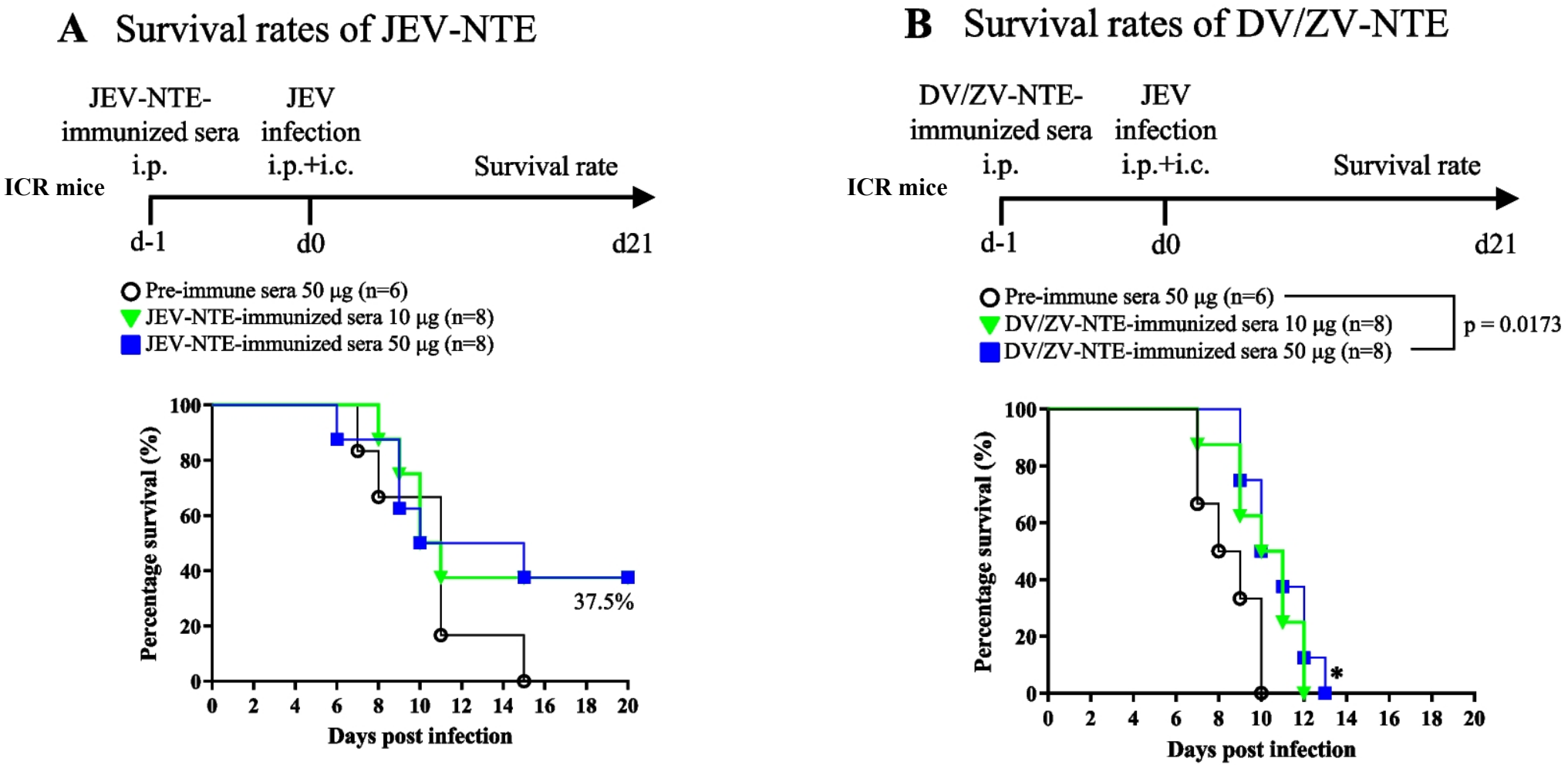
B Neutralization capability of DV/ZV-NTE



The results showed that JEV-NTE and DV/ZV-NTE-immune sera significantly stimulated the production of antibodies and conferred cross-neutralizing ability against the indicated flavivirus.

Data are the means ± SD of two independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 (by Two-way ANOVA). ns not significant.

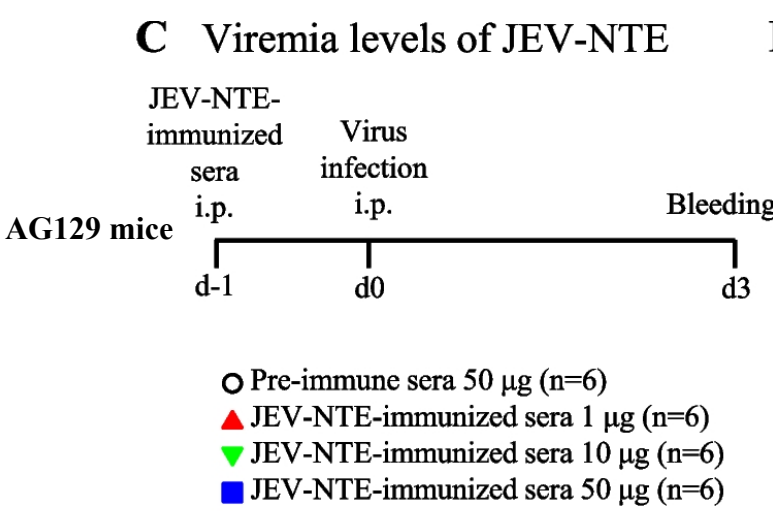
Objective: To evaluate the protective capabilities of JEV- NTE- or DV/ZV-NTE-immune sera *in vivo* against flavivirus

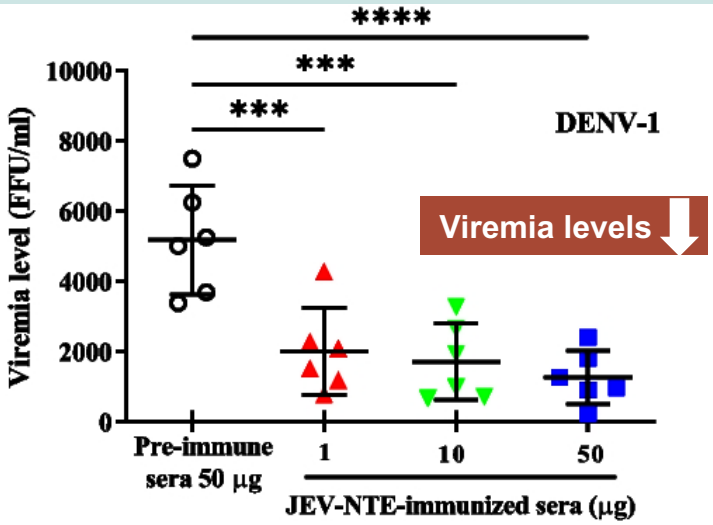


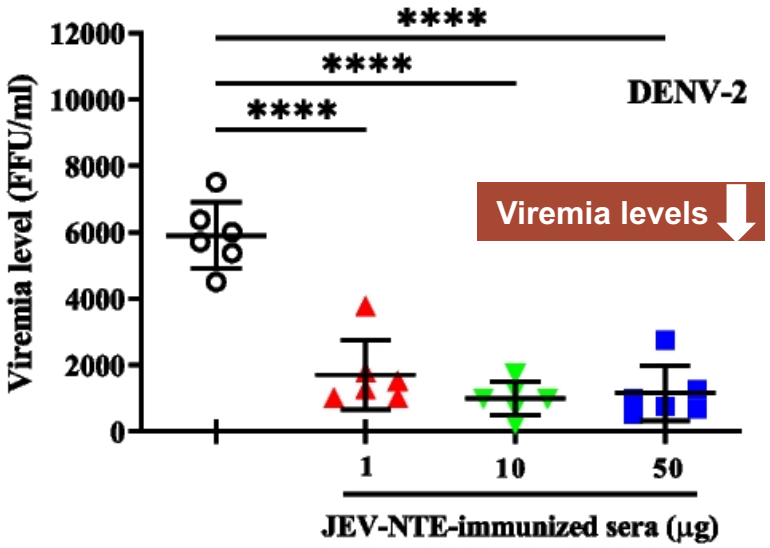
The passive immunization with JEV-NTE-immune sera could increase the survival rate. Notably, DV/ZV-NTE-immune sera could significantly prolong the survival rate in the JEV-challenged ICR mice

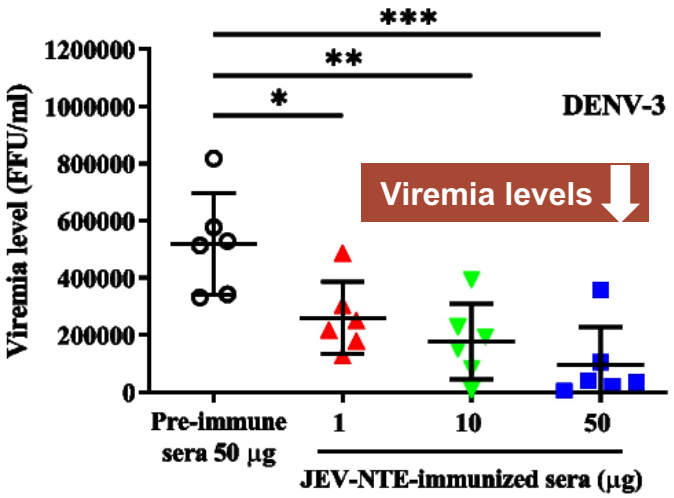
RESULT 3 Passive immunization with JEV-NTE- or DV/ ZV-NTE-immune sera

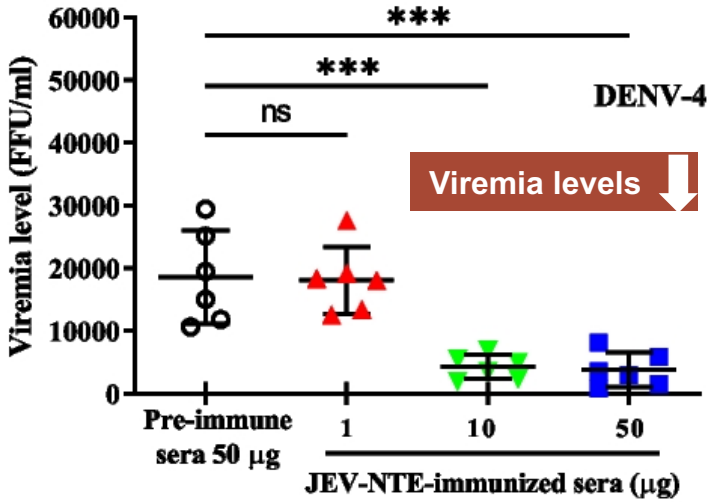
Objective: To evaluate the protective capabilities of JEV- NTE- or DV/ZV-NTE-immune sera *in vivo* against flavivirus

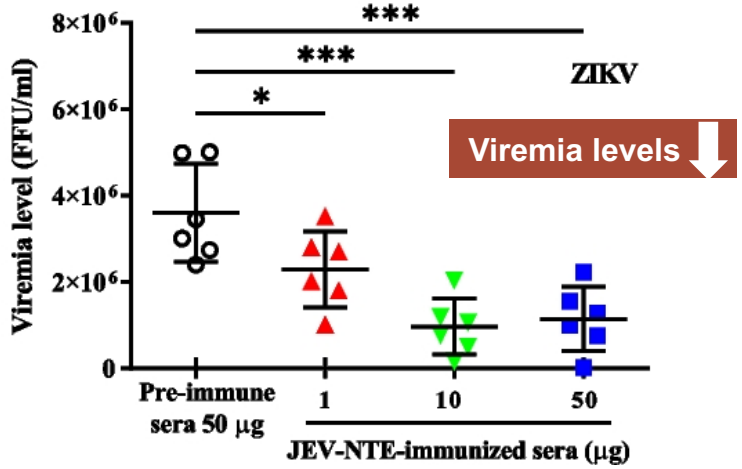








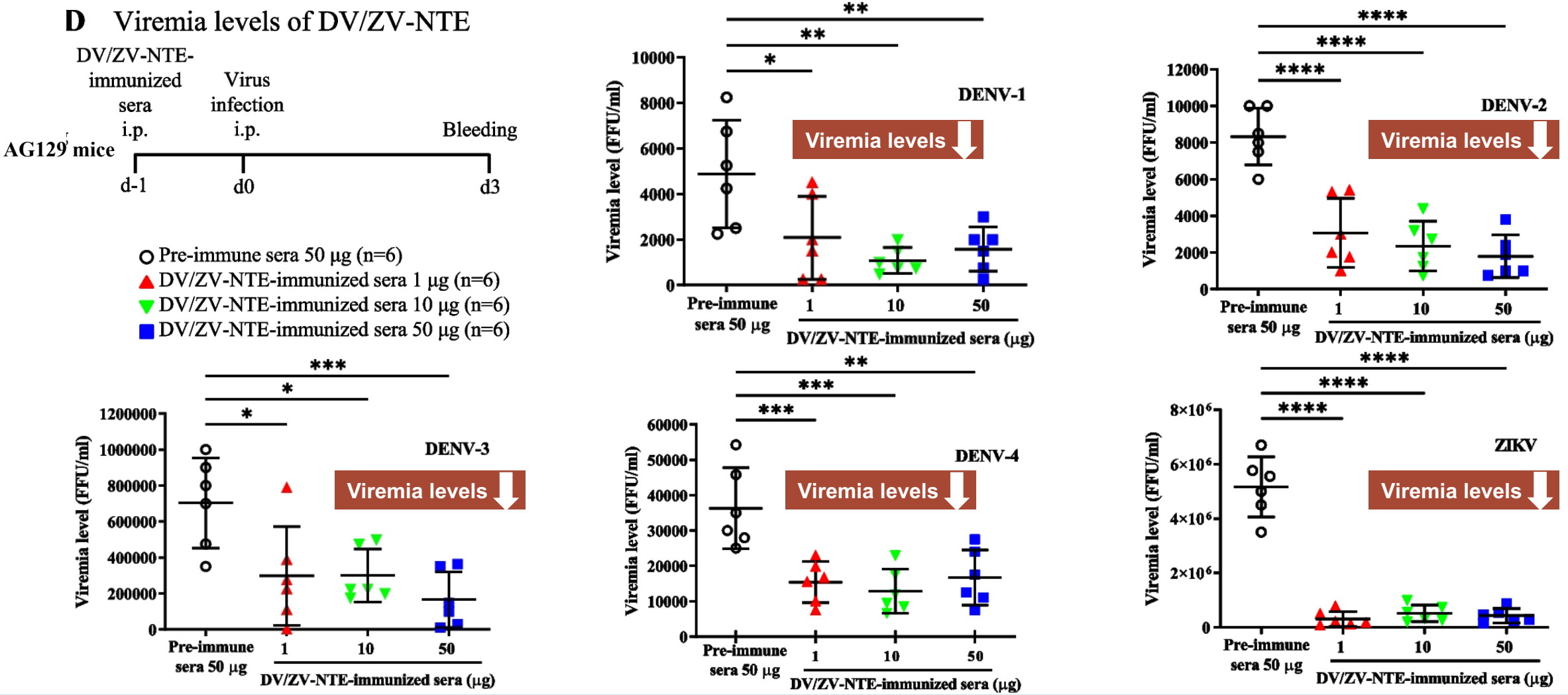




As a result, immunization with JEV-NTE immune sera significantly decreased the viremia levels in all serotypes of DENV and ZIKV-infected mice

Data are the means ± SD of two independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 [by Log-rank (Mantel-Cox) test or One-way ANOVA]. ns not significant.

Objective: To evaluate the protective capabilities of JEV- NTE- or DV/ZV-NTE-immune sera *in vivo* against flavivirus



As a results, immunization with DV/ZV-NTE-immune sera significantly decreased the viremia levels in all serotypes of DENV and ZIKV-infected mice

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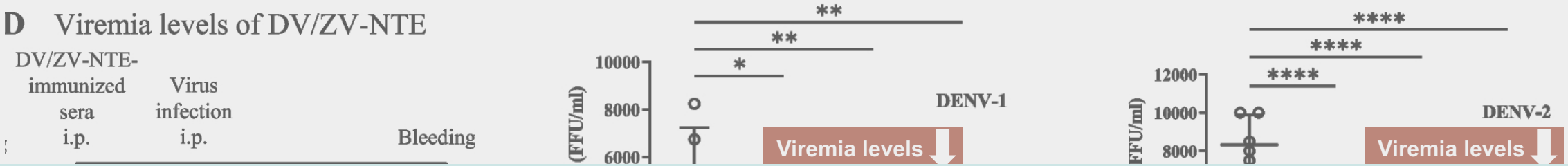
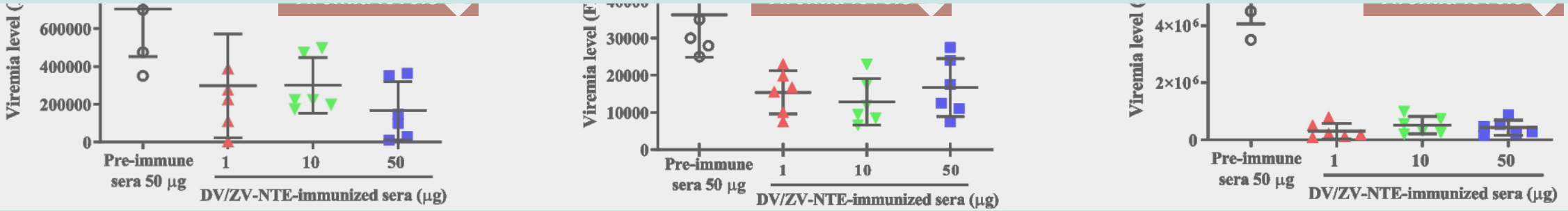
RESULT 3 Passive immunization with JEV-NTE- or DV/ ZV-NTE-immune sera

Objective: To evaluate the protective capabilities of JEV- NTE- or DV/ZV-NTE-immune sera *in vivo* against flavivirus



Overall, these findings demonstrate that passive transfer of antibodies induced by designed peptides can effectively protect mice against multiple flaviviruses.



As a results, immunization with DV/ZV-NTE-immune sera significantly decreased the viremia levels in all serotypes of DENV and ZIKV-infected mice

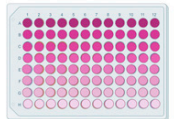
Data are the means ± SD of two independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 [by Log-rank (Mantel-Cox) test or One-way ANOVA]. ns not significant.

RESULT 4 *In vitro* ADE assay

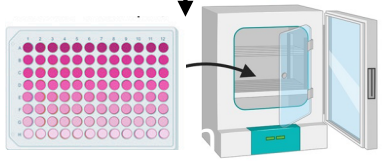
Objective: To evaluate whether JEV-NTE- or DV/ZV-NTE-immune sera induce ADE *in vitro*

Methods

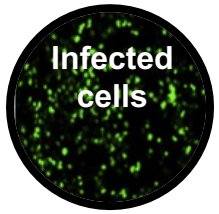
Immune complexes
(Serially diluted sera + Virus)



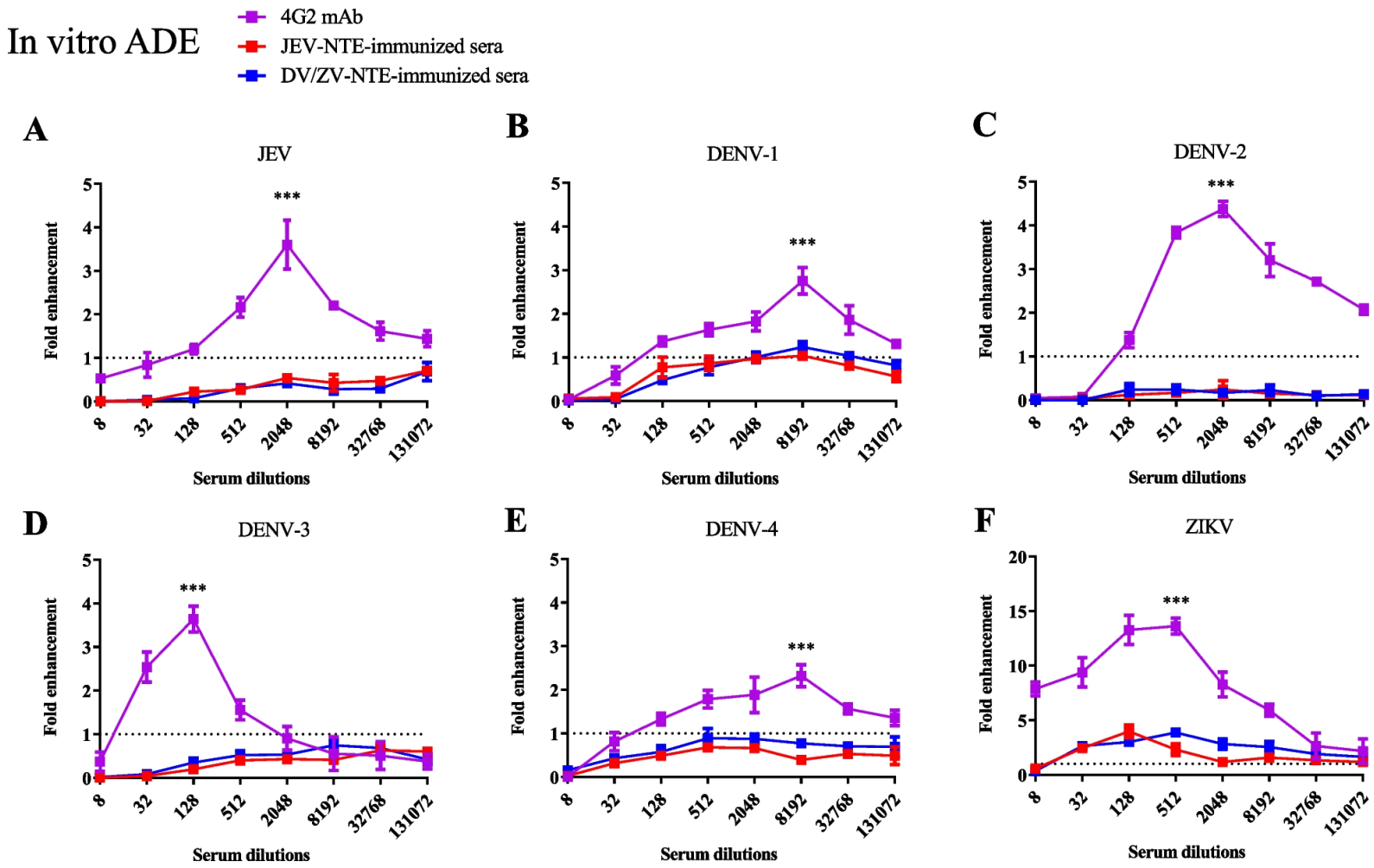
K562 cells were mixed with immune complexes



The cells were resuspened in fresh medium and incubated for 3 days at 37 °C



Focus-forming assay

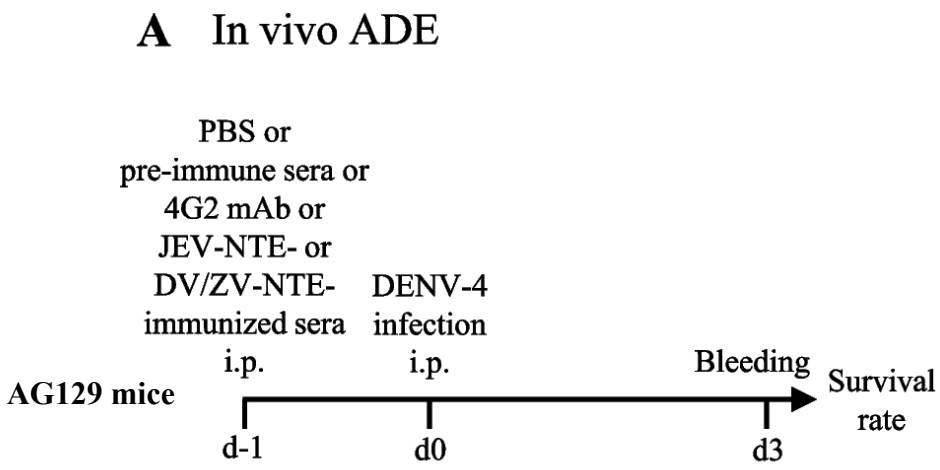


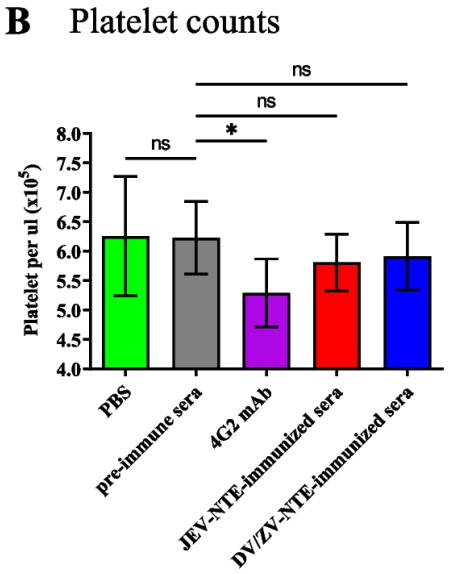
These results indicated that JEV-NTE- or DV/ZV-NTE-immune sera have little or no capacity for inducing ADE in vitro.

Data are presented as the means ± SD of two independent experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (by Two-way ANOVA).

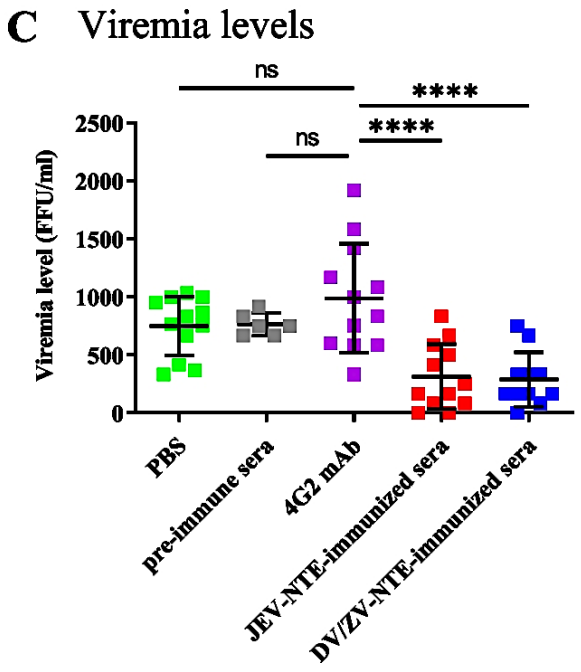
RESULT 5 *In vivo* ADE assay

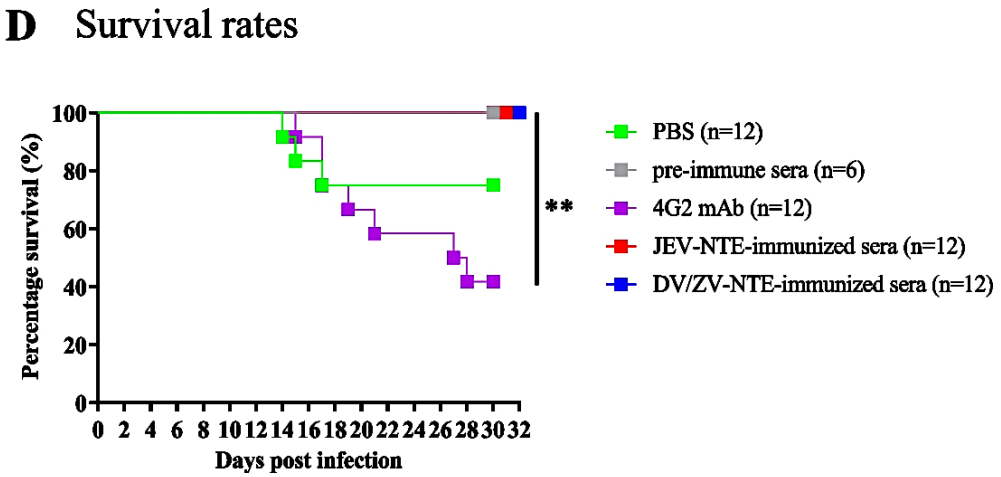
Objective: To evaluate whether JEV-NTE- or DV/ZV-NTE-immune sera induce ADE *in vivo*





These results indicated that antibodies elicited by JEV-NTE or DV/ZV-NTE have little capacity for inducing ADE *in vivo*.





Data are presented as the means ± SD of two independent experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ [by Log-rank (Mantel-Cox) test or One-way ANOVA]. *ns* not significant.

Conclusions

- The research showed for the **first time** that the **novel epitope sequence RCPTQGE** that located on the amino acids 73 to 79 of flavivirus E protein could confer **cross-neutralization** against JEV, DENV and ZIKV.
- These results highlighted that the **bc loop epitope** could be a potential target for flavivirus **vaccine development**.
- Although this demonstrated the potential of the bc loop as a vaccine target, its neutralizing potency was still **not strong enough**.

SECOND PAPER

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

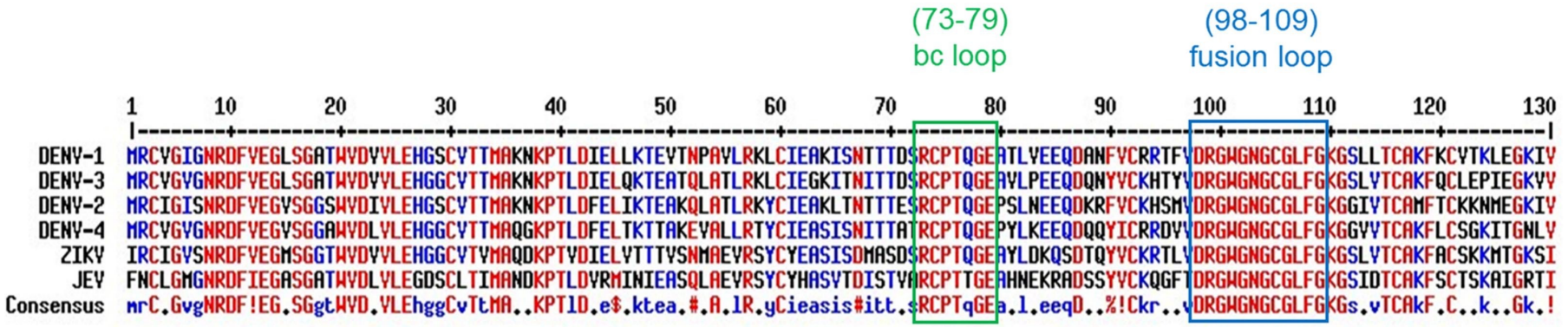
A novel immunogen comprising a bc loop and mutant fusion loop epitopes generates potent neutralization and protective abilities against flaviviruses without risk of disease enhancement

Shiu-Bii Lien [a,1], Qiao-Wen Yang [b,1], Hong-Wei Huang [b,1], Kuo-Chou Chiu [c,d,e], Sui-Lung Su [f], Ching-Len Liao [b,g], Li-Chen Yen [b,*]

Objective: To enhance the immunogen capable of robust neutralization and protection by combining the bc loop with a fusion loop region

Objective: To identify the bc loop and fusion loop sequence in the E proteins of flaviviruses (DENV-1 to DENV- 4, ZIKV, and JEV)

A Alignment of the E protein

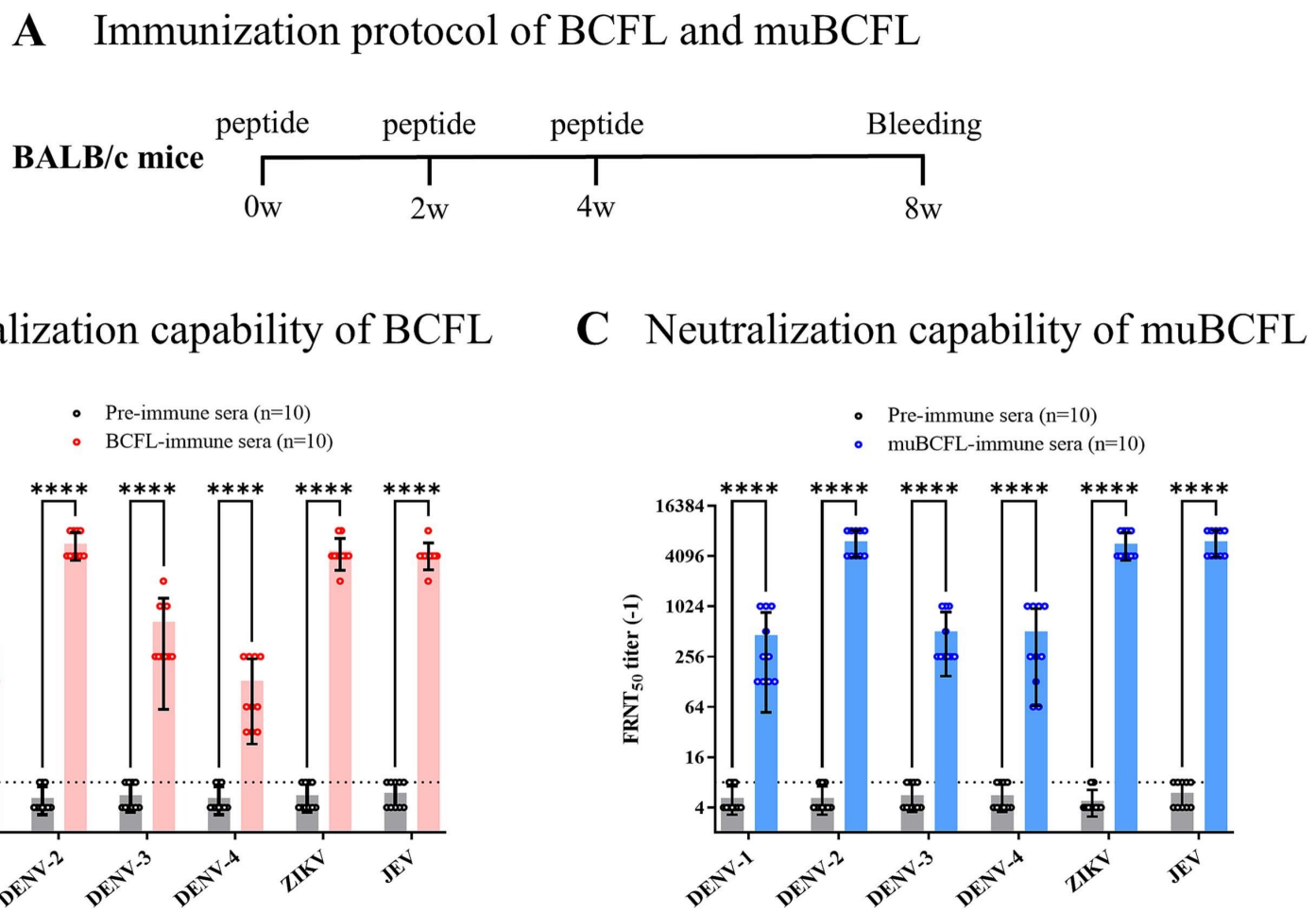


B Sequence of BCFL and muBCFL

The BCFL and muBCFL peptides were subsequently synthesized and evaluated for their neutralizing capacity, protective ability, and ADE risk through both *in vitro* and *in vivo* assays.

RESULT 2 Neutralization ability of BCFL- or muBCFL- immune sera against flaviviruses

Objective: To assess the neutralization capacity of BCFL- and muBCFL-immune sera

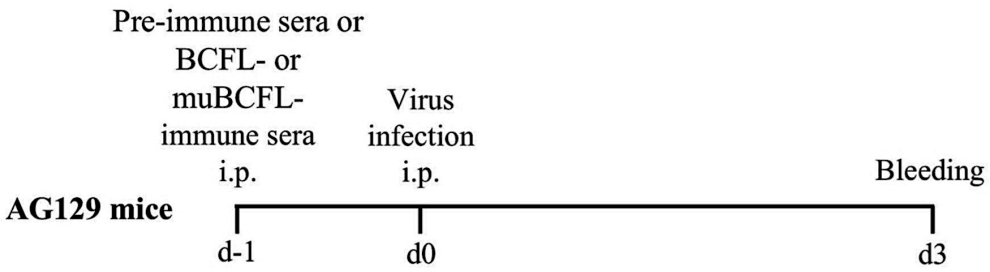


Both BCFL- and muBCFL-immune sera demonstrated comparable neutralizing activity, the differences observed in certain virus strains (e.g., DENV-4) prompted further investigation of their *in vivo* efficacy.

The detection limit was set at 1:4. Each symbol represents an individual mouse, and the data are expressed as the mean ± SD. ****$P < 0.0001$ (by Two-way ANOVA).

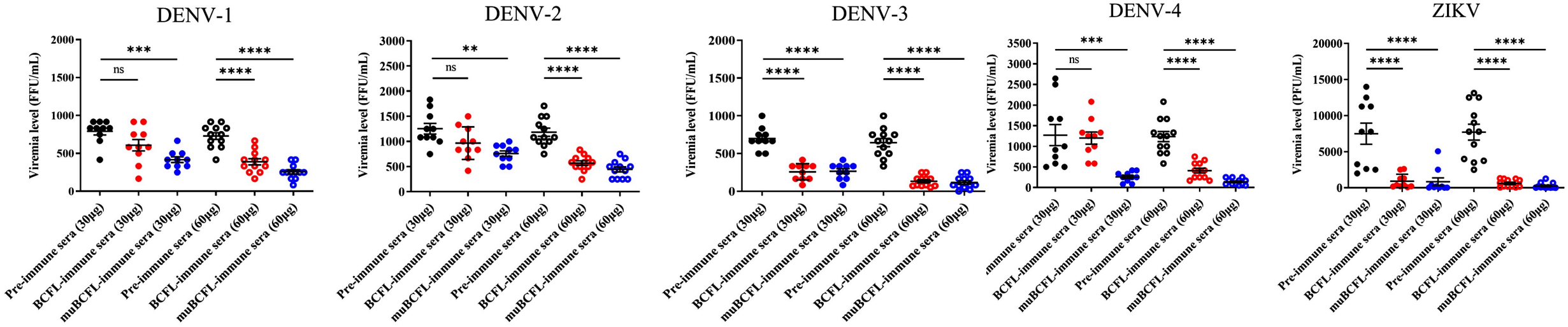
Objective: To examine the *in vivo* protective capabilities of BCFL- or muBCFL-immune sera against flaviviruses

(A) Scheme of the animal challenge experiment



- Pre-immune sera (30μg) (n=10)
- BCFL-immune sera (30μg) (n=10)
- muBCFL-immune sera (30μg) (n=10)
- Pre-immune sera (60μg) (n=12)
- BCFL-immune sera (60μg) (n=12)
- muBCFL-immune sera (60μg) (n=12)

(B) Viremia level of BCFL and muBCFL

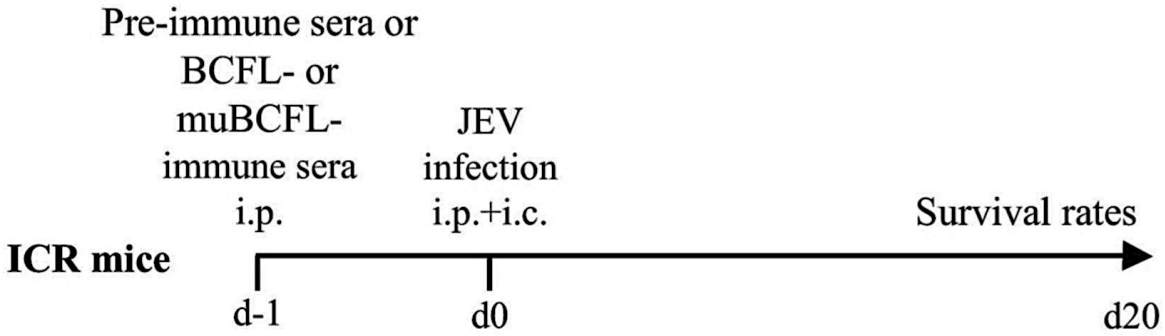


These results demonstrated that passive immunization with BCFL- or muBCFL-immune sera provided robust protection by significantly reducing viremia levels in all DENV serotypes and ZIKV-infected mice.

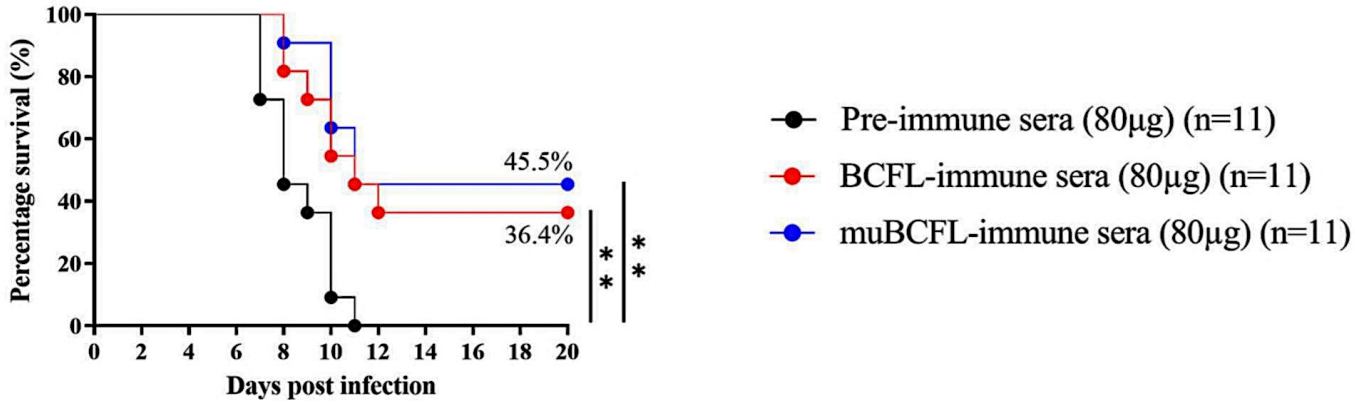
The data are presented as the mean ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ [by One-way ANOVA or Log-rank (Mantel-Cox) test]. ns: not significant.

Objective: To examine the *in vivo* protective capabilities of BCFL- or muBCFL-immune sera against flaviviruses

(C) Scheme of the animal challenge experiment



(D) Survival rates of BCFL and muBCFL



These results demonstrated that passive immunization with BCFL- or muBCFL-immune sera increased the survival rates in JEV- challenged mice.

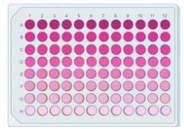
The data are presented as the mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 [by One-way ANOVA or Log-rank (Mantel-Cox) test]. ns: not significant.

RESULT 4 *In vitro* ADE assay

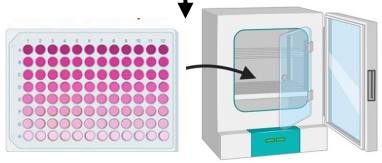
Objective: To determine whether BCFL- or muBCFL-immune sera have a potential risk of causing ADE *in vitro*

Methods

Immune complexes
(Serially diluted sera + Virus)

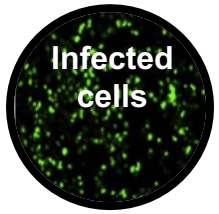


K562 cells were mixed with immune complexes

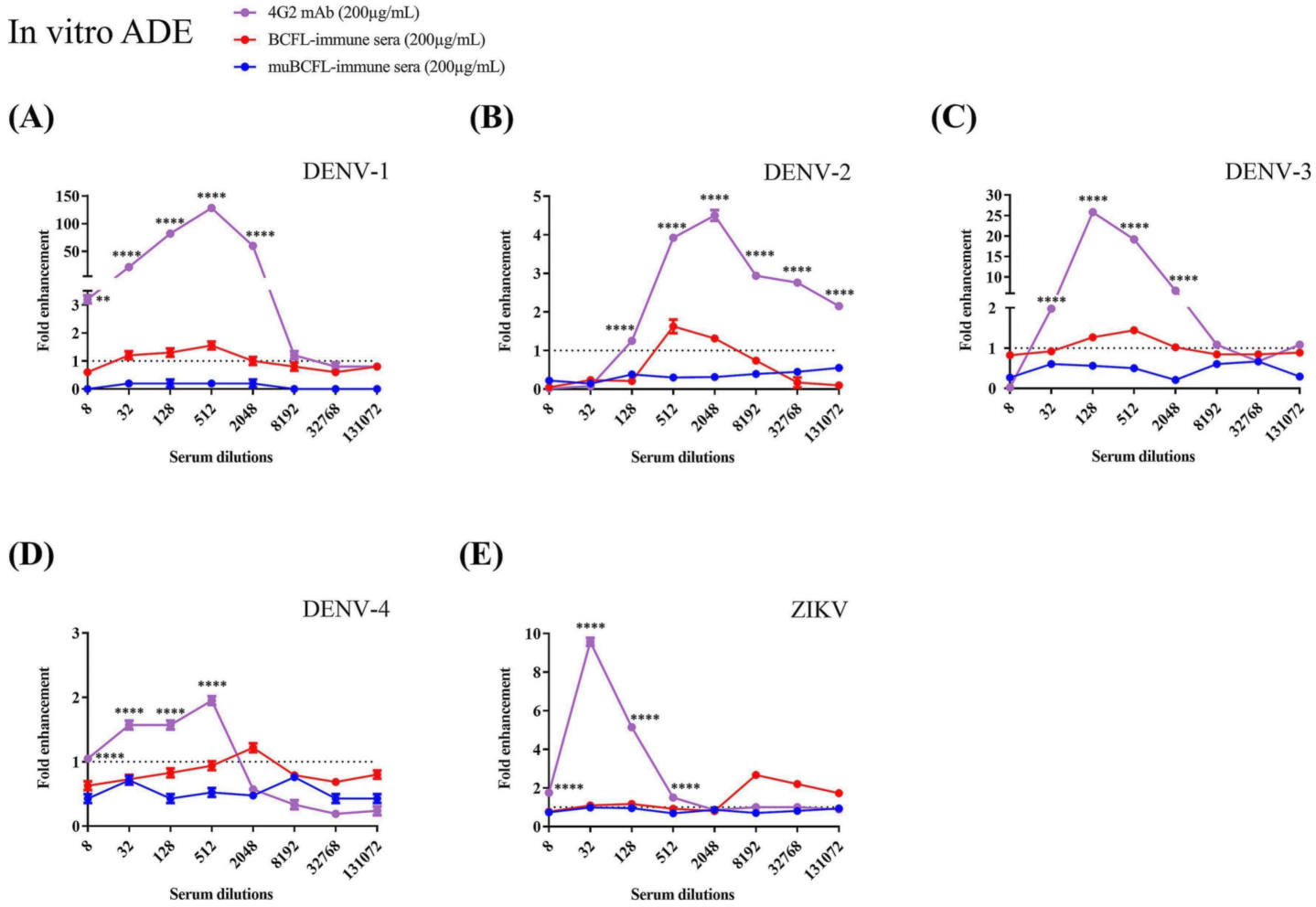


1.5 hr at 37°C, 5% CO_2

The cells were resuspened in fresh medium and incubated for 3 days at 37 °C



Focus-forming assay

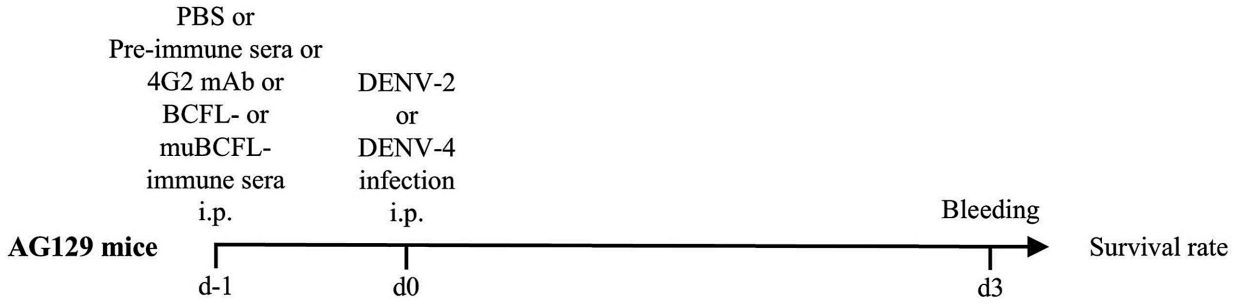


The fold enhancement values of muBCFL-immune sera were lower than those observed for the mAb 4G2, indicating a substantially reduced ADE potential.

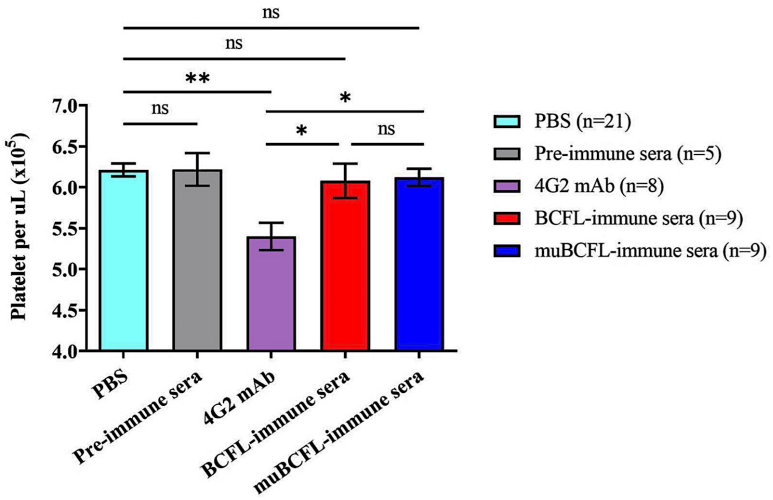
The data represents the results from two independent experiments and is expressed as the means ± SDs. **P < 0.01: ****P < 0.0001 (by Two-way ANOVA).

Objective: To determine whether BCFL- or muBCFL-immune sera have a potential risk of causing ADE *in vivo*

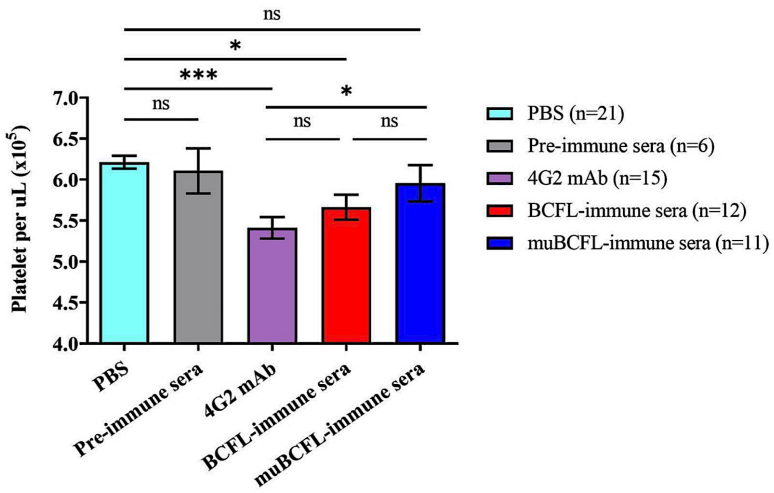
(A) In vivo ADE



(B) Platelet counts in DENV-2 group



(C) Platelet counts in DENV-4 group

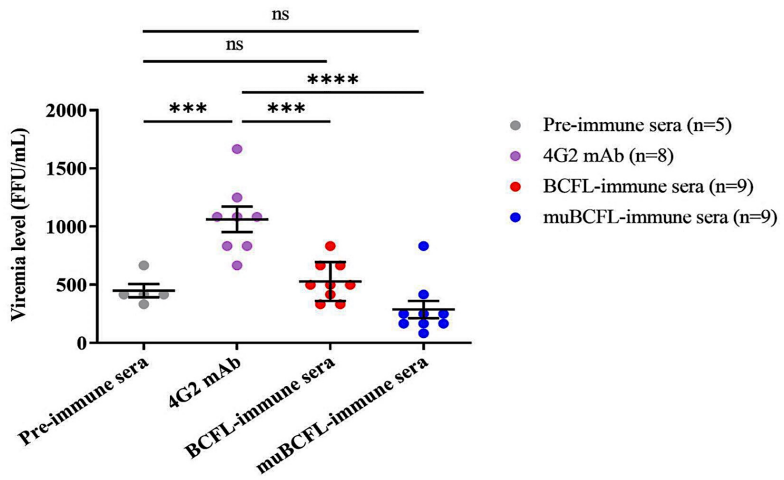


The platelet counts in the muBCFL group remained substantially higher than those of the mAb 4G2 group, closely approximating the levels seen in the PBS and pre-immune control groups.

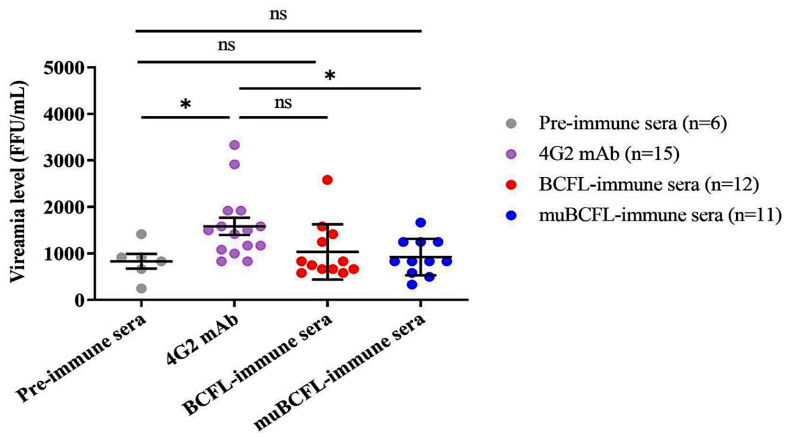
The platelet counts and viremia levels are presented as the means ± SEMs. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ [by One-way ANOVA or Log-rank (Mantel–Cox) test]. ns: not significant.

Objective: To determine whether BCFL- or muBCFL-immune sera have a potential risk of causing ADE *in vivo*

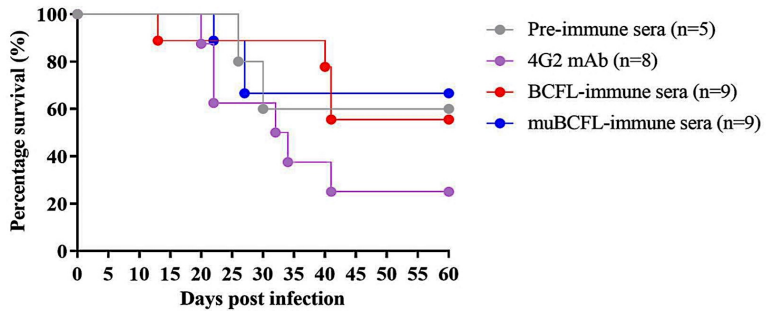
(D) Viremia level in DENV-2 group



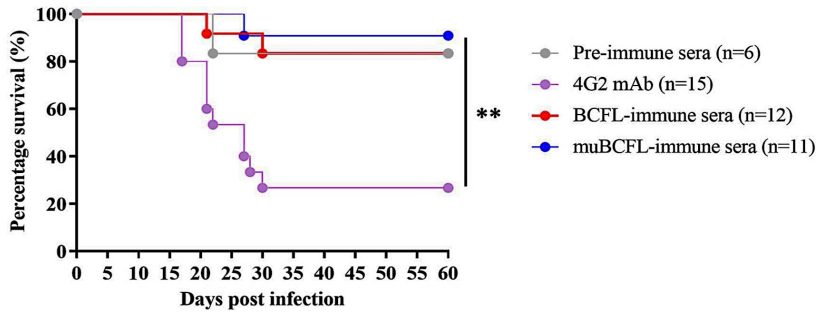
(E) Viremia level in DENV-4 group



(F) Survival rates in DENV-2 group



(G) Survival rates in DENV-4 group

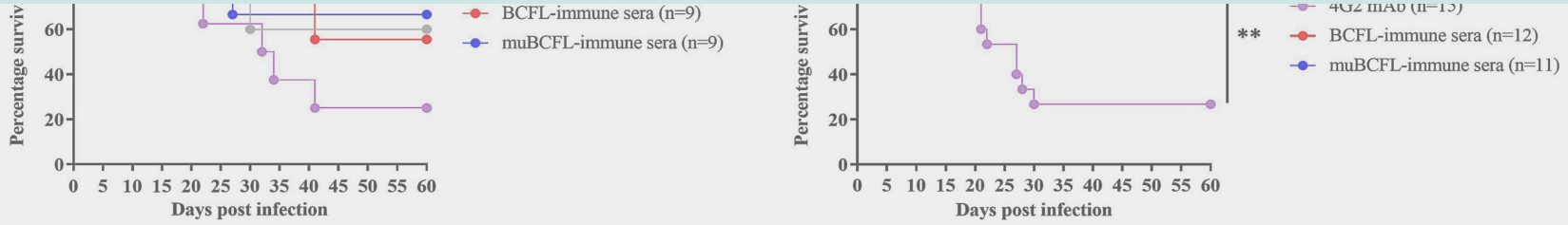


The results showed that the muBCFL group consistently exhibited significantly lower viremia levels and improved survival rates compared to the mAb 4G2 group.

The platelet counts and viremia levels are presented as the means ± SEMs. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ [by One-way ANOVA or Log-rank (Mantel–Cox) test]. ns: not significant.

Objective: To determine whether BCFL- or muBCFL-immune sera have a potential risk of causing ADE *in vivo*

Overall, the muBCFL-immune sera demonstrated minimal capacity to induce ADE *in vivo*, with better performance in maintaining platelet counts, reducing viremia, and improving survival rates compared to the mAb 4G2 group.



The results showed that the muBCFL group consistently exhibited significantly lower levels and improved survival rates compared to the mAb 4G2 group.

The platelet counts and viremia levels are presented as the means ± SEMs. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$ [by One-way ANOVA or Log-rank (Mantel–Cox) test]. ns: not significant.

Conclusions

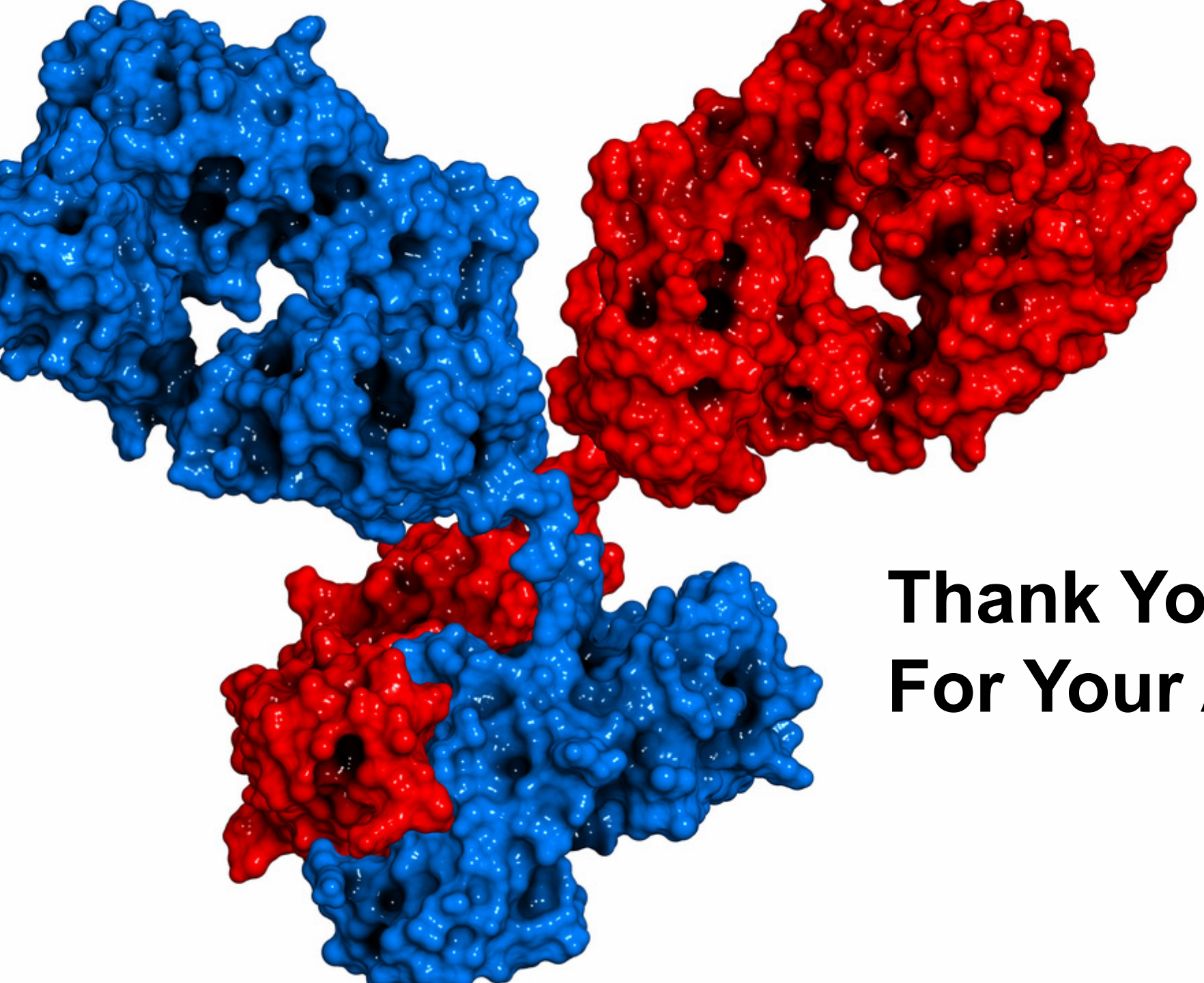
- This study indicated that the immune sera generated by novel muBCFL sequence could elicit **neutralizing antibodies against all four serotypes of DENV, ZIKV, and JEV**, and **produce protective efficacy** in mice infected with DENV, ZIKV, and JEV.
- The muBCFL- immune sera could **minimize the ADE effect** *in vitro* and *in vivo*.
- These findings revealed that the muBCFL sequence could be applied to develop as a **potent and safe flavivirus vaccine in the future**.

Criticisms

	First Paper	Second Paper
Strong Points	• The study presents a well-designed and comprehensive evaluation with appropriate controls.	• The study presents a well-designed and comprehensive evaluation with appropriate controls.
Weak Points	• *In vivo* ADE was evaluated only using DENV-4.	• *In vivo* ADE was evaluated only using DENV-2 and DENV-4.

Acknowledgement

Assist. Prof. Dr. Chonlatip Pipattanaboon (Advisor)
Department of Microbiology, Faculty of Medicine, KKU



Medical Microbiology

Thank You
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