Virology at integral molecular

Engineering ZIKV E protein to diminish anti-DENV immune response

Identifying the 7 residues that drive DENV-ZIKV cross-reactivity

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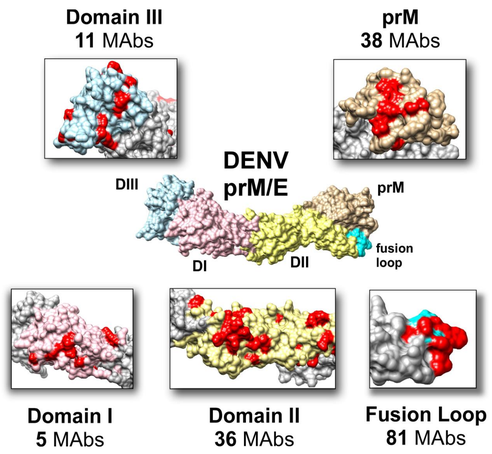
[1]Integral Molecular; [2]IBT Bioservices

The high homology between ZIKV and DENV envelope proteins results in immunological cross-reactivity that can exacerbate disease, complicating ZIKV vaccine development

Identifying the 7 ZIKV E protein residues responsible for cross-reactivity enabled engineering of a protective ZIKV immunogen with diminished anti-DENV immune response

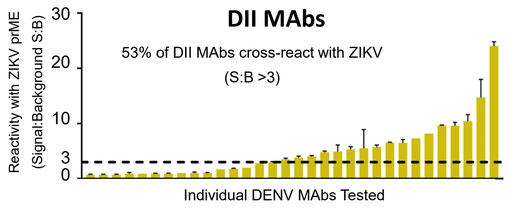
Grinyo-Escuer *et al.*, August 2025 *Cell Reports*

171 anti-DENV MAb epitopes



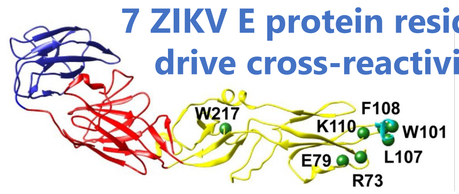
Epitope mapping of MAbs on DENV envelope proteins
Epitope residues shown (in red) summarize 171 MAbs mapped previously by shotgun mutagenesis.

71 MAbs cross-react with ZIKV



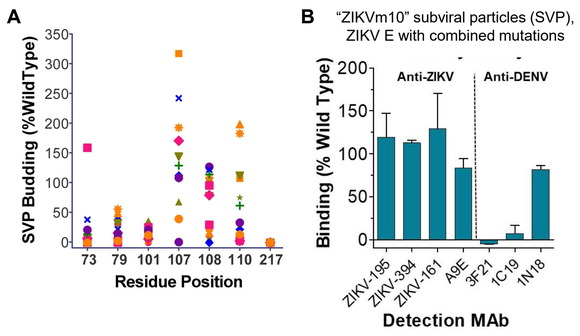
DENV MAb binding was tested against ZIKV prM/E expressed in HEK-293T cells (showing DII MAbs only)

7 ZIKV E protein residues drive cross-reactivity



Epitopes of 66 cross-reactive MAbs included at least 1 of 7 E protein residues, establishing these 7 residues as the major determinants of DENV-ZIKV cross-reactivity.

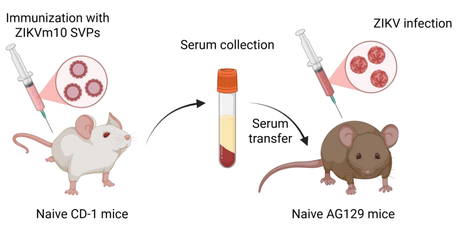
"ZIKVm10" E protein engineered to reduce MAb cross-reactivity



(**A**) The 7 ZIKV E residues responsible for cross-reactivity were mutated to all other amino acids, ELISA was used to identify changes that support SVP production.

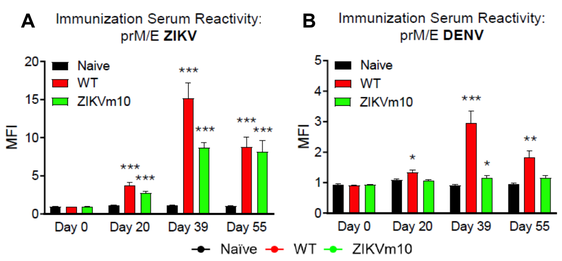
(**B**) Permissive mutations were combined to make "ZIKVm10", which was incorporated into SVPs and tested for binding to ZIKV and DENV MAbs.

ZIKVm10 SVPs used to immunize mice



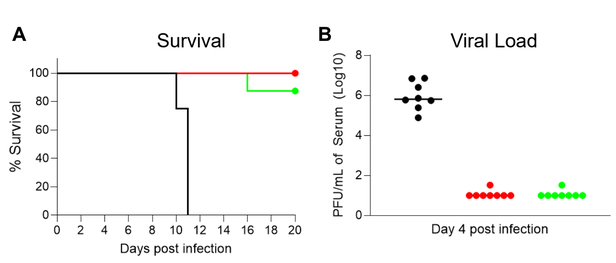
- CD-1 mice immunized with SVPs displaying WT or ZIKVm10 E protein
- Mouse sera tested for binding with cells expressing DENV or ZIKV prM/E
- Passive immunization of AG129 mice with sera from mice immunized with WT or ZIKVm10 SVPs, then challenged with live ZIKV

ZIKVm10 immunization reduces cross-reactivity



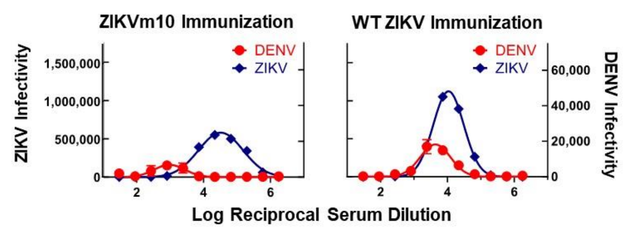
Mean reactivity of sera from 15 naïve CD-1 mice, or immunized with WT or ZIKVm10 SVPs, tested by flow cytometry against cells expressing prM/E from **A**) ZIKV and **B**) DENV2 (*p<0.05 vs. naïve, **p<0.01, ***p<0.001).

ZIKVm10 immunization maintains protection from live ZIKV

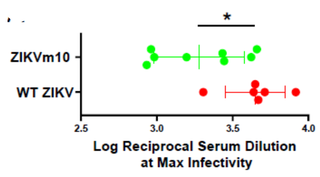


AG129 mice received passive immunizations of serum from naïve mice, mice immunized with WT SVPs or ZIKVm10 SVPs, and were then challenged with live ZIKV. Mice were monitored for survival (**A**), viral load in serum 4 days post-infection (**B**), body weight, and health score (not shown).

Sera from mice immunized with ZIKVm10 SVPs show reduced ADE of DENV infectivity



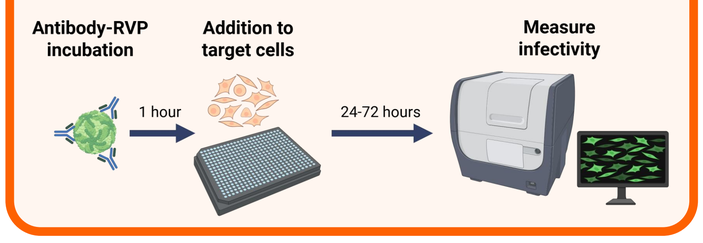
Representative ADE-mediated infectivity for sera from individual mice, immunized with WT ZIKV SVPs or ZIKVm10 SVPs, incubated with **DENV2** or **ZIKV** RVPs and K562 cells, mean ± range from duplicate assays.



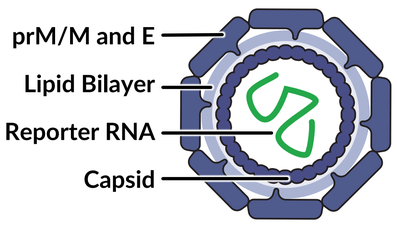
ZIKVm10 sera (n = 8) showed maximum ADE infectivity at concentrations significantly higher than WT ZIKV sera (n = 6) (*p<0.05, Student's t test)

For studying immune responses to flaviviruses, handling live virus can be time-consuming with limited assay format or readout options

Reporter Virus Particles are ready-to-use live virus replacements offering optical readouts and flexibility in assay format



Dengue Reporter Virus Particles (RVPs)

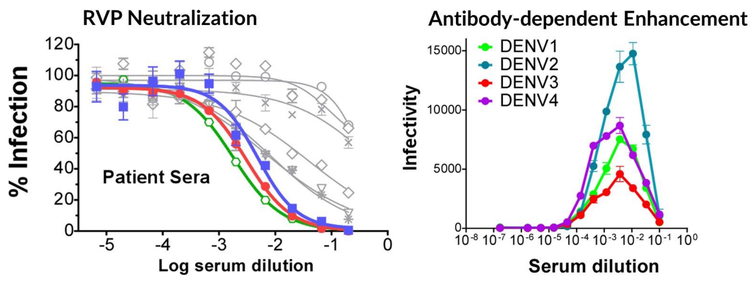


- Capsid, prM/E expressed with modified replicon
- Luciferase/GFP reporter RNA replaces replicon structural genes
- Replication-incompetent flavivirus particles
- Antigenically equivalent to live virus

Validated Flavivirus RVPs
- Dengue Virus (DENV) Serotypes I-4
- Zika Virus (ZIKV)
- Yellow Fever Virus (YFV)
- West Nile Virus (WNV)
- 100+ Custom Strains and Mutants

Applications
- ✓ Antibody neutralization
- ✓ Serum screening
- ✓ Automated workflows
- ✓ High-throughput assays

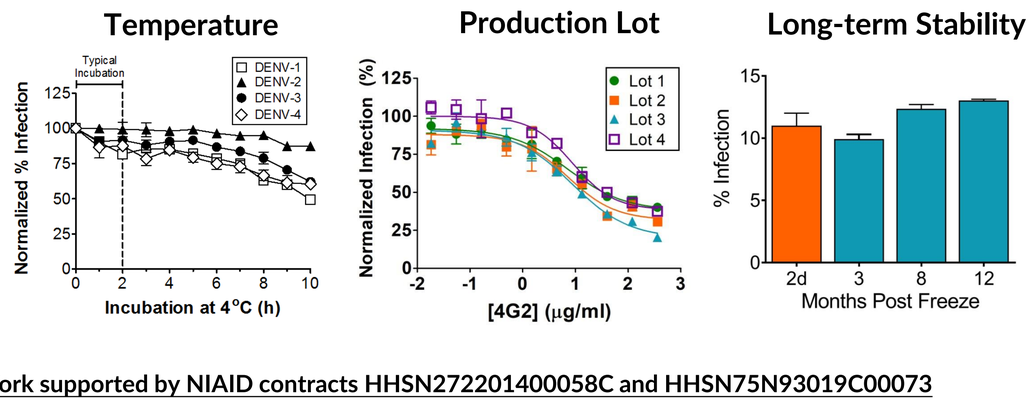
Dengue RVPs



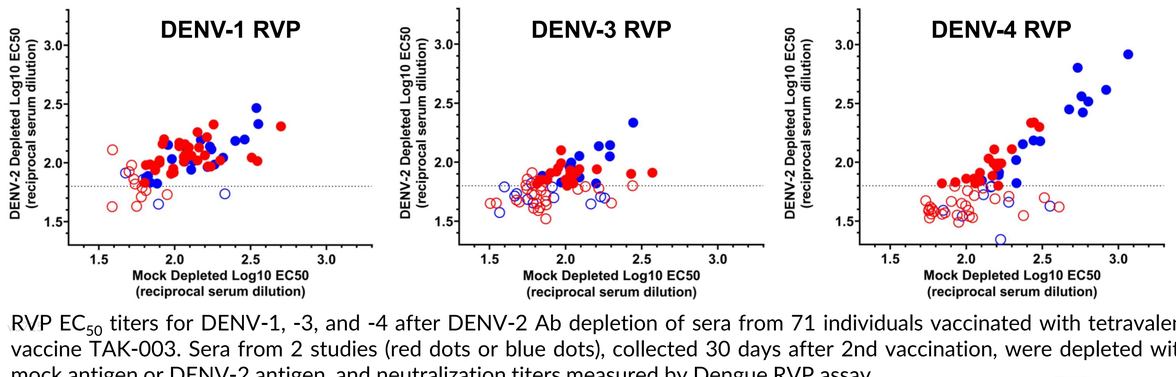
RVPs are highly flexible and have been used for MAb isolation and to study infectivity, neutralization, enhancement (ADE), binding by ELISA, and biosensor

Mattia *et al.*, 2011 *PLoS One*

Stable after thawing, lot-to-lot consistency, good cryopreservation



RVPs in analyses of response to a Tetravalent Dengue Vaccine



RVP EC_{50} titers for DENV-1, -3, and -4 after DENV-2 Ab depletion of sera from 71 individuals vaccinated with tetravalent vaccine TAK-003. Sera from 2 studies (red dots or blue dots), collected 30 days after 2nd vaccination, were depleted with mock antigen or DENV-2 antigen, and neutralization titers measured by Dengue RVP assay.

DeMaso *et al.*, 2022 *J Inf Disease*

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