Supplemental Material for: Delineating antibody recognition against Zika virus during natural infection

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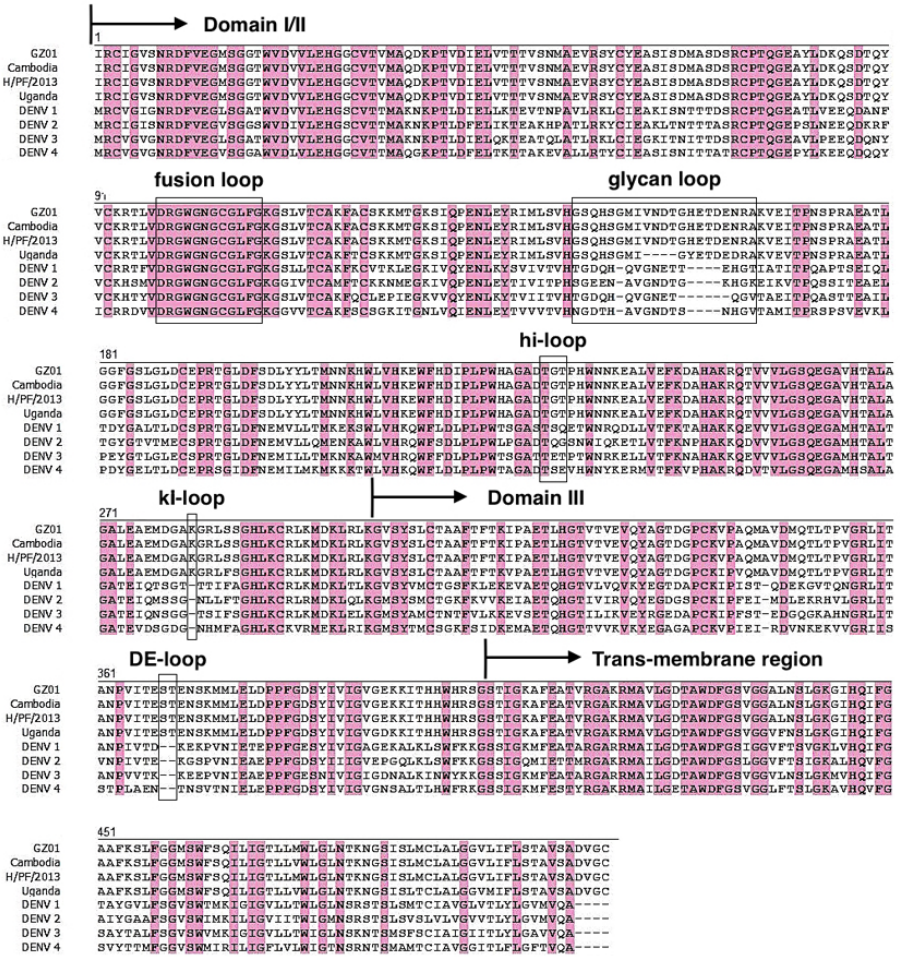
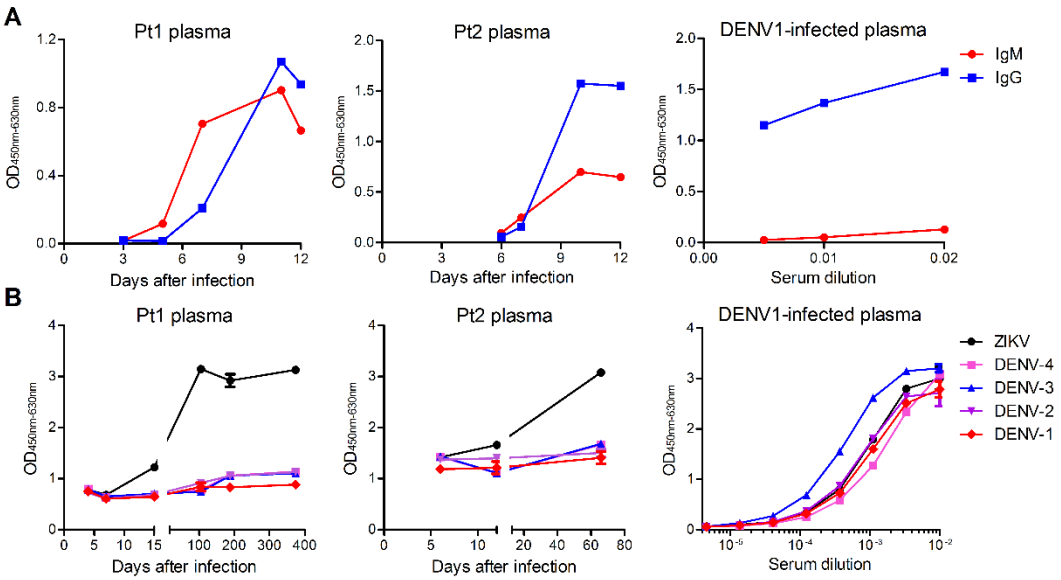
A

(%)	Cambodia	H/PF/2013	Uganda	DENV1	DENV2	DENV3	DENV4
GZ01	99%	99%	97%	58%	54%	58%	56%

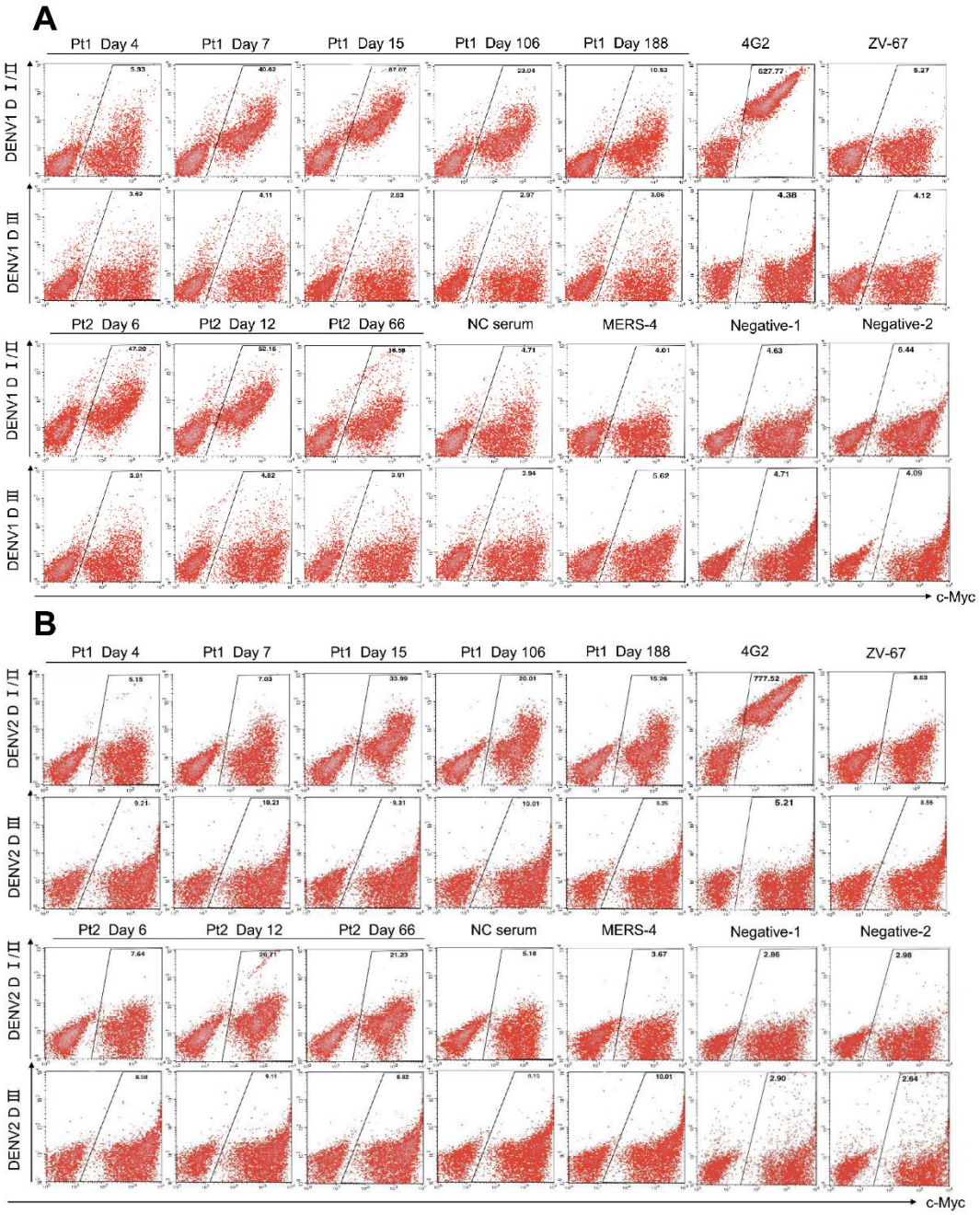
B



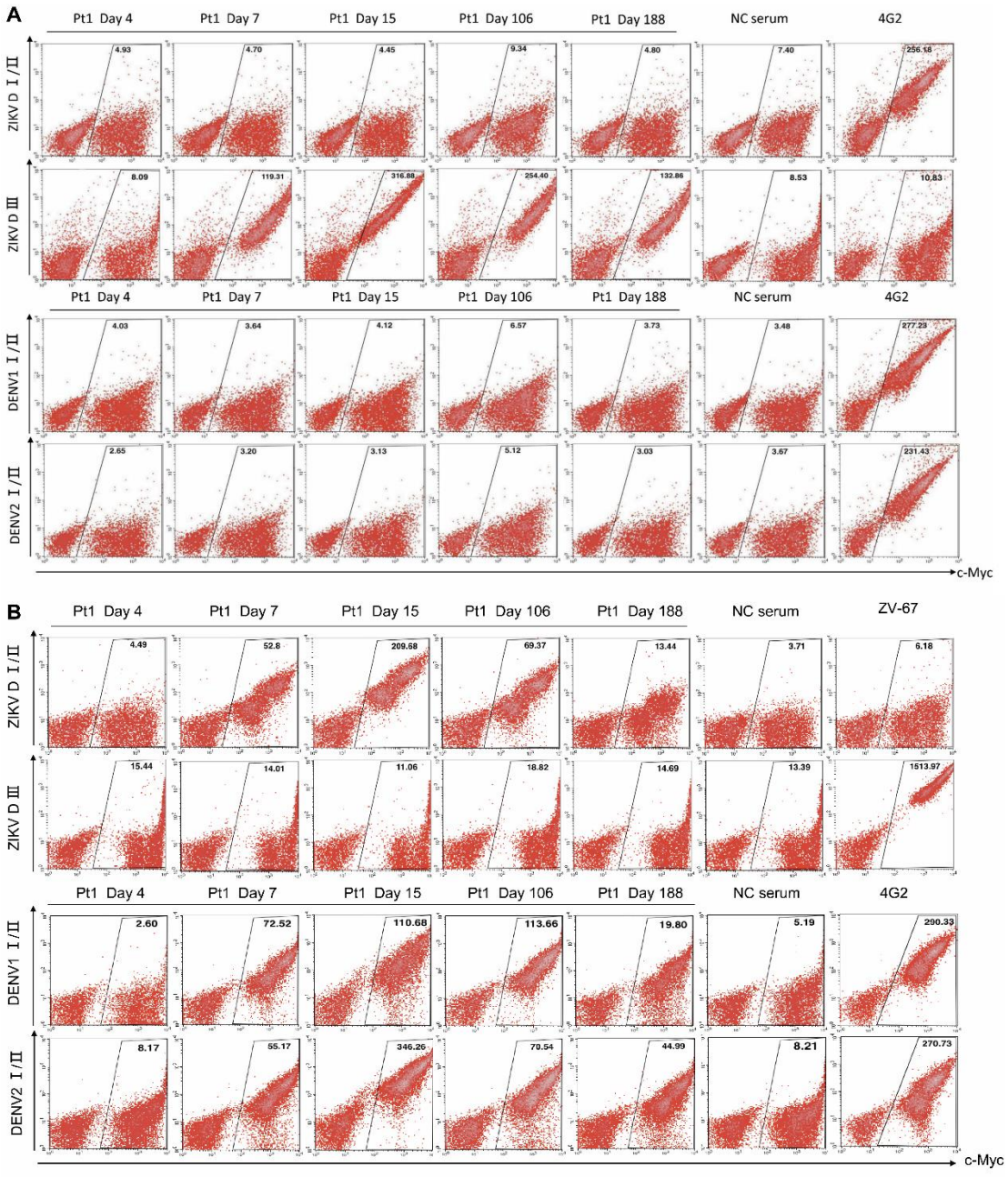
Supplemental Figure 1. Sequence alignment of E glycoprotein from ZIKV (GZ01, Cambodia, H/PF/2013, and Uganda), DENV1, DENV2, DENV3 and DENV4. (A) Overall amino acid identify of each viral strain to ZIKV GZ01 was presented on the top. (B) Conservative regions are highlighted in color together with some of the boxed regions previously shown with functional implications. The amino acid sequences encoding the DI/II (1-301aa) and DIII (302-404aa) for yeast surface display are shown.



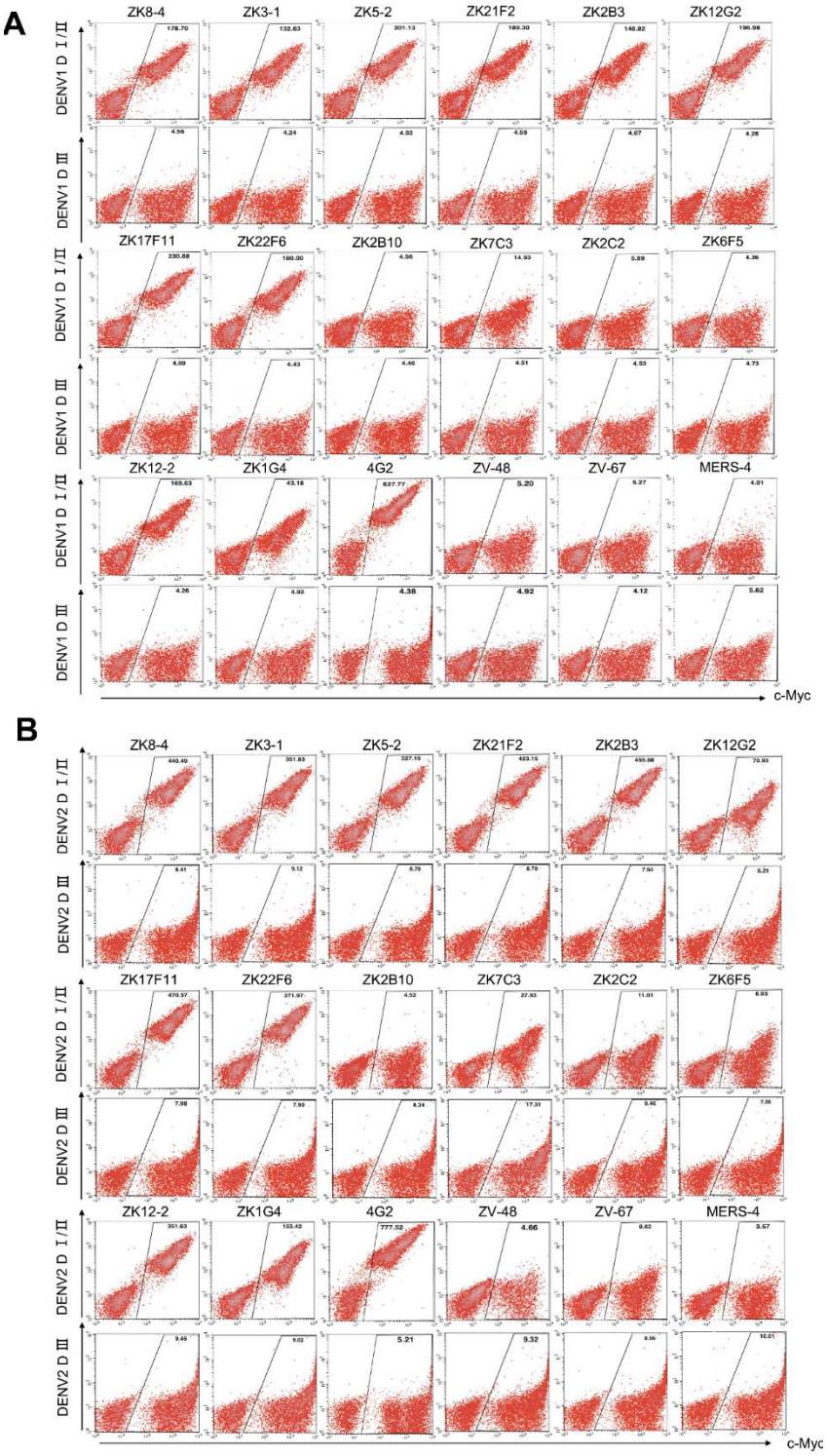
Supplemental Figure 2. Flavivirus immune status of patients analyzed by NS1 protein binding assay. Plasma from Pt1 and Pt2 and a DENV1-infected patients were applied to the ZIKV NS1-captured plate to detect the IgM and IgG binding activity, using a commercially available ELISA kit for diagnosis of ZIKV infection (EUROIMMUN, Germany) (A) or to full-length ZIKV and DENV1-4 NS1 proteins produced in our laboratory (B). Plasma from Pt1 and Pt2 were 1:100 diluted, and positive control were diluted as indication.



Supplemental Figure 3. Cross-binding of sequential plasma samples to DENV1 (A) or DENV2 (B) DI/DII- and DIII-expressing yeast clones. Sequential plasma samples (1:100 dilution) were incubated with domain-specific yeast clones and analyzed by FACS. Positive control mAbs 4G2 and ZV-67 were used for specific recognition of DI/II and DIII, respectively. Negative controls included plasma sample from a healthy individual (NC serum), mAbs MERS-4 previously isolated against MERS-CoV, and the cells staining without corresponding patients' plasma (Negative-1) or PE labeled anti-human IgG secondary antibody (Negative-2). C-Myc is a protein tag used for monitoring DI/II and DIII expression under the induction condition.



Supplemental Figure 4. Binding activity after aborption with ZIKV DI/DII- and DIII-expressing yeast clones. Sequential plasma samples (1:100 dilution) were absorbed with multiple rounds of ZIKV DI/II- (A) or DIII- (B) yeast clones before analyzed for residual binding to ZIKV and cross-binding to DENV1 and DENV2. Positive control mAbs 4G2 and ZV-67 were used for specific recognition of DI/II and DIII, respectively. Negative controls included plasma sample from a healthy individual NC serum). C-Myc is a protein tag used for monitoring DI/II and DIII expression under the induction condition.

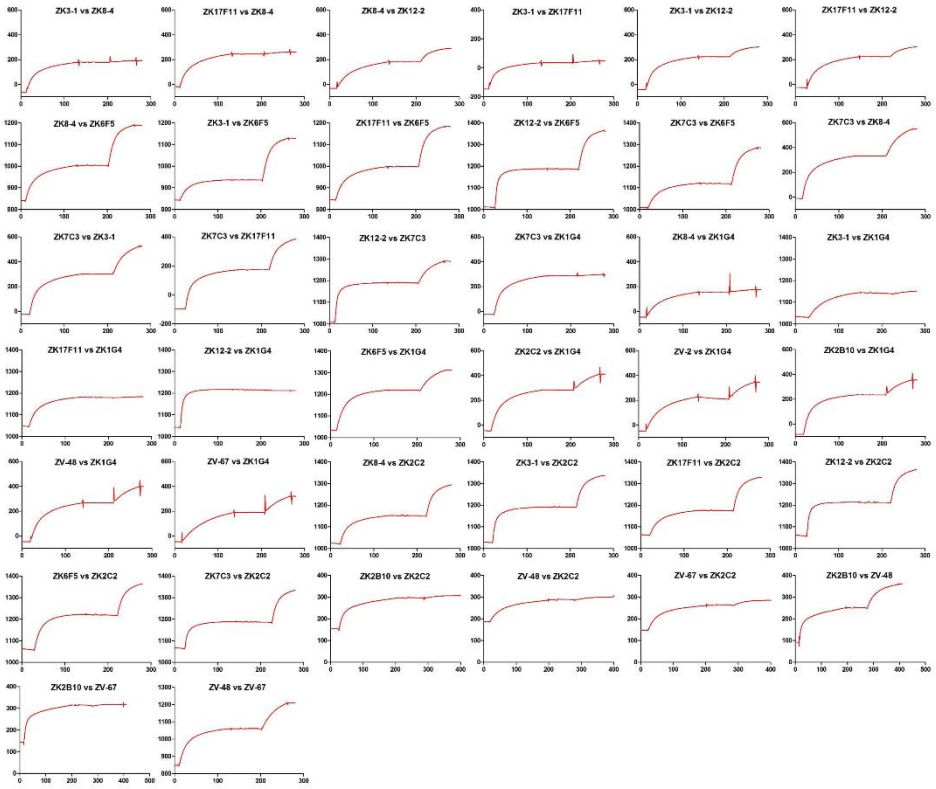


Supplemental Figure 5. Cross-binding activity of isolated mAbs to DI/DII and DIII of DENV1 and DENV2 E glycoprotein displayed on yeast surface. Each mAb (10 ug/ml) were incubated with domain-specific yeast clones and analyzed by FACS. Control mAbs included 4G2, ZV48 and ZV-67 for specific recognition of DI/II and ZIKV DIII, respectively as well as irrelevant control mAb MERS-4 previously isolated against MERS-CoV. C-Myc is a protein tag used for monitoring DI/II and DIII expression under the induction condition.

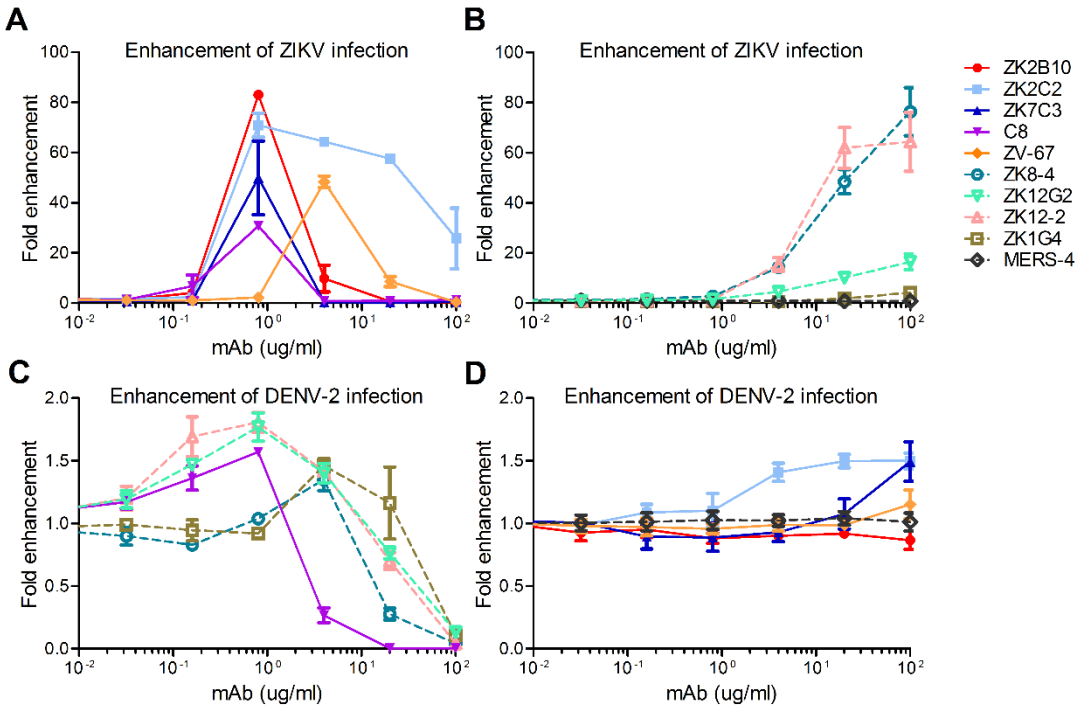
A

		DI/II						DI/II/III		DIII		
		ZK8-4	ZK3-1	ZK17F11	ZK12-2	ZK6F5	ZK7C3	ZK1G4	ZK2C2	ZK2B10	ZV-48	ZV-67
DI/II	ZK8-4	+	+	+	±	-	-	+	-			
	ZK3-1	+	+	+	±	-	-	+	-			
	ZK17F11	+	+	+	±	-	-	+	-			
	ZK12-2	±	±	±	+	-	-	+	-			
	ZK6F5	-	-	-	-	+	-	-	-			
	ZK7C3	-	-	-	-	-	+	+	-			
DI/II/III	ZK1G4	+	+	+	+	-	+	+	-	-	-	-
	ZK2C2	-	-	-	-	-	-	-	+	+	+	+
DIII	ZK2B10							-	+	+	-	+
	ZV-48							-	+	-	+	-
	ZV-67							-	+	+	-	+

B



Supplemental Figure 6. Epitope mapping of isolated mAbs through competitive binding measured by SPR. (A) Representative mAbs from domain-specific analysis presented in Figure 6 were further analyzed in competition assay using SPR. "**+**" indicates strong competing pairs (residual binding <30%); "±" intermediate (residual binding 30~70%), and "-" non-competing pairs (residual binding >70%). (B)The sensorgrams show distinct binding patterns when pairs of testing antibodies were sequentially applied to the purified ZIKV E protein coated onto a CM5 sensor chip, and the results of which were used to summarize (A).



Supplemental Figure 7. Representative mAbs showed differential ADE effects on ZIKV and DENV2 infections. K562 cells were infected with ZIKV (SZ-WIV01) or DENV2 (16681) in the presence or absence of serially diluted mAbs. Cells were harvested and intracellular stained with a pan-flavivirus antibody 4G2 (for ZIKV infection) or D1-11 (for DENV infection), and then analyzed by FACS. Antibodies were classified into two groups according to their ADE effects against ZIKV (A)(B) and DENV2 (C)(D).