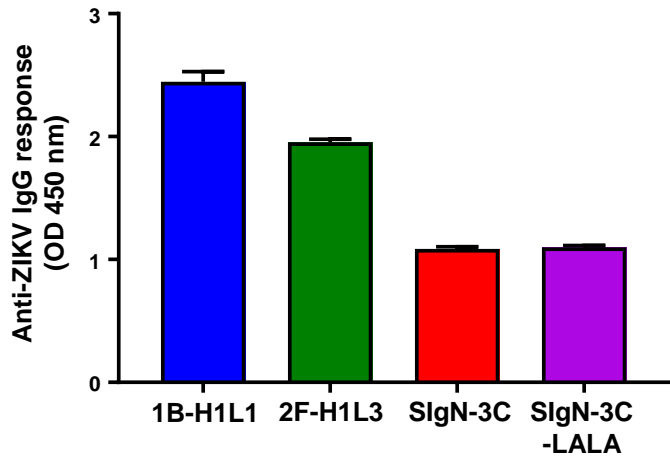
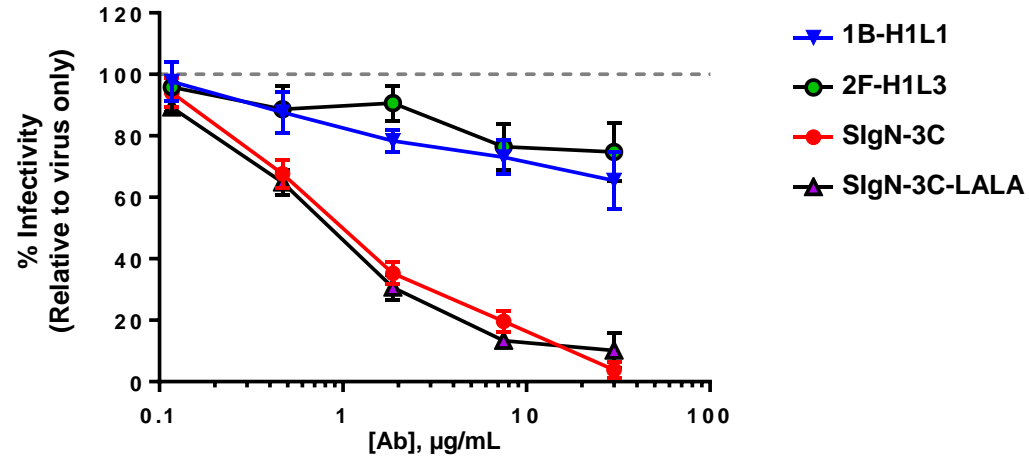
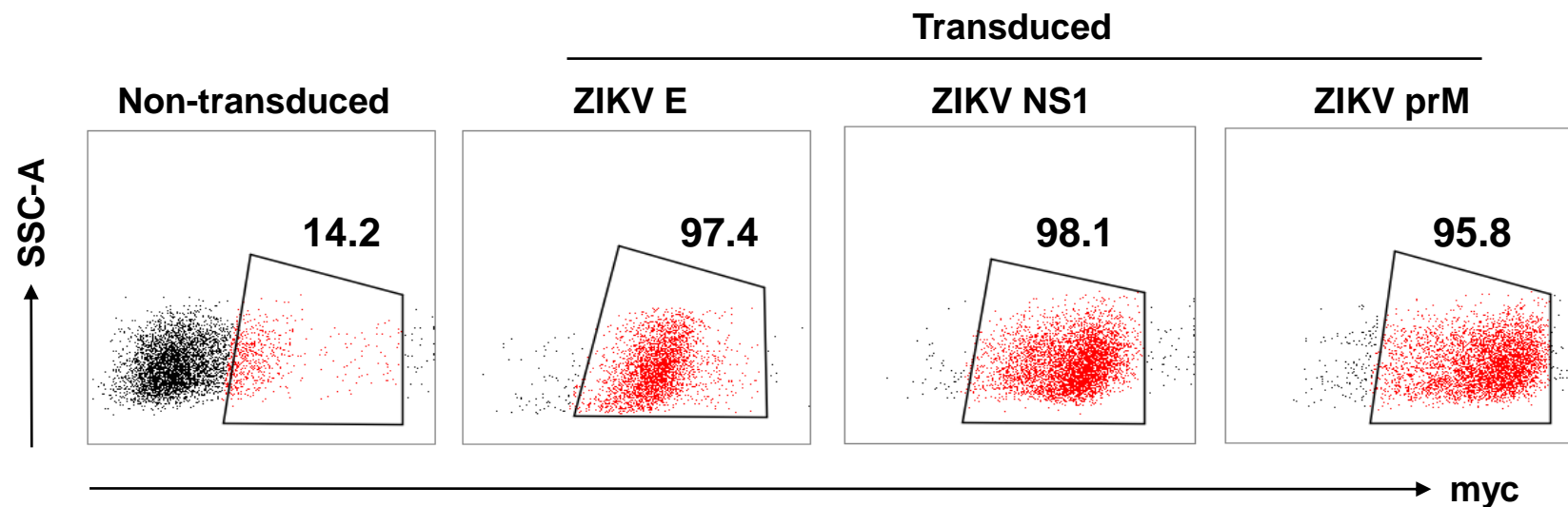
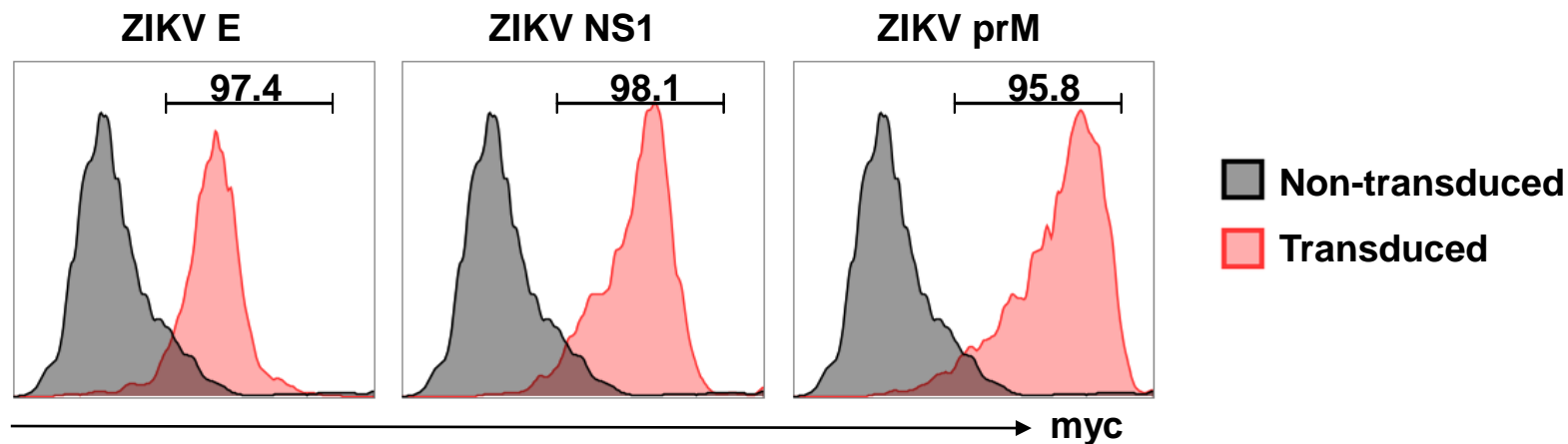


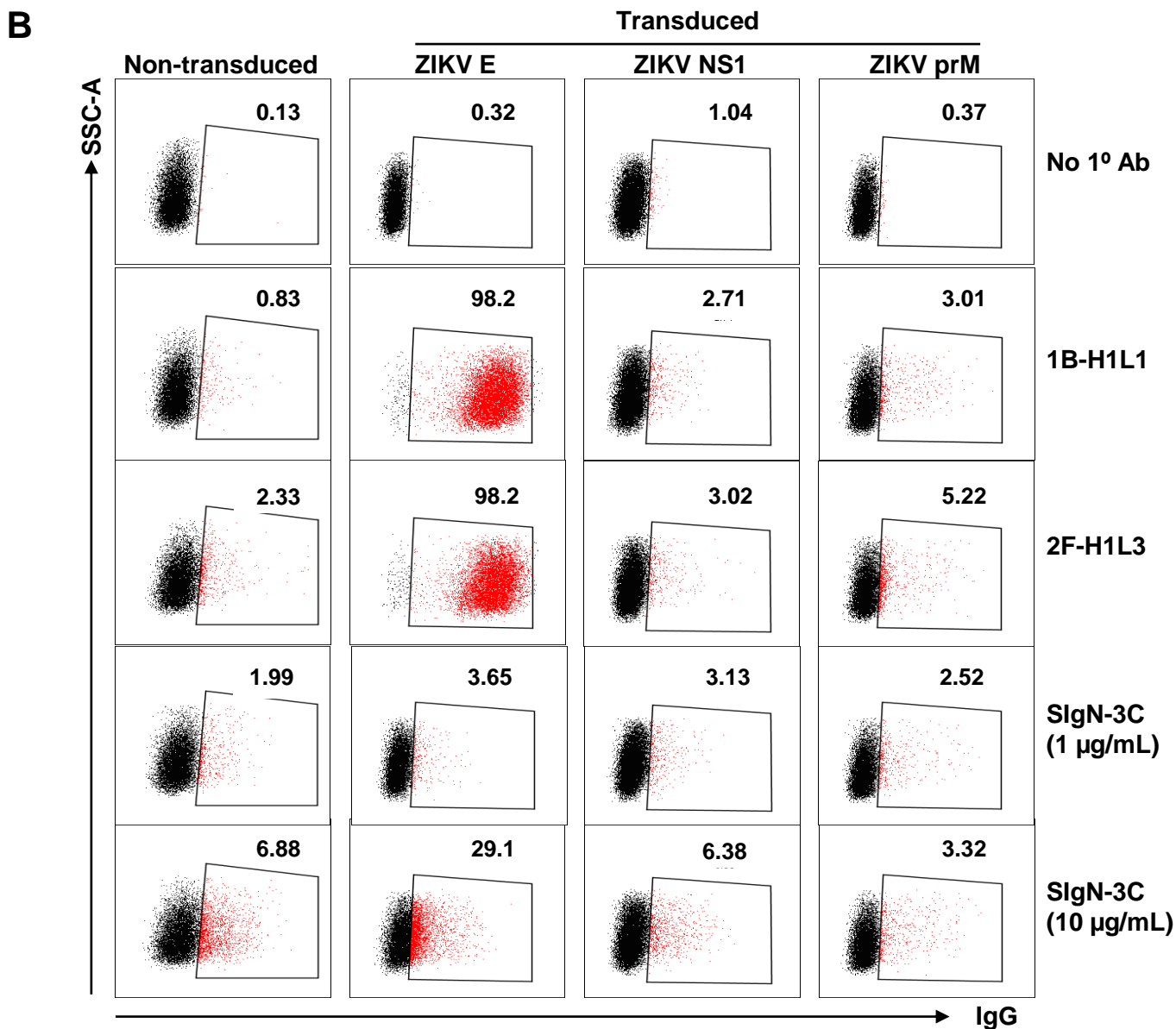
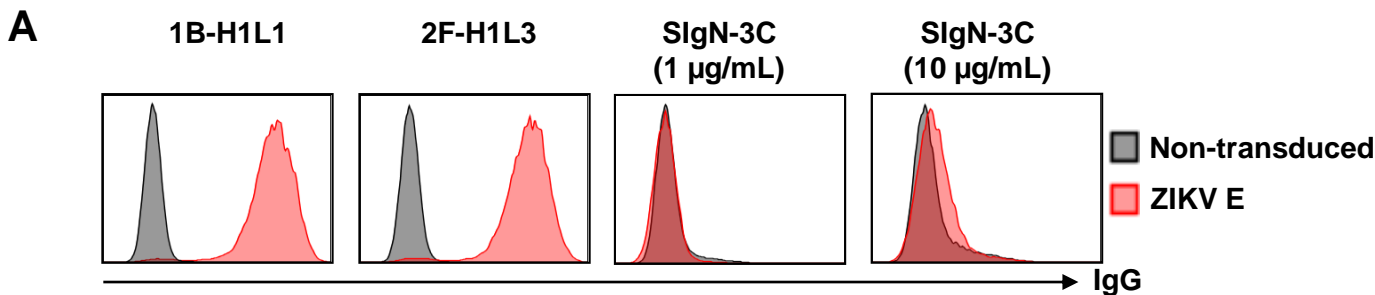
A**B**

Supplemental Figure 1

Binding and neutralizing activity of human DENV monoclonal antibodies against the Brazilian ZIKV PE243 strain. **(A)** Level of recognition of ZIKV whole virions by human DENV mAbs was all tested at 3 µg/mL ($n = 3$) and determined by ELISA using purified ZIKV PE243 strain virions. Data are presented as mean \pm SD. **(B)** Neutralizing capacities of selected human DENV mAbs against the Brazilian ZIKV PE243 strain *in vitro*. ZIKV PE243 strain was pre-incubated with serial dilutions of human DENV mAbs 1B-H1L1, 2F-H1L3, SlgN-3C, and SlgN-3C-LALA prior to infecting Vero-E6 cells at MOI of 10. Mock-infected and virus-only conditions were used as controls. Infectivity was quantified 48 h post-infection by immunofluorescence. Data are presented as mean \pm SEM of 3 independent experiments, normalized to virus-only control.

A**B****Supplemental Figure 2**

Successful generation and expression of ZIKV antigens on K562 cell surface. Non-transduced K562 cells and K562 cells surface-displaying the ZIKV E ectodomain, NS1 or prM were labeled with anti-rabbit myc antibody. An Alexa Fluor 647-conjugated goat anti-rabbit secondary antibody was used to quantify binding by flow cytometry. **(A)** Dot plots showing the percentage expression of myc. Gates were drawn based on the secondary only control for each cell line. **(B)** Red histograms of transduced K562 cells are overlaid over grey histograms of control untransduced K562 cells. Results are representative of 3 independent experiments.



Supplemental Figure 3

Binding of mAbs to cell surface-expressed ZIKV antigens. Untransduced K562 cells and K562 cells surface-displaying the ZIKV E ectodomain, prM or NS1 were labeled with each mAb at 1 µg/mL. SlgN-3C was also tested at 10 µg/mL. An Alexa Fluor 647-conjugated secondary antibody was used to quantify binding by flow cytometry. (A) Red histograms of K562-ZIKV E cells are overlaid over grey histograms of control untransduced K562 cells. (B) Gates were drawn based on the secondary only control for each cell line. Results are representative of 3 independent experiments.

E glycoprotein

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ZIKV H/PF/2013 1  I R C I G V S N R D F V E G M S G G T W V D V V L E H G G C V T V M A Q D K P T V D I E L V T T V S N M A E V R S Y C
DENV3          1  M R C V G V G N R D F V E G L S G A T W V D V V L E H G G C V T T M A K N K P T L D I E L Q K T E A T Q L A T L R K L C

61  Y E A S I S D M A S D S R C P T Q G E A Y L D K Q S D T Q Y V C K R T L V D R G W G N G C G L F G K G S L V T C A K F A
61  I E G K I T N I T T D S R C P T Q G E A V L P E E Q D Q N Y V C K H T Y V D R G W G N G C G L F G K G S L V T C A K F Q

121 C S K K M T G K S I Q P E N L E Y R I M L S V H G S Q H S G M I V N D T G H E T D E N R A K V E I T P N S P R A E A T L
121 C L E P I E G K V V Q Y E N L K Y T V I I T V H T G D Q H - - - - - Q V G N E T Q G - - V T A E I T P Q A S T T E A I L

181 G G F G S L G L D C E P R T G L D F S D L Y Y L T M N N K H W L V H K E W F H D I P L P W H A G A D T G T P H W N N K E
174 P E Y G T L G L E C S P R T G L D F N E M I L L T M K N K A W M V H R Q W F F D L P L P W T S G A T T E T P T W N R K E

241 A L V E F K D A H A K R Q T V V V L G S Q E G A V H T A L A G A L E A E M D G A K G R L S S G H L K C R L K M D K L R L
234 L L V T F K N A H A K Q E V V V L G S Q E G A M H T A L T G A T E I Q N S G G T S - I F A G H L K C R L K M D K L E L

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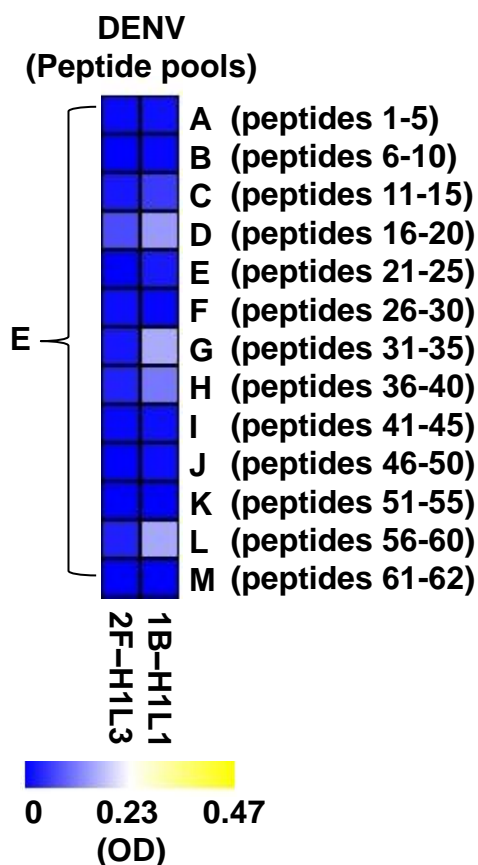
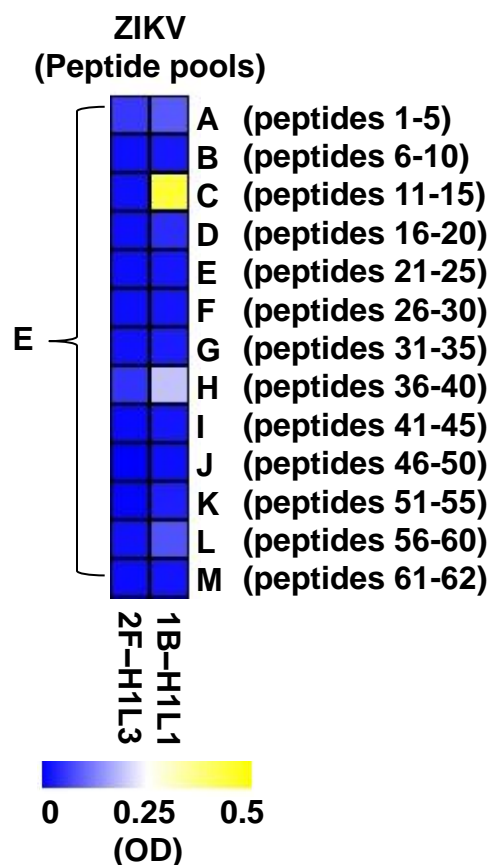
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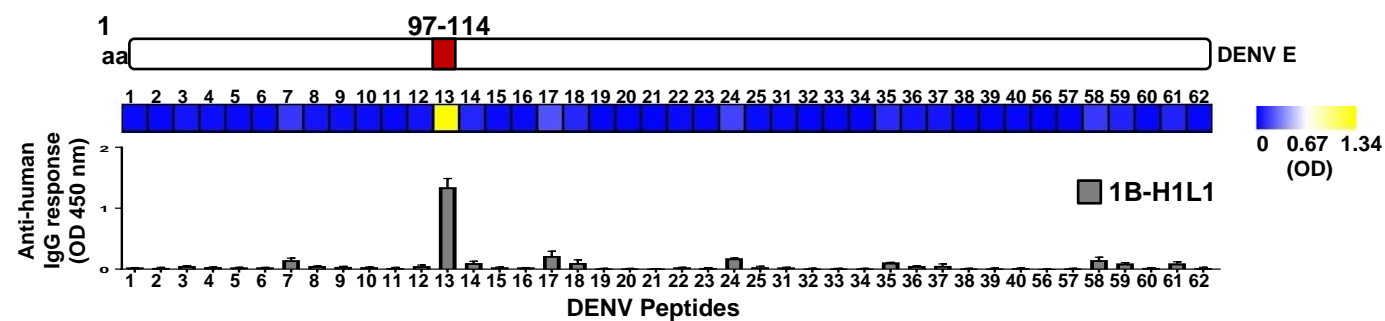
Supplemental Figure 4

Sequence alignment of the E glycoprotein from DENV and ZIKV. The consensus sequence of DENV3 and ZIKV Polynesia isolate (ZIKV H/PF/2013 – GenBank ID: KJ776791) used in the peptide library synthesis. Residues that are conserved between the DENV and ZIKV sequence are highlighted in red.

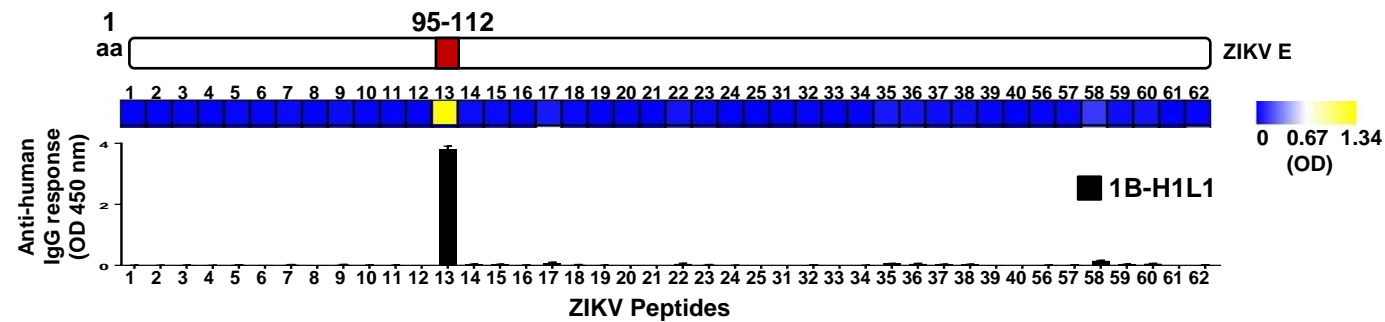
A**B****Supplemental Figure 5**

ELISA using peptide pools from DENV E (**A**) and ZIKV E (**B**) overlapping peptide libraries. Peptides from each library were grouped into 13 pools (A-M) as indicated to perform ELISA assays. Each mAb was tested at 1 μ g/ml. Selected human DENV monoclonal antibodies were subjected to (**A**) DENV (DENV peptide pools A – M) and (**B**) ZIKV (ZIKV peptide pools A – M) peptide-based ELISA assays corresponding to the DENV and ZIKV E glycoproteins. Data are presented in heat-map format with blue representing no binding and yellow color representing the highest OD. Results represent an average of two independent experiments.

A



B

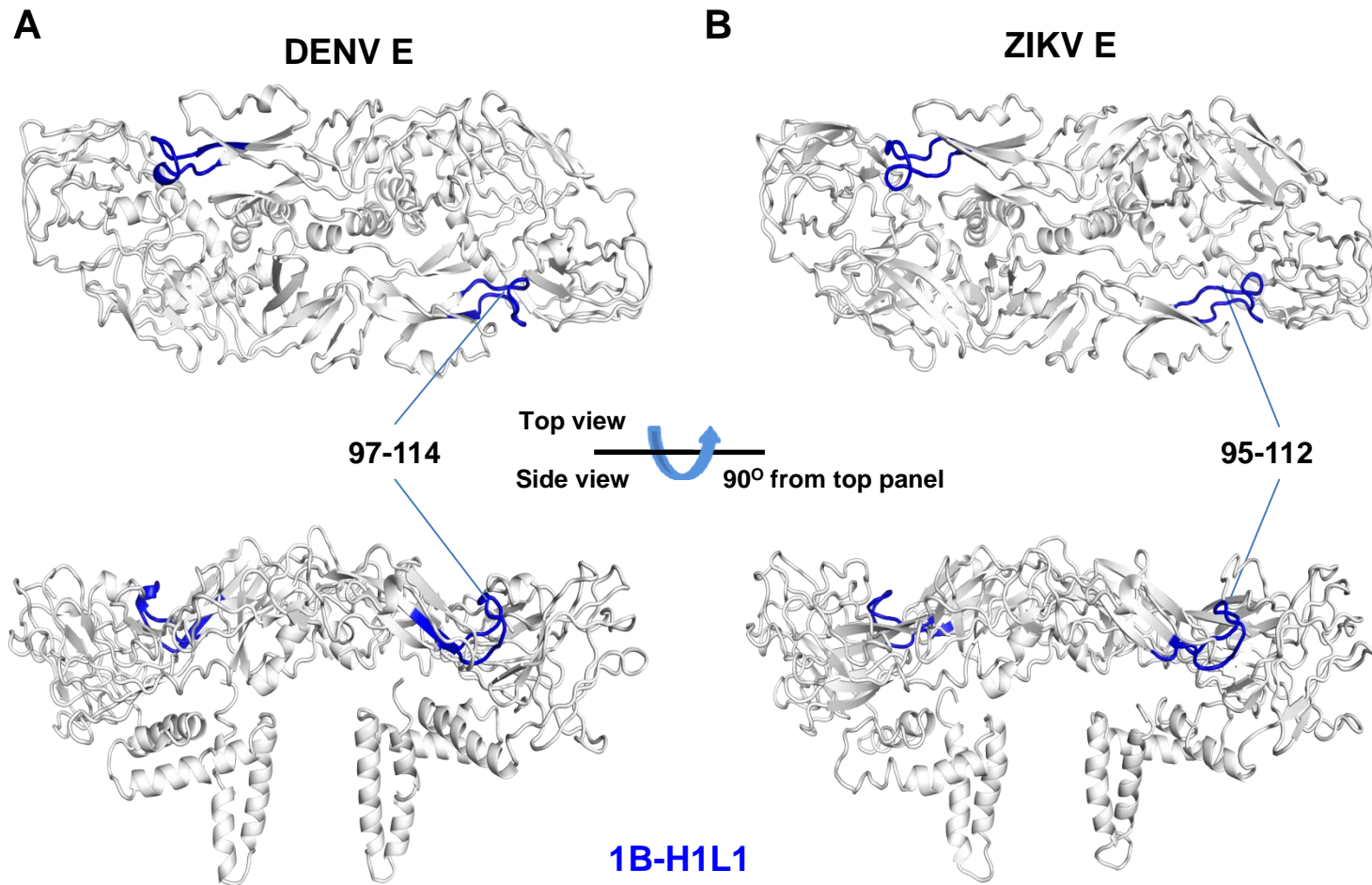


C

DENV E			ZIKV E	
Antibody	Peptide sequence	Amino acid	Peptide sequence	Amino acid
1B-H1L1	<u>VDRGWGNGCGLFGKGS</u> LV	97-114	TL <u>VDRGWGNGCGLFGKGS</u>	95-112

Supplemental Figure 6

Linear epitope mapping on ZIKV E glycoprotein. (**A** and **B**) 1B-H1L1 was tested for binding to selected DENV E and ZIKV E peptides by ELISA at 1 ug/mL respectively. Data are presented in heat-map format with blue representing no binding and yellow color representing the highest OD. Raw data were presented in bar-chart format below the corresponding heat-map. Results represent an average of two independent experiments. The regions of protein found to be important for antibody recognition are indicated in red above the heat map. (**C**) Sequences of the DENV E and ZIKV E peptides most strongly recognized by 1B-H1L1.



Supplemental Figure 7

Mapping of 1B-H1L1 epitope onto DENV E and ZIKV E structures. **(A)** Schematic diagrams showing the epitope position determined by peptide-ELISA in the DENV E glycoproteins based on the structural data retrieved from PDB records: 3J6U. **(B)** Schematic diagrams showing the epitope position determined by peptide-ELISA in the ZIKV E glycoproteins based on structural data retrieved from PDB records: 5IZ7. Peptide regions recognized by 1B-H1L1 are colored in blue.

Supplemental Table 1

Characterization of human DENV mAbs against ZIKV.

Antibody	ZIKV binding capacity ^a	ZIKV neutralizing capacity (IC50) ^b
1D-H4L1	Medium	> 150 µg/mL
2C-H3L2	Medium	Non-neutralizing
2F-H1L1	Strong	> 150 µg/mL
5A-H6L1	Strong	Non-neutralizing
5B-H1L1	Medium	Non-neutralizing
5D-H1L2	Medium	Non-neutralizing
6C-H8L1	Medium	Non-neutralizing
8F-H1L1	Strong	Non-neutralizing
1B-H1L1	Strong	19.25 µg/mL
1D-H8L1	Strong	Non-neutralizing
2F-H1L3	Strong	> 30 µg/mL
SIgN-3C	Medium	0.93 µg/mL
3H-H1L1	Strong	> 150 µg/mL
4B-H2L1	Strong	> 150 µg/mL
5A-H1L1	None	Non-neutralizing
5D-H6L2	Strong	Non-neutralizing
6E-H1L1	Strong	> 150 µg/mL
7A-H1L1	Medium	Non-neutralizing
7E-H1L1	Medium	Non-neutralizing
7H-H1L1	None	Non-neutralizing
9B-H1L1	Medium	Non-neutralizing
9E-H2L2	Strong	Non-neutralizing
11E-H1L1	Strong	> 150 µg/mL

^aHuman DENV mAbs were grouped into strong binders, medium binders and non-binders according to the ZIKV virion-based ELISA results.

^bHuman DENV mAbs that did not show any *in vitro* ZIKV neutralizing activity at 30 µg/mL testing concentration were classified as “Non-neutralizing”. For mAbs showing neutralizing activity within the testing ranges (0.029 µg/mL – 30 µg/mL), nonlinear regression fitting was used to determine the IC50 values.