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(54) Title: POTENT ZIKA VIRUS-SPECIFIC AND CROSS-NEUTRALIZING MONOCLONAL ANTIBODIES TO ZIKA AND DENGUE VIRUSES FOLLOWING ZIKV INFECTION OR VACCINATION

(57) Abstract: The invention described herein provides antibodies to Zika virus. The novel polypeptides are useful alone or as portions of larger molecules, such as antibodies or antibody fragments, that can be used to treat or prevent infection of Zika virus.

Potent Zika Virus-Specific and Cross-Neutralizing Monoclonal Antibodies to Zika and Dengue Viruses Following Zikv Infection or Vaccination

Statement Regarding Federally Sponsored Research or Development

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Reference to Sequence Listing

[0002] A computer readable text file entitled "Seuence Listing_044508-5089WO," created on or about April 22, 2019, with a file size of about 111 kb contains the sequence listing for this application and is hereby incorporated by reference in its entirety.

Background of the Invention

[0003] Zika virus (ZIKV) has caused significant worldwide disease. In 2016, ZIKV infected more than 500,000 people in Central and South America, and more than 5,000 people in the United States. Although the majority of ZIKV infections are asymptomatic or cause only a mild illness, ZIKV has become a significant public health concern due to its link with congenital neurological complications such as microcephaly and Guillain-Barré syndrome.

[0004] ZIKV was first isolated in 1947 from a febrile sentinel rhesus monkey in Uganda and is a member of the Flaviviridae family, which also includes West Nile virus (WNV), Yellow Fever virus (YFV), Japanese Encephalitis virus (JEV) and four dengue virus (DENV) serotypes (Dick et al. 1947). The first human ZIKV infection was observed in 1952. ZIKV is usually transmitted by *Aedes aegypti* mosquitoes, but can also spread by blood transfusions, sexual contact, and perinatal transmission. ZIKV. While the majority of ZIKV infections worldwide occur in DENV-endemic areas, most (97%) U.S. cases occurred in travelers returning from affected areas.

[0005] Identifying neutralizing monoclonal antibodies (mAbs) and their epitopes is a critical step towards understanding protective antibody responses, and further enables the development of antibody-based therapies and vaccines. Most neutralizing antibodies bind to the envelope (E) glycoprotein, which mediates ZIKV attachment to cells, cell entry, and fusion of the viral envelope with endosomal membranes. The E glycoprotein consists of three domains (DI, DII, and DIII) that are

connected by flexible hinges. The surface of a mature virion has exactly 90 E glycoprotein homodimers. Protomer-protomer interactions between homodimers facilitate the formation of a sophisticated icosahedral symmetry on the surface of the virus, with dimeric, trimeric, and pentameric vertices (Yu, 2017). Critical targets of ZIKV neutralizing antibodies are mainly conformational and quaternary epitopes that require higher-order structures on intact virions not fully available on individual E protomers (Wu, 2017).

[0006] Analysis of mAbs from ZIKV-infected humans and mice demonstrated that recognition of different domains is associated with differences in neutralizing activity in vitro and protective capacity in animal models. In general, mAbs against DIII were Zika-specific, highly neutralizing and protective. In addition, DI/II antibodies developed early in infection, whereas DIII antibodies developed later and were durably maintained (Yu, 2017).

[0007] The ZIKV E protein shares a considerable amount of sequence and structural similarity with DENV envelope (E) glycoprotein, resulting in immunological cross-reactivity. The majority of prior studies isolating monoclonal antibodies and defining their structural epitopes have focused on individuals with prior flavi-exposure (Collins, 2017). These individuals have B cells primed from pre-existing DENV immune responses. Flavivirus-exposure primes B cell responses and impacts the specificity of antibody responses to ZIKV in subsequent infection through original antigenic sin (Walker, 2017). Since DENV is also endemic in many areas with ZIKV infection, determining how previous DENV immunity affects subsequent ZIKV infection has been an important area of research.

[0008] Less is known about the antibodies targeting ZIKV E glycoprotein in flavi-naïve individuals. Analysis of the B cell antibody repertoire in a flavi-naïve human indicated that > 60% of B cell responses were to unknown regions of the E glycoprotein (Walker, 2017). Therefore, further studies are needed to delineate antibody responses in flavivirus naïve ZIKV infection. Understanding immune responses to Zika infection without prior flavi-exposure is of vital importance for travelers not living in endemic areas.

[0009] For humans, the induction of high titer neutralizing antibodies is a major goal of vaccination. Rapid vaccine development led to a number of candidates and modalities capable of eliciting high titers of ZIKV-neutralizing antibodies (reviewed in Morabito, 2017). However, these prior studies did not explain how ZIKV vaccination affects humans with prior exposure to DENV.

[0010] Therefore, the development of neutralizing or inhibiting antibodies and antibody fragments against ZIKV and DENV could have important implications for prophylaxis and passive immunotherapy. In addition, the characterization of the epitopes of the antibodies and antibody fragments and the mechanisms of neutralization and inhibition of ZIKV and DENV infection could provide helpful

information for development of candidate vaccines and drugs. Finally, such antibodies and antibody fragments could also be used for diagnosis and as research reagents.

Summary of the Invention

[0011] Non-human primates (NHPs) infected with ZIKV developed high titers of neutralizing antibody responses (McCracken, 2017). This application describes the isolation of eleven neutralizing antibodies from a convalescent ZIKV infected rhesus macaque using a unique B cell sorting strategy with whole ZIKV virions. All NHP mAbs were Zika-specific, originated from different B cell lineages, had low somatic hypermutation (SHM), and defined 4 new classes of antibodies targeting cross-protomer antigenic epitopes on the viral envelope. High-resolution crystal structures revealed targeting of cross-protomer epitopes at the inter- and intra- E-dimer interfaces, including a newly described E Tetramer Epitope (ETE). The three other antibody classes recognized different conformational epitopes and potently neutralized Zika on par with some of the most potent Zika-neutralizing mAbs described to date. In addition, in vivo passive transfer studies of these mAbs in mice demonstrated full protection against ZIKV infection. These are the first NHP monoclonal antibodies described to date, but the epitopes targeted were prevalent in both macaque and human ZIKV infections with and without flavi-priming. Overall, these results demonstrate targeting of the viral envelope by several different classes of Zika-specific neutralizing antibodies with distinct modes of recognition that have therapeutic potential. The findings described herein indicate that potent ZIKV neutralizing antibodies can be generated during acute infection in absence of pre-existing flavivirus immunity, which has broad implications for vaccine design.

[0012] Additional studies showed that vaccination of a DENV-experienced human with ZIKV purified inactivated vaccine (ZPIV) elicited potent cross-ZIKV-DENV immune responses after a single immunization. Using a unique sorting strategy, potently neutralizing antibodies were isolated and characterized, including one termed MZ4, which targeted a novel site of vulnerability. MZ4 neutralized ZIKV and DENV-2 with half-maximum inhibitory concentrations (IC50) in the low ng/ml range. Biophysical mapping and structural studies demonstrated that MZ4 binds to a conserved epitope centered on the E domain I/III linker region. MZ4 protected mice from viraemia following ZIKV challenge with a median effective dose (ED50) of 0.1275 mg/kg. In addition, only one ZPIV vaccination was required to achieve potent MZ4-like mAbs. These data demonstrate that ZPIV vaccination in DENV experienced individuals can elicit rapid potent neutralizing responses against ZIKV and boosts pre-

existing immunity through recall of protective cross-neutralizing DENV immune responses, which have significant implications for flavivirus vaccine design and prophylactic therapeutics.

[0013] The present application is directed to novel peptides, antibodies and antibody fragments that bind ZIKV. The present application is also directed to methods of using the novel peptides, antibodies and antibody fragments, such as methods on inhibiting ZIKV infection, methods of treatment, methods of prevention, diagnostic methods, and pharmaceutical compositions.

[0014] The present application also relates to nucleic acids encoding the novel peptides, antibodies and antibody fragments of the present application, including vectors and host cells containing the nucleic acids.

[0015] The present application also relates to a method of isolating an antibody that binds to cross-protomer epitopes of a virus. In this method, peripheral blood mononuclear cells from an infected primate are contacted first with the virus and then with a fluorescently-labeled antibody that binds the virus. Polynucleotides encoding the heavy and light chains of an antibody that binds to cross-protomer epitopes of a virus are isolated from the fluorescent PBMC. The polynucleotides are then used to express the antibody in a host cell.

[0016] In certain embodiments, the application relates to an antibody or fragment thereof comprising the CDR sequences of any row of Table 1. In certain embodiments, the application relates to an antibody or fragment thereof that selectively binds Zika virus, wherein the heavy chain CDR1 sequence differs from SEQ ID NO: 5 by four or less substitutions, wherein the heavy chain CDR2 sequence differs from SEQ ID NO: 6 by two or less substitutions, wherein the heavy chain CDR3 sequence differs from SEQ ID NO: 7 by five or less substitutions, wherein the light chain CDR1 sequence differs from SEQ ID NO: 8 by one or less substitutions, wherein the light chain CDR2 sequence differs from SEQ ID NO: 9 by three or less substitutions, and wherein the light chain CDR3 sequence differs from SEQ ID NO: 10 by one or less substitutions.

[0017] In certain embodiments, the application relates to an antibody or antibody fragment comprising the CDR sequences of any row of Table 1 that inhibits Zika virus infection, Dengue virus infection, Dengue virus serotype 2 infection, Dengue virus serotype 3 infection, Zika virus transmission from a pregnant female to her unborn child, and/or sexual transmission of a flavivirus.

[0018] In certain embodiments, the application relates to an antibody or antibody fragment comprising the CDR sequences from any row of Table 1 with an ED₅₀ for neutralizing Zika infection of less than less than 10 mg kg⁻¹, less than 5 mg kg⁻¹, less than 1 mg kg⁻¹, less than 0.5 mg kg⁻¹, less than 0.2 mg kg⁻¹, less than 0.1 mg kg⁻¹, less than 0.05 mg kg⁻¹, less than 0.02 mg kg⁻¹, or less than 0.01 mg kg⁻¹.

[0019] In certain embodiments, the application relates to an antibody or antibody fragment comprising the CDR sequences from any row of Table 1 with an IC₅₀ for neutralizing Zika infection of less than 10 μ M, less than 5 μ M, less than 2 μ M, less than 1 μ M, less than 500 nM, less than 200 nM, or less than 100 nM, and 100 μ g/ml of said antibody or antibody fragment does not neutralize infection by a flavivirus selected from the group of Dengue virus, Japanese Encephalitis virus, West Nile virus, or Yellow Fever virus.

[0020] In certain embodiments, the application relates to an antibody or antibody fragment comprising the CDR sequences from any of rows 1-11 of Table 1 and having an equilibrium dissociation constant (KD) is in the range from 10^{-7} to 10^{-9} molar and/or a (KD) of less than 10^{-7} molar.

[0021] In certain embodiments, the application relates to an Fd fragment, an Fab fragment, a single chain variable fragment, and/or a human or humanized antibody or antibody fragment comprising the CDR sequences of any row of Table 1.

[0022] In certain embodiments, the application relates to a polynucleotide comprising a nucleotide sequence that encodes an antibody or antibody fragment comprising the CDR sequences from any row of Table 1; a host cell comprising a polynucleotide comprising a nucleotide sequence that encodes an antibody or antibody fragment comprising the CDR sequences from any row of Table 1; and/or a method of isolating an antibody from a host cell comprising a polynucleotide comprising a nucleotide sequence that encodes an antibody or antibody fragment comprising the CDR sequences from any row of Table 1.

[0023] In certain embodiments, the application relates to a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibody or antibody fragment comprising the CDR sequences of any row of Table 1.

[0024] In certain embodiments, the application relates to a method for the prevention or treatment of flavivirus infection, a method for inhibiting or preventing transmission of a flavivirus infection from a pregnant female to her unborn child, a method for inhibiting or preventing sexual transmission of a flavivirus infection, and/or a method of reducing the likelihood of a subject developing a disease caused by flavivirus, wherein the method comprises administering a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibody or antibody fragment comprising the CDR sequences of any of rows 1-11 of Table 1, and wherein the flavivirus may be Zika virus, Dengue virus, Dengue virus serotype 2, Dengue virus serotype 3, West Nile virus, and/or Japanese Encephalitis virus.

[0025] In other embodiments, the application relates to a method for the prevention or treatment of Zika virus infection, a method for inhibiting or preventing transmission of a Zika virus infection from a

pregnant female to her unborn child, a method for inhibiting or preventing sexual transmission of a Zika virus infection, and/or a method of reducing the likelihood of a subject developing a disease caused by flavivirus, wherein the method comprises administering a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibody or antibody fragment comprising the CDR sequences of any of rows 12-21 of Table 1, and wherein the method is specific for Zika virus and has no effect on Dengue virus, Dengue virus serotype 2, Dengue virus serotype 3, West Nile virus, and/or Japanese Encephalitis virus.

[0026] In certain embodiments, the application relates to a method of detecting the presence of a flavivirus in a biological sample, the method comprising contacting an antibody or antibody fragment comprising the CDR sequences of any of rows 1-11 of Table 1 with the biological sample and detecting the binding of the antibody or antibody fragment to a flavivirus, wherein the flavivirus is Zika virus, Dengue virus, Dengue virus serotype 2, Dengue virus serotype 2, West Nile virus and/or Japanese Encephalitis virus.

[0027] In other embodiments, the application relates to a method of detecting the presence of Zika virus in a biological sample, the method comprising contacting an antibody or antibody fragment comprising the CDR sequences of any of rows 12-21 of Table 1 with the biological sample and detecting the binding of the antibody or antibody fragment to a Zika virus, and wherein the method is specific to Zika virus and does not detect any other flavivirus including Dengue virus, Dengue virus serotype 2, Dengue virus serotype 2, West Nile virus and/or Japanese Encephalitis virus.

[0028] In certain embodiments, the application relates to a kit for detecting the presence of a flavivirus such as Zika virus, Dengue virus, Dengue virus serotype 2, Dengue virus serotype 2, West Nile virus and/or Japanese Encephalitis virus in a biological sample comprising an antibody or fragment thereof comprising the CDR sequences of any of rows 1-11 of Table 1.

[0029] In other embodiments, the application relates to a kit for detecting the presence of a Zika virus in a biological sample comprising an antibody or fragment thereof comprising the CDR sequences of any of rows 12-21 of Table 1, wherein the kit does not detect any other flavivirus including Dengue virus, Dengue virus serotype 2, Dengue virus serotype 2, West Nile virus and/or Japanese Encephalitis virus.

[0030] In certain embodiments, the application relates to a method of diagnosing infection by a flavivirus, the method comprising obtaining a biological sample for a subject at risk of flavivirus infection; contacting the biological sample with an antibody or antibody fragment comprising the CDR sequences of any of rows 1-11 of Table 1; and determining if the antibody or antibody fragment has bound to a flavivirus antigen; wherein binding of the antibody or antibody fragment to a flavivirus

antigen indicates that the subject is infected with flavivirus; and wherein the flavivirus may be Zika virus, Dengue virus, Dengue virus serotype 2, Dengue virus serotype 3, West Nile virus, and/or Japanese Encephalitis virus.

[0031] In other embodiments, the application relates to a method of diagnosing infection by a Zika virus, the method comprising obtaining a biological sample for a subject at risk of flavivirus infection; contacting the biological sample with an antibody or antibody fragment comprising the CDR sequences of any of rows 12-21 of Table 1; and determining if the antibody or antibody fragment has bound to a Zika virus antigen; wherein binding of the antibody or antibody fragment to a Zika virus antigen indicates that the subject is infected with Zika virus.

[0032] In certain embodiments, the application relates to a method of detecting a latent infection by a flavivirus, the method comprising obtaining a biological sample for a subject at risk of flavivirus infection; stimulating the biological sample to induce viral outgrowth; contacting the biological sample with an antibody or antibody fragment comprising the CDR sequences of any of rows 1-11 of Table 1; and determining if the antibody or antibody fragment has bound to a flavivirus antigen; wherein binding of the antibody or antibody fragment to a flavivirus antigen indicates that the subject is infected with a flavivirus; and wherein the flavivirus may be Zika virus, Dengue virus, Dengue virus serotype 2, Dengue virus serotype 3, West Nile virus, and/or Japanese Encephalitis virus.

[0033] In other embodiments, the application relates to a method of detecting a latent infection by a Zika virus, the method comprising obtaining a biological sample for a subject at risk of flavivirus infection; stimulating the biological sample to induce viral outgrowth; contacting the biological sample with an antibody or antibody fragment comprising the CDR sequences of any of rows 12-21 of Table 1; and determining if the antibody or antibody fragment has bound to a Zika virus antigen; wherein binding of the antibody or antibody fragment to a Zika virus antigen indicates that the subject is infected with a Zika virus.

[0034] In certain embodiments, the application relates to a method of inducing immunity to a flavivirus in a subject at risk of flavivirus infection comprising injecting a single dose of Zika virus purified inactivated vaccine to the subject, wherein the subject was previously infected by a flavivirus, and wherein the flavivirus may be Zika virus, Dengue virus, Dengue virus serotype 2, Dengue virus serotype 3, West Nile virus, and/or Japanese Encephalitis virus.

[0035] In certain embodiments, the application relates to a method of measuring the efficacy of a flavivirus vaccine comprising contacting the vaccine with an antibody or antibody fragment comprising the CDR sequences of any of rows 1-11 of Table 1.

[0036] In other embodiments, the application relates to a method of measuring the efficacy of a Zika virus vaccine comprising contacting the vaccine with an antibody or antibody fragment comprising the CDR sequences of any of rows 12-21 of Table 1.

[0037] In certain embodiments, the application relates to an antibody or fragment thereof that binds to the DI-DIII linker domain of a Zika virus. The antibody or fragment thereof may have an MN50 in a 100 PFU Zika virus microneutralization assay of 100 ng, 50 ng, 20 ng, 10 ng, 5 ng, 2 ng, 1 ng, 0.5 ng, 0.2 ng or 0.1 ng. Binding to Zika Virus of the antibody or fragment thereof may be reduced by at least 70% when Zika virus E glycoprotein residue Tyrosine 305 is substituted with alanine.

[0038] In certain embodiments, the application relates to a method for isolating an antibody that binds to cross-protomer epitopes of a virus comprising, (a) immunizing a primate with an intact virus, (b) isolating peripheral blood mononuclear cells (PBMCs) from the primate, (c) contacting the PBMCs with intact virus to create PBMC-virus complexes, (d) contacting the PBMC-virus complexes with a fluorescently-labeled antibody that binds the virus, (e) isolating a fluorescent PBMC, (f) isolating polynucleotides encoding the heavy and light chains of an antibody from the fluorescent PBMC, (g) expressing the isolated polynucleotides in a host cell, and (h) isolating an antibody expressed by the host cell.

Brief Description of the Drawings

[0039] The accompanying drawings, which are included to provide a further understanding of the disclosure, are incorporated in and constitute a part of this specification. The drawings illustrate embodiments of the disclosure and, together with the detailed description, serve to explain the principles of the disclosure. No attempt is made to show structural details of the disclosure in more detail than may be necessary for a fundamental understanding of the disclosure and various ways in which it may be practiced.

FIGURE 1. Isolation of ZIKV reactive antibodies from a flavivirus naïve ZIKV-infected macaque using whole ZIKV. (A) Schematic of the sequential sorting strategy of ZIKV-specific B cells by (1) incubation of PBMCs with unlabeled whole ZIKV followed by (2) incubation with fusion-loop targeted 4G2-APC antibody-conjugate. (B) Isolation of ZIKV reactive activated B cells from peripheral blood of animal 10U032 at 14 days post-infection. Flow cytometry gates show the percentage of cells identified for each phenotypic population. CD19⁺CD38⁺4G2⁺ ZIKV-specific B cells that were sorted and sequenced are shown in the last gate, with cells from which the neutralizing antibodies were isolated are labeled with the matching antibody number. (C) Gene usage and characteristics of isolated mAbs that bound to ZIKV

sE using Biolayer Interference (BLI). V(D)J assignments were performed using IgBLAST. Antibodies positive in the ZIKV (PRVABC59) micro-neutralization screen are highlighted in gray.

FIGURE 2. Neutralizing and binding characteristics define four ZIKV-specific epitopes targeted on the E glycoprotein. (A) Neutralizing activities against ZIKV PR were assessed by micro-neutralization assay in Vero cells (MN50). Shown are neutralization curves compared to the EDE1-C8 and Z004 controls. (B) Summary of binding and neutralization activities. Antibodies were screened for binding to recombinant ZIKV sE by BLI. Values indicate mean binding responses calculated from two independent experiments. Neutralization results from the MN50 and a flow-based assay in U937-DC-SIGN cells (FlowNT50) against ZIKV PR and BR strains, respectively. Shown are mean values for the 50% inhibitory concentration (IC₅₀) in ng/mL averaged from at least two independent experiments. Screening for cross-neutralization was performed against a 7 flavivirus panel (DENV1-4, JEV, WNV and YFV). Shading represents binding or neutralization strength ranging from strong (dark) to weak (light); NT= not tested. (C, D) Binding activities observed against monomeric ZIKV sE (C) and whole ZIKV (D) by ELISA. Antibodies were titrated using 4-fold dilution series starting from 20µg/ml. Values indicate mean binding responses calculated from two independent experiments. (E) Relative ratio of sE over whole virus binding activities from C, D revealed quaternary targeting. To directly compare binding activities between isolated macaque antibodies and human controls, sE/whole virus relative binding ratios were calculated by using O.D. values obtained at 20µg/ml with their respective secondary antibodies. Antibodies with low ratio values were considered as targeting quaternary epitopes (such as EDE) whereas ratios closer to 1 would be characteristic of FLE antibody, such as 2A10G6, that bind well to both monomeric E and ZIKV. (F) Binding competition with a set of characterized control antibodies. Lek, control antibody epitopes mapped onto the ZIKV E dimer structure. Right, 4 groups of neutralizing antibodies were identified in a BLI-based competition assay for ZIKV sE glycoprotein. Values represent the % of residual binding of the indicated second antibody after prior saturation of ZIKV sE with the indicated first antibody. Shading from dark to light indicates competition strength ranging from strong (0-30%), intermediate (31-69%), to weak/none (70-100%). The negative control mAb, used as a non-ZIKV sE reactive antibody, was VRC01, an HIV-1-specific antibody.

FIGURE 3. Crystal structure of ZIKV-specific EDE antibody rhMZ107-B Fab in complex with Zika E glycoprotein. (A) Top view of the co-crystal structure of rhMZ107-B in complex with Zika E (PRVABC59). rhMZ107-B Fv heavy and light chains are colored dark, and light green respectively, and are shown in surface representation, while four Zika E protomers are shown in ribbon representation, and colored

blue, and gray, with glycan-154 shown in stick representation. (B) Antibody epitope footprint of rhMZ107-B heavy and light chain binding is shown as solid, and dashed lines, respectively, displayed on four Zika E protomers in surface representation with glycan-154 represented as above. (C) rhMZ107-B antibody recognition is modeled in the context of the mature ZIKV (PDB: 5IRE). Individual protomers of ZIKV are depicted in smooth surface colored green, blue, and gray, with rhMZ107-B Fv shown in atomic surface representation colored dark, and light green. (D) rhMZ107-B amino-acid sequence alignment with immunoglobulin heavy (HC) (VH3.63, HD4-1*01, and HJ6*01) and light (VL) chains (VL11.42 and JL6) germline gene-encoded sequences. Junction-encoded residues are colored light blue and residues that have undergone somatic hypermutation are colored red with HD-encoded residues boxed. Heavy and light chain CDR loop residues are highlighted in gray. Antibody heavy and light chain contacts are indicated with open circles (o) denoting antibody main-chain-only contacts, open circle with rays (⊙) denoting antibody side-chain-only contacts, and filled circles (●) denoting both main-chain and side-chain contacts. (E) Co-crystal structure of rhMZ107-B antibody in complex with Zika E glycoprotein. Inset panels (a-c) show rhMZ107-B antibody residues within 5 Å of Zika E glycoprotein represented as sticks and colored as in (D). Zika E is colored blue and gray with the exception of the B-strand (residues X – Y) which is highlighted in dark blue. (F) Surface representation of antibody rhMZ107-B, and ribbon representation of Zika E glycoprotein contact residues, viewed looking down at the CDRs at a 180° rotation about the horizontal axis from (E). (G) Overlay of the rhMZ107-B Zika E co-crystal structure with the EDE1-C8 Zika E co-crystal structure (PDB: 5LBS). rhMZ107-B and EDE1-C8 are depicted in ribbon representation and colored green and white respectively.

FIGURE 4. rhMZ antibody epitope mapping. (A) Shotgun mutagenesis epitope mapping of ZIKV E to identify critical residues that effect rhMZ134-B antibody binding. (B) Shotgun mutagenesis epitope mapping data for group D antibodies. The average of two experiments is plotted with standard deviation indicated by error bars.

FIGURE 5. Crystal structure of Inter-Dimer-Epitope Antibody rhMZ100-C Fab in Complex with ZIKV E Glycoprotein. (A) Co-crystal structure of rhMZ100-C in complex with ZIKV E (PRABC59). rhMZ100-C Fv heavy and light chains are colored dark, and light red respectively, and are shown in surface representation, modeled onto four ZIKV E protomers shown in ribbon representation colored blue, and gray, with glycan-154 shown in stick representation. (B) Antibody epitope footprint of rhMZ100-C heavy and light chain is shown as solid, and dashed lines, respectively displayed on four ZIKV E glycoproteins in surface representation and glycan-154 in stick representation colored as in (A). (C) rhMZ100-C antibody

recognition is modeled in the context of the mature ZIKV (PDB: 5IRE). Individual protomers of the ZIKV are depicted in smooth surface, colored blue, dark gray and light gray, with rhMZ100-C Fv shown in atomic surface representation colored dark, and light red. (D) rhMZ100-C amino-acid sequence alignment with immunoglobulin heavy chain (VH3.63, HD6-3*01, and HJ6*01) and light chain (VL11.42 and JLx1) germline genes. Residues that undergo somatic mutation, and junction-encoded residues are colored and highlighted as in Figure 3D. (E) Co-crystal structure of rhMZ100-C antibody in complex with ZIKV E glycoprotein. Inset panel (a-c) rhMZ100-C antibody residues within 5 Å of ZIKV E glycoprotein are shown as sticks and colored as in (D). The ZIKV E glycoprotein is colored blue, and gray with the exception of the B-strand (residues 63–73) which is highlighted in dark blue. (F) Surface representation of rhMZ100-C and ribbon representation of ZIKV E glycoprotein contact residues, viewed looking down at the CDRs at a 180° rotation about the horizontal axis from (E). Molecule 1 is colored gray, and molecule 2 is colored light blue, with the B strand colored dark blue. (G) Overlay of the rhMZ107-B ZIKV E co-crystal structure with the ZIKV-117 ZIKV E co-crystal structure (PDB: 5UHY). rhMZ100-C and antibody ZIKV-117 are depicted in ribbon representation and colored red, and dark teal respectively.

FIGURE 6. Crystal Structure of ZIKV-specific EDE Antibody rhMZ100-C in Complex with ZIKV E glycoprotein. (A) Top view of the co-crystal structure of rhMZ100-C in complex with ZIKV E (PRABC59). rhMZ100-C Fv heavy and light chains are shown in surface representation, colored dark, and light red respectively, while two ZIKV E protomers shown in ribbon representation, and colored blue. Antibody epitope footprint of rhMZ107-B is outlined on two ZIKV E protomers shown in surface representation. ZIKV E regions that interact with rhMZ107-B are indicated. (B) rhMZ107-B antibody residues within 5 Å of ZIKV E are shown as sticks and colored as in (A). ZIKV E is colored blue and gray with the b-strand (residues 63–73) highlighted in dark blue. (C) Overlay of the rhMZ107-B epitope and the ZIKV-117 antibody epitope (PDB: 5UHY).

FIGURE 7. Structure of Inter-Dimer-Epitope Antibody rhMZ104-D in Complex with ZIKV E Glycoprotein. (A) Top view of the co-crystal structure of rhMZ104-D in complex with ZIKV E (PRABC59). rhMZ104-D Fv heavy and light chains are shown in surface representation and are colored dark, and light orange respectively, while four ZIKV E protomers are shown in ribbon representation, and colored blue, and gray, with glycan-154 shown in stick representation. (B) Antibody epitope footprints of rhMZ104-D heavy, and light chain are shown as solid, and dashed lines, respectively, displayed on four ZIKV E glycoprotein protomers in surface representation with glycan-154 represented as above. (C) rhMZ107-B antibody recognition is modeled in the context of the mature ZIKV (PDB: 5IRE). Individual protomers of

the ZIKV are depicted in smooth surface colored blue, dark gray and light gray, with rhMZ104-D Fv shown in atomic surface representation colored dark, and light orange. (D) rhMZ104-D amino-acid sequence alignment with immunoglobulin heavy chain (VH3.15, HD2-2*01, and HJ4*01) and light chain (VL11.42, and JLx1) germline genes. Residues that undergo somatic mutation, and junction-encoded residues are colored and highlighted as in Figure 3D. (E) Co-crystal structure of antibody rhMZ104-D in complex with ZIKV E glycoprotein. Inset panel (a-b) rhMZ100-C antibody residues within 5 Å of ZIKV E glycoprotein are shown as sticks and colored as in (D). The ZIKV E glycoprotein is colored blue, and gray with the exception of the B-strand (residues 63–73) which is highlighted in dark blue. (F) Surface representation of rhMZ104-D and ribbon representation of ZIKV E glycoprotein contact residues, viewed looking down at the CDRs at a 180° rotation about the horizontal axis from (E). (G) Group D antibodies were assessed for binding using shotgun mutagenesis epitope mapping. Alanine or serine mutations which dramatically impacted group D antibody binding are shown in sphere representation on the ZIKV E structure and indicated on the right. Residues important for binding for all Group D mAb are highlighted in orange.

FIGURE 8. Structures of Antibodies rhMZ104-D and rhMZ119-D in Complex with ZIKV E Glycoprotein. (A,B) Top view of the co-crystal structures of rhMZ104-D (A) or rhMZ119-D (B) in complex with ZIKV E (PRABC59). Antibody Fv heavy and light chains are shown in surface representation colored tan, and light yellow (rhMZ119-D), dark and light orange (rhMZ119-D), while two ZIKV E protomers are shown in ribbon representation colored blue, and gray, with glycan-154 shown in stick representation. (right panels) Top view of the co-crystal structure of rhMZ119-D in complex with ZIKV E (PRABC59). Fv heavy and light chains are shown in surface representation and are colored dark, and light orange respectively, while two ZIKV E protomers are shown in ribbon representation, and colored blue, and gray, with glycan-154 shown in stick representation. ZIKV E regions that interact with the antibodies are indicated. (C, D) Co-crystal structure of antibody rhMZ104-D in complex with ZIKV E glycoprotein. Inset panel (a-b) rhMZ100-C antibody residues within 5 Å of ZIKV E glycoprotein are shown as sticks and colored as in (A, B). The ZIKV E glycoprotein is colored blue, and gray with the exception of the b-strand (residues 63–73) which is highlighted in dark blue. (E) Comparison of rhMZ104-D, and rhMZ119-D antibody recognition of ZIKV E glycoprotein. (F) Antibody epitope comparisons of rhMZ104-D, ZIKV195, and rhMZ119-D shown on two ZIKV E protomers. ZIKV E regions that interact with the antibodies are indicated as in (A, B). (G) Group D antibodies were assessed for binding using shotgun mutagenesis epitope mapping. Alanine or serine mutations which dramatically impacted group D antibody binding are shown in sphere

representation on the ZIKV E structure and indicated on the right. Residues important for binding for all Group D mAb are highlighted in orange.

FIGURE 9. Sequence differences between ZIKV and DENV. (A) Modeling of rhMZ antibodies in the context of DENV which contains a glycan at residue N67. rhMZ107-B Fab (left, green), rhMZ100-C Fab (middle, raspberry) and rhMZ104-D Fab (right, orange) bound on ZIKV E (PDB: 5IRE). ZIKV E protomers are in blue and white, Glycan-154 is colored brown and the modeled DENV glycan-N67 is shown in red. (B) Control mAbs that have been previously shown to bind to both ZIKV and DENV and can accommodate the glycan-67 are EDE1-C8 Fab (left, grey) and EDE2-A11 Fab (middle, olive). The binding site for antibody ZIKV-117 (right, teal) overlaps significantly with glycan-67. Coloring and labeling scheme for ZIKV E and glycans are as shown in (A). (C) Sequence differences between ZIKV and DENV (-1, -3 and -4) E, are mapped on the ZIKV E structure (PDB: 5IRE). White color indicates 100% identity, while light red indicates sequence differences compared to ZIKV. Glycan-67 and 153 are shown as spheres in raspberry and brown colors, respectively.

FIGURE 10. rhMZ antibody ZIKV E complex structure asymmetric unit contents, and modeling of higher order interactions. (A) Left rhMZ100-C - ZIKV E asymmetric contents. Center rhMZ100-C antibody modeled onto four ZIKV E protomers. Right rhMZ100-C epitope mapped onto four ZIKV E protomers. (B) Left rhMZ104-D - ZIKV E asymmetric contents. Center rhMZ104-D antibody modeled onto four ZIKV E protomers. Right rhMZ104-D epitope mapped onto four ZIKV E protomers. (C) Left rhMZ119-D - ZIKV E asymmetric contents. Center rhMZ119-D antibody binding to two ZIKV E protomers using symmetry related molecules. Right rhMZ119-D epitopes mapped onto two ZIKV E protomers. (D) rhMZ antibodies are modeled in the context of the mature ZIKV virus for the four antibody-E complex structures.

FIGURE 11. Prevalence of rhMZ antibody epitope targeting in ZIKV-infected humans and non-human primates. (A) Schematic of the BLI-based competition assay. Streptavidin sensors loaded with biotinylated ZIKV E proteins were incubated with ZIKV-immune or uninfected plasmas (step 1) and then dipped into the mAb of interest diluted in the matching plasma (step 2). For any given plasma, background binding obtained with a E non-reactive control antibody (VRC01, right) was subtracted from residual binding (center) for the rhMZ mAb. The % binding inhibition of rhMZ mAb due to the competing effect of plasma was calculated by taking the reciprocal of the corrected residual binding (center) divided by the maximum binding obtained in presence of a non-competing plasma control (left). (B) Human (left) and macaque (right) ZIKV immune plasmas compete binding of representative mAbs from

groups A-D to ZIKV E. Sensors loaded with ZIKV E were incubated with plasmas from ZIKV infected or uninfected macaques prior to binding to the indicated mAb in the BLI-based competition assay. The plotted % binding inhibition was calculated as described in (A). Each dot represents an individual plasma sample, and the geometric mean of all samples is indicated by a red, or black horizontal line.

FIGURE 12. Comparison of DENV and ZIKV Antibody Specificity. (A) Sequence difference map between ZIKV E and DENV2 E, mapped onto the ZIKV structure shown in surface representation (PDB: 5IRE). Sequence and positional differences between ZIKV and DENV-2 are colored light red, while identical residues are colored white. Glycan-154, and DENV glycan-67 are shown in sphere representation colored brown and raspberry, respectively. The antibody binding footprints of rhMZ107-B, and EDE1-C8 are shown on the left as green and white solid lines, respectively. Antibody binding epitopes of rhMZ100-C, rhMZ104-D, and ZIKV-117 are shown in the center as red, orange, and teal solid lines, respectively. (B) Neutralization (IC₅₀, µg/ml) of wildtype (WT) and D67N-A69T mutant ZIKV performed in the ZIKV/H/PF2013 background using a Reporter Virus Particle (RVP) assay. The addition of glycan-67 found on DENV to ZIKV interfered with epitope recognition and abrogated or eliminated neutralization. (C) Epitope mapping of structurally defined antibodies mapped onto four protomers of DENV (left) and ZIKV (second from left). Residues contacted by previously described mAbs are colored dark gray, and residues not previously identified prior to this study indicated in white. Only previously identified mAb structures with resolution greater than 4 Å were used since the contact residues are clearly interpretable. Newly identified residues contacted by rhMZ mAbs described in this study are colored red (right, and second from right). Glycan sites at positions 67, and 153 or 154 are indicated in rose, and brown color, respectively. (right)

FIGURE 13. Protection of ZIKV-specific Neutralizing mAbs. (A) Schematic of passive protection study experimental design. Antibodies were infused intravenously into groups of naïve recipient Balb/c mice (n=5/group) prior to ZIKV-BR challenge. Mice received 200 µg of antibody (10mg/kg) and were challenged with 10⁵ viral particles (10² plaque-forming units) of ZIKV-BR intravenously, 2 hours after infusion. (B) Complete or partial protection from ZIKV replication were observed for six representative neutralizing mAbs with 1 or 2 from each group. Following infusion with the indicated antibody, or saline (Sham), ZIKV viral loads were measured in serum post-challenge by RT-PCR daily until day 7. Viral load peaked at day 3 or 4. The six antibodies were tested in two sets of experiments, and a representative Sham from one experiment is shown. The most potent neutralizing mAbs completely protected mice from ZIKV replication (top row), while mAbs less potent in neutralization partially protected mice from

ZIKV replication (bottom row). (C) Viral dissemination in brain, spleen and lymph nodes (LN) was assessed at day 3 post challenge for 3 of the most potent mAbs (black circles) as compared to the Sham group (red circles). Error bars indicate mean $\pm$ s.e.m.

FIGURE 14. Binding and neutralization of 23 ZIKV-reactive mAbs isolated in this study. (A) Summary of all mAbs isolated in this study that were found to bind to ZIKV E or ZIKV. Highlighted in grey are mAbs that neutralized ZIKV that were further studied. Binding to ZIKV and DENV-2 E glycoproteins was assessed using both Biolayer Interference (BLI) and ELISA. Both E monomeric and dimeric epitopes were available using BLI which resulted in increased binding activity. Modest, if any, binding of was found to ZIKV monomeric sE using ELISA. The magnitude of binding obtained by BLI assays to ZIKV and DENV-2 immobilized E glycoproteins are indicated as follows: 0-1 nM (+); 1-2 nM (++); 2-3 nM (+++); >3nM (++++). For ZIKV and DENV E and whole virus ELISA, antibodies were used at 10 or 20 μ g/ml. Background nonspecific binding was obtained using an HIV-1 specific antibody, VRC01, and this background was subtracted from all values. The magnitude of binding measured in ELISA is indicated as follows: OD650 0-0.5 (+); 0.5-1.0 (++); 1.0-2.0 (+++); >2.0 (++++). Also shown are micro-neutralization activities obtained for antibodies at ≥ 100 μ g/ml against ZIKV, DENV1-4, JEV, WNV and YFV. Magnitude of neutralization is indicated as follows: IC₅₀ >10 μ g/ml (+); 1-10 μ g/ml (++), 0.1-1 μ g/ml (+++), <100 ng/ml (++++). In all cases, (-) indicates a lack of binding or neutralization in the assays tested. No binding or neutralization was detected against DENV-2 or other flaviviruses for the mAbs that neutralized ZIKV. *: Deng et al., 2011; **: Robbiani et al., 2017. (B) Among E binders, neutralizing mAbs demonstrated significantly higher binding responses than non-neutralizing mAbs (Mann-Whitney t test, $p=0.0005$). (C) Among neutralizers, no significant correlation in the magnitude of E binding and neutralization potency was observed (Spearman correlation, $r=-0.19$).

FIGURE 15. Characterization of isolated mAbs using FlowNT, PRNT, and ELISA. (A) Neutralization of ZIKV BR strain measured in the FlowNT assay of rhMZ134-B and rhMZ119-D compared to EDE1-C8 and Z004 controls. The 50% inhibitory concentration (IC₅₀) in μ g/ml is indicated in parenthesis for each mAb. (B) PRNT neutralization of ZIKV BR using the 5 most potent antibodies identified by MN and FlowNT assays compared to the Z004 control. The 50% inhibitory concentration (IC₅₀) in μ g/ml is indicated in parenthesis for each mAb. (C) PRNT neutralization of ZIKV Brazil, Thailand and Uganda strains using rhMZ134-B. In each graph, neutralization curves are shown with mean $\pm$ s.e.m. values from at least two independent experiments. The 50% inhibitory concentration (IC₅₀) in μ g/ml is indicated in parenthesis for each mAb. (D) Binding of control antibodies 2A10G6 (FLE) and EDE1-C8 (EDE1) to ZIKV E (left) and

whole ZIKV (right) measured by ELISA. Antibodies were titrated using 4-fold dilution series starting from 20 µg/ml. Values indicate mean binding responses calculated from two independent experiments. An anti-human secondary antibody was used to detect binding of 2A10G6 (humanized Fc domain) and EDE1-C8. These curves were not plotted on the same graph as the rhMZ mAbs due to difference in secondary Ab detection reagent.

FIGURE 16. Antibody somatic hypermutation, and structure analysis. (A) Alignment of rhMZ103-A, rhMZ107-B, rhMZ100-C, rhMZ104-D, and rhMZ119-D Ig heavy and light chain sequences with germline gene-encoded sequences. Junction-encoded residues are colored blue and residues that have undergone somatic hypermutation are colored red. HD-encoded residues are boxed. Heavy and light chain CDR loop residues are highlighted in gray. Antibody-ZIKV E contact residues are indicated below the sequences. (B-F) Crystal structures of rhMZ103-A, rhMZ107-B, rhMZ100-C, rhMZ104-D, and rhMZ119-D are shown in ribbon representation with spheres indicating residues that have undergone somatic hypermutation.

FIGURE 17. Shotgun mutagenesis epitope mapping data for group D mAbs.

FIGURE 18. Asymmetric unit components of rhMZ antibody ZIKV E complex structures. (A) The asymmetric unit of the rhMZ107-B ZIKV E complex contains four rhMZ107-B Fv molecules in complex with four ZIKV E protomers. (B) The asymmetric unit of the rhMZ100-C ZIKV E complex contains two rhMZ100-C Fab molecules and two ZIKV E dimers. (C) The asymmetric unit of the rhMZ104-D ZIKV E complex contains one rhMZ104-D Fab, one rhMZ104-D Fv, and a single ZIKV E dimer.

FIGURE 19. Isolation of neutralizing antibodies to ZIKV and DENV from a ZPIV-vaccinated individual. a, b, Vaccination schedule and plasma neutralizing antibody titers against (a) ZIKV and (b) other flaviviruses of participant #00015 of the WRAIR trial. ZIKV, ZIKV E and DENV-2 E triple reactive CD19+/IgG+ B cells were sorted at peak ZIKV neutralization, four weeks post second vaccination. c, Characteristics of the binding (ZIKV and DENV-2 virions) and neutralizing (ZIKV and DENV 1-4) monoclonal antibodies isolated at week 8. d, Gene assignment and characteristics of the ZIKV neutralizing antibodies performed with IgBlast (Ye J, 2013). MZ4 family members are shaded in dark grey. e, f, g, Neutralization activities (FlowNT) against ZIKV (Brazil/2015) (e), DENV-2 (S16803) (f), and for MZ4 against ZIKV and DENV 1-4 (g). Shown are neutralization curves obtained by 2-fold serial dilutions of the indicated antibodies and fitted using a 4-parameter logistic regression model. Data are mean ± SEM calculated from at least two-independent experiments performed in triplicate. The concentrations (ng/ml) at which 50% neutralization is observed

are indicated in parentheses next to antibody names. EDE1-C8, a potent cross-neutralizing antibody (Barba-Spaeth G, 2016) was used as reference. h, Binding competition with a set of characterized control antibodies. Top, identification of domain specificities using a BLI-based competition assay. Values represent the % residual binding of the indicated second antibody after saturation of ZIKV E with the indicated first antibody. Shading from dark to light indicates competition strength ranging from strong (0-30%), to intermediate (31-69%), to weak/none (70-100%). Control indicates a non-ZIKV E reactive antibody. Bottom, control antibody epitopes mapped onto the ZIKV E dimer structure. i, Shotgun mutagenesis epitope mapping. Top, residues critical for binding to ZIKV (black check mark) and DENV-2 (red check mark) prM/E (which substitution to alanine causes >70% loss in binding) are indicated for each mAb. Bottom, same critical residues mapped on the ZIKV E dimer.

FIGURE 20. Additional neutralizing characteristics of MZ4 family antibodies. a, Isolation of ZIKV, ZIKV E and DENV-2 E triple reactive CD19+/IgG+ B cells 4 weeks post second vaccination. Flow plots show the characteristics of individual B cells from which the most potent neutralizing antibodies were isolated. b, Neutralization activities of the MZ4 family antibodies obtained in the microneutralization assay (MN50) against ZIKV PR, DENV1-4, JEV, WNV and YFV. Data were generated from neutralization curves obtained by 3-fold serial dilutions from at least two independent experiments and fitted using a 4-parameter logistic regression model. Shown are the concentrations (ng/ml) at which 50% neutralization is observed. c, d, Neutralization activities (FlowNT) of MZ4 against ZIKV (Brazil/2015) (c) and DENV-2 (S16803) (d) compared to other potent ZIKV/DENV cross-neutralizing antibodies. Shown are neutralization curves obtained by 2-fold serial dilutions of the indicated antibodies and fitted using a 4-parameter logistic regression model. Data are mean $\pm$ SEM calculated from at least two-independent experiments performed in triplicate. The concentrations (ng/ml) at which 50% neutralization is observed are indicated in parentheses next to antibody names. e, Neutralization activities of MZ4 (PRNT) against ZIKV strains of American, Asian and African lineages. Data were generated as described in c. The concentrations (ng/ml) at which 50% neutralization is observed are indicated in parentheses next to virus names.

FIGURE 21. Additional binding characteristics and epitope mapping of MZ4 family antibodies. a-d, Binding activities to monomeric E proteins and whole virions for ZIKV and DENV-2, assessed by ELISA. a, Binding to ZIKV E (left) and virions (right). Antibodies were titrated using 4-fold dilution series starting from 20 μ g/ml. Values indicate mean binding responses calculated from two independent experiments. b, Relative ratio of binding to E over binding to whole ZIKV calculated from (a) at 20 μ g/ml. Antibodies

with low ratio values indicated targeting of quaternary epitopes (such as EDE) whereas ratios closer to 1 were characteristic of monomeric recognition similar to a FLE antibody, such as 2A10G6, that bind to both monomeric E and ZIKV. c, Binding to DENV-2 E (left) and virions (right), performed as described in (a). d, Relative ratio of binding to E over binding to whole DENV-2 calculated from (c) at 20µg/ml as described in (b). e, Binding to ZIKV E isolated DIII domain assessed by ELISA. Antibodies were titrated using 4-fold dilution series starting from 20µg/ml. Values indicate mean binding responses calculated from two independent experiments. f, Shotgun mutagenesis ZIKV E epitope analysis. Relative binding to ZIKV prM/E for individual mutations is plotted. Residues from which substitution to alanine causes >70% loss in binding (dotted line) were considered critical.

FIGURE 22. Crystal structure of human antibody MZ4 in complex with ZIKV E glycoprotein. a, MZ4 recognizes the DI/DIII linker and DIII domain on ZIKV E. MZ4 Fv is shown in ribbon representation (orange), and ZIKV E is shown in surface format (blue and white). Inset highlights the four major areas of contact. DI/DIII linker is shown in dark brown color. b, MZ4 contact residues are shown based on 1-CDRs H1, and H2; 2-CDR H3; 3-CDR L1; 4-CDR L2, and FR L3 antibody contacting regions. MZ4 escape mutants G182 and S368 are shown in red. Somatic hyper mutation residues, making contact with ZIKV E, are shown in dark pink color. c, MZ4 shown in surface representation is modeled in the context of the full ZIKV particle (PDB: 5IRE). d, e, Detailed interactions of antibody MZ4 at the 5-fold vertex (d), and inter-raft interface (e) on the mature ZIKV are shown. f, Sequence alignment of ZIKV, and DENV1-4 residues that were identified as MZ4 antibody contacts. Symbols ★, : and ., denote the same, very similar and less similar residues, respectively. Buried surface area (BSA) for contacting residues, calculated using PISA, is shown as bars. g, ZIKV BR/2015 escape from MZ4 neutralization identified two variants which reduced antibody neutralization significantly.

FIGURE 23. Structure comparison of human antibody MZ4 with known ZIKV and DENV antibodies. a, MZ4 recognizes the DI/DIII linker and DIII domain on ZikaE. MZ4 Fv is shown in ribbon representation (orange), and ZikaE is shown in surface format (light blue). Other Zika specific DIII domain targeting antibodies (ZV-2, ZV-64, ZV-67 and Z004) are shown in ribbon and are labeled appropriately. b, Dengue E is shown in surface format (gray). Other Dengue E targeting antibodies (EDE1-C8, Ab513, 4E11 and 5H2) are shown in ribbon and are labeled appropriately.

FIGURE 24. MZ4 and MZ1 protect mice *in vivo* from ZIKV replication. a, Schematic of passive protection study experimental design. Antibodies were infused intravenously into groups of naïve recipient Balb/c

mice (N=5/group) prior to ZIKV-BR challenge. Mice were infused with antibody or saline (sham) and 2 hours later, mice were challenged with 10^5 viral particles (10^2 plaque-forming units) of ZIKV-BR intravenously. Following infusion with the indicated antibody, or saline (sham), ZIKV viral loads were measured post-challenge by RT-PCR daily until peak viral load (day 4) and at day 7. b, MZ4 and MZ1 afford complete protection from ZIKV viremia at a single dose of 200 μg ($10 \text{ mg}^{-1} \text{ kg}^{-1}$). c, *In vivo* titration of MZ4 prophylactic dose. Kaplan-Meier plot of MZ4 dose-dependent protective effects on ZIKV replication. An ED50 of 2.55 μg (95% confidence interval = 2.139, 3.262) was calculated as the dose required to protect half of the animals using a 5-parameter non-linear regression analysis.

FIGURE 25. *In vivo* titration of MZ4 prophylactic dose. Antibodies were infused intravenously into groups of naïve recipient Balb/c mice (N=5/group) prior to ZIKV-BR challenge. Mice were infused with the indicated dose of MZ4 or saline (sham) and 2 hours later, mice were challenged with 10^5 viral particles (10^2 plaque-forming units) of ZIKV-BR intravenously. Following infusion with the indicated antibody, or saline (sham), ZIKV viral loads were measured post-challenge by RT-PCR daily until peak viral load (day 4) and at day 7.

FIGURE 26. A single ZPIV vaccination induces broad neutralization responses and elicits MZ2, a potent MZ4-like antibody. a, b, Plasma binding and neutralization activities of donor #00015 and flavivirus-naïve donors. a, Polyclonal binding and neutralizing responses to ZIKV and DENV-2 in donor #00015 (red). Binding responses to the indicated E protein (nM) were measured after 900s association time by BLI. and flavivirus-naïve donors (blue, average of 5 donors) plasma measured by BLI. Data are mean values $\pm$ SEM calculated from two independent experiments. Differences in neutralization titers between week 0 and 2 were statistically significant (Wilcoxon matched-pairs test). b, Neutralization activities (FlowNT) observed in plasma against ZIKV and all 4 DENV serotypes. Shown are reciprocal dilution at which 50% of neutralization (ID50) is achieved. Flavivirus-naïve donor plasmas were only tested against ZIKV and DENV-2. Data are mean values $\pm$ SEM calculated from two independent experiments performed in triplicate. Dotted line indicates lower limit of detection. c, Phylogenetic tree of MZ4 and the week 2 mAb, MZ2 VH sequences rooted on the germ line sequence. d, MZ2 shotgun mutagenesis epitope mapping. Residues critical for binding to ZIKV (black check mark) and DENV-2 (red check mark) prM/E are indicated. e, Binding and neutralization characteristics of MZ2. f, g, Neutralization activities (FlowNT) of MZ2 and MZ4 against ZIKV (Brazil/2015) (f), DENV-2 (S16803) (g). Shown are neutralization curves obtained by 2-fold serial dilutions of the indicated antibodies and fitted using a 4-parameter logistic regression model. Data are mean $\pm$ SEM calculated from at least two-independent experiments

performed in triplicate. The concentrations (ng/ml) at which 50% neutralization is observed are indicated in parentheses next to antibody names.

FIGURE 27. Additional plasma characteristics and isolation of a MZ4-like antibody, MZ2, two weeks after the first ZPIV vaccination. a, Polyclonal binding responses to ZIKV and DENV-2 E in donor plasma measured by BLI. Off-rates (s^{-1}) were calculated after a 1200s dissociation step and fitted with a 1:1 binding model. Data are mean values $\pm$ SEM calculated from two independent experiments. b, Longitudinal ZIKV neutralization titers in donor #00015 (red) and 15 flavivirus-naïve donors (blue) during the course of vaccination. Median value with 95% confidence interval is plotted for naïve donors. c, Binding of MZ4 and MZ2 to ZIKV and DENV-2 virions, assessed by ELISA. Antibodies were titrated using 4-fold dilution series starting from 20 μ g/ml. d, Binding kinetics of MZ4 and MZ2 to ZIKV (top) and DENV-2 (bottom) E proteins measured by BLI. Kinetic constants (table below) were calculated from binding curves (red [MZ4] and yellow [MZ2]) obtained from 4 serial dilutions of Fabs and fitted (grey curves) using a 1:1 binding model. e, Neutralization activities of the MZ4 and MZ2 antibodies obtained in the microneutralization assay (MN50) against ZIKV PR, DENV1-4, JEV, WNV and YFV. Data were generated from neutralization curves obtained by 3-fold serial dilutions from at least two independent experiments and fitted using a 4-parameter logistic regression model. Shown are the concentrations (ng/ml) at which 50% neutralization is observed.

FIGURE 28. Schematic of phage-display strategy to increase the neutralization breadth and potency of MZ4. (A) Schematic of structure-based phage display library design and selection of improved MZ4 variants. (B) Description of the two phage-display libraries based on the MZ4-E contact residues. (i) All wildtype (wt) template. MZ4 variants are introduced into a library where wildtype CDR loops will combine with library variants. (ii) All stop4 template. Each displayed MZ4 variant will be made up of combinations of variant CDR loops.

FIGURE 29. Structure-based design of MZ4 phage library variants using the MZ4-ZIKV E glycoprotein structure. Analysis of the MZ4 - ZIKV E and MZ1 - ZIKV E glycoprotein crystal structures allowed identification of critical contact residues. Modeling of MZ4 or MZ1 in complex with DENV1-4 E glycoproteins allowed identification of residue substitutions that may lead to increased affinity to DENV-1, and DENV-4 E glycoprotein and neutralization potency. Residue variants were designed for CDR H1, H2, H3, CDR L1, CDR L2, and FR L3. In addition, a set of insertions in CDR L1 were also designed.

FIGURE 30. Selection of MZ4 phage library variants using DENV-1 E glycoprotein. MZ4 variant displaying phage libraries ((i) stop-based and (ii) wild-type-based) were screened for variants with increased binding to DENV-1 E glycoprotein. Phage selection was carried out using increasing concentration of Tween, and increasing number of washes for four rounds. A set of 36 colonies from both libraries were grown overnight in the presence of helper phage. MZ4-displaying phage (MZ4 phage) was grown similarly. *E. coli* cells with no phage were used as a control (Blank). Phage binding to DENV-1 E glycoprotein or BSA was assessed by ELISA. Selected libraries show increased diversity of DENV-1 E glycoprotein binding levels, with some members showing significantly improved binding compared to MZ4.

FIGURE 31. Design of multi-specific Flavivirus neutralizing antibodies. Development of an immunotherapeutic with broad reactivity and protection against multiple flaviviruses may require combination of a set of antibody domains into a single immunotherapeutic. (A) a set of mutations in the Heavy constant 3 (T366Y, Y407T) create the “knob in hole” approach that allows partnering of two different heavy chains in a single antibody. (B) single-chain Fv design, allows the addition of multiple Fv regions with given specificities into a single antibody. (C) Hinge-insertion approach utilizes the linker region between Fv, and Fc, or between Fc1, and Fc2 to add additional scFvs. (D) Tail addition approach allows addition of either a Fab, or scFv(s) at the C-terminus of an antibody to allow additional specificities. (E) Examples of bi-specific tetravalent and bispecific bivalent antibodies are shown as examples.

FIGURE 32. Design of multi-specific Flavivirus neutralizing antibodies. Development of an immunotherapeutic with broad reactivity and protection against multiple flaviviruses may require combination of a set of antibody domains into a single immunotherapeutic. (A) FlowNT50 of a set of dengue and Zika neutralizing antibodies (MZ4, MZ20, EDE2-A11, and Ab513 (does not neutralize ZIKV)). (B) MZ4, Ab513, and EDE2-A11 antibodies have non-overlapping epitopes as shown on a ZIKV E dimer structure. Escape variants in multiple epitopes would be difficult to achieve and increases the protective potential of multi-specific antibodies.

FIGURE 33. Design and biochemical characterization of bi-specific tetravalent flavivirus neutralizing antibodies. (A) Schematic of Bi-specific tetravalent type A antibodies. Two examples are shown (i) BTA-A11-MZ4 and (ii) BTA-MZ20-MZ4. (B) BTA-A11-MZ4 design schematic, SDS-PAGE profile (reduced and

non-reduced), and gel filtration purification profile. (C) BTA-MZ20-MZ4 design schematic, SDS-PAGE profile following expression in 293 Expi cells, and gel filtration profile.

FIGURE 34. Antigenic and Neutralization properties of BTA-MZ4-A11 and BTA-MZ20-MZ4 multi-specific antibodies. (A) Binding and Neutralization of BTA-A11-MZ4 against ZIKV, DENV-1, DENV-2, and DENV-4. Binding was measured by biolayer interferometry using E glycoproteins. FlowNT neutralization was used to measure neutralization against ZIKV, and DENV1-4 viruses. EDE2-A11 and MZ4 monoclonal antibodies are included for comparison. (B) Binding and Neutralization of BTA-MZ20-MZ4 against ZIKV, DENV-1, DENV-2, and DENV-4. Binding was measured by biolayer interferometry using E glycoproteins. FlowNT neutralization was used to measure neutralization against ZIKV, and DENV1-4 viruses. EDE2-A11 and MZ4 monoclonal antibodies are included for comparison.

FIGURE 35. Design and biochemical characterization of bi-specific tetravalent flavivirus neutralizing antibodies. (A) Schematic of Bi-specific tetravalent type B antibodies. Two examples are shown (i) BTB-A11-MZ4 and (ii) BTB-MZ20-MZ4. (B) BTB-A11-MZ4 design schematic, SDS-PAGE profile (reduced and non-reduced), and gel filtration purification profile. (C) BTB-MZ20-MZ4 design schematic, SDS-PAGE profile following expression in 293 Expi cells, and gel filtration profile.

FIGURE 36. Antigenic and Neutralization properties of BTB-MZ4-A11 and BTB-MZ20-MZ4 multi-specific antibodies. (A) Binding and Neutralization of BTB-A11-MZ4 against ZIKV, DENV-1, DENV-2, and DENV-4. Binding was measured by biolayer interferometry using E glycoproteins. FlowNT neutralization was used to measure neutralization against ZIKV, and DENV1-4 viruses. EDE2-A11 and MZ4 monoclonal antibodies are included for comparison. Gray sphere is a 50-50 mixture of A11 and MZ4 monoclonal antibodies. (B) Binding and Neutralization of BTB-MZ20-MZ4 against ZIKV, DENV-1, DENV-2, and DENV-4. Binding was measured by biolayer interferometry using E glycoproteins. FlowNT neutralization was used to measure neutralization against ZIKV, and DENV1-4 viruses. EDE2-A11 and MZ4 monoclonal antibodies are included for comparison. Gray sphere is a 50-50 mixture of A11 and MZ4 monoclonal antibodies.

FIGURE 37. Design and biochemical characterization of bi-specific bivalent flavivirus neutralizing antibodies. (A) Schematic of Bi-specific bivalent Heavy chain-only knob in hole multi-specific antibodies. Four examples are shown on the left of possible combinations. In a given BB antibody, two chains are combined with the cartoon (right side) indicating the dual specificity and knob in hole design. (B) Schematic of Bi-specific bivalent Cross-Mab knob in hole multi-specific antibodies. An example is shown

on the left of combinations e.g. Ab513 with MZ4, with a cartoon (right side) indicating the dual specificity and knob in hole design.

FIGURE 38. Characterization of multi-specific Flavivirus neutralizing antibody BB-MZ4-A11. (A) Schematic of Bi-specific bivalent antibody BB-MZ4-A11, and cartoon. (B) BB-MZ4-A11 analysis by SDS-PAGE, and gel filtration profile. (C) Binding and Neutralization of BB-MZ4-A11 against ZIKV, DENV-1, DENV-2, and DENV-4. Binding was measured by biolayer interferometry using E glycoproteins. FlowNT neutralization was used to measure neutralization against ZIKV, and DENV1-4 viruses.

Detailed Description of the Invention

[0040] The following detailed description is presented to enable any person skilled in the art to make and use the invention. For purposes of explanation, specific nomenclature is set forth to provide a thorough understanding of the present invention. However, it will be apparent to one skilled in the art that these specific details are not required to practice the invention. Descriptions of specific applications are provided only as representative examples. The present invention is not intended to be limited to the embodiments shown, but is to be accorded the widest possible scope consistent with the principles and features disclosed herein.

[0041] An aspect of the present application relates to novel peptides as set forth in Table 1. The terms “peptide,” “polypeptide” and “protein” are used interchangeably herein. In particular, the present invention provides for peptides comprising amino acid sequences at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or even 100% identical to the specified amino acid sequences.

[0042] In other embodiments, the present application provides for peptides that consist essentially of, or consist of an amino acid sequence at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or even 100% identical to the specified amino acid sequences in Table 1. In other embodiments, the present invention provides polypeptides with CDR sequences that differ from the CDR sequences of table 1 by 0, 1, 2, 3, 4, 5, or 6 amino acids.

[0043] In certain select embodiments of the present application, the peptides of the present application comprise, consist essentially of, or consist of an amino acid sequence that includes but is not limited to the amino acid sequences referred to in Table 1.

Table 1: sequence identifiers of selected embodiments of the present invention

Row	Antibody	Variable domain polynucleotide sequence		Variable domain amino acid sequence		Heavy Chain CDR			Light Chain CDR		
		Heavy Chain	Light Chain	Heavy Chain	Light Chain	CDR1	CDR2	CDR3	CDR1	CDR2	CDR3
1	rhMZ100	1	2	3	4	5	6	7	8	9	10
2	rhMZ101	11	12	13	14	15	16	17	18	19	20
3	rhMZ103	21	22	23	24	25	26	27	28	29	30
4	rhMZ104	31	32	33	34	35	36	37	38	39	40
5	rhMZ107	41	42	43	44	45	46	47	48	49	50
6	rhMZ119	51	52	53	54	55	56	57	58	59	60
7	rhMZ121	61	62	63	64	65	66	67	68	69	70
8	rhMZ123	71	72	73	74	75	76	77	78	79	80
9	rhMZ124	81	82	83	84	85	86	87	88	89	90
10	rhMZ133	91	92	93	94	95	96	97	98	99	100
11	rhMZ134	101	102	103	104	105	106	107	108	109	110
12	MZ4	111	112	113	114	115	116	117	118	119	120
13	MZ1	121	122	123	124	125	126	127	128	129	130
14	MZ24	131	132	133	134	135	136	137	138	139	140
15	MZ20	141	142	143	144	145	146	147	148	149	150
16	MZ54	151	152	153	154	155	156	157	158	159	160
17	MZ56	161	162	163	164	165	166	167	168	169	170
18	MZ22	171	172	173	174	175	176	177	178	179	180
19	MZ18	181	182	183	184	185	186	187	188	189	190
20	MZ19	191	192	193	194	195	196	197	198	199	200
21	MZ23	201	202	203	204	205	206	207	208	209	210
22	MZ2	211	212	213	214	215	216	217	218	219	220

[0044] As disclosed herein, the novel peptides of the present invention comprising amino acid sequences of SEQ ID NO: 5-10, 15-20, 25-30, 35-40, 45-50, 55-60, 65-70, 75-80, 85-90, 95-100, 105-110, 115-120, 125-130, 135-140, 145-150, 155-160, 165-170, 175-180, 185-190, 195-200 and 205-210 are each useful as a complementarity determining region (CDR) of an antibody or antibody fragment that binds to ZIKV. In one embodiment, the novel peptides with amino acid sequences of any one of SEQ ID NO: 5-10, 15-20, 25-30, 35-40, 45-50, 55-60, 65-70, 75-80, 85-90, 95-100, 105-110, 115-120, 125-130, 135-140, 145-150, 155-160, 165-170, 175-180, 185-190, 195-200 and 205-210 of the present invention are, alone, considered to be an antibody fragment that could be useful in binding ZIKV.

[0045] For example, any of residues X1-8 of SEQ ID NO: 5 can be present or absent and can be any single amino acid. In select embodiments of the present invention, residue X1 can be glycine (G), alanine (A), proline (P), isoleucine (I), leucine (L) or valine (V). In additional select embodiments of the present invention, residue X2 can be phenylalanine (F), histidine (H), tryptophan (W), or tyrosine (Y). In additional select embodiments of the present invention, residue X3 can be threonine (T), cysteine (C), serine (S), methionine (M), asparagine (N), or glutamine (Q). In additional select embodiments of the present invention, residue X4 can be phenylalanine (F), histidine (H), tryptophan (W), or tyrosine (Y). In additional select embodiments of the present invention, residue X5 can be serine (S), cysteine (C), threonine (T), methionine (M), asparagine (N), or glutamine (Q). In additional select embodiments of the present invention, residue X6 can be serine (S), cysteine (C), threonine (T), methionine (M), asparagine (N), or glutamine (Q). In additional select embodiments of the present invention, residue X7 can be aspartic acid (D), glutamic acid (E), lysine (K), or arginine (R). In additional select embodiments of the present invention, residue X8 can be glycine (G), alanine (A), proline (P), isoleucine (I), leucine (L) or valine (V).

[0046] For example, any of residues X1-16 of SEQ ID NO: 117 can be present or absent and can be any single amino acid. In select embodiments of the present invention, residue X1 can be cysteine (C), serine (S), threonine (T), methionine (M), asparagine (N), or glutamine (Q). In additional select embodiments of the present invention, residue X2 can be alanine (A), glycine (G), proline (P), isoleucine (I), leucine (L) or valine (V). In additional select embodiments of the present invention, residue X3 can be glycine (G), alanine (A), proline (P), isoleucine (I), leucine (L) or valine (V). In additional select embodiments of the present invention, residue X4 can be leucine (L), glycine (G), alanine (A), proline (P), isoleucine (I) or valine (V). In additional select embodiments of the present invention, residue X5 can be aspartic acid (D), glutamic acid (E), lysine (K), or arginine (R). In additional select embodiments of the present invention, residue X6 can be arginine (R), aspartic acid (D), glutamic acid (E) or lysine (K). In additional select embodiments of the present invention, residue X7 can be aspartic acid phenylalanine (F), histidine (H), tryptophan (W), or tyrosine (Y). In additional select embodiments of the present invention, residue X8 can be asparagine (N), serine (S), cysteine (C), threonine (T), methionine (M), or glutamine (Q). In additional select embodiments of the present invention, residue X9 can be tryptophan (W), histidine (H), phenylalanine (F), or tyrosine (Y). In additional select embodiments of the present invention, residue X10 can be asparagine (N), serine (S), cysteine (C), threonine (T), methionine (M), or glutamine (Q). In additional select embodiments of the present invention, residue X11 can be aspartic acid (D), glutamic acid (E), lysine (K), or arginine (R). In additional select embodiments of the present invention, residue

X12 can be glutamic acid (E), aspartic acid (D), lysine (K), or arginine (R). In additional select embodiments of the present invention, residue X13 can be glycine (G), alanine (A), proline (P), isoleucine (I), leucine (L) or valine (V). In additional select embodiments of the present invention, residue X14 can be aspartic acid (D), glutamic acid (E), lysine (K), or arginine (R). In additional select embodiments of the present invention, residue X15 can be cysteine (C), serine (S), threonine (T), methionine (M), asparagine (N), or glutamine (Q). In additional select embodiments of the present invention, residue X16 can be tryptophan (W), histidine (H), phenylalanine (F), or tyrosine (Y).

[0047] The novel peptides of the present invention can serve as at least one CDR of an antibody or antibody fragment that can bind to a specific epitope present on ZIKV. The antibodies of the present invention can be monoclonal or polyclonal. As used herein, the term “antibody” means an immunoglobulin molecule or a fragment of an immunoglobulin molecule having the ability to specifically bind to a particular antigen. Antibodies are well known to those of ordinary skill in the science of immunology. As used herein, the term antibody includes fragments of full-length antibodies that specifically bind one or more antigens. Such fragments are also well known in the art and are regularly employed both in vitro and in vivo. Examples of fragments of full length antibodies that are encompassed by the term antibody include but are not limited to F(ab')₂, Fab, Fv, Fd fragments, as well as scFv peptides and the like.

[0048] In addition to Fabs, smaller antibody fragments and epitope-binding peptides, including the novel peptides of the present invention, that have binding specificity for the epitopes defined by the Zika antibodies are also contemplated by the present invention and can also be used to bind or neutralize the virus. For example, single chain antibodies can be constructed according to the method of U.S. Patent No. 4,946,778, which is incorporated by reference. Single chain antibodies comprise the variable regions of the light and heavy chains joined by a flexible linker moiety. Another smaller antibody fragment that the invention provides is the antibody fragment known as the single domain antibody or Fd, which comprises an isolated variable heavy chain domain. Techniques for obtaining a single domain antibody with at least some of the binding specificity of the full-length antibody from which they are derived are known in the art.

[0049] Complementarity determining regions (CDRs) are peptide regions within the antigen-binding portion of an antibody. CDRs may directly interact with the epitope of the antigen and are the main determinant of antibody specificity. The framework regions (FRs) are peptide regions in the antigen-binding portion of the antibody that maintain the tertiary structure of the paratope. In some embodiments, in both the heavy chain variable region (V_H) and the light chain variable region (V_L), there

are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, may be largely responsible for antibody specificity.

[0050] In one specific embodiment, the novel peptides of the present invention serve as the CDR1 portion of the heavy chain of an antibody or antibody fragment. In another specific embodiment, the novel peptides of the present invention serve as the CDR2 portion of the heavy chain of an antibody or antibody fragment. In another specific embodiment, the novel peptides of the present invention serve as the CDR3 portion of the heavy chain of an antibody or antibody fragment. In another specific embodiment, the novel peptides of the present invention serve as the CDR1 portion of the light chain of an antibody or antibody fragment. In another specific embodiment, the novel peptides of the present invention serve as the CDR2 portion of the light chain of an antibody or antibody fragment. In another specific embodiment, the novel peptides of the present invention serve as the CDR3 portion of the light chain of an antibody or antibody fragment.

[0051] In one embodiment, any of the novel peptides described can serve as a heavy chain CDR3 for an antibody or antibody fragment, with the antibody or antibody fragment further comprising at least one additional heavy chain CDR. In a more specific embodiment, any of the novel peptides described can serve as a heavy chain CDR3 for an antibody or antibody fragment, and a peptide comprising an amino acid sequence disclosed in Table 1 can serve as an additional heavy chain CDR, for example either CDR1 or CDR2. In another embodiment, any of the novel peptides described can serve as a heavy chain CDR3 for an antibody or antibody fragment, with the antibody or antibody fragment further comprising at least two additional heavy chain CDRs. In another specific embodiment, any of the novel peptides described can serve as a heavy chain CDR3 for an antibody or antibody fragment, and peptides comprising an amino acid sequences disclosed in Table 1 can each serve as two additional heavy chain CDRs, for example CDR1 and CDR2, or vice versa.

[0052] In additional embodiments, any of the novel peptides described can serve as a heavy chain CDR3 for an antibody or antibody fragment, with the antibody or antibody fragment further comprising at least one light chain CDR, and a peptide comprising an amino acid sequence disclosed in Table 1 can serve as either light chain CDR1, CDR2 or CDR3. In another embodiment, any of the novel peptides described can serve as a heavy chain CDR3 for an antibody or antibody fragment, with the antibody or antibody fragment further comprising at least two additional light chain CDRs. In another specific embodiment, any of the novel peptides described can serve as a heavy chain CDR3 for an antibody or antibody fragment, and peptides comprising any of the amino acid sequences of Table 1 can serve as

two additional light chain CDRs, for example light chain CDR1, CDR2 or CDR3. In particular, a peptide with the amino acid sequence of SEQ ID NO: 208 can serve as the light chain CDR1 and a peptide with an amino acid sequence of SEQ ID NO: 209 or SEQ ID NO: 210 can interchangeably serve as the light chain CDR2 or CDR3.

[0053] In another specific embodiment, any of the novel peptides described can serve as a heavy chain CDR3 for an antibody or antibody fragment, with the antibody or antibody fragment further comprising at least three additional light chain CDRs. In another specific embodiment, any of the novel peptides described can serve as a heavy chain CDR3 for an antibody or antibody fragment, and peptides comprising the amino acid sequences of SEQ ID NO: 8, SEQ ID NO: 9 or SEQ ID NO: 10 can serve as three additional light chain CDRs, for example light chain CDR1, CDR2 and CDR3. In particular, a peptide with the amino acid sequence of SEQ ID NO: 8 can serve as the light chain CDR1 and a peptide with an amino acid sequence of SEQ ID NO: 9 can serve as the light chain CDR2 and a peptide with an amino acid sequence of SEQ ID NO: 10 can serve as the light chain CDR3.

[0054] In additional embodiments, any of the novel peptides described can serve as a heavy chain CDR3 for an antibody or antibody fragment, with the antibody or antibody fragment further comprising at least one, two, three, four or five additional CDRs. In specific embodiments, any of the novel peptides described can serve as a heavy chain CDR for an antibody or antibody fragment, with the antibody or antibody fragment further comprising at least two additional CDRs. In another specific embodiment, any of the novel peptides described can serve as a heavy chain CDR for an antibody or antibody fragment, and peptides comprising the amino acid sequences of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10 can serve as at least one, two, three, four or five additional CDR(s). In particular, any of the novel peptides described can serve as a heavy chain CDR for an antibody or antibody fragment, and a peptide comprising the amino acid sequences of SEQ ID NO: 5 can serve as a heavy chain CDR1, a peptide comprising the amino acid sequence of SEQ ID NO: 6 can serve as a heavy chain CDR2, a peptide comprising the amino acid sequence of SEQ ID NO: 7 can serve as a heavy chain CDR3, a peptide with the amino acid sequence of SEQ ID NO: 8 can serve as a light chain CDR1, a peptide with an amino acid sequence of SEQ ID NO: 9 can serve as a light chain CDR2, and/or a peptide with an amino acid sequence of SEQ ID NO: 10 can serve as a light chain CDR3.

[0055] Any of the series of antibodies or antibody fragments in Table 1 above may or may not include one or more framework regions as well.

[0056] In specific embodiments, the antibodies or antibody fragments of the present invention comprise at least one CDR, wherein the amino acid sequence of the CDR comprises, consists essentially

of or consist of an amino acid sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or even 100% identical to the amino acid sequence of SEQ ID NO: 5-10, 15-20, 25-30, 35-40, 45-50, 55-60, 65-70, 75-80, 85-90, or 95-100. In more specific embodiments, the antibodies or antibody fragments comprise, consist essentially of or consist of at least two CDRs.

[0057] In particular, the present invention provides antibodies or antibody fragments that bind to cross-protomer epitopes on ZIKV. The antibodies may be monoclonal or polyclonal. The primary amino acid structure and the secondary and tertiary structures of the E glycoprotein of the ZIKV are well known.

[0058] A polypeptide having an amino acid sequence at least, for example, about 95% “identical” to a reference amino acid sequence, e.g., SEQ ID NO: 1, is understood to mean that the amino acid sequence of the polypeptide is identical to the reference sequence except that the amino acid sequence may include up to about five modifications per each 100 amino acids of the reference amino acid sequence. In other words, to obtain a peptide having an amino acid sequence at least about 95% identical to a reference amino acid sequence, up to about 5% of the amino acid residues of the reference sequence may be deleted or substituted with another amino acid or a number of amino acids up to about 5% of the total amino acids in the reference sequence may be inserted into the reference sequence. These modifications of the reference sequence may occur at the N- terminus or C-terminus positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among amino acids in the reference sequence or in one or more contiguous groups within the reference sequence.

[0059] As used herein, “identity” is a measure of the identity of nucleotide sequences or amino acid sequences compared to a reference nucleotide or amino acid sequence. In general, the sequences are aligned so that the highest order match is obtained. “Identity” per se has an art-recognized meaning and can be calculated using well known techniques. While there are several methods to measure identity between two polynucleotide or polypeptide sequences, the term “identity” is well known to skilled artisans (Carillo (1988) J. Applied Math. 48, 1073). Examples of computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux (1984) Nucleic Acids Research 12, 387), BLASTP, ExPASy, BLASTN, FASTA (Atschul (1990) J. Mol. Biol. 215, 403) and FASTDB. Examples of methods to determine identity and similarity are discussed in Michaels (2011) Current Protocols in Protein Science, Vol. 1, John Wiley & Sons.

[0060] In one embodiment of the present invention, the algorithm used to determine identity between two or more polypeptides is BLASTP. In another embodiment of the present invention, the algorithm used to determine identity between two or more polypeptides is FASTDB, which is based upon the algorithm of Brutlag (1990) *Comp. App. Biosci.* 6, 237-245). In a FASTDB sequence alignment, the query and reference sequences are amino sequences. The result of sequence alignment is in percent identity. In one embodiment, parameters that may be used in a FASTDB alignment of amino acid sequences to calculate percent identity include, but are not limited to: Matrix=PAM, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject amino sequence, whichever is shorter.

[0061] If the reference sequence is shorter or longer than the query sequence because of N-terminus or C-terminus additions or deletions, but not because of internal additions or deletions, a manual correction can be made, because the FASTDB program does not account for N-terminus and C-terminus truncations or additions of the reference sequence when calculating percent identity. For query sequences truncated at the N- or C- termini, relative to the reference sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C- terminus to the reference sequence that are not matched/aligned, as a percent of the total bases of the query sequence. The results of the FASTDB sequence alignment determine matching/alignment. The alignment percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score can be used for the purposes of determining how alignments “correspond” to each other, as well as percentage identity. Residues of the reference sequence that extend past the N- or C-termini of the query sequence may be considered for the purposes of manually adjusting the percent identity score. That is, residues that are not matched/aligned with the N- or C-termini of the comparison sequence may be counted when manually adjusting the percent identity score or alignment numbering.

[0062] For example, a 90 amino acid residue query sequence is aligned with a 100 residue reference sequence to determine percent identity. The deletion occurs at the N-terminus of the query sequence and therefore, the FASTDB alignment does not show a match/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the reference sequence (number of residues at the N- and C-termini not matched/total number of residues in the reference sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched (100% alignment) the final percent identity would be 90% (100% alignment – 10% unmatched overhang). In another example, a 90 residue query sequence is compared with a 100

reference sequence, except that the deletions are internal deletions. In this case the percent identity calculated by FASTDB is not manually corrected, since there are no residues at the N- or C-termini of the subject sequence that are not matched/aligned with the query. In still another example, a 110 amino acid query sequence is aligned with a 100 residue reference sequence to determine percent identity. The addition in the query sequence occurs at the N-terminus of the query sequence and therefore, the FASTDB alignment may not show a match/alignment of the first 10 residues at the N-terminus. If the remaining 100 amino acid residues of the query sequence have 95% identity to the entire length of the reference sequence, the N-terminal addition of the query would be ignored and the percent identity of the query to the reference sequence would be 95%.

[0063] As used herein, the terms “corresponds to” and “corresponding to” as they relate to sequence alignment, are intended to mean enumerated positions within the reference protein and those positions in the modified peptide that align with the positions on the reference protein. Thus, when the amino acid sequence of a subject or query peptide is aligned with the amino acid sequence of a reference peptide, e.g., SEQ ID NO: 3, the amino acids in the subject sequence that “correspond to” certain enumerated positions of the reference sequence are those that align with these positions of the reference sequence, e.g., SEQ ID NO: 3, but are not necessarily in these exact numerical positions of the reference sequence. Methods for aligning sequences for determining corresponding amino acids between sequences are described herein. Accordingly, the invention provides novel peptides whose sequences correspond to the sequence of SEQ ID NO: 3.

[0064] Variants resulting from insertion of a polynucleotide encoding the novel peptides into an expression vector system are also contemplated. For example, variants (usually insertions) may arise from when the amino terminus and/or the carboxy terminus of a novel peptide is/are fused to another polypeptide.

[0065] In another aspect, the invention provides deletion variants wherein one or more amino acid residues in the novel peptides are removed. Deletions can be effected at one or both termini of the peptides, or with removal of one or more non-terminal amino acid residues.

[0066] Within the confines of the disclosed percent identities, the invention also relates to substitution variants of disclosed peptides of the invention. Substitution variants include those polypeptides wherein one or more amino acid residues of an amino acid sequence are removed and replaced with alternative residues. Knowledge of the three-dimensional structure of an antibody, as disclosed herein, as well as the structures of the Zika virus, the Zika virus E glycoprotein, and the Zika virus E glycoprotein in complex with monoclonal antibodies (Dai, Cell Host and Microbe 19(5) 2016, pp 696-704; Sirohi, Science

352(6284) 2016 pp 467-470; Zhau, Cell 166(4) 2-16, pp 1016-1027) provides guidance regarding which positions within the amino acid sequences of the antibodies disclosed herein can be substituted without loss of binding or neutralization activity.

[0067] In one aspect, the substitutions are conservative in nature; however, the invention embraces substitutions that are also non-conservative. Conservative substitutions for the purposes of the present invention may be defined as set out in the tables below. Amino acids can be classified according to physical properties and contribution to secondary and tertiary protein structure. A conservative substitution is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are set out in Table 2.

Table 2: Conservative Substitutions

Side Chain Characteristic	Amino Acid
Aliphatic	
Non-polar	Gly, Ala, Pro, Iso, Leu, Val
Polar-uncharged	Cys, Ser, Thr, Met, Asn, Gln
Polar-charged	Asp, Glu, Lys, Arg
Aromatic	His, Phe, Trp, Tyr
Other	Asn, Gln, Asp, Glu

[0068] Alternatively, conservative amino acids can be grouped as described in Lehninger (1975) Biochemistry, Second Edition; Worth Publishers, pp. 71-77, as set forth below.

Table 3: Conservative Substitutions

Side Chain Characteristic	Amino Acid
Non-polar (hydrophobic)	
Aliphatic:	Ala, Leu, Iso, Val, Pro
Aromatic:	Phe, Trp
Sulfur-containing:	Met
Borderline:	Gly
Uncharged-polar	
Hydroxyl:	Ser, Thr, Tyr
Amides:	Asn, Gln
Sulfhydryl:	Cys
Borderline:	Gly
Positively Charged (Basic):	Lys, Arg, His
Negatively Charged (Acidic)	Asp, Glu

[0069] And still other alternative, exemplary conservative substitutions are set out below.

Table 4: Conservative Substitutions

Original Residue	Exemplary Substitution
Ala (A)	Val, Leu, Ile
Arg (R)	Lys, Gln, Asn
Asn (N)	Gln, His, Lys, Arg
Asp (D)	Glu
Cys (C)	Ser
Gln (Q)	Asn
Glu (E)	Asp
His (H)	Asn, Gln, Lys, Arg
Ile (I)	Leu, Val, Met, Ala, Phe
Leu (L)	Ile, Val, Met, Ala, Phe
Lys (K)	Arg, Gln, Asn
Met (M)	Leu, Phe, Ile
Phe (F)	Leu, Val, Ile, Ala
Pro (P)	Gly
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser
Val (V)	Ile, Leu, Met, Phe, Ala

[0070] It is now well-established in the art that the non-CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of “humanized” antibodies in which non-human CDRs are covalently joined to human framing regions (FRs) and/or Fc/pFc’ regions to produce a functional antibody or antibody fragment. For example, PCT International Publication Number WO 92/04381 teaches the production and use of humanized murine RSV antibodies in which at least a portion of the murine FR regions have been replaced by FR regions of human origin. It is also possible, in accordance with the present invention, to produce chimeric antibodies including non-human sequences. For example, murine, ovine, equine, bovine, non-human primate or other mammalian Fc or FR sequences can be used to replace some or all of the Fc or FR regions of Zika antibodies.

[0071] The present invention also provides for F(ab’)₂, Fab, Fv and Fd fragments of Zika antibodies, as well as chimeric antibodies or antibody fragments in which the Fc and/or FR and/or, CDR1 and/or CDR2 and/or CDR3 light chain or heavy chain regions of the Zika monoclonal have been replaced by homologous human or non-human sequences. For example, the invention provides chimeric Fab and/or

F(ab')₂ fragments in which the FR and/or CDR1 and/or CDR2 and/or CDR3 light chain or heavy chain regions of the Zika antibodies have been replaced by homologous human or non-human sequences. The invention also provides for chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or CDR3 heavy chain regions have been replaced by homologous human or non-human sequences. Such CDR grafted or chimeric antibodies or antibody fragments can be effective in prevention and treatment of ZIKV infection.

[0072] In select embodiments, the chimeric antibodies or antibody fragments of the invention are fully human monoclonal antibodies including at least the novel peptides of the present invention, which can be used as heavy chain CDR3 regions in the antibodies or antibody fragments. As noted above, such chimeric antibodies may be produced in which some or all of the FR regions of the Zika antibodies or antibody fragments have been replaced by other homologous human FR regions. In addition, the Fc portions may be replaced so as to produce IgA or IgM as well as IgG antibodies bearing some or all of the CDRs of the Zika antibodies or antibody fragments. In select embodiments, administration of the antibodies, antibody fragments, chimeric antibodies or chimeric antibody fragments will not evoke an immune response.

[0073] It is possible to determine, without undue experimentation, if any of the antibodies or antibody fragments described herein have specificity towards at least a portion of the ZIKV using standard techniques well known to one of skill in the art. For example, the antibody or antibody fragment can be tested for its ability to compete with known ZIKV antibodies to bind to ZIKV, e.g., as demonstrated by a decrease in binding of the known ZIKV antibodies. Screening of ZIKV antibodies or antibody fragments can also be carried out by utilizing ZIKV and determining whether the test antibodies or antibody fragments neutralize the virus.

[0074] By using the antibodies or antibody fragments of the invention, it is also possible to produce anti-idiotypic antibodies which can be used to screen other antibodies to identify whether the antibody has the same binding specificity as an antibody of the invention. In addition, such anti-idiotypic antibodies can be used for active immunization (Herlyn, 1986 Science 232:100-102). Such anti-idiotypic antibodies can be produced using well-known hybridoma techniques (Kohler, 1975 Nature 256:495-497). An anti-idiotypic antibody is an antibody which recognizes unique determinants present on an antibody produced by the cell line of interest. These determinants are located in the hypervariable region of the antibody. It is this region which binds to a given epitope and, thus, is responsible for the specificity of the antibody. An anti-idiotypic antibody can be prepared by immunizing an animal with the monoclonal antibody of interest. The immunized animal will recognize and respond to the idiotype

determinants of the immunizing antibody and produce an antibody to these idiotypic determinants. By using the anti-idiotypic antibodies of the immunized animal, which are specific for the monoclonal antibodies of the invention, it is possible to identify other clones with the same idio type as the antibody of the hybridoma used for immunization. Idiotypic identity between monoclonal antibodies of two cell lines demonstrates that the two monoclonal antibodies are the same with respect to their recognition of the same epitopic determinant. Thus, by using anti-idiotypic antibodies, it is possible to identify other hybridomas expressing monoclonal antibodies having the same epitopic specificity.

[0075] The present invention also provides nucleic acids encoding the novel peptides of the present invention as well as proteins and peptides comprising the novel peptides of the present invention. Such nucleic acids may or may not be operably joined to other nucleic acids forming a recombinant vector for cloning or for expression of the peptides of the present invention. The present invention thus includes any recombinant vector containing coding sequences of the novel peptides of the present invention, or part thereof, whether for prokaryotic or eukaryotic transformation, transfection or gene therapy. Such vectors may be prepared using conventional molecular biology techniques, known to those with skill in the art. Recombinant techniques would include but are not limited to utilizing DNA coding sequences for the immunoglobulin V-regions of the flavivirus antibodies or antibody fragments, including framework and CDRs or parts thereof, and a suitable promoter either with (Whittle 1987 Protein Eng 1:499-505 and Burton 1994 Science 266:1024-1027) or without (Marasco, 1993. Proc Natl Acad Sci USA 90:7889-7893 and Duan, 1994 Proc Natl Acad Sci USA 91:5075-5079) a signal sequence for export or secretion. Such vectors may be transformed or transfected into prokaryotic (Huse, 1989 Science 246:1275-1281; Ward, 1989 Nature 341:544-546; Marks, 1991 J Mol Biol 222:581-597; and Barbas, 1991 Proc Natl Acad Sci USA 88:7978-7982) or eukaryotic (Whittle, 1987 Protein Eng 1:499-505 and Burton, 1994 Science 266:1024-1027) cells or used for gene therapy (Marasco, 1993 Proc Natl Acad Sci USA 90:7889-7893 and Duan, 1994 Proc Natl Acad Sci USA 91:5075-5079) by conventional techniques, known to those with skill in the art.

[0076] As used herein, a “vector” may be any of a number of nucleic acids into which a desired sequence may be inserted by restriction and ligation for transport between different genetic environments or for expression in a host cell. Vectors are typically composed of DNA although RNA vectors are also available. Vectors include, but are not limited to, plasmids and phagemids. A cloning vector is one which is able to replicate in a host cell, and which is further characterized by one or more endonuclease restriction sites at which the vector may be cut in a determinable fashion and into which a desired DNA sequence may be ligated such that the new recombinant vector retains its ability to

replicate in the host cell. In the case of plasmids, replication of the desired sequence may occur many times as the plasmid increases in copy number within the host bacterium or just a single time per host before the host reproduces by mitosis. In the case of phage, replication may occur actively during a lytic phase or passively during a lysogenic phase. An expression vector is one into which a desired DNA sequence may be inserted by restriction and ligation such that it is operably joined to regulatory sequences and may be expressed as an RNA transcript. Vectors may further contain one or more marker sequences suitable for use in the identification and selection of cells which have been transformed or transfected with the vector. Markers include, for example, genes encoding proteins which increase or decrease either resistance or sensitivity to antibiotics or other compounds, genes which encode enzymes whose activities are detectable by standard assays known in the art, e.g., β -galactosidase or alkaline phosphatase, and genes which visibly affect the phenotype of transformed or transfected cells, hosts, colonies or plaques. Some vectors that may be utilized include but are not limited to vectors that are capable of autonomous replication and expression of the structural gene products present in the DNA segments to which they are operably joined.

[0077] As used herein, a coding sequence and regulatory sequences are said to be “operably joined” or “operably connected” when they are covalently linked in such a way as to place the expression or transcription of the coding sequence under the influence or control of the regulatory sequences. If it is desired that the coding sequences be translated into a functional protein, two DNA sequences are said to be operably joined if induction of a promoter in the 5' regulatory sequences results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a promoter region would be operably joined to a coding sequence if the promoter region were capable of effecting transcription of that DNA sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

[0078] The precise nature of the regulatory sequences needed for gene expression may vary between species or cell types, but in general include but are not limited to 5' non-transcribing and 5' non-translating sequences involved with initiation of transcription and translation respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. In particular, a 5' non-transcribing regulatory sequence may include a promoter region which includes a promoter sequence for transcriptional

control of the operably joined coding sequence. Regulatory sequences may also include enhancer sequences or upstream activator sequences, as desired.

[0079] The vectors of the present invention may or may not be expression vectors. Expression vectors include regulatory sequences operably joined to a nucleotide sequence encoding one of the novel peptides, antibodies or antibody fragments of the invention. As used herein, the term “regulatory sequences” means nucleotide sequences necessary for or conducive to the transcription of a nucleotide sequence encoding a desired peptide and/or which are necessary for or conducive to the translation of the resulting transcript into the desired peptide. Regulatory sequences include, but are not limited to, 5' sequences such as operators, promoters and ribosome binding sequences, and 3' sequences such as polyadenylation signals. The vectors of the invention may optionally include 5' leader or signal sequences, 5' or 3' sequences encoding fusion products to aid in protein purification, and various markers which aid in the identification or selection of transformants. The choice and design of an appropriate vector is within the ability and discretion of one of ordinary skill in the art. The subsequent purification of the antibodies may be accomplished by any of a variety of standard means known in the art.

[0080] The present invention also provides for host cells, both prokaryotic and eukaryotic comprising at least one nucleic acid encoding the novel peptides of the present invention, including but not limited to the vectors of the present invention.

[0081] In one embodiment using a prokaryotic expression host, the vector utilized includes a prokaryotic origin of replication or replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extrachromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such origins of replication are well known in the art.

[0082] One method of achieving high levels of gene expression in *E. coli* includes but is not limited to the use of strong promoters to generate large quantities of mRNA and also ribosome binding sites to ensure that the mRNA is efficiently translated. For example, ribosome binding sites in *E. coli* include an initiation codon (AUG) and a sequence 3-9 nucleotides long located 3-11 nucleotides upstream from the initiation codon (Shine 1975 Nature 254:34-38). The sequence, which is called the Shine-Dalgarno (SD) sequence, is complementary to the 3' end of *E. coli* 16S rRNA. Binding of the ribosome to mRNA and the sequence at the 3' end of the mRNA can be affected by several factors: the degree of complementarity between the SD sequence and 3' end of the 16S rRNA, the spacing lying between the SD sequence and the AUG and even the nucleotide sequence following the AUG, which affects ribosome binding. The 3'

regulatory sequences may or may not define at least one termination (stop) codon in frame with and operably joined to the heterologous fusion polypeptide.

[0083] In addition, those embodiments that include a prokaryotic replicon may or may not include a gene whose expression confers a selective advantage, such as drug resistance, to a bacterial host transformed therewith. Typical bacterial drug resistance genes are those that confer resistance to ampicillin, tetracycline, neomycin/kanamycin or chloramphenicol. Vectors typically also contain convenient restriction sites for insertion of translatable DNA sequences. Exemplary vectors are the plasmids pUC18 and pUC19 and derived vectors such as those that are commercially available.

[0084] The antibodies or antibody fragments of the present invention may additionally, of course, be produced by eukaryotic cells such as CHO cells, human or mouse hybridomas, immortalized B-lymphoblastoid cells, and the like. In this case, a vector is constructed in which eukaryotic regulatory sequences are operably joined to the nucleotide sequences encoding one or more peptides of the present invention. The design and selection of an appropriate eukaryotic vector is within the ability and discretion of one of ordinary skill in the art. The subsequent purification of the antibodies may be accomplished by any of a variety of standard means known in the art.

[0085] The antibodies or antibody fragments of the present invention may furthermore, of course, be produced in plants. In 1989, Hiatt et al. (Nature 342:76-78 (1989)) first demonstrated that functional antibodies could be produced in transgenic plants. Since then, a considerable amount of effort has been invested in developing plants for antibody (or "plantibody") production (for reviews see Giddings, 2000 Nat. Biotechnol., 18:1151-1155; Fischer, 2000 Transgenic Res., 9:279-299).

[0086] One vector useful for screening monoclonal antibodies is a recombinant DNA molecule containing a nucleotide sequence that codes for and is capable of expressing a fusion polypeptide containing, in the direction of amino- to carboxy-terminus, (1) a prokaryotic secretion signal domain, (2) a peptide of the invention, and, optionally, (3) a fusion protein domain. The vector includes DNA regulatory sequences for expressing the fusion polypeptide, for example prokaryotic regulatory sequences. Such vectors can be constructed by those of ordinary skill in the art and have been described by Smith, 1985 Science 228:1315-1317; Clackson, 1991 Nature 352:624-628; Batbas 1991 Proc Natl Acad Sci USA 88:7978-7982; Roberts, 1992 Proc Natl Acad Sci USA 89:2429-2433.

[0087] A fusion polypeptide may be useful for purification of the antibodies of the invention. The fusion domain may, for example, include a His tag that allows for purification of the peptide, or a maltose binding protein of the commercially available vector pMAL (New England BioLabs). A fusion domain that

may be useful is a filamentous phage membrane anchor that is particularly useful for screening phage display libraries of monoclonal antibodies.

[0088] A secretion signal is a leader peptide domain of a protein that targets the protein to a region, such as the plasma membrane, of the host cell. For example, one secretion signal is the E. coli pelB secretion signal. The leader sequence of the pelB protein has previously been used as a secretion signal for fusion proteins (Better, 1988 Science 240:1041-1043; Sastry, 1989 Proc Natl Acad Sci USA 86:5728-5732; and Mullinax, 1990 Proc Natl Acad Sci USA 87:8095-8099. Amino acid residue sequences for other secretion signal polypeptide domains from E. coli useful in this invention can be found in Neidhard, (ed.), 1987 in *Escherichia coli and Salmonella Typhimurium: Typhimurium Cellular and Molecular Biology*, American Society for Microbiology.

[0089] When the antibodies or antibody fragments of the invention include heavy chain and light chain sequences, these sequences may be encoded on separate vectors or, more conveniently, may be expressed by a single vector. The heavy and light chain may, after translation or after secretion, form the heterodimeric structure of natural antibody molecules. Such a heterodimeric antibody may or may not be stabilized by disulfide bonds between the heavy and light chains.

[0090] A vector for expression of heterodimeric antibodies, such as full-length antibodies or antibody fragments of the invention, is a recombinant DNA molecule adapted for receiving and expressing translatable first and second DNA sequences. That is, a DNA expression vector for expressing a heterodimeric antibody or antibody fragment provides a system for independently cloning (inserting) two or more translatable DNA sequences into two or more separate cassettes present in the vector, to form two or more separate cistrons for expressing the first and second polypeptides of a heterodimeric antibody or antibody fragment. The DNA expression vector for expressing two cistrons is referred to as a dicistronic expression vector.

[0091] In general, a dicistronic expression vector comprises a first cassette that includes upstream and downstream DNA regulatory sequences operably joined via a sequence of nucleotides adapted for directional ligation to an insert DNA. The upstream translatable sequence may encode the secretion signal as described above. The cassette also may include DNA regulatory sequences for expressing the first peptide that is produced when an insert translatable DNA sequence (insert DNA) is directionally inserted into the cassette via the sequence of nucleotides adapted for directional ligation.

[0092] The dicistronic expression vector may also contain a second cassette for expressing the second peptide. The second cassette may also include a second translatable DNA sequence that encodes a secretion signal, as described above, that may be operably joined at its 3' terminus via a sequence of

nucleotides adapted for directional ligation to a downstream DNA sequence of the vector that typically defines at least one stop codon in the reading frame of the cassette. The second translatable DNA sequence can be operably joined at its 5' terminus to DNA regulatory sequences forming the 5' elements. Upon insertion of a translatable DNA sequence (insert DNA), the second cassette is capable of expressing the second fusion polypeptide comprising a secretion signal with a polypeptide coded by the insert DNA.

[0093] The invention also provides for methods of making any of the novel, inventive peptides of the present invention. In certain embodiments, the methods of making the novel peptides of the present invention include making antibodies or antibody fragments that comprise at least one novel peptide of the present invention. The methods of making the novel peptides, or making antibodies or antibody fragments comprising the novel peptides, include but are not limited to culturing the novel, inventive host cells of the present invention under conditions suitable for protein expression and isolating the peptides from culture. The host cells used in the methods of making peptides of the present invention may or may not include nucleic acids that encode antibodies or antibody fragments comprising the novel peptides of the present invention. The produced peptides or produced antibodies or antibody fragments may or may not be substantially pure.

[0094] As used herein with respect to polypeptides, the term "substantially pure" is used to mean that the polypeptides are essentially free of other substances with which they may be found in nature or in vivo systems to an extent practical and appropriate for their intended use. In particular, the polypeptides are sufficiently pure and are sufficiently free from other biological constituents of their host cells so as to be useful in, for example, generating antibodies, sequencing, or producing pharmaceutical preparations. By techniques well known in the art, substantially pure polypeptides may be produced in light of the nucleic acid and amino acid sequences disclosed herein. Because a substantially purified polypeptide of the invention may be admixed with a pharmaceutically acceptable carrier in a pharmaceutical preparation, the polypeptide may comprise only a certain percentage by weight of the preparation. The polypeptide is nonetheless substantially pure in that it has been substantially separated from the substances with which it may be associated in living systems.

[0095] As used herein with respect to nucleic acids, the term "isolated" means: (i) amplified in vitro by, for example, polymerase chain reaction (PCR); (ii) recombinantly produced by cloning; (iii) purified, as by cleavage and gel separation; or (iv) synthesized by, for example, chemical synthesis. An isolated nucleic acid is one which is readily manipulable by recombinant DNA techniques well known in the art. Thus, a nucleotide sequence contained in a vector in which 5' and 3' restriction sites are known or for which

polymerase chain reaction (PCR) primer sequences have been disclosed is considered isolated but a nucleic acid sequence existing in its native state in its natural host is not. An isolated nucleic acid may be substantially purified, but need not be. For example, a nucleic acid that is isolated within a cloning or expression vector is not pure in that it may comprise only a tiny percentage of the material in the cell in which it resides. Such a nucleic acid is isolated, however, as the term is used herein because it is readily manipulable by standard techniques known to those of ordinary skill in the art.

[0096] Methods of culturing host cells to produce proteins, including antibodies or antibody fragments comprising the novel peptides of the present invention, are well known in the art and such methods need not be repeated herein. One of skill in the art will readily recognize that the culture conditions necessary for protein production depend upon, among other things, the type of host cell being cultured, the nature of the protein or peptide being produced and the quantity desired.

[0097] The invention also provides methods for preparing diagnostic or pharmaceutical compositions comprising the peptides of the present invention, which may or may not be part of an antibody or antibody fragment. The invention also provides methods for preparing diagnostic or pharmaceutical compositions comprising the novel nucleic acid sequences encoding the novel peptides of the invention or part thereof. The pharmaceutical compositions of the present invention can be used for treating symptoms of ZIKV Disease in a subject in need thereof, or can be used for treating Zika Disease itself in a subject in need thereof.

[0098] Accordingly, the present invention provides methods of treating a subject with a ZIKV infection comprising administering a therapeutically effective amount of at least one peptide of the present invention to a subject in need thereof. In a more specific embodiment, the invention provides for methods of treating a subject with a ZIKV infection comprising administering a therapeutically effective amount at least one antibody or antibody fragment, wherein the antibody or antibody fragment comprises, consists essentially of or consists of at least one novel peptide of the present invention to a subject in need thereof.

[0099] As used herein, a “therapeutically effective amount” of the peptides, antibodies or antibody fragments of the invention is a dosage large enough to produce the desired effect in which the symptoms of Zika Disease are ameliorated or the likelihood of infection is decreased. A therapeutically effective amount is generally not a dose so large as to cause adverse side effects, such as but not limited to hyperviscosity syndromes, pulmonary edema, congestive heart failure, and the like. Generally, a therapeutically effective amount may vary with the subject’s age, condition, and sex, as well as the extent of the disease in the subject and can be determined by one of skill in the art. The dosage of the

therapeutically effective amount may be adjusted by the individual physician or veterinarian in the event of any complication. A therapeutically effective amount may vary from about 0.01 mg/kg to about 50 mg/kg, specifically from about 0.1 mg/kg to about 20 mg/kg, more specifically from about 0.2 mg/kg to about 2 mg/kg. The peptides, antibodies or antibody fragments may be administered once or more than once in a single day or over a period of days. When administered to a pregnant female, a therapeutically effective amount of the peptides, antibodies or antibody fragments of the invention prevents transmission, or reduces the extent of transmission, from an infected mother to her unborn child.

[00100] The present invention also provides prophylactic methods as well. Indeed, the present invention provides methods of preventing or reducing the likelihood of acquiring a ZIKV infection and preventing or reducing the likelihood of acquiring a disease or condition associated with ZIKV infection. The prevention methods comprise administering a prophylactically effective amount of at least one peptide of the present invention to a subject. In a more specific embodiment, the invention provides for methods of reducing the likelihood of acquiring a condition or disease associated with ZIKV infection comprising administering a prophylactically effective amount of at least one antibody or antibody fragment, wherein the antibody or antibody fragment comprises, consists essentially of or consists of at least one novel peptide of the present invention to a subject. The subject on which the prevention or prophylactic methods are practiced may or may not be a higher risk of acquiring a condition or disease associated with ZIKV infection than another subject from a different population.

[00101] As used herein, a “prophylactically effective amount” of the peptides, antibodies or antibody fragments of the invention is a dosage large enough to produce the desired effect in the protection of individuals against flavivirus infection for a reasonable period of time, such as one to two months or longer following administration. Generally, a prophylactically effective amount may vary with the subject’s age, condition, and sex, as well as the extent of the disease in the subject and can be determined by one of skill in the art. The dosage of the prophylactically effective amount may be adjusted by the individual physician or veterinarian in the event of any complication. A prophylactically effective amount may vary from about 0.01 mg/kg to about 50 mg/kg, specifically from about 0.1 mg/kg to about 20 mg/kg, more specifically from about 0.2 mg/kg to about 2 mg/kg, in one or more administrations (priming and boosting). When administered to a pregnant female, a prophylactically effective amount of the peptides, antibodies or antibody fragments of the invention prevents infection, or reduces the severity of infection, of the mother and her unborn child.

[00102] The treatment and prevention methods herein may or may not include screening a subject to determine if the subject has been infected with ZIKV or is at risk of being infected with ZIKV.

[00103] As used herein, “administer” or variations thereof is used to mean bringing the one or more novel peptides into proximity with a cell or group of cells, including cells comprised within a living, whole organism, such that the one or more novel peptides can exert a biological effect on the cells. Of course, “administering” the novel peptides of the present invention can be achieved by administering an antibody or antibody fragment comprising one or more novel peptides to a subject in need thereof. Thus, in one embodiment of the present invention, “administer” can mean a stable or transient transfection of DNA or RNA molecule(s) into cells, where the cells may or may not be part of a living, whole organism. In another embodiment, the peptides or antibodies or antibody fragments comprising the novel peptides can be administered repeatedly to the subject.

[00104] As used herein, the term “Zika Virus Disease” refers to diseases or conditions caused, directly or indirectly, by infection from ZIKV. Symptoms of Zika Virus Disease include congenital and neurological complications such as microcephaly and Guillain-Barré syndrome.

[00105] The pharmaceutical preparation includes a pharmaceutically acceptable carrier. Such carriers, as used herein, means a material that does not interfere with the effectiveness of the biological activity of the active ingredients. The term “physiologically acceptable” refers to a material that is compatible with a biological system such as a cell, cell culture, tissue, or organism. The characteristics of the carrier will depend on the route of administration. Physiologically and pharmaceutically acceptable carriers include diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials which are well known in the art.

[00106] The peptides, antibodies or antibody fragments of the invention can be administered by injection or by gradual infusion over time. The administration of the peptides, antibodies or antibody fragments of the invention may, for example, be intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, or transdermal. Techniques for preparing injectate or infusate delivery systems containing antibodies are well known to those of skill in the art. Generally, such systems should utilize components which will not significantly impair the biological properties of the peptides, antibodies or antibody fragments such as the paratope binding capacity (see, for example, Remington’s Pharmaceutical Sciences, 2017, Mack Publishing). Those of skill in the art can readily determine the various parameters and conditions for producing injectates or infusates without resort to undue experimentation.

[00107] For example, preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents include but are not limited to propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include but are not limited to water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include but are not limited to sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include but are not limited to fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and the like.

[00108] The peptides, antibodies or antibody fragments of the invention are suited for in vitro use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the peptides, antibodies or antibody fragments in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize the peptides, antibodies or antibody fragments of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of antigens using the monoclonal antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

[00109] The anti-Zika peptides, antibodies or antibody fragments of the invention may be labeled by a variety of means for use in diagnostic and/or pharmaceutical applications. There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include but are not limited to enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds and bioluminescent compounds. One of ordinary skill in the art will readily be able to determine suitable labels for binding to the peptides, antibodies or antibody fragments of the invention. Furthermore, the binding of these labels to the peptides, antibodies or antibody fragments of the invention can be done using standard techniques common to those of ordinary skill in the art.

[00110] Another labeling technique which may result in greater sensitivity consists of coupling the peptides, antibodies or antibody fragments to low molecular weight haptens. These haptens can then be specifically altered by means of a second reaction. For example, it is common to use haptens such as

biotin, which reacts with avidin, or dinitrophenol, pyridoxal, or fluorescein, which can react with specific anti-hapten antibodies.

[00111] The peptides, antibodies or antibody fragments of the invention can be bound to many different carriers and used to detect the presence of flavivirus. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylase, natural and modified cellulose, polyacrylamide, agarose and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding peptides, antibodies or antibody fragments, or will be able to ascertain such, using routine experimentation.

[00112] For purposes of the invention, ZIKV may be detected by the peptides, antibodies or antibody fragments of the invention when present in biological fluids and tissues. Any sample containing a detectable amount of ZIKV can be used. A sample can be a liquid such as urine, saliva, cerebrospinal fluid, blood, serum or the like; a solid or semi-solid such as tissues, feces, or the like; or, alternatively, a solid tissue such as those commonly used in histological diagnosis.

[00113] The invention also provides for methods of diagnosis and in vivo detection of ZIKV using the peptides, antibodies or antibody fragments of the present invention. In using the peptides, antibodies or antibody fragments of the invention for the in vivo detection of antigen, the detectably labeled peptides, antibodies or antibody fragments are given in a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled peptides, antibodies or antibody fragments are administered in sufficient quantity to enable detection of the site having the flavivirus antigen for which the peptides, antibodies or antibody fragments are specific.

[00114] The concentration of detectably labeled peptide, antibody or antibody fragment which is administered should be sufficient such that the binding to flavivirus is detectable compared to the background.

[00115] As a rule, the dosage of detectably labeled peptides, antibodies or antibody fragments for in vivo diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. The dosage of peptides, antibodies or antibody fragments can vary from about 0.01 mg/kg to about 50 mg/kg, specifically from about 0.1 mg/kg to about 20 mg/kg, more specifically from about 0.1 mg/kg to about 2 mg/kg. Such dosages may vary, for example, depending on whether multiple injections are given, on the tissue being assayed, and other factors known to those of skill in the art.

[00116] For in vivo diagnostic imaging, the type of detection instrument available is a one factor in selecting an appropriate label, such as but not limited to a radioisotope. For example, the radioisotope

chosen must have a type of decay which is detectable for the given type of instrument. Still another factor in selecting an appropriate label for in vivo diagnosis is that the half-life of the label must be long enough such that it is still detectable at the time of maximum uptake by the target, but short enough such that any deleterious effect to the host is acceptable.

[00117] For in vivo diagnosis, the label(s) may be bound to the peptides, antibodies or antibody fragments of the invention either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind labels, such as for example radioisotopes, can exist as metallic ions and may be bifunctional chelating agents such as diethylenetriaminepentaacetic acid (DTPA) and ethylenediaminetetra-acetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the peptides, antibodies or antibody fragments of the invention are ^{111}In , ^{97}Ru , ^{67}Ga , ^{68}Ga , ^{72}As , ^{89}Zr and ^{201}Tl to name a few.

[00118] The peptides, antibodies or antibody fragments of the invention can also be labeled with a paramagnetic isotope for purposes of in vivo diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include but are not limited to ^{157}Gd , ^{55}Mn , ^{162}Dy , ^{52}Cr and ^{56}Fe .

[00119] The peptides, antibodies or antibody fragments of the invention can be used in vitro and in vivo to monitor the course of flavivirus disease therapy. Thus, for example, by measuring the increase or decrease in the number of cells infected with ZIKV over time, i.e., measuring at a first and second time point, or changes in the concentration of ZIKV present in the body or in various body fluids over time, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating flavivirus disease is effective.

[00120] The materials for use in the diagnostic assays that the invention provides are ideally suited for the preparation of a kit. Such a kit may comprise a carrier that is compartmentalized to receive in close confinement one or more containers such as vials, tubes, and the like, with each of the container comprising one of the separate elements to be used in the method. For example, one of the containers may comprise a peptide, antibody or antibody fragment of the invention that is, or can be, detectably labeled. The kit may also have containers containing buffer(s) and/or a container comprising a reporter, such as but not limited to a biotin-binding protein, such as avidin or streptavidin, bound to a reporter molecule, such as an enzymatic or fluorescent label.

[00121] Measuring the ability of the peptides, antibodies or antibody fragments of the present invention to inhibit fusion mediated by HeV envelope glycoprotein (Env) expressing cells with cells that we had previously identified as fusion-competent can be used to test the neutralizing activity of the peptides, antibodies or antibody fragments of the present invention. Fusion can be measured by two assays--a reporter gene assay and a syncytia formation assay. Methods of measuring fusion of a virus are reported in U.S. Patent No. 7,988,971, which is incorporated by reference in its entirety.

[00122] Neutralization assays utilizing infectious Zika and Dengue viruses can also be used to test the inhibitory activity of the peptides, antibodies or antibody fragments. Such neutralization assays are reported in U.S. Patent No. 7,988,971.

[00123] One aspect of the present application relates to an antibody or fragment thereof that selectively binds whole Zika virus, wherein said antibody comprises: (a) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 5 for CDR1, SEQ ID NO: 6 for CDR2, and SEQ ID NO: 7 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 8 for CDR1, SEQ ID NO: 9 for CDR2 and SEQ ID NO: 10 for CDR3; or (b) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 15 for CDR1, SEQ ID NO: 16 for CDR2, and SEQ ID NO: 17 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 18 for CDR1, SEQ ID NO: 19 for CDR2 and SEQ ID NO: 20 for CDR3; or (c) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 25 for CDR1, SEQ ID NO: 26 for CDR2, and SEQ ID NO: 27 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 28 for CDR1, SEQ ID NO: 29 for CDR2 and SEQ ID NO: 30 for CDR3; or (d) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 35 for CDR1, SEQ ID NO: 36 for CDR2, and SEQ ID NO: 37 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 38 for CDR1, SEQ ID NO: 39 for CDR2 and SEQ ID NO: 40 for CDR3; or (e) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 45 for CDR1, SEQ ID NO: 46 for CDR2, and SEQ ID NO: 47 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 48 for CDR1, SEQ ID NO: 49 for CDR2 and SEQ ID NO: 50 for CDR3; or (f) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 55 for CDR1, SEQ ID NO: 56 for CDR2, and SEQ ID NO: 57 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 58 for CDR1, SEQ ID NO: 59 for CDR2 and SEQ ID NO: 60 for CDR3; or (g) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 65 for CDR1, SEQ ID NO: 66 for CDR2, and SEQ ID NO: 67 for CDR3; and a light chain variable region comprising CDRs having amino

acid sequences of SEQ ID NO: 68 for CDR1, SEQ ID NO: 69 for CDR2 and SEQ ID NO: 70 for CDR3; or (h) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 75 for CDR1, SEQ ID NO: 76 for CDR2, and SEQ ID NO: 77 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 78 for CDR1, SEQ ID NO: 79 for CDR2 and SEQ ID NO: 80 for CDR3; or (i) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 85 for CDR1, SEQ ID NO: 86 for CDR2, and SEQ ID NO: 87 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 88 for CDR1, SEQ ID NO: 89 for CDR2 and SEQ ID NO: 90 for CDR3; or (j) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 95 for CDR1, SEQ ID NO: 96 for CDR2, and SEQ ID NO: 97 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 98 for CDR1, SEQ ID NO: 99 for CDR2 and SEQ ID NO: 100 for CDR3; or (k) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 105 for CDR1, SEQ ID NO: 106 for CDR2, and SEQ ID NO: 107 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 108 for CDR1, SEQ ID NO: 109 for CDR2 and SEQ ID NO: 110 for CDR3; or (l) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 115 for CDR1, SEQ ID NO: 116 for CDR2, and SEQ ID NO: 117 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 118 for CDR1, SEQ ID NO: 119 for CDR2 and SEQ ID NO: 120 for CDR3; or (m) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 125 for CDR1, SEQ ID NO: 126 for CDR2, and SEQ ID NO: 127 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 128 for CDR1, SEQ ID NO: 129 for CDR2 and SEQ ID NO: 130 for CDR3; or (n) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 135 for CDR1, SEQ ID NO: 136 for CDR2, and SEQ ID NO: 137 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 138 for CDR1, SEQ ID NO: 139 for CDR2 and SEQ ID NO: 140 for CDR3; or (o) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 145 for CDR1, SEQ ID NO: 146 for CDR2, and SEQ ID NO: 147 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 148 for CDR1, SEQ ID NO: 149 for CDR2 and SEQ ID NO: 150 for CDR3; or (p) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 155 for CDR1, SEQ ID NO: 156 for CDR2, and SEQ ID NO: 157 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 158 for CDR1, SEQ ID NO: 159 for CDR2 and SEQ ID NO: 160 for CDR3; or (q) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 165 for CDR1, SEQ ID NO: 166 for CDR2, and SEQ ID NO: 167 for CDR3; and a light chain variable region comprising

CDRs having amino acid sequences of SEQ ID NO: 168 for CDR1, SEQ ID NO: 169 for CDR2 and SEQ ID NO: 170 for CDR3; or (r) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 175 for CDR1, SEQ ID NO: 176 for CDR2, and SEQ ID NO: 177 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 178 for CDR1, SEQ ID NO: 179 for CDR2 and SEQ ID NO: 180 for CDR3; or (s) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 185 for CDR1, SEQ ID NO: 186 for CDR2, and SEQ ID NO: 187 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 188 for CDR1, SEQ ID NO: 189 for CDR2 and SEQ ID NO: 190 for CDR3; or (t) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 195 for CDR1, SEQ ID NO: 196 for CDR2, and SEQ ID NO: 197 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 198 for CDR1, SEQ ID NO: 199 for CDR2 and SEQ ID NO: 200 for CDR3; or (u) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 205 for CDR1, SEQ ID NO: 206 for CDR2, and SEQ ID NO: 207 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 208 for CDR1, SEQ ID NO: 209 for CDR2 and SEQ ID NO: 210 for CDR3; or (v) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 215 for CDR1, SEQ ID NO: 216 for CDR2, and SEQ ID NO: 217 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 218 for CDR1, SEQ ID NO: 219 for CDR2 and SEQ ID NO: 220 for CDR3.

[00124] In some embodiments, said antibody comprises: (a) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 5 for CDR1, SEQ ID NO: 6 for CDR2, and SEQ ID NO: 7 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 8 for CDR1, SEQ ID NO: 9 for CDR2 and SEQ ID NO: 10 for CDR3; or (b) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 15 for CDR1, SEQ ID NO: 16 for CDR2, and SEQ ID NO: 17 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 18 for CDR1, SEQ ID NO: 19 for CDR2 and SEQ ID NO: 20 for CDR3; or (c) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 25 for CDR1, SEQ ID NO: 26 for CDR2, and SEQ ID NO: 27 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 28 for CDR1, SEQ ID NO: 29 for CDR2 and SEQ ID NO: 30 for CDR3; or (d) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 35 for CDR1, SEQ ID NO: 36 for CDR2, and SEQ ID NO: 37 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 38 for CDR1, SEQ ID

NO: 39 for CDR2 and SEQ ID NO: 40 for CDR3; or (e) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 45 for CDR1, SEQ ID NO: 46 for CDR2, and SEQ ID NO: 47 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 48 for CDR1, SEQ ID NO: 49 for CDR2 and SEQ ID NO: 50 for CDR3; or (f) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 55 for CDR1, SEQ ID NO: 56 for CDR2, and SEQ ID NO: 57 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 58 for CDR1, SEQ ID NO: 59 for CDR2 and SEQ ID NO: 60 for CDR3; or (g) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 65 for CDR1, SEQ ID NO: 66 for CDR2, and SEQ ID NO: 67 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 68 for CDR1, SEQ ID NO: 69 for CDR2 and SEQ ID NO: 70 for CDR3; or (h) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 75 for CDR1, SEQ ID NO: 76 for CDR2, and SEQ ID NO: 77 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 78 for CDR1, SEQ ID NO: 79 for CDR2 and SEQ ID NO: 80 for CDR3; or (i) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 85 for CDR1, SEQ ID NO: 86 for CDR2, and SEQ ID NO: 87 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 88 for CDR1, SEQ ID NO: 89 for CDR2 and SEQ ID NO: 90 for CDR3; or (j) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 95 for CDR1, SEQ ID NO: 96 for CDR2, and SEQ ID NO: 97 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 98 for CDR1, SEQ ID NO: 99 for CDR2 and SEQ ID NO: 100 for CDR3; or (k) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 105 for CDR1, SEQ ID NO: 106 for CDR2, and SEQ ID NO: 107 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 108 for CDR1, SEQ ID NO: 109 for CDR2 and SEQ ID NO: 110 for CDR3.

[00125] In some embodiments, said antibody comprises: (a) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 115 for CDR1, SEQ ID NO: 116 for CDR2, and SEQ ID NO: 117 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 118 for CDR1, SEQ ID NO: 119 for CDR2 and SEQ ID NO: 120 for CDR3; or (b) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 125 for CDR1, SEQ ID NO: 126 for CDR2, and SEQ ID NO: 127 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 128 for CDR1, SEQ ID NO: 129 for CDR2 and SEQ ID NO: 130 for CDR3; or (c) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 135 for CDR1, SEQ ID NO: 136 for CDR2, and SEQ ID NO: 137 for CDR3; and a light chain variable region

comprising CDRs having amino acid sequences of SEQ ID NO: 138 for CDR1, SEQ ID NO: 139 for CDR2 and SEQ ID NO: 140 for CDR3; or (d) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 145 for CDR1, SEQ ID NO: 146 for CDR2, and SEQ ID NO: 147 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 148 for CDR1, SEQ ID NO: 149 for CDR2 and SEQ ID NO: 150 for CDR3; or (e) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 155 for CDR1, SEQ ID NO: 156 for CDR2, and SEQ ID NO: 157 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 158 for CDR1, SEQ ID NO: 159 for CDR2 and SEQ ID NO: 160 for CDR3; or (f) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 165 for CDR1, SEQ ID NO: 166 for CDR2, and SEQ ID NO: 167 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 168 for CDR1, SEQ ID NO: 169 for CDR2 and SEQ ID NO: 170 for CDR3; or (g) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 175 for CDR1, SEQ ID NO: 176 for CDR2, and SEQ ID NO: 177 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 178 for CDR1, SEQ ID NO: 179 for CDR2 and SEQ ID NO: 180 for CDR3; or (h) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 185 for CDR1, SEQ ID NO: 186 for CDR2, and SEQ ID NO: 187 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 188 for CDR1, SEQ ID NO: 189 for CDR2 and SEQ ID NO: 190 for CDR3; or (i) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 195 for CDR1, SEQ ID NO: 196 for CDR2, and SEQ ID NO: 197 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 198 for CDR1, SEQ ID NO: 199 for CDR2 and SEQ ID NO: 200 for CDR3; (j) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 205 for CDR1, SEQ ID NO: 206 for CDR2, and SEQ ID NO: 207 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 208 for CDR1, SEQ ID NO: 209 for CDR2 and SEQ ID NO: 210 for CDR3; or (k) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 215 for CDR1, SEQ ID NO: 216 for CDR2, and SEQ ID NO: 217 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 218 for CDR1, SEQ ID NO: 219 for CDR2 and SEQ ID NO: 220 for CDR3.

[00126] In particular embodiments, said antibody or antibody fragment comprises a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 55 for CDR1, SEQ ID NO: 56 for CDR2,

and SEQ ID NO: 57 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 58 for CDR1, SEQ ID NO: 59 for CDR2 and SEQ ID NO: 60 for CDR3.

[00127] In particular embodiments, said antibody or antibody fragment comprises a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 95 for CDR1, SEQ ID NO: 96 for CDR2, and SEQ ID NO: 97 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 98 for CDR1, SEQ ID NO: 99 for CDR2 and SEQ ID NO: 100 for CDR3.

[00128] In particular embodiments, said antibody or antibody fragment comprises a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 105 for CDR1, SEQ ID NO: 106 for CDR2, and SEQ ID NO: 107 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 108 for CDR1, SEQ ID NO: 109 for CDR2 and SEQ ID NO: 110 for CDR3.

[00129] In particular embodiments, said antibody or antibody fragment comprises a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 115 for CDR1, SEQ ID NO: 116 for CDR2, and SEQ ID NO: 117 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 118 for CDR1, SEQ ID NO: 119 for CDR2 and SEQ ID NO: 120 for CDR3.

[00130] In particular embodiments, said antibody or antibody fragment comprises a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 215 for CDR1, SEQ ID NO: 216 for CDR2, and SEQ ID NO: 217 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 218 for CDR1, SEQ ID NO: 219 for CDR2 and SEQ ID NO: 220 for CDR3.

[00131] Another aspect of the present application relates to an antibody or fragment thereof that selectively binds Zika virus wherein, the heavy chain CDR1 sequence differs from SEQ ID NO: 55 by four or less substitutions, the heavy chain CDR2 sequence differs from SEQ ID NO: 56 by two or less substitutions, the heavy chain CDR3 sequence differs from SEQ ID NO: 57 by five or less substitutions, the light chain CDR1 sequence differs from SEQ ID NO: 58 by one or less substitutions, the light chain CDR2 sequence differs from SEQ ID NO: 59 by three or less substitutions, and the light chain CDR3 sequence differs from SEQ ID NO: 60 by one or less substitutions.

[00132] Another aspect of the present application relates to an antibody or fragment thereof that selectively binds Zika virus wherein, the heavy chain CDR1 sequence differs from SEQ ID NO: 95 by four or less substitutions, the heavy chain CDR2 sequence differs from SEQ ID NO: 96 by two or less substitutions, the heavy chain CDR3 sequence differs from SEQ ID NO: 97 by five or less substitutions, the light chain CDR1 sequence differs from SEQ ID NO: 98 by one or less substitutions, the light chain CDR2 sequence differs from SEQ ID NO: 99 by three or less substitutions, and the light chain CDR3 sequence differs from SEQ ID NO: 100 by one or less substitutions.

[00133] Another aspect of the present application relates to an antibody or fragment thereof that selectively binds Zika virus wherein, the heavy chain CDR1 sequence differs from SEQ ID NO: 105 by four or less substitutions, the heavy chain CDR2 sequence differs from SEQ ID NO: 106 by two or less substitutions, the heavy chain CDR3 sequence differs from SEQ ID NO: 107 by five or less substitutions, the light chain CDR1 sequence differs from SEQ ID NO: 108 by one or less substitutions, the light chain CDR2 sequence differs from SEQ ID NO: 109 by three or less substitutions, and the light chain CDR3 sequence differs from SEQ ID NO: 110 by one or less substitutions.

[00134] Another aspect of the present application relates to an antibody or fragment thereof that selectively binds Zika virus wherein, the heavy chain CDR1 sequence differs from SEQ ID NO: 115 by four or less substitutions, the heavy chain CDR2 sequence differs from SEQ ID NO: 116 by two or less substitutions, the heavy chain CDR3 sequence differs from SEQ ID NO: 117 by five or less substitutions, the light chain CDR1 sequence differs from SEQ ID NO: 118 by one or less substitutions, the light chain CDR2 sequence differs from SEQ ID NO: 119 by three or less substitutions, and the light chain CDR3 sequence differs from SEQ ID NO: 120 by one or less substitutions.

[00135] In some embodiments, an antibody or antibody fragment as described herein inhibits Zika virus infection.

[00136] In some embodiments, an antibody or antibody fragment as described herein inhibits Dengue virus infection.

[00137] In some embodiments, an antibody or antibody fragment as described herein inhibits infection by Dengue virus serotype 2.

[00138] In some embodiments, an antibody or antibody fragment as described herein inhibits infection by Dengue virus serotype 3.

[00139] In some embodiments, an antibody or antibody fragment as described herein inhibits Zika virus transmission from a pregnant female to a fetus.

[00140] In some embodiments, an antibody or antibody fragment as described herein inhibits sexual transmission of Zika virus.

[00141] In some embodiments, an antibody or antibody fragment as described herein inhibits or prevents infection of human testes.

[00142] In some embodiments, an antibody or antibody fragment as described herein has an ED₅₀ for neutralizing Zika infection of less than less than 10 mg kg⁻¹, less than 5 mg kg⁻¹, less than 1 mg kg⁻¹, less than 0.5 mg kg⁻¹, less than 0.2 mg kg⁻¹, less than 0.1 mg kg⁻¹, less than 0.05 mg kg⁻¹, less than 0.02 mg kg⁻¹, or less than 0.01 mg kg⁻¹.

[00143] In some embodiments, an antibody or antibody fragment as described herein has an IC₅₀ for neutralizing Zika infection of less than 10 μ M, less than 5 μ M, less than 2 μ M, less than 1 μ M, less than 500 nM, less than 200 nM, or less than 100 nM.

[00144] In some embodiments, 100 μ g/ml of an antibody or antibody fragment as described herein does not neutralize infection by a flavivirus selected from the group of Dengue virus, Japanese Encephalitis virus, West Nile virus, or Yellow Fever virus.

[00145] In some embodiments, an antibody or antibody fragment as described herein has an equilibrium dissociation constant (K_D) is in the range from 10^{-7} to 10^{-9} molar.

[00146] In some embodiments, an antibody or antibody fragment as described herein has an equilibrium dissociation constant (K_D) of less than 10^{-7} molar.

[00147] In some embodiments, an antibody or antibody fragment as described herein comprises an Fd fragment.

[00148] In some embodiments, an antibody fragment as described herein is a Fab fragment.

[00149] In some embodiments, an antibody fragment as described herein is a single chain variable fragment (ScFv).

[00150] In some embodiments, an antibody or antibody fragment as described herein is a human antibody, humanized antibody or humanized antibody fragment.

[00151] Another aspect of the present application relates to a polynucleotide comprising a nucleotide sequence encoding an antibody or antibody fragment as described herein.

[00152] Another aspect of the present application relates to a host cell comprising a polynucleotide comprising a nucleotide sequence encoding an antibody or antibody fragment as described herein.

[00153] Another aspect of the present application relates to a method of making an antibody or antibody fragment as described herein comprising isolating antibody secreted by a host cell comprising a polynucleotide comprising a nucleotide sequence encoding an antibody or antibody fragment as described herein.

[00154] Another aspect of the present application relates to a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibody or antibody fragment as described herein.

[00155] Another aspect of the present application relates to a method for the prevention or treatment of a flavivirus infection comprising administering to a patient a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibody or antibody fragment as described herein.

[00156] Another aspect of the present application relates to a method for inhibiting or preventing transmission of a flavivirus infection from a pregnant female to her fetus comprising administering to the pregnant female a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibody or antibody fragment as described herein.

[00157] Another aspect of the present application relates to a method for inhibiting or preventing sexual transmission of a flavivirus infection comprising administering a therapeutically effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibody or antibody fragment as described herein.

[00158] Another aspect of the present application relates to a method of reducing the likelihood of a subject developing a disease caused by Zika virus or a flavivirus, the method comprising administering a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an antibody or antibody fragment as described herein to a subject prior to a flavivirus infection.

[00159] Another aspect of the present application relates to a method of detecting the presence of a flavivirus in a biological sample, the method comprising contacting an antibody or antibody fragment as described herein with the biological sample and detecting the binding of the antibody or antibody fragment to a flavivirus.

[00160] Another aspect of the present application relates to a kit for detecting the presence of a flavivirus in a biological sample, the kit comprising an antibody or antibody fragment as described herein.

[00161] Another aspect of the present application relates to a method of diagnosing infection by a flavivirus, the method comprising: obtaining a biological sample for a subject at risk of a flavivirus infection; contacting the biological sample with an antibody or antibody fragment as described herein; and determining if the antibody or antibody fragment has bound to a flavivirus antigen; wherein binding of the antibody or antibody fragment to a flavivirus antigen indicates that the subject is infected with a flavivirus.

[00162] Another aspect of the present application relates to a method of detecting a latent infection by a flavivirus, the method comprising: obtaining a biological sample for a subject at risk of a flavivirus infection; stimulating the biological sample to induce viral outgrowth; contacting the biological sample with an antibody or antibody fragment as described herein; and determining if the antibody or antibody fragment has bound to a flavivirus antigen; wherein binding of the antibody or antibody fragment to a flavivirus antigen indicates that the subject is infected with a flavivirus.

[00163] Another aspect of the present application relates to a method of inducing immunity to a flavivirus in a human subject at risk of flavivirus infection comprising, administering a single dose of Zika virus purified inactivated vaccine in the human subject, wherein the subject was previously infected by a flavivirus.

[00164] In some embodiments, the flavivirus is a Zika virus.

[00165] In some embodiments, the flavivirus is a Dengue virus.

[00166] In some embodiments, the flavivirus is Dengue virus serotype 2.

[00167] In some embodiments, the flavivirus is Dengue virus serotype 3.

[00168] In some embodiments, the flavivirus is a West Nile virus.

[00169] In some embodiments, the flavivirus is a Japanese Encephalitis virus.

[00170] In other embodiments, the flavivirus that the human subject was previously exposed to was not a Zika virus.

[00171] Another aspect of the present application relates to a method of measuring the efficacy of a vaccine batch comprising contacting an aliquot of the vaccine batch with an antibody or antibody fragment as described herein, and detecting the binding of the antibody or antibody fragment.

[00172] A method of determining whether a flavivirus vaccine comprises a DI/DIII linker domain comprising contacting the vaccine with an antibody or antibody fragment comprising: (a) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 115 for CDR1, SEQ ID NO: 116 for CDR2, and SEQ ID NO: 117 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 118 for CDR1, SEQ ID NO: 119 for CDR2 and SEQ ID NO: 120 for CDR3; or (b) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 125 for CDR1, SEQ ID NO: 126 for CDR2, and SEQ ID NO: 127 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 128 for CDR1, SEQ ID NO: 129 for CDR2 and SEQ ID NO: 130 for CDR3; or (c) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 135 for CDR1, SEQ ID NO: 136 for CDR2, and SEQ ID NO: 137 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 138 for CDR1, SEQ ID NO: 139 for CDR2 and SEQ ID NO: 140 for CDR3; or (d) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 145 for CDR1, SEQ ID NO: 146 for CDR2, and SEQ ID NO: 147 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 148 for CDR1, SEQ ID NO: 149 for CDR2 and SEQ ID NO: 150 for CDR3; or (e) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 155 for CDR1, SEQ ID NO: 156 for CDR2, and SEQ ID NO: 157 for CDR3; and a light chain variable region comprising

CDRs having amino acid sequences of SEQ ID NO: 158 for CDR1, SEQ ID NO: 159 for CDR2 and SEQ ID NO: 160 for CDR3; or (f) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 165 for CDR1, SEQ ID NO: 166 for CDR2, and SEQ ID NO: 167 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 168 for CDR1, SEQ ID NO: 169 for CDR2 and SEQ ID NO: 170 for CDR3; or (g) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 175 for CDR1, SEQ ID NO: 176 for CDR2, and SEQ ID NO: 177 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 178 for CDR1, SEQ ID NO: 179 for CDR2 and SEQ ID NO: 180 for CDR3; or (h) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 185 for CDR1, SEQ ID NO: 186 for CDR2, and SEQ ID NO: 187 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 188 for CDR1, SEQ ID NO: 189 for CDR2 and SEQ ID NO: 190 for CDR3; or (i) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 195 for CDR1, SEQ ID NO: 196 for CDR2, and SEQ ID NO: 197 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 198 for CDR1, SEQ ID NO: 199 for CDR2 and SEQ ID NO: 200 for CDR3; (j) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 205 for CDR1, SEQ ID NO: 206 for CDR2, and SEQ ID NO: 207 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 208 for CDR1, SEQ ID NO: 209 for CDR2 and SEQ ID NO: 210 for CDR3; or (k) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 215 for CDR1, SEQ ID NO: 216 for CDR2, and SEQ ID NO: 217 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 218 for CDR1, SEQ ID NO: 219 for CDR2 and SEQ ID NO: 220 for CDR3..

[00173] Another aspect of the present application relates to a method of purifying a flavivirus E glycoprotein comprising contacting the flavivirus E glycoprotein with an antibody or antibody fragment as described herein.

[00174] Another aspect of the present application relates to an antibody or antibody fragment as described herein, wherein the antibody or antibody fragment binds to the DI-DIII linker domain of a Zika virus.

[00175] In some embodiments, 100 ng, 50 ng, 20 ng, 10 ng, 5 ng, 2 ng, 1 ng, 0.5 ng, 0.2 ng or 0.1 ng of the antibody or fragment thereof can neutralize at least 50% of the infectious activity of 100 PFU of Zika virus in a microneutralization assay.

[00176] In some embodiments, binding to Zika virus is reduced by at least 70% when Zika virus E glycoprotein residue Tyrosine 305 is substituted with alanine.

[00177] Another aspect of the present application relates to a method for isolating an antibody that binds to cross-protomer epitopes of a virus comprising: (a) immunizing a subject with a viral immunogen, (b) isolating peripheral blood mononuclear cells (PBMCs) from the subject, (c) contacting the PBMCs with intact virus to create PBMC-virus complexes, (d) contacting the PBMC-virus complexes with a fluorescently-labeled antibody that binds the virus, (e) isolating a fluorescent PBMC, (f) isolating polynucleotides encoding the heavy and light chains of an antibody from the fluorescent PBMC, (g) expressing the isolated polynucleotides in a host cell, and (h) isolating an antibody expressed by the host cell.

[00178] In some embodiments, the subject is a primate.

[00179] In some embodiments, the viral immunogen is an intact virus.

[00180] In some embodiments, the viral immunogen is a flavivirus immunogen. In some further embodiments, the flavivirus immunogen is a Zika virus immunogen. In other further embodiments, the flavivirus immunogen is a Dengue virus immunogen. In still other further embodiments, the flavivirus immunogen is a West Nile virus immunogen.

[00181] Another aspect of the present application relates to a multispecific antibody comprising a first binding site that binds to a flavivirus and a second binding site that binds to a flavivirus, wherein the first binding site binds to a different epitope than the second binding site.

[00182] In some embodiments, the multispecific antibody comprises a plurality of first binding sites and a plurality of second binding sites.

[00183] In some embodiments, the multispecific antibody comprises a third binding site that binds to a different flavivirus epitope than the first binding site or the second binding site. In some still further embodiments, the multispecific antibody comprises a fourth binding site that binds to a different flavivirus epitope. In some still further embodiments, the multispecific antibody comprises a fifth binding site that binds to a different flavivirus epitope. In some still further embodiments, the multispecific antibody comprises a sixth binding site that binds to a different flavivirus epitope. In some still further embodiments, the multispecific antibody comprises a seventh binding site that binds to a different flavivirus epitope. In some still further embodiments, the multispecific antibody comprises an eighth binding site that binds to a different flavivirus epitope. In some still further embodiments, the multispecific antibody comprises a ninth binding site that binds to a different flavivirus epitope. In some

still further embodiments, the multispecific antibody comprises a tenth binding site that binds to a different flavivirus epitope.

[00184] In some embodiments, the first binding site comprises CDR sequences of a heavy chain variable domain or a fragment thereof and CDR sequences of a light chain variable domain or a fragment thereof.

[00185] In some embodiments, the second binding domain comprises CDR sequences of a single chain variable fragment (ScFv).

[00186] In some embodiments, the constant region of a heavy chain polypeptide comprising CDR sequences of the first binding site comprises a Threonine 366 to Tyrosine substitution and the constant region of a heavy chain polypeptide comprising CDR sequences of the second binding site comprises a Tyrosine 407 to Threonine substitution.

[00187] In some embodiments, a ScFv comprising the second binding site is inserted into the hinge region of a heavy chain polypeptide comprising CDR sequences of the first binding site.

[00188] In some embodiments, a ScFv comprising the second binding site is inserted within a constant region of a heavy chain polypeptide comprising CDR sequences of the first binding site.

[00189] In some embodiments, the first binding site has relatively higher affinity for a Zika virus E glycoprotein than for a Dengue virus E glycoprotein and the second binding site has relatively higher affinity for a Dengue virus E glycoprotein than for a Zika virus E glycoprotein.

[00190] In some embodiments, wherein the first binding site comprises CDR sequences from an antibody selected from the group comprising MZ4, MZ20, EDE2-A11 and Ab513.

[00191] In some embodiments, the second binding site comprises CDR sequences from an antibody selected from the group comprising MZ4, MZ20, EDE2-A11 and Ab513.

[00192] In some embodiments, wherein the mean binding response as measured by BioLayer Interferometry for ZIKV, DENV1, DENV2, DENV3, and DENV4 is less than the mean binding response as measured by BioLayer Interferometry for ZIKV, DENV1, DENV2, DENV3, and DENV4 of an antibody comprising only a first binding site or only a second binding site.

[00193] In some embodiments, the mean of IC_{50} values for neutralization of ZIKV, DENV1, DENV2, DENV3, and DENV4 is less than the mean of IC_{50} values for neutralization of ZIKV, DENV1, DENV2, DENV3, and DENV4 of an antibody comprising only a first binding site or only a second binding site.

[00194] In some embodiments, the multispecific antibody comprises a first polypeptide comprising an MZ4 heavy chain variable domain and an MZ4 light chain variable domain and a second polypeptide comprising an EDE2-A11 heavy chain variable domain and an EDE2-A11 light chain variable domain.

[00195] In some embodiments, the multispecific antibody comprises a polypeptide comprising an MZ4 heavy chain variable domain, an MZ4 light chain variable domain, an EDE2-A11 heavy chain variable domain and an EDE2-A11 light chain variable domain.

[00196] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

[00197] Aspects and embodiments of the present application are further illustrated by the following non-limiting examples.

Example 1. General Methods

[00198] Cell lines: D1-4G2-4-15 mouse hybridoma (ATCC #HB-112), C6/36 (ATCC #CRL-1660), Vero (ATCC #CCL-81), Expi293F (ThermoFisher Scientific), DS-2 (ThermoFisher Scientific), and U937-DC-SIGN (ATCC) cell lines were utilized in this study. These lines were verified to be authentic, using short tandem repeat profiling, morphology, and cytochrome C oxidase I testing, and free of contamination by mycoplasma prior to use.

[00199] Preparation of ZIKV and DENV: C6/36 mosquito cells were grown in T75 flasks and infected with ZIKV strain (Paraiba_01 strain, GenBank KX280026) or DENV-2 (S16803, GenBank GU289914) at a multiplicity of infection of approximately 0.1 PFU/cell. The infected cell culture supernatant was harvested on day 5 postinfection. Cell debris was removed by centrifugation at 5,000 rpm for 30 min at 4°C. The supernatant was layered on top of a 30% sucrose solution containing 10 mM Tris, 100 mM NaCl, and 1 mM EDTA. The virus was pelleted by ultracentrifugation in a swinging-bucket rotor at 26,000 rpm for 4 hr at 4°C to remove low-molecular-weight contaminants such as soluble proteins. The supernatant was removed, and the tubes were briefly left upside down on chromatography paper in order to remove excess liquid from the side of the tubes. The virus pellet was resuspended in phosphate-buffered saline. The purity of the viral preparations was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

[00200] Sorting of whole ZIKV positive B cells from non-human primates: Approximately 10 million cryopreserved peripheral blood mononuclear cells (PBMCs) were obtained from a flavivirus-naïve, five-year-old male, rhesus macaque previously described in McCracken, 2017. This animal was not previously

infected and confirmed by testing for ZIKV, JEV, WNV, YFV, and DENV1-4 antibodies by a sensitive screening virus neutralization assay prior to initial infection (McCracken, 2017). The PBMC sample was collected 14 days following challenge with ZIKV (Brazil-ZKV2015 strain, GenBank KU497555). PBMCs were thawed in warm medium containing benzonase, then washed with phosphate-buffered saline (PBS) and stained for viability using Invitrogen Aqua Live/Dead stain. Cells were incubated at 4°C for 30 minutes with a cocktail of antibodies including CD3 BV510, CD14 BV510, and CD56 BV510 (BioLegend) as dump channel markers, and CD19 ECD (Beckman Coulter), CD20 APC-Cy7 (BioLegend), CD38 PE (NHP Reagent Resource) and CXCR5 PE-Cy7 (eBioscience) as positive gating markers. To obtain monoclonal antibodies that target quaternary epitopes, primary staining also included a 1/10 dilution of live whole ZIKV (Paraiba_01) produced in C6/36 cells (see above). ZIKV-reactive B cells were identified by secondary staining using 4G2 (Biovest) conjugated to APC (ThermoFisher). Cells were selected by sorting based on negative expression of CD3, CD14 and CD56, positive expression of CD19, mid to high expression of CD38, and positive sequential staining with 4G2. Cells were sorted directly into lysis buffer (murine RNase inhibitor (New England Biolabs), DTT and SuperScript III First Strand Buffer (ThermoFisher), Igpal (Sigma) and carrier RNA (Qiagen) at one cell per well into polypropylene PCR plates using a FACSAria (Becton Dickinson) and stored at -80°C until subsequent reverse transcription.

[00201] Antibody sequencing and production: RNA from single B cells was reverse-transcribed using random primers and the SuperScriptIII kit (ThermoFisher). Antibody V (D) J genes were amplified from the cDNA by nested PCR, using the HotStar Taq DNA Polymerase kit (Qiagen) and a combination of primer. V (D) J gene assignment, somatic hypermutation and CDR3 determinations was performed with IgBlast (Ye, 2013). For the non-human primate experiments, antibody variable regions were synthesized and cloned (Genscript) into CMVR expression vectors (a gift from Kevin Saunders) between a murine Ig leader (GenBank DQ407610) and the constant regions of rhesus macaques IgG1 (GenBank AAF14058), Ig κ light chain (GenBank AAD02577) or Ig λ light chain (GenBank ADX62855) (Saunders, 2015). Variable regions for control antibodies EDE1-C8, Z3L1 (Wang, 2016), 2A10G6 (Deng, 2011) and Z004 were synthesized as above and cloned into CMVR expression vectors carrying human IgG1, Ig κ and Ig λ constant regions. For human antibodies, antibody variable regions were synthesized and cloned (Genscript) into CMVR expression vectors (NIH AIDS reagent program) between a murine Ig leader (GenBank DQ407610) and the constant regions of human Ig γ 1 (GenBank AAA02914), Ig κ (GenBank AKL91145) or Ig λ (GenBank AAA02915). Plasmids encoding heavy and light chains were co-transfected into Expi293F cells (ThermoFisher) according to the manufacturer's instructions. After 5 days, antibodies were purified from cleared culture supernatants with Protein A agarose (ThermoFisher) using standard

procedures, buffer exchanged to Phosphate-buffered saline (PBS) and quantified from A280 measurements. SDS-PAGE and Coomassie staining in both reducing and non-reducing conditions assessed purity and stability of the purified antibodies.

[00202] Fab production: Freshly prepared Fab digestion buffer containing 20 mM sodium phosphate, 10 mM EDTA and 20 mM of cysteine-HCl, pH 7.4 was added to papain slurry (Thermo Scientific) and incubated with MZ antibodies (IgGs) at 20 mg/ml. Reaction was allowed to proceed for five hours to overnight in a shaker incubator at 37°C temperature and 100 rpm shaking speed. Resin was separated from the supernatant by centrifugation at 3000×g. Digestion was assessed by SDS-PAGE and upon completion, the reaction mixture was passed through protein-A beads (0.5-1 ml beads) 3 times and the final flow through was assessed by SDS-PAGE for purity.

[00203] Production of recombinant proteins: Recombinant ZIKV soluble E (sE) protein (1-404) from strain PRVABC59 (GenBank KX087101) and DENV-2 sE (1-396) from strain 16681-PDK53 (GenBank M84728) were produced with C-terminal AviTag and poly-histidine tags from Expi293F cells. The coding sequence for prM/sE was synthesized (Genscript) and cloned into the pcDNA3.4 vector (ThermoFisher) downstream a murine Ig leader sequence. Following transient co-transfection with a furin (Genbank BC012181) expression vector, mature sE proteins were purified from cell culture supernatants using a Ni-NTA (Qiagen) affinity column. An isolated E domain III (303-404) was expressed by deleting the prM and domains I-II from the full-length prM/sE pcDNA3.4 construct. A cleavable twin-strep-tagged ZIKV sE version was also expressed from stably transfected S2 cells using the Drosophila Expression system (ThermoFisher) according to the manufacturer's instructions. Briefly, a DNA fragment encoding for the first 405 residues of E from strain PRVABC59 was synthesized (Genscript) with a C-terminal HRV-3C cleavage site followed by a twin-strep tag (IBA) and cloned into the pMT-BiP vector (ThermoFisher). The L107C and A319C mutations were introduced using the Quikchange Lightning site-directed mutagenesis kit (Agilent). S2 cells were co-transfected with the pMT-BiP-ZIKV sE expression vector and the pCoBlast selection vector at a 19:1 (w/w) ratio. Stably transfected cells were selected with Blasticidin and adapted to suspension and serum-free medium (Lonza Insect Xpress). ZIKV E expression was induced with 0.5mM CuSO₄ and culture supernatants were harvested after 7 days. The insect-produced ZIKV sE was purified on a StrepTactin XT column (IBA) following the manufacturer's instructions followed by gel filtration on an Enrich SEC 650 column (Bio-Rad) or GE Sephadex S200 column to obtain pure monomeric (WT sE) or dimeric (mutant sE) ZIKV sE proteins. ZIKV (Suriname) NS1 was purchased from The Native Antigen Company.

[00204] Biolayer interferometry binding and competition assays: Real-time interactions between purified sE proteins and antibodies were monitored on an Octet RED96 instrument (Pall FortéBio) at 30°C. Avi-tagged purified ZIKV or DENV-2 sE proteins, biotinylated with the BirA biotinylation kit (Avidity), were diluted in kinetics buffer (0.1% [w/v] bovine serum albumin (BSA), 0.02% [v/v] Tween-20 in PBS; Pall ForteBio) and immobilized on streptavidin (SA) biosensors (Pall ForteBio) at ~50% of the sensor maximum binding capacity. Baseline was established in kinetics buffer. In the screening assay for NHP antibodies, loaded biosensors were then dipped into wells containing the antibodies diluted to 400 nM in kinetics buffer. Binding responses were measured after 450s of association using the Data analysis software 9.0 (Pall ForteBio). For measurement of human antibody binding activities in plasma, loaded biosensors were dipped into wells containing plasma diluted at 1/40 in kinetic buffer for 450s, to obtain binding responses, followed by a dissociation step in buffer. Off-rates were calculated by fitting dissociation curves to a 1:1 binding model. For full characterization of Fabs affinity, loaded biosensors were dipped into wells containing serial dilutions of the antibody Fab fragments for 450s. sE:Fab complexes were then allowed to dissociate for 1200s in buffer. After reference subtraction, binding kinetic constants were determined, from at least 4 concentrations of Fab, by fitting the curves to a 1:1 binding model using the Data analysis software 9.0 (Pall ForteBio). Finally, in the binding competition assay, sensors loaded with ZIKV sE, as described above, were immersed into wells containing the first competing antibody at a concentration (ranging from 100 to 800 nM) necessary to reach binding saturation after 900 s. Next, biosensors were dipped into wells containing the second antibody, in presence of the first competing antibody, and binding was measured after 900 s of association. Residual binding signal of the second antibody was expressed as a percent of the signal obtained in presence of a non-competing control antibody (VRC01), ran in parallel, and further corrected for the binding signal obtained with the first antibody alone after 1800 s. As some competing antibodies did not reach saturation after the first 900 s association and continue to contribute to binding signal together with the second antibody, a set of controls were run independently with all first competing antibodies alone for a 1800 s association. The difference in signal obtained between $t = 1800$ s and $t = 900$ s was subtracted from the signal obtained in presence of the second antibody to generate a corrected residual binding signal. Antibodies were defined as competing when binding signal of the second antibody was reduced to less than 30% of its maximum binding capacity and non-competing when binding was greater than 70%. Intermediate competition was defined by binding levels of 30-70%. Control monoclonal antibodies included the monoclonal 4G2 purified from hybridoma2, A10G6 (Deng, 2011), expressed with a human Fc domain, Z3L1 (Wang, 2016), EDE1-C8 (Dejnirattisai, 2015), and Z004 (Robbiani, 2017), all expressed

and purified from Expi293F cells. The HIV-1 specific VRC01 monoclonal antibody (also expressed in Expi293F cells) served as negative control.

[00205] Plasma competition assays were performed similarly to the mAb competition assays described above with the following modifications. Sensors loaded with ZIKV sE were immersed into wells containing plasma from ZIKV infected macaques (McCracken, 2017) and humans (Seracare), as well as control naïve plasma from the two species at dilutions (ranging from 1/10 to 1/200) necessary to reach near binding saturation after 900 s. Next, biosensors were dipped into wells containing the indicated monoclonal antibody, in presence of competing plasma, and binding was measured after 30 s of association. Residual binding signal of the monoclonal antibody was expressed as a percent of the signal obtained in presence of a non-competing matrix control of IgG-depleted human serum (BBI solutions), ran in parallel. Binding of monoclonal antibodies was further corrected for the binding signal obtained with plasma-only controls that ran simultaneously. Finally, results were expressed as percentage of binding inhibition defined as the inverse of residual binding.

[00206] Measurement of antibody binding affinity: Determination of affinity constant was performed on the Octet RED96 instrument. Disulfide-stabilized ZIKV sE was biotinylated at a 2:1 molar ratio using EZ-link NHS-PEG4-biotin (ThermoFisher), following manufacturer's instructions. A single buffer (1X kinetics buffer [Pall ForteBio]) was used for all dilution, baseline and dissociation steps. Streptavidin biosensors, loaded with ZIKV sE dimer at ~50% of maximum binding capacity, were dipped into wells containing two-fold serial dilutions of the antibody Fab fragments for 450 s with starting concentrations ranging from 1 to 10 μ M. ZIKV sE:Fab complexes were then allowed to dissociate for 1200 s in buffer. After reference subtraction, binding kinetic constants were determined, from at least 4 concentrations of Fab, by fitting the curves to a 1:1 binding model using the Data analysis software 9.0 (Pall ForteBio).

[00207] Whole virus ELISA assay: Binding of antibodies to whole ZIKV or DENV-2 viruses was measured using a capture ELISA assay. ELISA plates were coated overnight at 4°C with the capture antibody (4G2) at 100ng per well in borate saline pH9.0 buffer. After washes in PBS-T (PBS with 0.05% Tween-20), plates were blocked with 1% (v/v) normal goat serum, 0.25% (w/v) BSA, 0.1% (v/v) Tween-20 for 30min at 37°C. Washes in PBS-T were performed after each subsequent steps and all dilutions were made in blocking buffer. Previously titrated purified viruses (ZIKV/Brazil/2015 or DENV-2 16681) were diluted and added at 50 μ l per well and incubated for 2h at 37°C. Serial 4-fold dilutions of antibodies (starting at 20 μ g/ml) were added to the plate and incubated for 2h at 37°C. Secondary HRP-conjugated antibodies anti-mouse, human and monkey IgG were added for 1h at 37°C and plates were developed using 3,3',5,5'-Tetramethylbenzidine (TMB) peroxidase substrate (KPL) and read at 650nm. After background

(average blank + 2 standard deviation) subtraction, the binding curves were fitted using a 4-parameter logistic regression model in the Prism 7 software (GraphPad).

[00208] Recombinant ZIKV sE binding ELISA assay: Binding of antibodies to recombinant ZIKV sE protein was also performed in a standard ELISA assay. ELISA plates were coated overnight at 4°C with 100ng of purified ZIKV sE (produced in DS-2 cells) in sodium bicarbonate/carbonate pH9.4 buffer. Plates were then blocked with 5% (w/v) nonfat dry milk, 1% (w/v) BSA in PBS for 1h at 37°C. Washes in between each steps were performed with 0.1% (v/v) Triton-X100 in PBS. Serial 4-fold dilutions of antibodies (starting at 20µg/ml) made in 5% (v/v) Fetal Bovine Serum, 2% (w/v) BSA, 1% (v/v) Triton X-100 in PBS were added to the plate and incubated for 1 hour at RT. Secondary HRP-conjugated antibodies anti-mouse, human and monkey IgG were added for 1h at 37°C and plates were developed using TMB peroxidase substrate (KPL) and read at 650nm. Data were analyzed as described for the whole virus ELISA.

[00209] In vivo protection studies: Female Balb/c mice were purchased from commercial vendors and housed at Beth Israel Deaconess Medical Center. Indicated monoclonal macaque antibody was infused intravenously into groups of naïve recipient Balb/c mice (N=5/group) prior to ZIKV-BR challenge. Mice received 100 µl (200 µg) of a 2 mg/ml solution of purified monoclonal antibody and 2 hr after infusion, mice were challenged with 10⁵ viral particles (VP) [10² plaque-forming units (PFU)] ZIKV-BR intravenously. RT-PCR assays were utilized to monitor viral loads, essentially as previously described (Larocca, 2016). RNA was extracted from serum samples with a QIAcube HT (Qiagen). The wildtype ZIKV BeH815744 Cap gene was utilized as a standard. RNA was purified (Zymo Research). Log dilutions of the RNA standard were reverse transcribed and included with each RT-PCR assay. Viral loads were calculated as virus particles (VP) per ml with a sensitivity of 100 copies/ml.

[00210] Shotgun Mutagenesis Epitope Mapping: Epitope mapping was performed by shotgun mutagenesis. A ZIKV prM/E expression construct (strain ZikaSPH2015) was subjected to high-throughput alanine scanning mutagenesis to generate a comprehensive library of individual mutations where each residue within prM/E was changed to alanine, with alanine mutated to serine. In total, 672 ZIKV prM/E mutants were generated (100% coverage), sequence confirmed, and arrayed into 384-well plates. Each prM/E mutant was transfected into HEK-293T cells and allowed to express for 22 hrs. Cells were fixed in 4% (v/v) paraformaldehyde (Electron Microscopy Sciences), permeabilized with 0.1% (w/v) saponin (Sigma-Aldrich) in PBS plus calcium and magnesium (PBS++), then incubated with purified mAbs diluted in PBS++, 10% (v/v) normal goat serum (NGS) (Sigma), 0.1% (v/v) saponin. Primary mAb screening concentrations were determined using an independent immunofluorescence titration curve against

wild-type ZIKV prM/E to ensure that signals were within the linear range of detection. MAb binding was detected using 3.75 µg/ml AlexaFluor488-conjugated secondary antibody (Jackson ImmunoResearch Laboratories) in 10% NGS/0.1% saponin. Cells were washed 3 times with PBS++/0.1% saponin followed by 2 washes in PBS. Mean cellular fluorescence was detected using a high throughput flow cytometer (HTFC, Intellicyt). MAb reactivities against each mutant prM/E clone were calculated relative to wild-type prM/E reactivity by subtracting the signal from mock-transfected controls and normalizing to the signal from wild-type prM/E-transfected controls. Mutations within clones were identified as critical to the mAb epitope if they did not support reactivity of the test mAb, but supported reactivity of other ZIKV mAbs. This counter-screen strategy facilitates the exclusion of prM/E mutants that are locally misfolded or have an expression defect.

[00211] Zika Virus Microneutralization (MN): The method of Larocca et al., 2016 was used to perform microneutralization assays. Plasma or purified antibodies at 1 to 2 mg/ml were serially diluted 3-fold in 96-well micro-plates, and 100 µl of ZIKV containing 100 PFU were added to 100 µl of each serum dilution and incubated at 35°C for 2 hr. Supernatants were then transferred to microtiter plates containing confluent Vero cell monolayers (World Health Organization, NICSC-011038011038). After incubation for 4 days, cells were fixed with absolute ethanol: methanol for 1 hr at -20 °C and washed three times with PBS. The pan flavivirus monoclonal antibody 6B6-C1 conjugated to HRP was then added to each well, incubated at 35°C for 2 hr, and washed with PBS. Plates were washed, developed with TMB substrate for 50 min at room temperature, stopped with 1:25 phosphoric acid, and absorbance was read at 450 nm. Assays were validated using the following criteria: the average absorbance at 450 nm of three non-infected control wells had to be ≤ 0.5 , and virus-only control wells had to be ≥ 0.9 . Normalized absorbance values were calculated, and the concentration to achieve 50% neutralization (MN50 or IC50) titer was calculated using a 4-parameter logistic regression analysis in GraphPad Prism 7.

[00212] FlowNT50 Zika virus neutralization assay: Serial dilutions of mAb or plasma were mixed with an equal volume of virus, diluted to achieve 10–15% infection of cells/well, and incubated for 1 hr at 37°C. After 1 hr of incubation, an equal volume of medium (RPMI-1640 supplemented with 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine (200 mM), and 1% non-essential amino acids (10 mM)) containing 5x10⁴ U937-DC-SIGN cells were added to each serum-antibody mixture and incubated 18–20 hr overnight in a 37°C, 5% CO₂, humidified incubator. Following overnight incubation, the cells were fixed, permeabilized and immunostained with flavivirus group-reactive mouse monoclonal antibody 4G2 (Envigo Bioproducts) and secondary polyclonal goat anti-mouse IgG PE-conjugated antibody. The

percentage of infected cells was quantified on a BD Accuri C6 Plus flow cytometer (BD Biosciences). Data were analyzed by nonlinear regression in GraphPad Prism to determine 50% neutralization titers.

[00213] PRNT50 Zika Virus neutralization assay: Serial dilutions of mAb were mixed with an equal volume of virus and incubated for 1 hr at 37°C followed by infection of Vero- cell monolayers in triplicate. Plaques were visualized by staining with neutral red. Data were analyzed by nonlinear regression using asymmetric five-parameter logistic equation in GraphPad Prism to determine 50% neutralization titers.

[00214] Reporter Virus Particle (RVP): Neutralization of wildtype and mutant Zika (strain H/PF/2013) by mAbs was measured using a reporter virus particle (RVP) assay as described previously (Dowd et al., 2016). Briefly, mAbs were serially diluted 5-fold from 50 µg and incubated with 100 µl of virus for 1 hr at 37°C, after which 50 µl of target Vero cells (400,000 cells/ml) was added. Input virus dilution was calculated from titration experiments to ensure sufficient luciferase output within the linear portion of the titration curve. Cell only and virus only controls were included on each plate, and all mAbs (and virus-only) were run in triplicate. After a 48 hr incubation, luciferase activity was measured, and neutralization curves were calculated by averaging luciferase units from triplicates, subtracting cell-only control background and calculating the percent difference in serum samples to virus-only controls. Data was fit by nonlinear regression using the asymmetric five-parameter logistic equation in GraphPad Prism. The 50%, 80% and 90% inhibitory dilutions (ID50, ID80 and ID90, respectively) were defined as the reciprocal sera dilution resulting in a 50%, 80% or 90% reduction in infectivity.

Example 2. X-ray crystallography methods for non-human primate antibodies

[00215] Protein Purification: A construct encoding ZikaE glycoprotein spanning residues 1 to 405 with a C-terminal HRV-3c cleavage site followed by a StrepTagII peptide, was expressed in DS-2 insect cells as described above. Protein was purified from cell supernatant by StrepTagII affinity chromatography. The C-terminal StrepTagII peptide was removed using HRV-3c at 4 °C overnight, followed by gel filtration chromatography using a S200 Superdex 16/60 column. Monoclonal antibodies used in crystallization studies were expressed in Expi293F using transient co-transfection of constructs encoding the IgG heavy and light chains, respectively. Cultures were supplemented with fresh 293FreeStyle media (Life Technologies) 4 hr post-transfection and with HyClone SFM4HEK293 enriched medium (HyClone) containing valproic acid (4 mM final concentration) 24 hr after transfection. Cultures were incubated at 33°C for six days, after which supernatants were harvested. IgG protein was purified from the clarified supernatant using Protein A affinity chromatography and dialyzed against PBS, pH 7.4. The antigen

binding fragments (Fab) were proteolytically cleaved from the crystallizable fragment regions (Fc) using Pierce™ Fab Preparation Kit (Thermo Scientific 44985) at 37°C for 12 hr. The resulting Fc molecules and any remaining uncleaved IgG were removed from the reaction mixture using Protein A or Protein G chromatography.

[00216] X-ray crystallography and structure analysis: Purified Fabs were concentrated to 7-10 mg/ml and used for crystallization screening. For complexes, Fabs and ZikaE were mixed in an equimolar ratio at 7 mg/ml and incubated at 4 °C for 1 hr prior to crystallization screening using an Art Robbins Gryphon crystallization robot. A set of 1200 crystal growth conditions prepared using an Art Robbins Scorpion robot, were assessed by mixing 0.2 µl of protein complex with 0.2 µl of reservoir solution using the sitting-drop vapor diffusion method at 20°C. Once initial crystal conditions were observed, further crystallization trials to improve crystal size and shape were carried out by hand, using a 1:1 ratio of protein and reservoir solution. Optimized crystals were briefly soaked in mother liquor supplemented with a cryoprotectant and frozen in liquid nitrogen prior to x-ray diffraction data collection.

[00217] Crystals of the rhMZ103-A Fab were obtained at ~8 mg/ml protein concentration and a reservoir solution containing 20% PEG 4000, 0.2M sodium acetate, 0.1 M sodium citrate (pH 5.6). Crystals of the rhMZ107-B Fab were obtained at ~7 mg/ml protein concentration and a reservoir solution containing 23.5% PEG 4K, 0.2 M (NH₄)₂SO₄. Crystals of the rhMZ107-B Fab in complex with Zika E were obtained at 7 mg/ml protein concentration and a reservoir solution of 15% PEG 6000, 5% MPD, 0.1 M MES (pH 6.5). Crystals of the rhMZ100-C Fab were obtained at 8.1 mg/ml protein concentration and a reservoir solution containing 22.5% PEG 4000, 22.5% isopropanol, 0.1 M sodium citrate (pH 5.6). Crystals of the rhMZ100-C Fab in complex with Zika E were obtained using the hanging drop vapor diffusion method at 7.5 mg/ml protein concentration and a reservoir solution of 12% PEG 8000, 0.2 M (NH₄)₂SO₄, 0.1 M Tris (pH 8.5). Crystals of the rhMZ104-D Fab were obtained using the hanging drop vapor diffusion method at 8 mg/ml protein concentration and a reservoir solution containing 26% PEG 8000, 0.2 M zinc acetate, 0.1 M Tris-HCl (pH 8.5). Crystals of the rhMZ104-D Fab in complex with ZikaE were obtained at 7 mg/ml protein concentration and a reservoir solution of 12% PEG 8000, 0.2 M (NH₄)₂SO₄, 0.1 M Tris (pH 8.5). Crystals of the rhMZ119-D Fab were obtained at ~8.5 mg/ml protein concentration and a reservoir solution containing 18% PEG 8000, 0.2 M calcium acetate hydrate, 0.1 M sodium cacodylate trihydrate (pH 6.5). All crystals were cryoprotected with mother liquor supplemented with 25% (v/v) glycerol prior to flash-cooling.

[00218] Data for all crystals were collected at 0.97-1.00 Å wavelength at APS, ANL (Advanced Photon Source, Argonne National Laboratory) beamlines 19-BM, 22-BM, 19-ID, 22-ID and 24-ID-E and data collection and refinement statistics are provided in Tables 5 and 6.

Table 5. Crystallographic Data Collection and Refinement Statistics (rhMHRPZ Fab Complexes)

	rhMZ100-C/ ZIKV E	rhMZ104-D/ ZIKV E	rhMZ107-B/ ZIKV E	rhMZ119-D/ ZIKV E
Data collection	APS 19-ID	APS 19-ID	APS 22-BM	AMX 17-ID-1
Crystallization conditions	12% PEG 8000, 0.2 M (NH ₄) ₂ SO ₄ , 0.1M Tris (pH 8.5)	12% PEG 8000, 0.2 M (NH ₄) ₂ SO ₄ , 0.1M Tris-HCl (pH 8.5)	15% PEG 6000, 5% MPD, 0.1 M MES (pH 6.5)	0.06M Nitrate Phosphate Sulfate, 0.1M Sodium HEPES and MOPS (acid) pH 7.5, 20% Ethylene glycol, 10 % PEG 8000 + 2% w/v Benzamidine hydrochloride
Space group	P2 ₁	P2 ₁	P 1	P2 ₁ 2 ₁ 2
Cell dimensions				
a,b,c (Å)	101.84,126.68,140.5	85.3,130.4,109.7	92.0,105.6,132.4	51.7,103.7,196.7
α,β,γ (°)	90.0,90.0,90.0	90.0,104.2,90.0	82.5,70.5,81.0	90.0,90.0,90.0
Resolution (Å)	50.0-2.8	50.0-2.8	50.0-3.2	29.4-3.58
R _{sym}	8.3 (68.9)	8.9 (48.2)	25.3 (90.6)	21.1 (104.8)
I / σI	11.7 (1.24)	14.5 (1.4)	3.2 (0.8)	9.97 (2.18)
Reflections (tot/uni)	160,066/62,002	233,950/50533	148,912/70,659	86,432/13,100
Completeness (%)	70.8 (72.0)	89.0 (33.6)	92.3 (74.6)	98.6 (93.0)
Redundancy	2.6 (2.3)	4.6 (2.9)	2.1 (1.8)	-
CC(1/2)	49.9	79.8	32.2	99.5
R _{pim}	5.8 (52.1)	4.5 (27.1)	22.2 (85.7)	-
Refinement				
Resolution (Å)	10.0-2.9	15.0-2.82	50.0-3.5	15-3.58
No. reflections	53,190	40,826	47,550	12,826
R _{work} / R _{free} *	24.1/29.5	23.8/29.0	28.8/33.0	25.3/31.1
R _{free} percentile/ Total entries	14.5/2792	48.6/3177	14.5/1159	31.4/1163
Ramachandran favored/allowed/outliers	92.0/8.0/0.0	89.0/9.0/2.0	91.9/7.7/0.4	93.0/7.0/0.0
B-Factor				
Protein/ion/water	65.6/0.0/-	69.0/0.0/-	48.7/0.0/-	105.4/0.0/-

R.m.s deviations				
Bond lengths (Å)	0.003	0.003	0.002	0.002
Bond angles (°)	0.743	0.715	0.610	0.584

Values in parentheses are for highest-resolution shells.

* R_{free} was calculated using ~5% randomly selected reflections.

Table 6. Crystallographic Data Collection and Refinement Statistics (rhMHRPZ Fabs)

	Z100	Z103	Z104	Z107	Z119
Crystallization condition	22.5% PEG 4K, 22.5% IPA, 0.1M Na Citrate (pH5.6)	20.0% PEG 4K, 0.2M NaAc, 0.1M Na Citrate (pH5.6)	26% PEG 8K, 0.2M ZnAc, 0.1M Tris-HCl (pH8.5)	23.5% PEG 4K, 0.2 M $(\text{NH}_4)_2\text{SO}_4$	18% PEG 8K, 0.2M CaAc hydrate, 0.1M Na Cacodylate trihydrate (pH6.5)
Data collection					
Space group	$P2_12_12_1$	$C222_1$	$P3_22_1$	$P2_12_12_1$	$P12_11$
Cell dimensions					
a,b,c (Å)	56.0,71.2,114.2	71.5,80.1,175.5	71.7,71.7,159.9	41.2,118.7,119.5	70.2,71.0,103.7
α,β,γ (°)	90.0,90.0,90.0	90.0,90.0,90.0	90.0,90.0,120.0	90.0,90.0,90.0	90.0,90.0,102.4
Resolution (Å)	50.0-2.19	19.7-1.87	50.0-2.48	50.0-2.1	50.0-1.67
R_{sym}^a	12.7 (59.2)	5.2 (43.7)	19.4 (96.0)	12.3 (62.9)	6.2 (111.8)
$I / \sigma I$	7.6 (1.8)	11.9 (1.9)	1.3 (9.2)	9.4 (1.1)	21.2 (1.0)
Reflections (tot/uni)	91,755/22,274	91,481/37,647	102,754/17,381	129,620/32,733	423,438/114,507
Completeness (%)	92.5 (83.5)	90.5 (92.1)	98.4 (89.1)	93.2 (84.1)	99.3 (97.4)
Redundancy	4.1 (3.5)	2.4 (2.4)	5.9 (3.9)	4.0 (2.5)	3.7 (3.2)
CC(1/2)	98.8	99.8	46.1	54.3	35.4
R_{pim}	9.6 (53.1)	4.3 (34.5)	8.2 (52.7)	6.5 (46.0)	3.7 (74.1)
Refinement					
Resolution (Å)	45.0-2.19	15.0-1.87	10.0-2.55	15.0-2.10	35.0-1.67
No. reflections	20,504	37,560	14,291	32,599	106,010
$R_{\text{work}} / R_{\text{free}}^*$	20.7/24.8	16.4/19.7	21.7/27.1	17.9/20.8	18.2/19.8
Ramachandran favored/allowed/outliers	95.0/3.0/2.0	96.3/3.0/0.7	96.5/3.0/0.5	96.6/2.94/0.45	95.2/4.2/0.6
B-Factor Protein	39.5	36.5	39.8	35.2	20.9

R.m.s deviations	0.003	0.006	0.009	0.007	0.003
Bond lengths (Å)					
Bond angles (°)	0.626	0.862	0.611	1.210	0.734

[00219] All diffraction data were processed with the HKL2000 suite. Diffraction data for all ZikaE complexes was anisotropic and data were corrected using the UCLA Diffraction Anisotropy Server (Strong et al., 2006). Diffraction resolution of the structures are reported as the highest resolution shell with greater than 69% completeness and an $I/\sigma I$ of 1.0 or higher. Structures were solved by molecular replacement using PHASER, and iterative model building and refinement were performed in COOT, and Phenix or BUSTER, respectively. Prior to refinement, a cross validation (R_{free}) test set consisting of 5% of the reflections was selected and used to assess the model accuracy throughout the refinement process. For rhMZ103-A Fab, the heavy chain of 4FQQ and light chain of 2J6E PDBs were used as a search model. For all other Fab structures, rhMZ103-A was used as a search model, with ZikaE from PDB 5JHL used as the search model for ZikaE in the complex structures.

[00220] The Ramachandran plot as determined by MOLPROBITY showed 91-97% of all residues in favored regions and 97-99% of all residues in allowed regions. Interactive surfaces were analyzed using PISA (www.ebi.ac.uk/pdbe/pisa/). Structure figures were prepared using PyMOL (The PyMOL Molecular Graphics System (DeLano Scientific)).

Example 3. Crystallization methods for human antibodies

[00221] Crystallization: All proteins were crystallized by hanging-drop vapor diffusion at 273 K. MZ1 Fab (~7.5 mg ml⁻¹), MZ4 Fab (~6.5 mg ml⁻¹), MZ1 Fab + Zika E glycoprotein (~6.0 mg ml⁻¹), MZ4 Fab + Zika E glycoprotein (~6.8 mg ml⁻¹) were screened for crystallization using a set of 1200 conditions using an Art Robbins Gryphon crystallization robot and crystal drops were observed daily using a Jan Scientific UVEX-PS with automated UV and brightfield plate scanning. Initial crystal growth conditions were optimized manually and crystals used for data collection grew as follows. MZ1 Fab crystals were grown in 0.2 M ammonium sulfate, 0.1 M sodium acetate trihydrate (pH 4.6) and 25% (w/v) polyethylene glycol 4,000. Crystals of MZ4 Fab were grown in 0.2 M ammonium sulfate, 0.1 M HEPES (pH 7.5) and 25% (w/v) polyethylene glycol 3,350. Crystals of MZ1-ZikaE complex were grown in 0.1 M magnesium chloride, 0.1 M imidazole (pH 6.5), 0.1 M MES monohydrate (pH 6.5), 20% (v/v) ethylene glycol and 10% (w/v)

polyethylene glycol 8000. Crystals of MZ4-ZikaE complex were grown in 1.26 M ammonium sulfate, 0.1 M CHES/NaOH (pH 9.5) and 0.2 M sodium chloride.

[00222] Diffraction data collection and processing: Single crystals were transferred to mother liquor containing 22% glycerol, and cryo-cooled in liquid nitrogen prior to data collection. All diffraction data were collected at Advanced Photon Source, Argonne National Laboratory beamlines. Diffraction data for MZ1 and MZ4 Fabs were collected at beamline 19-ID to a final resolution of 1.95 Å and 2.85 Å, respectively, using a Q315r CCD detector. Diffraction data for MZ1-ZikaE complex were collected at 24-ID-E beamline and measured using a DECTRIS EIGER 16M PIXEL detector to a final resolution of 4.0 Å. Diffraction data for MZ4-ZikaE complex were collected at 19-BM beamline and measured using ADSC Quantum 210r CCD detector to a final resolution of 4.2 Å. Diffraction data for both antibody-E complexes were anisotropic and data were corrected using the UCLA Diffraction Anisotropy Server. Data indexing, integration and scaling were carried out using the HKL2000 suite. Data collection statistics are reported in Table 7.

[00223] Structure solution and refinement: All the structures reported here were solved by molecular replacement using the program Phaser. For MZ1 and MZ4 Fab crystal structures, a hybrid search model was prepared using heavy and light chains from two previously reported crystal structures (heavy chain: PDB code 4FQQ; light chain: PDB code 26JE). Refinement was carried out with Phenix refine with positional, individual isotropic B-factor refinement and TLS. Manual model building was performed in Coot. One protomer of ZikaE (PDB code 5IRE) was used in combination with either the MZ1 or MZ4 Fab structure to find a molecular replacement solution for the antibody-ZikaE complexes. Refinement was carried out using Phenix with positional, global isotropic B-factor refinement and TLS. Manual model building was performed in Coot. The later stages of refinement were performed with release of all non-crystallographic symmetry (NCS) restraints. Structure quality was assessed with MolProbity (Chen et al., 2010). The final refinement statistics for all the structures are presented in Table 7. Structure figures were prepared using PyMOL (The PyMOL Molecular Graphics System (DeLano Scientific)).

Table 7. Crystallographic Data Collection and Refinement Statistics

	MZ1 Fab (PDB: 6MTX)	MZ4 Fab (PDB: 6MTY)	MZ24 Fab (PDB: 6NIS)	MZ1 – ZIKV E complex (PDB: 6NIP)	MZ4 – ZIKV E complex (PDB: 6NIU)
Crystallization conditions	0.2 M ammonium sulfate, 0.1 M sodium acetate trihydrate (pH 4.6) and 25% (w/v) polyethylene glycol 4,000	0.2 M ammonium sulfate, 0.1 M HEPES (pH 7.5), 25% (w/v) polyethylene glycol 3,350	0.1 M Citric acid/NaOH (pH 4.0), 1M LiCl and 20% polyethylene glycol 6,000	0.06 M MgCl ₂ , 0.1M Imidazole MES monohydrate (pH 6.5), 20% Ethylene glycol and 10% polyethylene glycol 8,000	1.26 M ammonium sulfate, 0.1 M CHES/NaOH (pH 9.5) and 0.2 M sodium chloride
Data collection					
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	C 1 2 1	P 1
Cell dimensions					
a,b,c (Å)	59.6,66.8,137.5	60.4,67.6,138.0	63.8,66.7,134.4	417.4, 69.3, 212.6	113.5,136.7,137.4
α,β,γ (°)	90.0,90.0,90.0	90.0,90.0,90.0	90.0,90.0,90.0	90.0, 113.0, 90.0	80.4,65.5,65.8
Resolution (Å)	50.0-1.95 (2.2-2.10, 2.10-2.02, 2.02-1.95)	50.0-2.85 (3.07-2.95, 2.95-2.85)	28.8-2.11	50.0-4.1 (4.42-4.25, 4.25-4.1)	50.0-4.2 (4.52-4.35, 4.35-4.2)
R _{sym} ^a	20.3 (76.2, 58.4, 67.4)	28.6 (69.9, 93.5)	10.4 (73.5)	27.1 (59.4,78.2)	23.9 (34.5,51.5)
I / σI	9.0 (1.4, 2.1, 1.1)	7.4 (1.6, 1.1)	12.8 (2.5)	3.7 (1.6, 1.0)	2.64 (1.8, 1.2)
Reflections (uni/tot)	39,609/198,398	13,145/65,812	33,620/224,335	32,942/77,494	28,644/46,190
Completeness (%)	94.8 (94.2, 85.6, 79.6)	94.4 (84.1, 78.1)	99.7 (98.9)	75.6 (77.1, 77.5)	53.2 (54.5, 54.9)
Redundancy	5.0 (3.3, 2.4, 2.1)	5.0 (3.2, 2.8)	-***	2.4 (2.3, 2.3)	1.6 (1.5, 1.5)
CC(1/2)	(63.0, 52.2, 39.3)**	(42.4, 44.1)**	99.7 (81.5)	(64.1, 39.2)**	(83.3, 69.3)**
R _{pim}	8.1 (34.6, 45.2, 54.4)	12.4 (42.6, 60.8)	-***	19.5 (43.2, 56.7)	23.9 (34.5, 51.5)
Refinement					
Resolution (Å)	15.0-2.00	15.0-2.85	15.0-2.11	15.0-4.16	15.0-4.20
No. reflections	32,475	10,863	33,455	28,024	16,181
R _{work} / R _{free} *	18.1/20.8	24.0/27.9	17.4/19.9	21.1/25.8	29.3/35.8
R _{free} percentile/ Total entries	83.0/2220	22.0/3791	95.0/5449	88.0/1004	14.0/1006
Ramachandran allowed/outliers	100/0.0	100/0.0	100/0.0	100 /0.0	98.5/1.5

B-Factor					
Protein	25.2	55.0	43.4	108.5	118.4
R.m.s deviations					
Bond lengths (Å)	0.003	0.002	0.004	0.002	0.004
Bond angles (°)	0.715	0.596	0.727	0.533	1.041

Values in parentheses are for highest-resolution shells. *Rfree was calculated using ~5% randomly selected reflections. **HKL2000 was used for data reduction and scaling which did not calculate overall CC(1/2) for the listed data sets. ***XDS was used for data reduction and scaling, which did not calculate redundancy and Rpim.

Example 4. Isolation of ZIKV reactive antibodies from a flavivirus naïve ZIKV-infected macaque using whole ZIKV

[00224] Monoclonal antibodies were isolated from a flavi-naïve macaque infected Brazil ZK2015 (ZIKA-BR) previously described in McCracken, 2017.

[00225] To cast a wide net on ZIKV-reactive B cells and, in particular, capture B cells recognizing quaternary structure neutralizing epitopes, a sequential sorting strategy was developed based on whole ZIKV (Figure 1A-B). Unlabeled Zika virions were incubated with peripheral blood mononuclear cells (PBMCs) from a flavivirus naïve ZIKV-infected rhesus macaque at day 14 post-infection where high titers of neutralization were observed. None of the flavi-naïve macaques within this study developed cross-neutralizing antibodies to DENV 1-4 following ZIKV infection, except one animal that had very low levels of detectable neutralizing antibody to DENV-2 only.

Table 8. MN50 titers of flavivirus serologically-naïve NHP pre- and post- ZIKV infection.

	Sample	10U001	10U003	10U021	10U030	10U032	10U036	10U043	10U047	M230
ZIKV	pre-	<10	<10	<10	<10	<10	<10	<10	<10	<10
	day 14	>7290	1867	1655	810	>7290	3622	>7290	>7290	1970
	day 28	N/A	1659	1874	>7290	N/A	>7290	4122	3987	5759
YFV	pre-	<10	<10	<10	<10	<10	<10	<10	<10	<10
	day 14	<10	<10	<10	<10	<10	<10	<10	<10	<10
	day 28	N/A	<10	<10	<10	N/A	<10	<10	<10	<10
DENV-1	pre-	<20	<20	<20	<20	<20	<40	<40	<20	ND
	day 14	<20	<20	<20	<20	<20	<20	<20	<20	<20
	day 28	N/A	<20	<20	<20	N/A	<20	<20	<20	<20

DENV-2	pre-	<20	<20	<20	<20	<20	<40	<40	<20	ND
	day 14	<20	<20	<20	<20	<20	<20	<20	20	<20
	day 28	N/A	<20	<20	<20	N/A	<20	<20	22	<20
DENV-3	pre-	<20	<20	<20	<20	<20	<40	<40	<20	ND
	day 14	<20	<20	<20	<20	<20	<20	<20	<20	<20
	day 28	N/A	<20	<20	<20	N/A	<20	<20	<20	<20
DENV-4	pre-	<20	<20	<20	<20	<20	<40	<40	<20	ND
	day 14	<20	<20	<20	<20	<20	<20	<20	<20	<20
	day 28	N/A	<20	<20	<20	N/A	<20	<20	<20	<20

[00226] ZIKV-reactive activated CD19⁺ B cells (CD19⁺ CD38⁺, CXCR5^{hi}/lo) were identified by secondary staining using 4G2, a fusion loop targeted monoclonal antibody (Figure 1A-B). Antibody heavy and light chain V (D) J gene segments were amplified from single B cells using nested RT-PCR and sequenced. Primers for both IgG and IgM were used to generate a total of 40 matched pairs with the majority being IgG. All mAbs were expressed as rhesus macaque IgG1 and screened for binding to recombinant ZIKV soluble E protein (sE) and ZIKV neutralization. Twenty-three mAbs were found to bind to ZIKV sE, and 11 out of the 23 neutralized ZIKV (Figures 1C, 2A, 14A). Sequence analysis of the antibody variable regions revealed that the 23 ZIKV sE-reactive antibodies belonged to 19 independent clonal families that displayed low levels of somatic hypermutation (SHM) (Figure 1C, Table 9).

Table 9. Analysis of the sequences of NHP antibodies that bind ZIKV soluble E protein.

mAb ID	Heavy chain IgBlast	Identity (%)	D gene	J gene	Light chain IgBlast	Identity (%)	J gene	Ig type
rhMZ100-C	VH3.63	95.9	IGHD1-7*01	IGHJ6*01	VL11.42	98.4	JLx1	IgG
rhMZ101-B	VH3.15	95.6	IGHD1-8*01	IGHJ4*01	VL11.42	99.4	JL6	IgG
rhMZ103-A	VH4.40	94.3	IGHD3-1*01	IGHJ4*01	VL1.27	100	JL3	IgG
rhMZ104-D	VH3.15	99.7	IGHD2-2*01	IGHJ4*01	VL11.42	100	JLx1	IgG
rhMZ105	VH3.31	91.2	IGHD4-2*01	IGHJ6*01	VL11.42	95.8	JL6	IgG
rhMZ106	VH4.26	99.3	IGHD3-2*01	IGHJ2*01	VL5.28	99	JLx1	IgG
rhMZ107-B	VH4.34	100.0	IGHD4-1*01	IGHJ6*01	VL11.42	99.4	JL6	IgG
rhMZ113	VH7.21	82.3	IGHD1-7*01	IGHJ1*01	VL11.42	80.1	JL2	IgM
rhMZ115	VH5.7	82.1	IGHD6-1*01	IGHJ4*01	VL11.42	78.8	JLx1	IgM
rhMZ118	VH3.52	99.7	IGHD6-1*01	IGHJ4*01	VL5.28	92.3	JLx1	IgG
rhMZ119-D	VH5.7	99.3	IGHD6-3*01	IGHJ5-2*02	VL3.4	99.3	JL2	IgG
rhMZ120	VH3.58	95.2	IGHD3-2*01	IGHJ5-1*01	VL5.28	92.3	JLx1	IgG
rhMZ121-A	VH4.34	100.0	IGHD3-2*01	IGHJ4*01	VL3.15	100	JLx1	IgG
rhMZ123-A	VH5.7	96.9	IGHD6-1*01	IGHJ4*01	VL2.44	99	JL1	IgG
rhMZ124-D	VH4.34	95.3	IGHD6-3*01	IGHJ4*01	VL11.42	94.6	JL2	IgG
rhMZ125	VH4.34	96.3	IGHD3-1*01	IGHJ4*01	VL2.13	90.2	JL1	IgG
rhMZ129	VH5.7	94.9	IGHD2-2*01	IGHJ5-1*01	VL11.42	99.0	JL1	IgM
rhMZ130	VH3.58	95.5	IGHD6-1*01	IGHJ4*01	VL11.42	99.7	JL2	IgM
rhMZ132	VH7.21	91.8	IGHD2-2*01	IGHJ4*01	VL2.51	100.0	JL2	IgM
rhMZ133-C	VH3.30	99.0	IGHD2-2*02	IGHJ6*01	VL2.44	98.6	JLx1	IgM
rhMZ134-B	VH3.15	99.7	IGHD2-3*01	IGHJ6*01	VL3.46	97.2	JL3	IgG
rhMZ136	VH3.52	99.0	IGHD3-2*01	IGHJ4*01	VL11.42	100.0	JL3	IgM
rhMZ140	VH3.58	95.5	IGHD1-8*01	IGHJ4*01	VL11.42	99.7	JLx1	IgM

[00227] The observation of low somatically hypermutated antibodies were in agreement with previous reports of ZIKV infection in flavivirus-naïve humans (Stettler K 2016, Yu L 2017, Rogers T 2017). A few had unexpectedly high SHM levels over 15%, in particular some of the IgM clones, which is likely the result of incorrect V gene assignment in IgBlast due to the poor coverage of the rhesus V gene database. ZIKV sE-reactive antibodies showed great diversity in VH gene usage and CDRH3 length, ranging from 10

to 30 residues, with the longest CDRH3 lengths correlating with the ability to neutralize ZIKV (Figure 1C). Interestingly, VL gene usage was strongly biased toward VL11.42, which was present in a majority of the clones. As 90% of the neutralizers derived from IgG-expressing B cells, it is worth noting that only one IgM-derived antibody (rhMZ133-C) demonstrated neutralization activity, when expressed as IgG1 (Figure 1C).

Example 5. Neutralizing and binding characteristics define four ZIKV-specific epitopes targeted on the E glycoprotein

[00228] Eleven mAbs were identified as capable of ZIKV neutralization by screening in a micro-neutralization (MN) assay using Vero-produced ZIKV and Vero cells as target cells (Figure 1C, 14A). Although neutralizers showed significantly higher binding responses than non-neutralizers in the BLI assay ($P=0.0005$, Mann Whitney test), binding and neutralizing activities did not significantly correlate among the mAbs that neutralized (Figure 14 B-C). While most mAbs displayed modest micro-neutralization activity against a Puerto Rican strain with IC_{50} in the $\mu\text{g/ml}$ range, two mAbs, rhMZ133-C and rhMZ134-B (short for rhesus macaque MHRP Zika 133-C and 134-B, respectively), demonstrated greater potencies matching or surpassing the EDE1-C8 control, a potent DENV cross-neutralizing antibody (Dejnirattisai, 2015; Barba-Spaeth, 2016) (Figure 2A-B). Of note, rhMZ134 was also on par with Z004, one of the most potent ZIKV neutralizing antibodies reported to date (Robbiani 2017) with IC_{50} s of 3.8 ng/mL and 1.4 ng/mL, respectively (Figure 2A-B). To further confirm the observed neutralization properties in an assay that would better reflect the initial ZIKV infection events, we employed a flow cytometry-based assay (FlowNT50) measuring single cell infection of human monocytes by a Brazilian strain of ZIKV produced in mosquito cells. Comparable to the results of the Vero-based MN assays, the majority of antibodies performed similarly in the FlowNT50 assay (Figure 2B). rhMZ134-B was again the best neutralizing antibody with an IC_{50} of 22 ng/ml (Figure 2B, 15A), rhMZ119 was the second most potent antibody with an IC_{50} of 31ng/ml, while rhMZ133-C did not perform as well in this FlowNT50 neutralization assay (Figure 2B, 15A). To compare to other mAbs previously published in the literature, a third neutralization assay was performed using the plaque reduction neutralization test (PRNT) with the top 5 neutralizers identified by MN or FlowNT50 neutralization assays, where similar trends were obtained and rhMZ134 potency was again similar to Z004 (Figure 14B). When tested against non-American ZIKV strains, rhMZ134-B demonstrated broad neutralization activities against Ugandan and Thai strains (Figure 14C). Finally, to investigate whether the isolated neutralizing antibodies recognize ZIKV-specific or flavivirus cross-reactive epitopes, we performed a neutralization screen against a panel of 7 flaviviruses, including all 4 DENV serotypes, JEV, WNV and YFV. None of the isolated antibodies,

displayed neutralization activity in this assay even when starting at a high concentration ($\geq 100\mu\text{g/ml}$), indicating that all antibodies neutralized ZIKV specifically (Figure 2B). Consistent with these results, weak to no binding to DENV-2 sE or whole DENV-2 was detected by BLI and ELISA, respectively, confirming the mAb specificity for ZIKV (Figure 14).

[00229] To gain insight into the epitopes targeted with this set of neutralizing mAbs, the mAbs were titrated in binding assays using ELISA to monomeric sE glycoprotein and whole ZIKV. Binding to an immobilized ZIKV sE was performed in conditions where quaternary antibodies, such as EDE1-C8, are poorly reactive (Rouvinski 2017) while antibodies to the monomeric E protein, such as the FLE-directed mAb 2A10G6, bound robustly (Figure 15D left). However, since whole ZIKV displays both monomeric and quaternary epitopes, both EDE1-C8 and 2A10G6 bound robustly in the ELISA (Figure 15D right). When testing the isolated ZIKV-specific macaque mAbs, the majority of them did not demonstrate detectable binding monomeric ZIKV sE in ELISA (Figure 2C). Some reactivity was observed for rhMZ104-D, rhMZ119-D and rhMZ124-D, which were also identified as the strongest binders by BLI (Figure 2B-C). Next, binding was assessed in the context of the native viral particle by capture ELISA using whole purified ZIKV. All neutralizing antibodies bound to the whole ZIKV, suggesting that they recognized quaternary epitopes (Figure 2D). This was not surprising, as whole ZIKV was used to isolate antigen-specific B cells (Figures 1B). The differences in binding observed between the mAbs mirrored binding responses to sE obtained in the BLI assay (Figure 14A), suggesting that both monomeric and E dimer epitopes are available using the BLI binding assay. Interestingly, rhMZ104-D, rhMZ119-D and rhMZ124-D were again the strongest binders, reaching saturation at lower concentrations than their counterparts. In contrast, the most potent neutralizer, rhMZ134-B, bound ZIKV rather poorly and appeared to reach saturation at much lower optical density, suggesting that fewer rhMZ134-B epitopes might be present on ZIKV. To compare these binding responses to control mAbs 2A10G6 and EDE1-C8 where each had different secondary antibodies, we calculated ZIKV sE/whole virus binding ratios to assess the ability of these mAbs to recognize quaternary epitopes relative to the FLE control antibody (2A10G6), in which a ratio of 1 reflected a strong affinity for monomeric sE glycoprotein (Figure 2E). In contrast, the binding of the EDE1-C8 control, with a ratio of 0.1, was strongly biased towards whole virus, consistent with its quaternary epitope specificity. Intermediate binding ratios of approximately 0.2-0.4 were obtained for rhMZ104-D, rhMZ119-D and rhMZ124-D, which indicated that they were not as potent as the FLE control antibody in engaging monomeric sE. This data suggested that their epitopes may include a

quaternary component. All the other neutralizing antibodies had a ratio less than 0.1 (Figure 2E), indicating that they exclusively targeted quaternary epitopes found on the whole ZIKV.

[00230] Next, binding competition experiments were performed to delineate the antigenic sites targeted by these neutralizing antibodies (Figure 2F). Control antibodies directed against previously identified sites of vulnerability such as the fusion loop epitope (FLE, mAb 2A10G6), domain I/II (DI/II, mAb Z3L1), the E-dimer epitope-1 (EDE1, mAb EDE1-C8) and domain III (DIII, mAb Z004) were used in competition assays for real-time binding to ZIKV sE. When the eleven ZIKV-specific macaque neutralizing mAbs were used in these competition assays, their pattern of competition fell into four groups with overlapping, but discrete features (Figure 2F). As such, we labeled these mAbs accordingly with the letter of the group within the nomenclature of each neutralizing mAb (Figure 1C, 14A). Antibodies within group A was competed by all 4 control mAbs to varying degrees. Group B resembled the competition profile obtained with the EDE1 control, suggesting that these antibodies might represent new ZIKV-specific EDE members. Group C was competed by EDE1 and DIII mAbs, whereas group D was only sensitive to EDE1 mAb competition. Group C and D antibodies were of particular interest as their unique competition profiles indicated that they might target uncharacterized neutralizing epitopes. All together, these eleven neutralizing mAbs defined 4 ZIKV-specific epitopes that targeted quaternary epitopes on the E glycoprotein surface.

Example 6. Crystal structure of ZIKV-specific EDE antibody rhMZ107-B Fab in complex with ZikaE glycoprotein

[00231] To understand the structural basis for the recognition of the ZIKV-specific EDE-like antibodies, crystal structures of representative mAbs from each group were determined alone in and complex with the Zika sE glycoprotein. From antigenic specificity group B, the crystal structure of rhMZ107-B alone (2.1 Å resolution), and in complex with Zika sE glycoprotein at 3.2 Å resolution were determined (Figures 3A-D; Figure 16; Tables 9-10). Within the asymmetric unit, four rhMZ107-B, and four Zika sE glycoproteins were observed, with each Fv binding an epitope that spans three protomers with a contact region of X, X, and X Å² buried surface area (BSA), within the site of recognition. The largest antibody contact area was on the DII of one protomer, followed by recognition of the DIII of a second protomer, adjacent to the glycan-154, with minor contacts on the DI of a third protomer (Figure 3A-B). The heavy chain and light chain of the antibody shared approximately the same BSA (X Å heavy chain; X Å light chain). In the context of the mature ZIKV (PDB: 5IRE), rhMZ107-B was able to bind 180 sites with

no structural rearrangement required of the E glycoproteins (Figure 3C). Antibody rhMZ107-B recognition of ZikaE used 100 % of germline-encoded residues in both the HV, and HD,HJ encoded regions while in the light chain a single complementarity-determining region CDR L3 residue glycine94 which made a single main chain contact was mutated from the VL11.42 gene-encoded serine (Figure 3D).

[00232] Antibody rhMZ107-B recognized the DII of Zika E utilizing CDRs H2, H3, L1, L2 and L3 with the area of recognition focused on the B strand (residues X-Y) located in the center of the epitope. Additional recognition of the DIII from an adjacent protomer was through the light chain framework 3 (FR3), while recognition of the DI from the third protomer was facilitated by CDR L1, and L2 (Figures 3E-F). Features of the antibody recognition were reminiscent of EDE1-C8 recognition, which had broad reactivity against ZIKV, and multiple strains of DENV. Interestingly group B mAbs inclusive of rhMZ107-B were ZIKV-specific (Figure 3G). Analysis of the epitope-contact residues shows similar contact residues (Figure 3, and Tables 9-10), with only the interaction with residues adjacent to glycan-67 (DENV) showing major differences (Figure 3H). In addition to rhMZ107-B, we also screened the group B mAb rhMZ134-B for recognition of Zika E glycoprotein by shotgun alanine/serine-scanning mutagenesis and residues W101, F108, V257, G259, K316, M375 were highlighted as contact residues similar to rhMZ107-B, as well as other EDE1-like antibodies.

Table 10. rhMZ107 interface with E glycoprotein of Zika virus.

rhMZ107	Zika E glycoprotein	Distance (Å)
Hydrogen bonds		
H:TYR 34[OH]	B:MET 68[O]	3.87
H:SER 56[OG]	B:SER 66[OG]	3.19
H:SER 56[OG]	B:ASP 67[N]	3.76
H:VAL 98[O]	B:SER 70[N]	2.27
H:TYR 100A[OH]	B:CYS 105[SG]	3.22
H:TYR 100A[OH]	B:GLY 104[O]	3.20
H:TYR 100E[OH]	B:SER 70[O]	3.55
L:ASN 32[ND2]	B:ASP 71[OD1]	3.73
L:SER 52[OG]	B:GLN 77[NE2]	3.41
L:ASP 54A[OD1]	B:CYS 74[N]	3.42
L:ASP 54A[OD2]	B:CYS 74[N]	3.15
L:ASP 54A[OD1]	B:CYS 105[SG]	3.04
L:LYS 54B[N]	B:GLY 104[O]	3.86
L:LYS 66[O]	F:ASN 52[ND2]	2.68
L:SER 67[N]	F:ASN 52[OD1]	3.76
L:SER 67[OG]	F:ASN 52[OD1]	3.84

Table 11. Buried surface area of rhMZ107 antibody in complex with Zika virus.

Residues that form hydrogen bonds and salt bridges are indicated.

Residue	Bond type	Accessible Surface Area (Å ²)	Buried Surface Area (Å ²)
H:SER30		75.7	11.8
H:TYR34	H	35.2	33.7
H:SER52		5.9	2.3
H:SER53		74.1	14.4
H:SER54		84.8	30.9
H:SER56	H	33.4	19.4
H:TYR58		118.0	46.8
H:ARG97		71.9	14.1
H:VAL98	H	83.1	46.3
H:GLY99		62.5	49.5
H:SER100		63.0	46.8
H:TYR100A	H	155.4	127.3
H:PRO100B		70.2	20.0
H:TYR100C		151.5	1.5

H:TYR100E	H	64.4	28.8
L:SER27		118.4	1.7
L:ASP27A		88.3	29.2
L:LEU27B		24.5	1.8
L:SER27C		68.3	33.7
L:GLY29		32.2	20.4
L:SER30		71.4	40.5
L:LYS31		36.1	11.4
L:ASN32	H	49.8	35.9
L:TYR49		49.8	4.5
L:TYR51		64.9	46.6
L:SER52	H	36.6	13.7
L:ASP53		89.3	11.7
L:SER54		103.8	82.4
L:ASP54A	H	73.1	55.5
L:LYS54B	H	86.5	24.7
L:GLN54C		122.9	34.9
L:ASN60		135.7	49.3
L:LYS66	H	42.9	16.6
L:GLU66A		89.4	9.5
L:THR66B		89.7	53.0
L:SER67	H	115.3	38.8
L:TYR91		80.7	14.5
L:ASP92		42.1	14.0
L:GLY93		72.9	37.4
L:SER94		98.2	21.2

Zika virus E glycoprotein

Residue	Bond type	Accessible Surface Area (Å²)	Buried Surface Area (Å²)
B:ILE65		36.1	3.1
B:SER66	H	64.0	30.6
B:ASP67	H	87.0	60.8
B:MET68	H	130.1	87.8
B:ALA69		49.9	31.0
B:SER70	H	61.6	59.5
B:ASP71	H	23.7	23.1
B:SER72		54.4	54.4
B:ARG73		103.1	74.3
B:CYS74	H	17.9	17.9
B:PRO75		18.5	1.8
B:GLN77	H	122.9	67.6

B:LEU82		41.0	11.1
B:ASP83		96.9	43.6
B:LYS84		95.7	36.0
B:ARG99		56.7	31.0
B:TRP101		187.2	1.0
B:GLY102		77.9	4.9
B:ASN103		67.7	3.0
B:ASN103		67.7	17.8
B:GLY104	H	57.9	57.9
B:CYS105	H	55.5	41.3
B:GLY106		45.5	15.6
B:LEU107		102.2	10.3
B:LEU113		5.0	3.4
B:THR115		6.1	0.2
B:LYS118		78.8	3.2
B:LYS251		61.8	37.8
B:ARG252		53.2	14.5
B:GLN253		16.5	1.3
F:ASN52	H	140.9	82.7
F:MET53		38.5	20.6
F:ALA54		70.1	38.1
F:GLU55		76.1	8.0
F:GLN131		88.3	13.1
F:ASN134		79.3	10.9
F:GLY228		64.2	9.1
F:ALA229		88.7	34.4
F:ASP230		115.8	46.9
F:GLY232		87.4	12.1
F:ALA280		93.1	28.6
C:HIS148		163.5	0.6
C:ASP278		130.0	10.7
C:LYS316		120.1	4.2
C:GLN331		39.8	5.1
C:LYS373		88.8	43.5

Example 7. Crystal structure of inter-dimer-epitope antibody rhMZ100-C Fab in complex with Zika sE glycoprotein

[00233] To understand the structural basis for the recognition of the ZIKV-specific antibodies from antigenic specificity group C, the crystal structure of rhMZ100-C was determined alone (2.1 Å

resolution), and in complex with ZikaE glycoprotein at 2.8 Å resolution (Figures 5A-D). Within the asymmetric unit, two rhMZ100-C Fab molecules, and four ZikaE glycoprotein protomers were observed, with each Fab binding to a single protomer with a contact region of X Å² BSA through the light chain and X Å² from the heavy chain focused on the DI/DII (Figures 5A-B). We then modeled rhMZ100-C binding in the context of the mature ZIKV and were able to identify additional significant contacts in the DI/DII of an adjacent Zika E protomer increasing the heavy chain contact region to X Å². Since this epitope spanned across the center of two dimers, within the context of the ZIKV, only 60 antibody binding sites were accessible since only half of the epitope would be available on the dimers that form the raft-raft interface (Figure 5C). Antibody rhMZ100-C recognition of ZikaE uses X0 % of germline-encoded residues in the heavy chain and 100% in the light chain (Figure 5D).

[00234] MAb rhMZ100-C recognized the DII of ZikaE utilizing all CDRs with the area of recognition demarcated on either side by the ZikaE B strand (residues X-Y) (Figure 5E). Additional recognition of chain A, and C form the center of the epitope in an almost perfect mirror image (Figures 3E, and 3F). Features of the antibody recognition are reminiscent of the recently described Z117 antibody, which is highly potent against ZIKV (Figure 5G). However, there were distinct differences between the sites of recognition, with Z117 recognizing an inter-dimer epitope that is less centered across the inter-dimer interface with a predominant recognition of one protomer (X Å²) as compared to rhMZ100-C. Analysis of the epitope-contact residues showed dissimilar contact residues, with the rhMZ100-C epitope centered over the glycan-67 (DENV) site.

Table 12: rhMZ100 interface with E glycoprotein of Zika virus.

	rhMZ100	Zika E glycoprotein	Distance (Å)
Hydrogen bonds			
	H:GLY 100[O]	G:LYS 84[NZ]	2.93
	L:LYS 31[NZ]	G:ASP 83[OD1]	3.33
	L:TYR 51[OH]	G:TYR 90[OH]	3.86
Salt bridges			
	L:LYS 31[NZ]	G:ASP 83[OD2]	3.16
	L:LYS 31[NZ]	G:ASP 83[OD1]	3.33

Table 13: Buried surface area of rhMZ100 antibody in complex with Zika virus.

Residues that form hydrogen bonds and salt bridges are indicated.

rhMZ100 antibody			
Residue	Bond type	Accessible Surface Area (Å²)	Buried Surface Area (Å²)
H:THR97		110.71	5.35
H:ALA98		28.43	2.21
H:PRO99		145.03	19.55
H:GLY100	H	83.37	58.14
H:ARG100A		153.22	2.76
H:ASN100B		106.68	14.13
L:ASP27A		87.17	31.91
L:LEU27B		40.61	35.04
L:ASN27C		85.15	10.8
L:GLY29		37.97	6.77
L:THR30		88.93	74
L:LYS31	HS	15.47	15.13
L:ASN32		54.93	38.35
L:TYR49		45.38	6.83
L:TYR51	H	75.59	65.13
L:SER54		114.79	8.79
L:ASP54A		58.96	26.91
L:TYR91		76.02	20.71
L:ASP92		37.98	11.04
L:ASN93		136.93	78.85
L:SER94		99.1	1.31
Zika virus PF E glycoprotein			
Residue	Bond type	Accessible Surface Area (Å²)	Buried Surface Area (Å²)
G:ASP 67		94.31	22.06
G:ALA 69		45.29	17.41
G:SER 70		34.10	1.14
G:ASP 71		74.65	47.56
G:ARG 73		88.86	45.02
G:GLY 78		57.19	1.68
G:GLU 79		117.73	18.85
G:ALA 80		6.97	0.25
G:TYR 81		127.61	97.69
G:LEU 82		34.06	28.71
G:ASP 83	HS	97.14	88.4
G:LYS 84	H	89.81	89.81
G:SER 86		98.91	21.89
G:ASP 87		36.12	30.97

G:THR 88		74.75	11.40
G:GLN 89		61.77	0.88
G:TYR 90	H	32.29	15.63
G:LYS 118		64.63	11.93

H: Hydrogen bond
S: Salt bridge

Example 8. Structure of inter-dimer-epitope antibody rhMZ104-D in complex with ZikaE glycoprotein.

[00235] To understand the structural basis for the recognition of the ZIKV-specific antibodies from antigenic specificity group D, we determined the crystal structures of rhMZ119-D (1.7 Å resolution), and rhMZ104-D alone (2.5 Å resolution), and in complex with ZikaE glycoprotein at 3.2 Å resolution (Figures 7A-D; Figure 18). Within the asymmetric unit, one rhMZ104-C Fab, and one Fv molecule, and two ZikaE glycoprotein protomers were observed, with each rhMZ100-C binding to the DII region of a single protomer with a contact region of X Å² BSA through the light chain and X Å² from the heavy chain (Figure 7A-B). We then modeled rhMZ104-D recognition in the context of the mature ZIKV and were able to identify significant additional heavy chain contacts in the DI/DII of two adjacent ZikaE protomers increasing the heavy chain contact region to X Å². Since this epitope spanned across the center of two dimers similar to rhMZ100-C in the context of the ZIKV (Figure 5C), only 60 antibody binding sites were accessible since only half of the epitope would be available on the dimers that form the raft-raft interface (Figure 5C, 7C). Antibody rhMZ104-D recognition of the crystallographic ZikaE protomer utilized 100 % germline-encoded residues in the heavy chain and 100% in the light chain (Figure 7D).

[00236] Antibody rhMZ104-D recognized the DII of ZikaE utilizing all light chain CDRs and CDRH3 with the ZikaE B strand (residues X-Y) the major antigen contact region for the light chain, alongside a continuous stretch of residues from position 77 to position 90 that is recognized by both heavy and light chains (Figures 7E and 7F). Comparison of the rhMZ104-B recognition of ZikaE compared to the most related flaviviruses (DENV1-4) highlighted the epitope recognition as focused on a non-conserved region of the glycoprotein distal to the conserved epitope of EDE1-C8 (Figure 7G). In addition to the structural data describing the rhMZ104-D epitope, we also screened all group D antibodies rhMZ104-D, rhMZ119-D, and rhMZ124-D for recognition of ZikaE glycoprotein by shotgun alanine/serine-scanning mutagenesis (Figures 7H and 17). The scanning mutations identified 3 – 6 residues per antibody that significantly altered ZikaE recognition. In all cases residue D71 was highlighted as a significant contact residue, and D67, D83, D84, and G228 residues knocked out binding of two of the three group D mAbs (Figures 7G, and 8G).

Table 14. rhMZ104 interface with E glycoprotein of Zika virus.

	rhMZ104	Zika E glycoprotein	Distance (Å)
Hydrogen bonds			
	H:CYS 100C[O]	G:THR 88[OG1]	3.83
	H:CYS 100C[O]	G:THR 88[N]	3.47
	H:CYS 100C[N]	G:SER 86[O]	3.71
	H:CYS 100C[SG]	G:SER 86[O]	3.75
	H:ALA 100E[O]	G:LYS 84[NZ]	3.11
	L:LYS 31[NZ]	G:ASP 83[OD2]	2.36
	L:ASN 32[N]	G:ASP 83[OD2]	3.42
	L:TYR 49[OH]	G:LYS 84[NZ]	3.25
	L:TYR 51[OH]	G:TYR 90[OH]	3.65
	L:TYR 51[OH]	G:ASP 67[OD1]	3.05
	L:ASP 54A[OD2]	G:LYS 84[NZ]	2.99
	L:ASP 92[OD1]	G:ARG 73[NH2]	2.88
Salt bridges			
	L:LYS 31[NZ]	G:ASP 83[OD1]	3.61
	L:LYS 31[NZ]	G:ASP 83[OD2]	2.36
	L:ASP 92[OD1]	G:ARG 73[NH2]	2.88
	L:ASP 54A[OD2]	G:LYS 84[NZ]	2.99

Table 15. Buried surface area of rhMZ104 antibody in complex with Zika virus.

Residues that form hydrogen bonds and salt bridges are indicated			
Residue	Bond type	Accessible Surface Area (Å ²)	Buried Surface Area (Å ²)
H:CYS 98		31.01	1.48
H:GLY 100A		69.80	17.57
H:VAL 100B		102.16	34.22
H:CYS 100C	H	34.52	23.91
H:TYR 100D		188.90	54.79
H:ALA 100E	H	84.02	45.07
H:GLY 100F		45.58	34.24
H:LYS 100H		129.48	51.60
L:ASP 27A		97.90	14.80
L:LEU 27B		19.54	6.52
L:GLY 29		44.60	4.17
L:SER 30		82.48	70.48
L:LYS 31	HS	31.90	20.95
L:ASN 32	H	62.93	37.50
L:TYR 34		48.02	2.81
L:TYR 49	H	41.10	4.00
L:TYR 51	H	73.62	65.27
L:SER 52		35.73	2.13
L:SER 54		112.56	9.85
L:ASP 54A	HS	55.04	19.34
L:TYR 91		103.57	38.80
L:ASP 92	HS	30.15	25.61
L:SER 93		103.98	46.23
L:SER 94		90.96	3.53
E glycoprotein Residue	Bond type	Accessible Surface Area (Å ²)	Buried Surface Area (Å ²)
G:ASP 67	H	82.67	29.98
G:ALA 69		50.73	20.85
G:SER 70		34.97	2.33
G:ASP 71		29.65	5.28
G:ARG 73	HS	81.47	46.65
G:GLN 77		117.60	2.02
G:GLU 79		124.94	10.74
G:TYR 81		107.16	96.27
G:LEU 82		28.22	20.06

G:ASP 83	HS	108.11	108.11
G:LYS 84	HS	92.55	92.55
G:GLN 85		62.36	0.49
G:SER 86	H	102.26	84.60
G:ASP 87		33.78	28.64
G:THR 88	H	84.19	58.51
G:GLN 89		59.68	21.23
G:TYR 90	H	23.54	8.13
G:LYS 118		67.11	14.60
G:GLY 232		75.04	0.61
G:THR 233		89.36	18.92
G:HIS 235		86.03	3.17

H: Hydrogen
bond,
S: Salt bridge

Example 9. Neutralizing antibodies afford complete or partial protection from ZIKV replication in mice
[00237] Passive protection experiments were conducted in mice to determine whether representative neutralizing antibodies would confer protection in vivo. Six neutralizing antibodies of various potencies were infused to groups of naïve Balb/c mice (N=5/group) at a single dose (200µg). Mice were then challenged with 10⁵ viral particles (10² plaque-forming units) of ZIKV-BR intravenously and viral replication was monitored using a PCR-based assay, as previously described (Larocca R 2016) (Figure 13A). The top neutralizers, rhMZ134-B, rhMZ133-C and rhMZ119-D, conferred total protection from ZIKV as compared with the SHAM-infused control mice where ZIKV viral load peaked at day 3 post-challenge (Figure 13B). Partial protection was also observed with the less potent neutralizing antibodies (IC50s within the 1-3 µg/mL range in FlowNT50 assays), with one (rhMZ103-A, rhMZ100-C) or two (rhMZ107-B) mice out of 5 showing detectable viremia.

Example 10. Prevalence and mapping of ZIKV-specific humoral immune responses.

[00238] Having identified and characterized new ZIKV-specific neutralizing antibodies, we next evaluated the prevalence of these antibodies in other ZIKV-infected rhesus macaques and humans. To this end, we performed binding competition experiments to the ZIKV sE protein between plasma from infected donors and representative antibodies from each group. Remarkably, plasma response was almost completely ablated in the presence of the group A - D antibodies, as compared to control (non-flavivirus-exposed) sera in humans, suggesting that antibodies with similar specificities are commonly elicited during the course of natural ZIKV infection. Interestingly, most of the human plasma samples were also

found to cross-react with DENV, suggesting that these sites are targeted during ZIKV-infection even with prior DENV exposure or other cross reactive antibodies are elicited during ZIKV-infection. Similar observations were also seen with plasma from other ZIKV-infected rhesus macaques at day 14 post-infection (Figure 11B).

[00239] Despite the E glycoprotein sequence differences between ZIKV, and DENV1-4, many of the cross-protomer epitopes targeted by the ZIKV-specific mAbs curiously overlapped with the glycan at position 67 present on DENV E glycoprotein. Therefore, we also wanted to assess the role of the glycan at position 67 on antigenic recognition of ZIKV compared to DENV with the hypothesis that the glycan may cause interference with ZIKV-specific epitope recognition. In the case of DENV, and other flaviviruses, e.g. X and Y, this glycan is highly prevalent even in large sequence datasets. We assessed our full set of eleven NHP mAbs for neutralization of wild-type ZIKV (H/PF/2013), and an E mutant (D67N, A69T) lacking the glycan at position 67. In all cases, the introduction of the glycan at position 67 resulted in loss of neutralization ranging from a two-fold reduction for rhMZ133-C, five-fold reduction for rhMZ103-B, ten-fold for rhMZ134-B, to complete ablation of neutralization for the remaining eight of the eleven antibodies (Figure 12B). This data suggests that the glycan at position 67 on DENV interferes with antigenic recognition of these mAbs, which in turn yields ZIKV-specificity. To analyze the structural differences in immune responses to ZIKV, compared to DENV and other flaviviruses, we mapped these binding epitopes onto the viral raft in comparison with other previously identified mAbs from DENV-primed individuals that cross-neutralized both DENV and ZIKV. These mAbs were identified as recognizing the ZIKV E glycoprotein DII and DIII regions in areas distal to viral glycan sites (Figure 12C). However, in the case of ZIKV-infection without prior flavivirus exposure, the antibody response can be seen to target the full length of the Zika E glycoprotein as evident by the newly identified neutralization epitopes described within this study.

Human antibodies

[00240] Monoclonal antibodies were isolated from a flavi-experienced participant of the ZPIV Z001 BIDMC Phase 1 vaccine clinical trial previously described in Modjarrad et al., Lancet 391:563-571, 2018.

Example 11. Isolation of ZIKV and DENV neutralizing antibodies

[00241] Individuals were administered 5.0 µg doses of the ZPIV vaccine by intramuscular injected at week 0 and week 4 (Fig. 19a). Two ZPIV injections elicited neutralizing antibody responses capable of

protection in passive transfer studies. Participant #000015 showed remarkably high peak ZIKV neutralization at week 8, four weeks following the second ZPIV vaccination (Fig. 19a). Upon further investigation, it was found that this individual had previous exposure to DENV and WNV, as high titers of neutralization to DENV-1-4 and West Nile virus (WNV) were detected prior to ZPIV vaccination (Fig. 19b). Following the first ZPIV vaccination, neutralization titers increased to DENV 1-4, WNV, as well as Japanese Encephalitis virus (JEV) and ZIKV, but not Yellow Fever virus (YFV) (Fig. 19b,c).

[00242] To characterize these neutralizing responses elicited by ZPIV, a unique B cell sorting strategy was performed using a combination of fluorescently-labeled whole ZIKV virions, and ZIKV E and DENV-2 E proteins to isolate antigen-specific B cells (Fig. 20a). Viable PBMCs obtained from Participant #000015 at week 8 were incubated with a mixture of Brilliant Violet 510 (BV510) conjugated negative selection antibodies (CD3, CD4, CD8, CD14, CD16 and CD56) and CD19 ECD, CD20 APC-Cy7, ZIKV E PE and DENV-2 E PE-Cy7 positive selection markers. Cells were also sorted for binding to live whole ZIKV stained with a 462-APC secondary stain. Cells were selected by sorting based on negative expression of CD3, CD14 and CD56, positive expression of CD19, mid to high expression of CD38, and positive sequential staining with 4G2, ZIKV E and DENV E. Each positive B cell was sorted directly into lysis buffer in an isolated well of a PCR plate.

[00243] Antibody heavy and light chain V(D)J gene segments were amplified from single B cells by nested RT-PCR and sequenced. A total of 116 monoclonal antibodies (mAbs) were isolated and expressed as human IgG1, screened for binding to whole ZIKV and tested for neutralization in a qualified microneutralization assay (MN50). 75% of the mAbs bound to ZIKV and/or DENV, and 53% were found to neutralize at least one DENV serotype (Fig. 20b). Six potent ZIKV neutralizing antibodies isolated from B cells that stained positive for all three antigens (Fig 19d) were identified. They belong to four different clonal families, with the VH4-59/VL1-44 family consisting of three members sharing similar CDR3 sequences (Fig. 19d). Neutralization of one mAb, termed MZ4 (MHRP ZIKV-1004), demonstrated high potency against both ZIKV and DENV-2 with an IC_{50} of 8.3 and 20.9 ng ml⁻¹, respectively, in a flow-based assay using human monocytes as targets (Fig. 19e, f). MZ4 was able to match some of the most potent ZIKV and DENV-2 antibodies described to date and exhibited remarkably strong dual-neutralizing activities against ZIKV and DENV-2 as a single molecule. MZ4 was the most potent antibody against ZIKV, with similar potency as Z004 (Fig. 20c, d), a previously identified mAb from a ZIKV-infected individual (Robbiani et al, Cell 169:597-609, 2017). In addition, MZ4 surpassed the related MZ1 and EDE1 control (Barba-Spaeth et al, Nature 536:48-53, 2016), and was on par with other VH4-59 antibodies as well as the EDE2 control for DENV-2 neutralization (Fig. 19e, f and Fig. 20c, e). Potent activity against DENV-3

was also observed in this assay as well as weak, but detectable, neutralizing capabilities against DENV-1 and -4 (Fig. 19g, Fig. 20c). MZ4 had broad neutralizing activities against ZIKV strains from American, Asian and African lineages (Fig. 20f) but no inhibitory effects against other flaviviruses, such as West Nile (WNV), Yellow Fever (YFV) and Japanese Encephalitis (JEV) viruses (Fig. 20c).

Example 12. Characterization and structural analysis of ZIPV and DENV neutralizing antibodies

[00244] Epitope-mapping experiments delineated the epitope specificities of these antibodies induced by ZPIV-vaccination. First, binding activities against ZIKV and DENV-2 sE proteins and virions were measured to determine whether neutralizing epitopes were present only on the intact viral particles (Fig. 21a-d). Similar to the fusion loop epitope (FLE) control, antibodies such as MZ20, 54 and 56 bound equally well to E and whole virus in this assay, indicating that they recognize an epitope within the E monomer (Fig. 21a, c). However, antibodies from the MZ4 family bound better to ZIKV and DENV-2 virions than to their respective E proteins, suggesting that their epitopes have more quaternary characteristics (Fig. 21b, d). To gain insights into the target of these antibodies, binding competition experiments were carried out using biolayer interferometry (BLI) with a set of well-characterized controls (Fig. 19h). Antibodies within the MZ4 family were only competed by the DIII-directed antibody Z004, indicating that their epitope was within or overlapped with DIII (Fig. 19h). However, none of the MZ4 family member mAbs were able to bind to the isolated ZIKV DIII domain by ELISA, suggesting that their epitope lies near but not within DIII (Fig. 21e). However, MZ54, 56, and to some extent MZ20, displayed competition profiles consistent with those of FLE antibodies (Fig. 19h). A shotgun mutagenesis screen (Davidson E 2014) performed on ZIKV and DENV-2 prM/E proteins identified the FLE to be the target of MZ54 and MZ56, while MZ20 targeted DII (Fig 19i, Fig. 21f). MZ4 family of mAbs targeted Interestingly, the MZ4 family of mAbs targeted an epitope within the DI-DIII hinge or linker region in both ZIKV and DENV-2, suggesting a conserved mode of recognition (Fig 19i, Fig. 21f).

[00245] To understand the broad recognition and potent neutralization of MZ4, the structure of MZ4 was determined in complex with the ZIKV E glycoprotein (ZIKV E) at a resolution of 4.2 Å (Fig. 22). The structure was refined to an Rfactor of 27.8 % and an Rfree of 35.6 % with two ZIKV E dimers, and four antibody Fragment variable (Fv) domains observed in the asymmetric unit (Fig. 22a). MZ4 binds to the E glycoprotein domain I (DI) and DIII centered on the DI-DIII linker region (residues 299-306) with buried surface areas of 472.2 Å² and 137.4 Å² for the antibody heavy and light chains, respectively (Fig. 22a, b). MZ4 interacts with the E glycoprotein at four major sites (Fig. 22b, Table 16). First, the CDR H1 and CDR H2 interact with DI, and the DI-DIII linker via a set of germline gene-encoded Tyr residues that form

hydrogen bonds with the main chain atoms of Gly 182 and the side chain of Arg 299. Second, the CDR H3 binds to the full DI-DIII linker with 342.9 Å² of BSA, forming hydrogen bonds with Arg 299, Ser 304, and Tyr 305, while also interacting with the DI Gly 182. Third, the CDR L1 buries 128.3 Å² of surface area of the glycoprotein DIII, utilizing residues that have undergone somatic mutation. And fourth, the CDR L2 residues 50-52 have all undergone somatic hypermutation and bind to the DI-DIII linker and DIII while the FR L3 utilizes germline encoded residues to interact with the DIII BC- and DE-loops.

Table 16. Interface of MZ4 with ZIKV E glycoprotein

MZ4	Zika E glycoprotein	Distance (Å)
H:TYR 33 [OH]	G:GLY 182 [O]	3.73
H:SER 99 [OG]	G:TYR 305 [OH]	2.88
H:SER 30 [O]	G:ARG 299 [NH1]	3.42
H:SER 30 [O]	G:ARG 299 [NH2]	3.28
H:TYR 52 [OH]	ARG 299 [NH1]	3.62
H:TYR 98 [O]	G:TYR 305 [OH]	2.89
H:TYR 98 [OH]	G:ARG 299 [NH1]	2.69
H:SER 99 [O]	G:SER 304 [OG]	3.02
H:SER 99 [O]	G:SER 304 [N]	3.62
H:SER 99 [O]	G:VAL 303 [N]	3.67
L:ASN 50 [ND2]	G:SER 304 [O]	2.25
L:SER 67 [OG]	G:THR 335 [O]	2.30
L:GLY 68 [N]	G:THR 335 [O]	3.03
L:SER 67 [OG]	G:THR 335 [OG1]	2.45
L:GLY 68 [N]	G:THR 335 [OG1]	2.31
L:LYS 66 [NZ]	G:PRO 338 [O]	2.85
L:GLY 29 [O]	G:SER 368 [N]	3.84
L:ASN 50 [OD1]	G:SER 304 [OG]	2.69
L:GLY 68 [O]	G:SER 368 [OG]	3.02

Table 17A. MZ1 interface with ZIKV E glycoprotein

Hydrogen bonds	MZ1	ZIKV E PRVABC59	Distance (Å)
	H:SER 30[O]	Z:ARG 299[NH1]	3.56
	H:ASN 100A[OD1]	Z:SER 304[OG]	2.34
	L:SER 50[OG]	Z:SER 304[O]	2.49
	L:ARG 53[NH1]	Z:SER 304[O]	3.60
	L:SER 50[OG]	Z:SER 306[OG]	3.03
	L:LYS 66[NZ]	Z:GLY 337[O]	2.44
	L:LYS 66[NZ]	Z:SER 368[OG]	3.47
	L:GLY 29[O]	Z:SER 368[N]	3.72

L:SER 50[O]	Z:SER 306[OG]	2.29
L:SER 50[OG]	Z:SER 306[N]	3.02
L:ASN 52[OD1]	Z:SER 306[OG]	2.76

Table 17B. Buried surface area of MZ1 antibody in complex with ZIKV E glycoprotein

MZ1 antibody residues	Bond type	Accessible Surface Area (Å ²)	Buried Surface Area (Å ²)
H:SER 30	H	37.09	9.47
H:ASN 31		62.75	26.21
H:TYR 52		52.40	4.48
H:TYR 53		86.45	31.98
H:THR 54		90.97	7.14
H:PHE 98		98.68	55.53
H:ASN 99		123.39	66.98
H:TRP 100		170.56	128.41
H:ASN 100A		56.63	13.61
H:ASP 100B	H	151.25	37.76
H:GLY 100D		29.97	1.73
L:LEU 28		1.35	1.35
L:GLY 29		43.29	34.72
L:ARG 30		179.46	26.21
L:ASN 31		41.02	12.03
L:THR 32		71.68	38.45
L:TYR 49		70.32	13.40
L:SER 50		40.71	30.20
L:ASN 51	H	22.68	20.68
L:ASN 52		97.88	39.46
L:ARG 53		137.07	35.18
L:LYS 66		76.79	58.63
L:SER 67		88.85	45.67
L:ASP 68		72.79	21.05

H: Hydrogen bond

Table 17C. Buried surface area of ZIKV E glycoprotein in complex with MZ1

ZIKV E residue	Bond type	Accessible Surface Area (Å ²)	Buried Surface Area (Å ²)
Z:GLY 181	H	67.66	7.97
Z:GLY 182		40.52	33.48
Z:PHE 183		36.04	3.83
Z:GLY 184		22.61	5.71
Z:LYS 297		113.24	32.29
Z:ARG 299		153.92	101.10
Z:LEU 300		48.30	4.84
Z:LYS 301		91.81	34.88

Z:GLY 302		31.69	30.56
Z:VAL 303		83.30	38.74
Z:SER 304	H	106.70	106.7
Z:TYR 305		68.46	68.46
Z:SER 306	H	64.77	61.42
Z:LEU 307		98.17	2.29
Z:THR 309		105.14	5.02
Z:THR 335		127.70	55.36
Z:ASP 336		57.40	38.74
Z:GLY 337	H	10.22	9.71
Z:PRO 338		59.21	59.04
Z:THR 366		70.38	16.40
Z:GLU 367		104.85	1.48
Z:SER 368	H	75.95	57.39
Z:THR 369		76.89	0.50

H: Hydrogen bond

[00246] Structure alignment of the MZ4-ZIKV E structure with the Zika virus structure (5IRE), revealed additional quaternary contact sites at the pentamer vertex, and at the inter-raft interface (Fig. 22c). MZ4 binding at the pentamer vertex would generate additional contacts with the domain III and glycan N154 from a second protomer, and with the fusion-loop of a third protomer (Fig. 22d). Both antibody heavy and light chain contacts are involved in the additional recognition, through germline gene encoded residues of the heavy chain FR H3 and H4, and the CDR L2, and FR L3. The additional contacts result in increased contact regions with MZ4 heavy and light chains. This additional recognition also includes interaction with the N154-glycan with $\times \text{\AA}^2$ of BSA. In the context of the fully closed mature particle, only two antibodies can bind at the pentameric vertex due to steric hindrance, with the caveat that this vertex has been known to “breath” allowing increased stoichiometric binding (PMID: 28938115). MZ4 binding at the inter-raft interface also allows binding to the domain II of a neighboring protomer (Fig. 22e). These additional contacts result in an increase in the BSA for MZ4 heavy and light chains. These additional contacts are focused on the CDR H1, H3, FR H3, and the CDR L2.

[00247] To understand why MZ4 shows ng ml⁻¹ neutralization of ZIKV, and DENV2, but lower values against DENV1, and DENV4, the MZ4 epitope was mapped onto DENV1-4. Analysis of the site conservation, and assessment of critical contact residues indicates that across the five viruses, the MZ4 epitope contact residues are well conserved (9/19 identical; 8/19 similar) with only 2 residues (182 and 336) having significant (Fig. 22f). Critical residues identified in the shotgun mutagenesis experiments, R299 and Y305 are well conserved across viral strains and form five hydrogen bonds with CDR H3

residues. Viral escape analysis of ZIKV in the presence of MZ4 antibody identified escape mutations G182D, and S368N. In the context of the ZIKV, and DENV, residue 182 varies from Gly to Asp/Glu and appears to be critical for MZ4 recognition and viral neutralization potency.

[00248] To further understand the MZ4 site of vulnerability, the epitope was compared to previously described ZIKV- or DENV-targeting antibodies (Fig. 23). The majority of the DIII-targeting antibodies have overlapping epitopes with MZ4 to varying degrees, with antibodies ZV64 being the most similar. However, both these antibodies are highly strain specific for ZIKV (ZV64) or DENV2 (5H2) due to the lack of sequence conservation within their epitopes. Structure analysis suggests that one of the factors driving the potent neutralizing activity of MZ4 is its ability to bind to DI, DII, and DIII of the ZIKV (Fig. 22a), and thereby locking the virus in a prefusion form incompatible with viral-cell fusion.

Example 13. The MZ4 and MZ1 mAbs provide passive immunity against ZIPV infection

[00249] To investigate whether antibodies to the DI/DIII linker would protect from ZIKV replication *in vivo*, passive protection studies were performed in a wild-type mouse (Fig. 24a). A single dose of 200 μ g (10 mg kg⁻¹) of MZ4 or MZ1 fully protected mice from ZIKV viremia, as demonstrated by undetectable viral replication over the course of the experiment while viral loads peaked at three days post-challenge in the sham control group (Fig. 24b). To probe the minimal dose of MZ4 required for sterile protection as well as explore the *in vivo* effects of low plasma concentration of antibodies, we titrated down the MZ4 dose until protection was lost (Fig. 25). While protection decreased gradually with the antibody dose, eighty percent of the animals (4/5 mice) were still protected from viral replication at the low dose of 3.7 μ g (0.185 mg kg⁻¹) giving an estimated ED₅₀ of 2.55 μ g (0.1275 mg kg⁻¹).

[00250] The question of how exposure to flavivirus prior to vaccination shaped the ZPIV-elicited responses was investigated next. An analysis of longitudinal plasma samples from Participant #00015 for neutralization activity, strikingly showed that high magnitude ZIKV and DENV-2 binding and neutralizing antibody responses were observed two weeks after injection of the first ZIPV dose (Fig. 26a). All these responses were maintained to a similar level following the second ZPIV dose. Binding responses and off-rates to ZIKV and DENV-2 E proteins followed the same kinetics (Fig. 27a). In contrast, flavivirus naïve donors (n=5) that received the same vaccine regimen demonstrated modest titers 2 weeks following the first vaccination, with peak binding and neutralizing responses detected at week 8 after two immunizations (Fig. 26b, Fig. 27b), and those responses were specific to ZIKV with no cross-reactivity detected to DENV (Fig. 26b).

[00251] Since high binding and neutralizing titers were detected in serum from Participant #00015 after only one ZPIV vaccination, it was next determined if these were a result of the induction of MZ4-like antibodies. Therefore, the same sorting strategy was utilized to isolate antigen-positive B cells from cryopreserved PBMCs collected from Participant #00015 at 2 weeks following the first vaccination. One isolated antibody, called 'MZ2', for 2 weeks post the first vaccination, had high sequence similarity to MZ4 and similar heavy and light chain characteristics (Fig. 26d). MZ2 yielded nearly identical binding, neutralization and epitope mapping as MZ4, suggesting that a single dose of ZPIV was sufficient to elicit potent cross-reactive neutralizing antibodies to ZIKV and DENV (Fig. 26e-g, Fig. 27c-f).

[00252] While aspects of the present disclosure been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims. The claims are intended to cover the components and steps in any sequence which is effective to meet the objectives there intended, unless the context specifically indicates the contrary.

Claims

1. An antibody or fragment thereof that selectively binds whole Zika virus, wherein said antibody comprises:

(a) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 5 for CDR1, SEQ ID NO: 6 for CDR2, and SEQ ID NO: 7 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 8 for CDR1, SEQ ID NO: 9 for CDR2 and SEQ ID NO: 10 for CDR3; or

(b) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 15 for CDR1, SEQ ID NO: 16 for CDR2, and SEQ ID NO: 17 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 18 for CDR1, SEQ ID NO: 19 for CDR2 and SEQ ID NO: 20 for CDR3; or

(c) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 25 for CDR1, SEQ ID NO: 26 for CDR2, and SEQ ID NO: 27 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 28 for CDR1, SEQ ID NO: 29 for CDR2 and SEQ ID NO: 30 for CDR3; or

(d) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 35 for CDR1, SEQ ID NO: 36 for CDR2, and SEQ ID NO: 37 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 38 for CDR1, SEQ ID NO: 39 for CDR2 and SEQ ID NO: 40 for CDR3; or

(e) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 45 for CDR1, SEQ ID NO: 46 for CDR2, and SEQ ID NO: 47 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 48 for CDR1, SEQ ID NO: 49 for CDR2 and SEQ ID NO: 50 for CDR3; or

(f) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 55 for CDR1, SEQ ID NO: 56 for CDR2, and SEQ ID NO: 57 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 58 for CDR1, SEQ ID NO: 59 for CDR2 and SEQ ID NO: 60 for CDR3; or

(g) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 65 for CDR1, SEQ ID NO: 66 for CDR2, and SEQ ID NO: 67 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 68 for CDR1, SEQ ID NO: 69 for CDR2 and SEQ ID NO: 70 for CDR3; or

(h) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 75 for CDR1, SEQ ID NO: 76 for CDR2, and SEQ ID NO: 77 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 78 for CDR1, SEQ ID NO: 79 for CDR2 and SEQ ID NO: 80 for CDR3; or

(i) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 85 for CDR1, SEQ ID NO: 86 for CDR2, and SEQ ID NO: 87 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 88 for CDR1, SEQ ID NO: 89 for CDR2 and SEQ ID NO: 90 for CDR3; or

(j) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 95 for CDR1, SEQ ID NO: 96 for CDR2, and SEQ ID NO: 97 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 98 for CDR1, SEQ ID NO: 99 for CDR2 and SEQ ID NO: 100 for CDR3; or

(k) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 105 for CDR1, SEQ ID NO: 106 for CDR2, and SEQ ID NO: 107 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 108 for CDR1, SEQ ID NO: 109 for CDR2 and SEQ ID NO: 110 for CDR3; or

(l) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 115 for CDR1, SEQ ID NO: 116 for CDR2, and SEQ ID NO: 117 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 118 for CDR1, SEQ ID NO: 119 for CDR2 and SEQ ID NO: 120 for CDR3; or

(m) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 125 for CDR1, SEQ ID NO: 126 for CDR2, and SEQ ID NO: 127 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 128 for CDR1, SEQ ID NO: 129 for CDR2 and SEQ ID NO: 130 for CDR3; or

(n) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 135 for CDR1, SEQ ID NO: 136 for CDR2, and SEQ ID NO: 137 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 138 for CDR1, SEQ ID NO: 139 for CDR2 and SEQ ID NO: 140 for CDR3; or

(o) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 145 for CDR1, SEQ ID NO: 146 for CDR2, and SEQ ID NO: 147 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 148 for CDR1, SEQ ID NO: 149 for CDR2 and SEQ ID NO: 150 for CDR3; or

(p) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 155 for CDR1, SEQ ID NO: 156 for CDR2, and SEQ ID NO: 157 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 158 for CDR1, SEQ ID NO: 159 for CDR2 and SEQ ID NO: 160 for CDR3; or

(q) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 165 for CDR1, SEQ ID NO: 166 for CDR2, and SEQ ID NO: 167 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 168 for CDR1, SEQ ID NO: 169 for CDR2 and SEQ ID NO: 170 for CDR3; or

(r) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 175 for CDR1, SEQ ID NO: 176 for CDR2, and SEQ ID NO: 177 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 178 for CDR1, SEQ ID NO: 179 for CDR2 and SEQ ID NO: 180 for CDR3; or

(s) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 185 for CDR1, SEQ ID NO: 186 for CDR2, and SEQ ID NO: 187 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 188 for CDR1, SEQ ID NO: 189 for CDR2 and SEQ ID NO: 190 for CDR3; or

(t) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 195 for CDR1, SEQ ID NO: 196 for CDR2, and SEQ ID NO: 197 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 198 for CDR1, SEQ ID NO: 199 for CDR2 and SEQ ID NO: 200 for CDR3; or

- (u) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 205 for CDR1, SEQ ID NO: 206 for CDR2, and SEQ ID NO: 207 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 208 for CDR1, SEQ ID NO: 209 for CDR2 and SEQ ID NO: 210 for CDR3; or
- (v) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 215 for CDR1, SEQ ID NO: 216 for CDR2, and SEQ ID NO: 217 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 218 for CDR1, SEQ ID NO: 219 for CDR2 and SEQ ID NO: 220 for CDR3.
2. The antibody or fragment thereof of claim 1, wherein said antibody comprises:
- (a) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 5 for CDR1, SEQ ID NO: 6 for CDR2, and SEQ ID NO: 7 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 8 for CDR1, SEQ ID NO: 9 for CDR2 and SEQ ID NO: 10 for CDR3; or
- (b) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 15 for CDR1, SEQ ID NO: 16 for CDR2, and SEQ ID NO: 17 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 18 for CDR1, SEQ ID NO: 19 for CDR2 and SEQ ID NO: 20 for CDR3; or
- (c) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 25 for CDR1, SEQ ID NO: 26 for CDR2, and SEQ ID NO: 27 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 28 for CDR1, SEQ ID NO: 29 for CDR2 and SEQ ID NO: 30 for CDR3; or
- (d) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 35 for CDR1, SEQ ID NO: 36 for CDR2, and SEQ ID NO: 37 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 38 for CDR1, SEQ ID NO: 39 for CDR2 and SEQ ID NO: 40 for CDR3; or

(e) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 45 for CDR1, SEQ ID NO: 46 for CDR2, and SEQ ID NO: 47 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 48 for CDR1, SEQ ID NO: 49 for CDR2 and SEQ ID NO: 50 for CDR3; or

(f) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 55 for CDR1, SEQ ID NO: 56 for CDR2, and SEQ ID NO: 57 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 58 for CDR1, SEQ ID NO: 59 for CDR2 and SEQ ID NO: 60 for CDR3; or

(g) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 65 for CDR1, SEQ ID NO: 66 for CDR2, and SEQ ID NO: 67 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 68 for CDR1, SEQ ID NO: 69 for CDR2 and SEQ ID NO: 70 for CDR3; or

(h) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 75 for CDR1, SEQ ID NO: 76 for CDR2, and SEQ ID NO: 77 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 78 for CDR1, SEQ ID NO: 79 for CDR2 and SEQ ID NO: 80 for CDR3; or

(i) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 85 for CDR1, SEQ ID NO: 86 for CDR2, and SEQ ID NO: 87 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 88 for CDR1, SEQ ID NO: 89 for CDR2 and SEQ ID NO: 90 for CDR3; or

(j) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 95 for CDR1, SEQ ID NO: 96 for CDR2, and SEQ ID NO: 97 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 98 for CDR1, SEQ ID NO: 99 for CDR2 and SEQ ID NO: 100 for CDR3; or

(k) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 105 for CDR1, SEQ ID NO: 106 for CDR2, and SEQ ID NO: 107 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 108 for CDR1, SEQ ID NO: 109 for CDR2 and SEQ ID NO: 110 for CDR3.

3. The antibody or fragment thereof of claim 1, wherein said antibody comprises:

(a) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 115 for CDR1, SEQ ID NO: 116 for CDR2, and SEQ ID NO: 117 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 118 for CDR1, SEQ ID NO: 119 for CDR2 and SEQ ID NO: 120 for CDR3; or

(b) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 125 for CDR1, SEQ ID NO: 126 for CDR2, and SEQ ID NO: 127 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 128 for CDR1, SEQ ID NO: 129 for CDR2 and SEQ ID NO: 130 for CDR3; or

(c) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 135 for CDR1, SEQ ID NO: 136 for CDR2, and SEQ ID NO: 137 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 138 for CDR1, SEQ ID NO: 139 for CDR2 and SEQ ID NO: 140 for CDR3; or

(d) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 145 for CDR1, SEQ ID NO: 146 for CDR2, and SEQ ID NO: 147 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 148 for CDR1, SEQ ID NO: 149 for CDR2 and SEQ ID NO: 150 for CDR3; or

(e) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 155 for CDR1, SEQ ID NO: 156 for CDR2, and SEQ ID NO: 157 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 158 for CDR1, SEQ ID NO: 159 for CDR2 and SEQ ID NO: 160 for CDR3; or

(f) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 165 for CDR1, SEQ ID NO: 166 for CDR2, and SEQ ID NO: 167 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 168 for CDR1, SEQ ID NO: 169 for CDR2 and SEQ ID NO: 170 for CDR3; or

(g) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 175 for CDR1, SEQ ID NO: 176 for CDR2, and SEQ ID NO: 177 for CDR3; and a light

chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 178 for CDR1, SEQ ID NO: 179 for CDR2 and SEQ ID NO: 180 for CDR3; or

(h) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 185 for CDR1, SEQ ID NO: 186 for CDR2, and SEQ ID NO: 187 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 188 for CDR1, SEQ ID NO: 189 for CDR2 and SEQ ID NO: 190 for CDR3; or

(i) a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 195 for CDR1, SEQ ID NO: 196 for CDR2, and SEQ ID NO: 197 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 198 for CDR1, SEQ ID NO: 199 for CDR2 and SEQ ID NO: 200 for CDR3;

(j) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 205 for CDR1, SEQ ID NO: 206 for CDR2, and SEQ ID NO: 207 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 208 for CDR1, SEQ ID NO: 209 for CDR2 and SEQ ID NO: 210 for CDR3; or

(k) a heavy chain variable region comprising complementarily-determining regions (CDRs) having amino acid sequences SEQ ID NO: 215 for CDR1, SEQ ID NO: 216 for CDR2, and SEQ ID NO: 217 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 218 for CDR1, SEQ ID NO: 219 for CDR2 and SEQ ID NO: 220 for CDR3.

4. The antibody or fragment thereof of claim 1 wherein said antibody or antibody fragment comprises a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 55 for CDR1, SEQ ID NO: 56 for CDR2, and SEQ ID NO: 57 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 58 for CDR1, SEQ ID NO: 59 for CDR2 and SEQ ID NO: 60 for CDR3.

5. The antibody or fragment thereof of claim 1 wherein said antibody or antibody fragment comprises a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 95 for CDR1, SEQ ID NO: 96 for CDR2, and SEQ ID NO: 97 for CDR3; and a light chain variable region

comprising CDRs having amino acid sequences of SEQ ID NO: 98 for CDR1, SEQ ID NO: 99 for CDR2 and SEQ ID NO: 100 for CDR3.

6. The antibody or fragment thereof of claim 1 wherein said antibody or antibody fragment comprises a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 105 for CDR1, SEQ ID NO: 106 for CDR2, and SEQ ID NO: 107 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 108 for CDR1, SEQ ID NO: 109 for CDR2 and SEQ ID NO: 110 for CDR3.

7. The antibody or fragment thereof of claim 1 wherein said antibody or antibody fragment comprises a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 115 for CDR1, SEQ ID NO: 116 for CDR2, and SEQ ID NO: 117 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 118 for CDR1, SEQ ID NO: 119 for CDR2 and SEQ ID NO: 120 for CDR3.

8. The antibody or fragment thereof of claim 1 wherein said antibody or antibody fragment comprises a heavy chain variable region comprising CDRs having amino acid sequences SEQ ID NO: 215 for CDR1, SEQ ID NO: 216 for CDR2, and SEQ ID NO: 217 for CDR3; and a light chain variable region comprising CDRs having amino acid sequences of SEQ ID NO: 218 for CDR1, SEQ ID NO: 219 for CDR2 and SEQ ID NO: 220 for CDR3.

9. An antibody or fragment thereof that selectively binds Zika virus wherein,
the heavy chain CDR1 sequence differs from SEQ ID NO: 55 by four or less substitutions,
the heavy chain CDR2 sequence differs from SEQ ID NO: 56 by two or less substitutions,
the heavy chain CDR3 sequence differs from SEQ ID NO: 57 by five or less substitutions,
the light chain CDR1 sequence differs from SEQ ID NO: 58 by one or less substitutions,
the light chain CDR2 sequence differs from SEQ ID NO: 59 by three or less substitutions, and
the light chain CDR3 sequence differs from SEQ ID NO: 60 by one or less substitutions.

10. An antibody or fragment thereof that selectively binds Zika virus wherein,
the heavy chain CDR1 sequence differs from SEQ ID NO: 95 by four or less substitutions,
the heavy chain CDR2 sequence differs from SEQ ID NO: 96 by two or less substitutions,
the heavy chain CDR3 sequence differs from SEQ ID NO: 97 by five or less substitutions,
the light chain CDR1 sequence differs from SEQ ID NO: 98 by one or less substitutions,

- the light chain CDR2 sequence differs from SEQ ID NO: 99 by three or less substitutions, and the light chain CDR3 sequence differs from SEQ ID NO: 100 by one or less substitutions.
11. An antibody or fragment thereof that selectively binds Zika virus wherein, the heavy chain CDR1 sequence differs from SEQ ID NO: 105 by four or less substitutions, the heavy chain CDR2 sequence differs from SEQ ID NO: 106 by two or less substitutions, the heavy chain CDR3 sequence differs from SEQ ID NO: 107 by five or less substitutions, the light chain CDR1 sequence differs from SEQ ID NO: 108 by one or less substitutions, the light chain CDR2 sequence differs from SEQ ID NO: 109 by three or less substitutions, and the light chain CDR3 sequence differs from SEQ ID NO: 110 by one or less substitutions.
12. An antibody or fragment thereof that selectively binds Zika virus wherein, the heavy chain CDR1 sequence differs from SEQ ID NO: 115 by four or less substitutions, the heavy chain CDR2 sequence differs from SEQ ID NO: 116 by two or less substitutions, the heavy chain CDR3 sequence differs from SEQ ID NO: 117 by five or less substitutions, the light chain CDR1 sequence differs from SEQ ID NO: 118 by one or less substitutions, the light chain CDR2 sequence differs from SEQ ID NO: 119 by three or less substitutions, and the light chain CDR3 sequence differs from SEQ ID NO: 120 by one or less substitutions.
13. The antibody or fragment thereof of any of claims 1-12 wherein said antibody or antibody fragment inhibits Zika virus infection.
14. The antibody or fragment thereof of any of claims 1, 3, 7 or 12 wherein said antibody or antibody fragment inhibits Dengue virus infection.
15. The antibody or fragment thereof of any of claims 1, 3, 7 or 12 wherein said antibody or antibody fragment inhibits infection by Dengue virus serotype 2.
16. The antibody or fragment thereof of any of claims 1, 3, 7 or 12 wherein said antibody or antibody fragment inhibits infection by Dengue virus serotype 3.
17. The antibody or fragment thereof of any of claims 1-12 wherein said antibody or antibody fragment inhibits Zika virus transmission from a pregnant female to a fetus.

18. The antibody or fragment thereof of any of claims 1-12 wherein said antibody or antibody fragment inhibits sexual transmission of Zika virus.
19. The antibody or fragment thereof of any of claims 1-12 wherein said antibody or antibody fragment inhibits or prevents infection of human testes.
20. The antibody or fragment thereof of any of claims 1, 3, 7 or 12 wherein said antibody or antibody fragment has an ED50 for neutralizing Zika infection of less than less than 10 mg kg⁻¹, less than 5 mg kg⁻¹, less than 1 mg kg⁻¹, less than 0.5 mg kg⁻¹, less than 0.2 mg kg⁻¹, less than 0.1 mg kg⁻¹, less than 0.05 mg kg⁻¹, less than 0.02 mg kg⁻¹, or less than 0.01 mg kg⁻¹.
21. The antibody or fragment thereof of any of claims 1-2, 4-6, or 9-11 wherein said antibody or antibody fragment has an IC50 for neutralizing Zika infection of less than 10 µM, less than 5 µM, less than 2 µM, less than 1 µM, less than 500 nM, less than 200 nM, or less than 100 nM.
22. The antibody or fragment thereof of claim 1, 3, 7 or 12 wherein 100 µg/ml of said antibody or antibody fragment does not neutralize infection by a flavivirus selected from the group of Dengue virus, Japanese Encephalitis virus, West Nile virus, or Yellow Fever virus.
23. The antibody or fragment thereof of any of claims 1-12 wherein said antibody or antibody fragment has an equilibrium dissociation constant (KD) is in the range from 10⁻⁷ to 10⁻⁹ molar.
24. The antibody or fragment thereof of any of claims 1-12 wherein said antibody or antibody fragment has an equilibrium dissociation constant (KD) of less than 10⁻⁷ molar.
25. The antibody or antibody fragment of any of claims 1-12 wherein said antibody or antibody fragment comprises an Fd fragment.
26. The antibody or antibody fragment of any of claims 1-12 wherein said antibody fragment is a Fab fragment.
27. The antibody or antibody fragment of any of claims 1-12 wherein said antibody fragment is a single chain variable fragment (ScFv).
28. The antibody or antibody fragment of any of claims 1-12 wherein said antibody or antibody fragment is a human antibody, humanized antibody or humanized antibody fragment.

29. A polynucleotide comprising a nucleotide sequence encoding the antibody or antibody fragment of any of claims 1-12.
30. A host cell comprising the polynucleotide of claim 29.
31. A method of making the antibody of claim 1 comprising isolating antibody secreted by the host cell of claim 28.
32. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the antibody or antibody fragment of any of claims 1-27 or 62-76.
33. A method for the prevention or treatment of a flavivirus infection comprising administering to a patient a therapeutically effective amount of the pharmaceutical preparation of claim 32.
34. A method for inhibiting or preventing transmission of a flavivirus infection from a pregnant female to her fetus comprising administering to the pregnant female a therapeutically effective amount of the pharmaceutical preparation of claim 32.
35. A method for inhibiting or preventing sexual transmission of a flavivirus infection comprising administering a therapeutically effective amount of the pharmaceutical composition of claim 32.
36. A method of reducing the likelihood of a subject developing a disease caused by Zika virus or a flavivirus, the method comprising administering the pharmaceutical preparation of claim 32 to a subject prior to a flavivirus infection.
37. A method of detecting the presence of a flavivirus in a biological sample, the method comprising contacting the antibody or antibody fragment of any of claims 1-27 or 62-76 with the biological sample and detecting the binding of the antibody or antibody fragment to a flavivirus.
38. A kit for detecting the presence of a flavivirus in a biological sample, the kit comprising the antibody or antibody fragment of any of claims 1-27.
39. A method of diagnosing infection by a flavivirus, the method comprising:
obtaining a biological sample for a subject at risk of a flavivirus infection;
contacting the biological sample with the antibody or antibody fragment of any of claims 1-27;
and

determining if the antibody or antibody fragment has bound to a flavivirus antigen;
wherein binding of the antibody or antibody fragment to a flavivirus antigen indicates that the subject is infected with a flavivirus.

40. A method of detecting a latent infection by a flavivirus, the method comprising:
obtaining a biological sample for a subject at risk of a flavivirus infection;
stimulating the biological sample to induce viral outgrowth;
contacting the biological sample with the antibody or antibody fragment of any of any of claims 1-12; and

determining if the antibody or antibody fragment has bound to a flavivirus antigen;
wherein binding of the antibody or antibody fragment to a flavivirus antigen indicates that the subject is infected with a flavivirus.

41. A method of inducing immunity to a flavivirus in a human subject at risk of flavivirus infection comprising, administering a single dose of Zika virus purified inactivated vaccine in the human subject, wherein the subject was previously infected by a flavivirus.

42. The method of claim 41 wherein the flavivirus that the human subject was previously exposed to was not a Zika virus.

43. The method of any of claims 31-39 wherein the flavivirus is a Zika virus.

44. The method of any of claims 31-40 wherein the flavivirus is a Dengue virus.

45. The method of any of claims 31-40 wherein the flavivirus is Dengue virus serotype 2.

46. The method of any of claims 29-38 wherein the flavivirus is Dengue virus serotype 3.

47. The method of any of claims 31-40 wherein the flavivirus is a West Nile virus.

48. The method of any of claims 31-40 wherein the flavivirus is a Japanese Encephalitis virus.

49. A method of measuring the efficacy of a vaccine batch comprising contacting an aliquot of the vaccine batch with the antibody or antibody fragment of any of claims 1-27, and detecting the binding of the antibody or antibody fragment.

50. A method of determining whether a flavivirus vaccine comprises a DI/DIII linker domain comprising contacting the vaccine with the antibody or antibody fragment of claim 3.
51. A method of purifying a flavivirus E glycoprotein comprising contacting the flavivirus E glycoprotein with the antibody or antibody fragment of any of claims 1-10.
52. The antibody or fragment thereof of any of claims 1-27, wherein the antibody or fragment thereof binds to the DI-DIII linker domain of a Zika virus.
53. The antibody or fragment thereof of claim 52 wherein 100 ng, 50 ng, 20 ng, 10 ng, 5 ng, 2 ng, 1 ng, 0.5 ng, 0.2 ng or 0.1 ng of the antibody or fragment thereof can neutralize at least 50% of the infectious activity of 100 PFU of Zika virus in a microneutralization assay.
54. The antibody or fragment thereof of claim 52 wherein binding to Zika virus is reduced by at least 70% when Zika virus E glycoprotein residue Tyrosine 305 is substituted with alanine.
55. A method for isolating an antibody that binds to cross-protomer epitopes of a virus comprising:
- (a) immunizing a subject with a viral immunogen,
 - (b) isolating peripheral blood mononuclear cells (PBMCs) from the subject,
 - (c) contacting the PBMCs with intact virus to create PBMC-virus complexes,
 - (d) contacting the PBMC-virus complexes with a fluorescently-labeled antibody that binds the virus,
 - (e) isolating a fluorescent PBMC,
 - (f) isolating polynucleotides encoding the heavy and light chains of an antibody from the fluorescent PBMC,
 - (g) expressing the isolated polynucleotides in a host cell, and
 - (h) isolating an antibody expressed by the host cell.
56. The method of claim 55, wherein the subject is a primate.
57. The method of claim 55 or 56, wherein the viral immunogen is an intact virus.
58. The method of any one of claims 55-57, wherein the viral immunogen is a flavivirus immunogen.
59. The method of 58, wherein the flavivirus immunogen is a Zika virus immunogen.

60. The method of 58, wherein the flavivirus immunogen is a Dengue virus immunogen.
61. The method of 58, wherein the flavivirus immunogen is a West Nile virus immunogen.
62. A multispecific antibody comprising a first binding site that binds to a flavivirus and a second binding site that binds to a flavivirus, wherein the first binding site binds to a different epitope than the second binding site.
63. The multispecific antibody of claim 62 comprising a plurality of first binding sites and a plurality of second binding sites.
64. The multispecific antibody of claims 62-63 comprising a third binding site that binds to a different flavivirus epitope than the first binding site or the second binding site.
65. The multispecific antibody of claims 62-64 wherein the first binding site comprises CDR sequences of a heavy chain variable domain or a fragment thereof and CDR sequences of a light chain variable domain or a fragment thereof.
66. The multispecific antibody of claims 62-65 wherein the second binding domain comprises CDR sequences of a single chain variable fragment (ScFv).
67. The multispecific antibody of claims 62-66 wherein the constant region of a heavy chain polypeptide comprising CDR sequences of the first binding site comprises a Threonine 366 to Tyrosine substitution and the constant region of a heavy chain polypeptide comprising CDR sequences of the second binding site comprises a Tyrosine 407 to Threonine substitution.
68. The multispecific antibody of claims 62-67 wherein a ScFv comprising the second binding site is inserted into the hinge region of a heavy chain polypeptide comprising CDR sequences of the first binding site.
69. The multispecific antibody of claims 62-67 wherein a ScFv comprising the second binding site is inserted within a constant region of a heavy chain polypeptide comprising CDR sequences of the first binding site.

70. The multispecific antibody of claims 62-69 wherein the first binding site has relatively higher affinity for a Zika virus E glycoprotein than for a Dengue virus E glycoprotein and the second binding site has relatively higher affinity for a Dengue virus E glycoprotein than for a Zika virus E glycoprotein.

71. The multispecific antibody of claims 62-70 wherein the first binding site comprises CDR sequences from an antibody selected from the group comprising MZ4, MZ20, EDE2-A11 and Ab513.

72. The multispecific antibody of claim 71 wherein the second binding site comprises CDR sequences from an antibody selected from the group comprising MZ4, MZ20, EDE2-A11 and Ab513.

73. The multispecific antibody of claims 62-72 wherein the mean binding response as measured by BioLayer Interferometry for ZIKV, DENV1, DENV2, DENV3, and DENV4 is less than the mean binding response as measured by BioLayer Interferometry for ZIKV, DENV1, DENV2, DENV3, and DENV4 of an antibody comprising only a first binding site or only a second binding site.

74. The multispecific antibody of claims 62-73 wherein the mean of IC_{50} values for neutralization of ZIKV, DENV1, DENV2, DENV3, and DENV4 is less than the mean of IC_{50} values for neutralization of ZIKV, DENV1, DENV2, DENV3, and DENV4 of an antibody comprising only a first binding site or only a second binding site.

75. The multispecific antibody of claims 62-74 comprising a first polypeptide comprising an MZ4 heavy chain variable domain and an MZ4 light chain variable domain and a second polypeptide comprising an EDE2-A11 heavy chain variable domain and an EDE2-A11 light chain variable domain.

76. The multispecific antibody of claims 62-75 comprising a polypeptide comprising an MZ4 heavy chain variable domain, an MZ4 light chain variable domain, an EDE2-A11 heavy chain variable domain and an EDE2-A11 light chain variable domain.

Figure 1

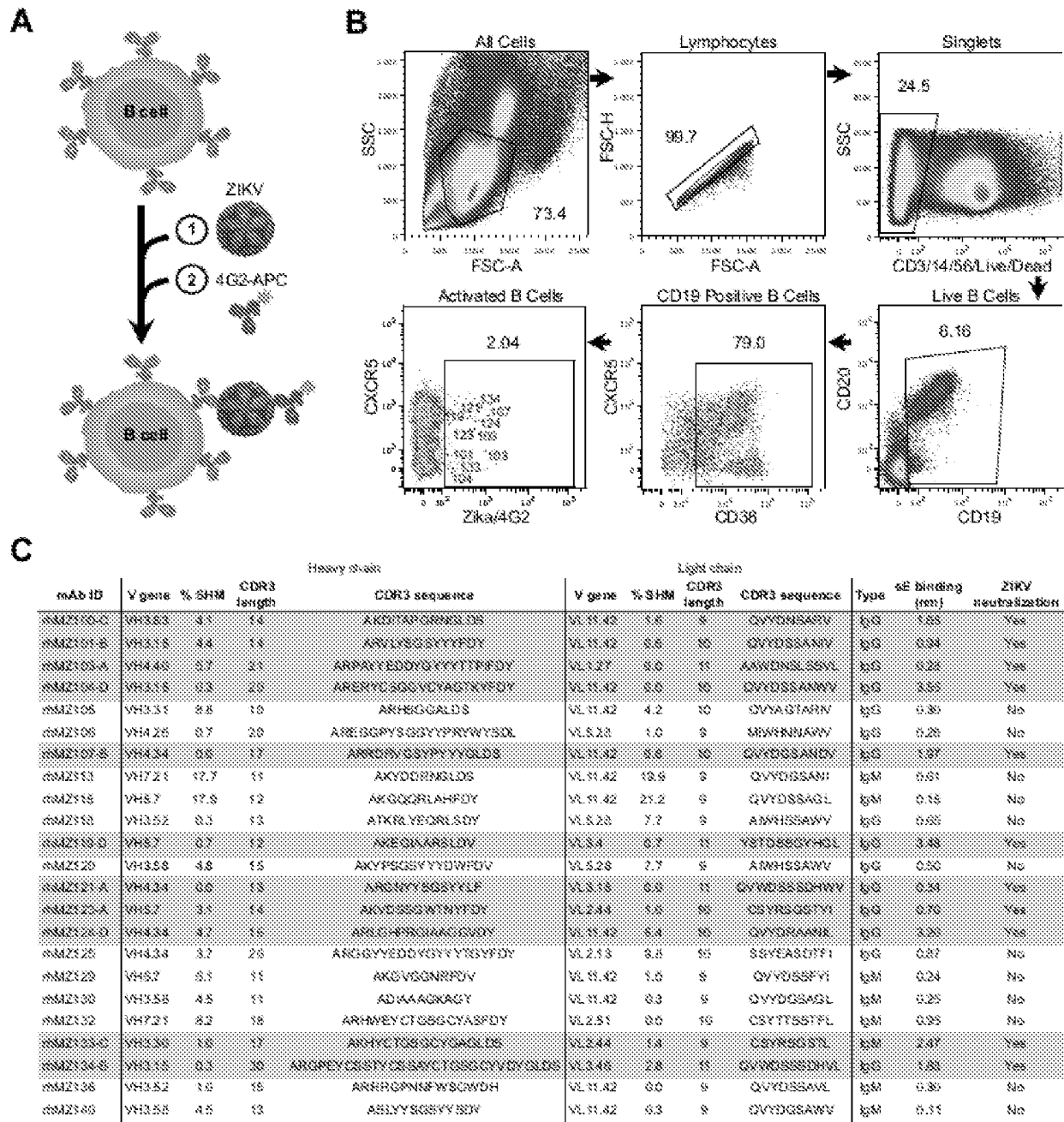


Figure 2

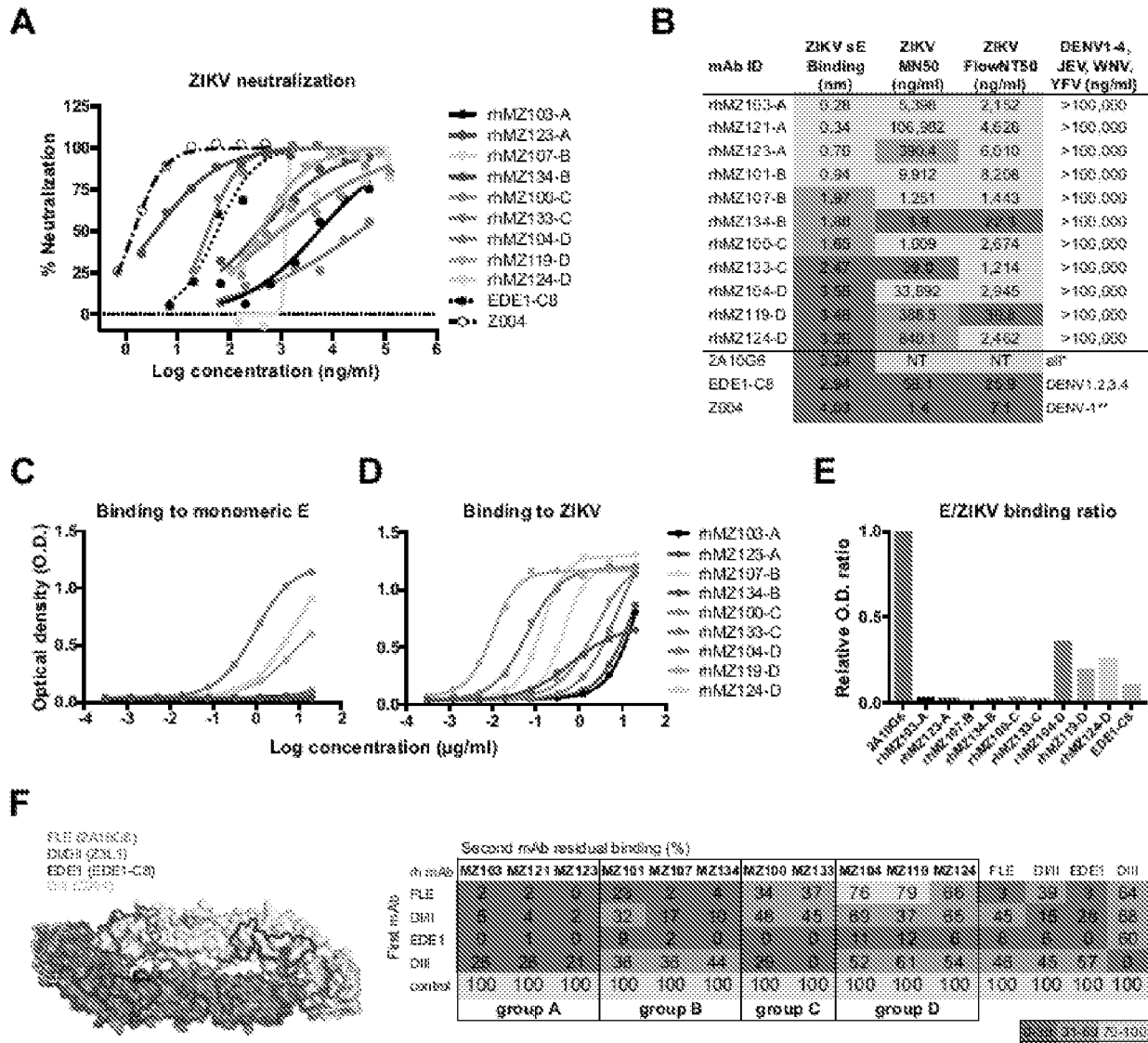


Figure 3

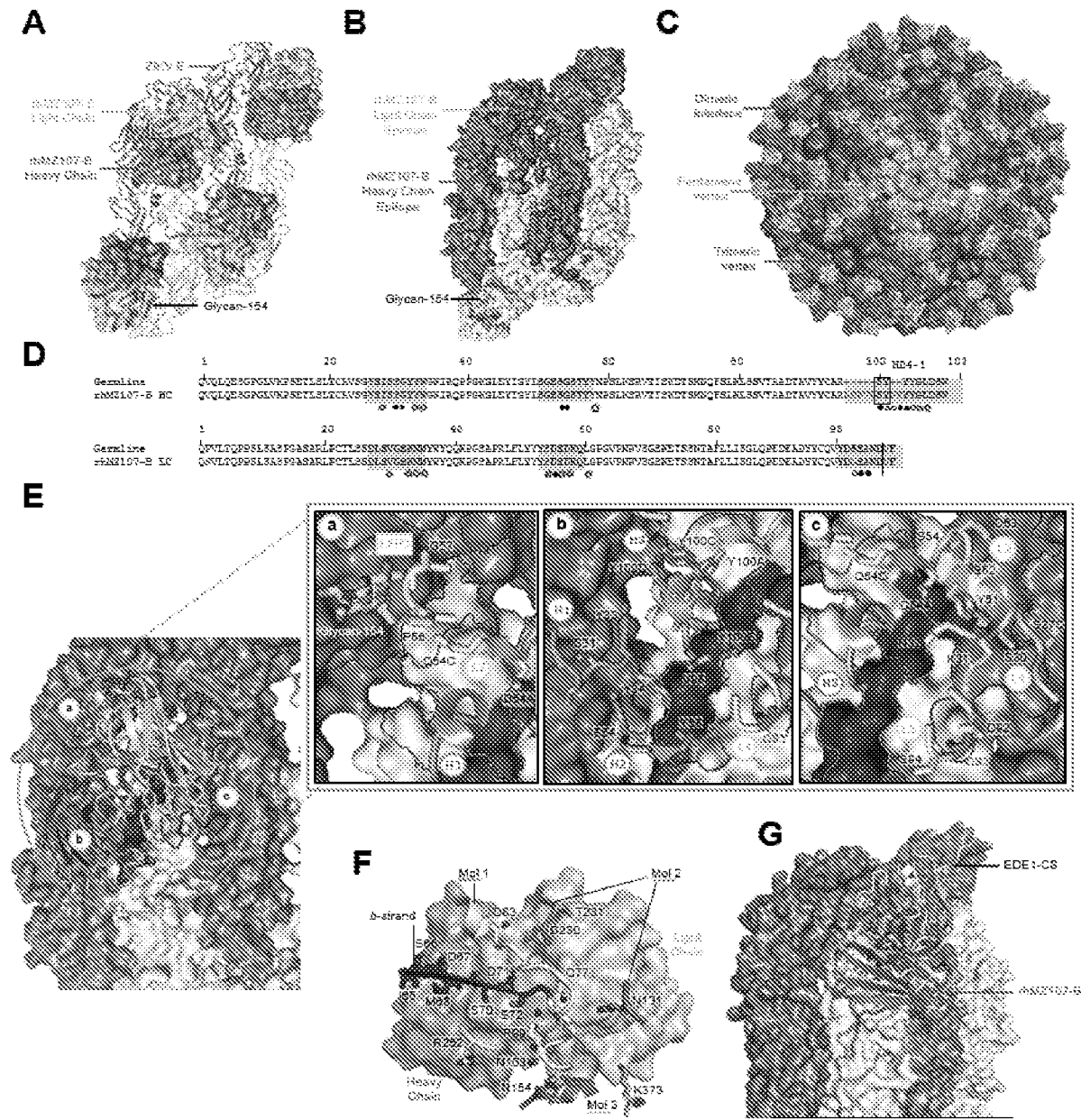
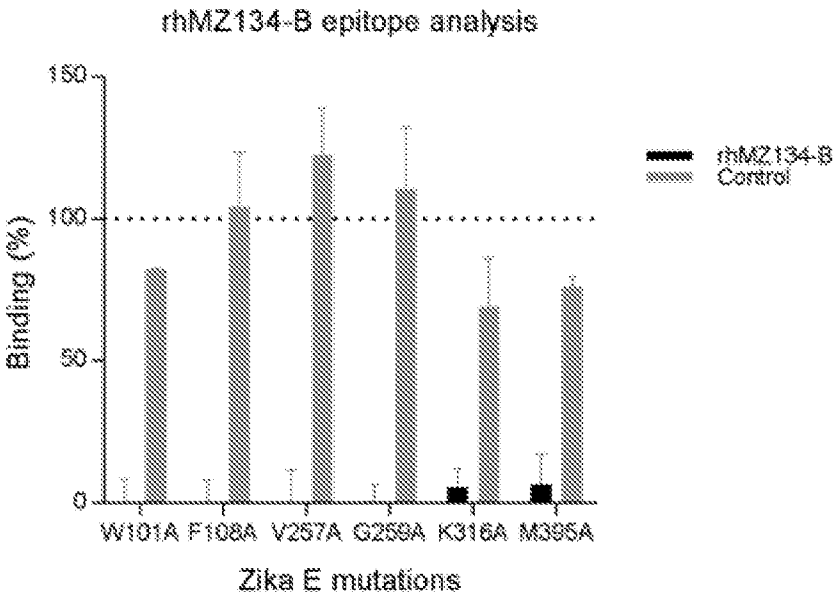
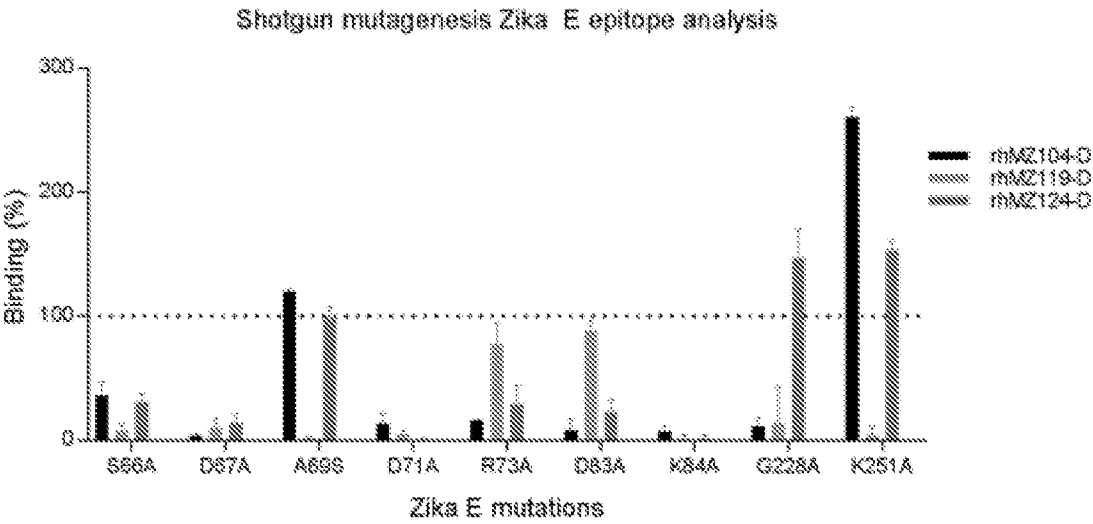


Figure 4

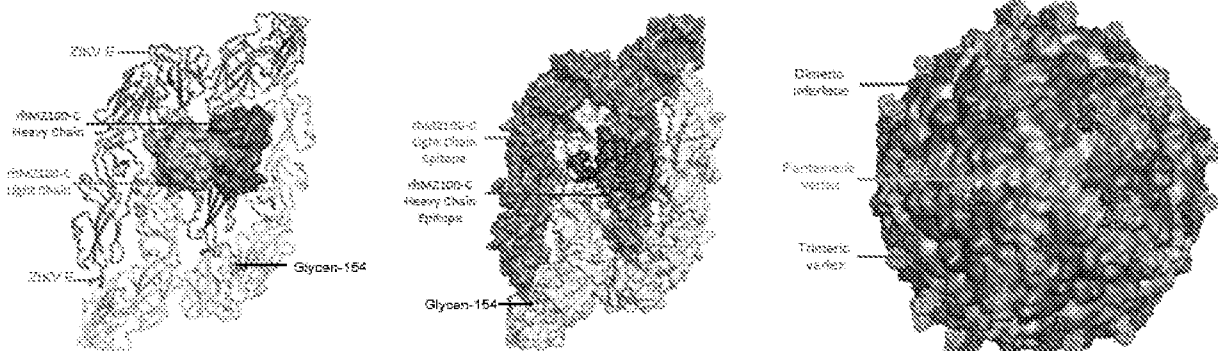
A



B



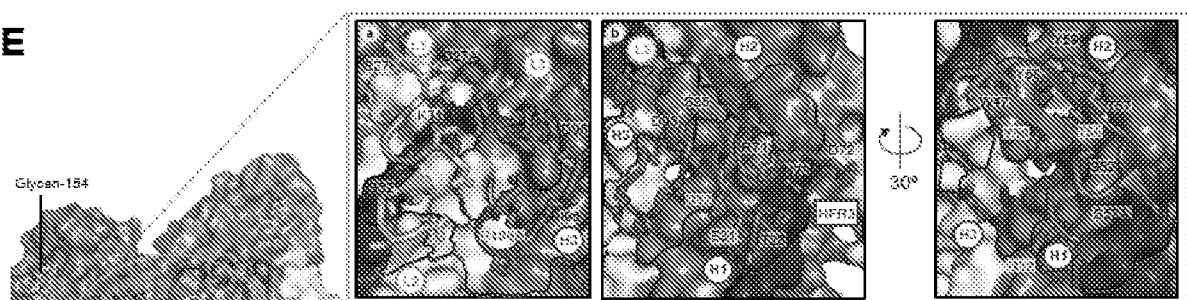
C



D

[illegible]

E

**F**

G

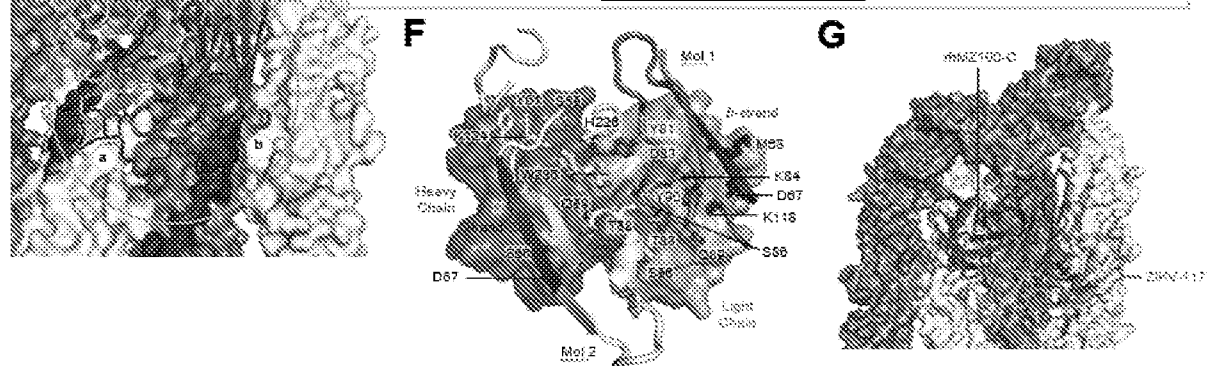


Figure 6

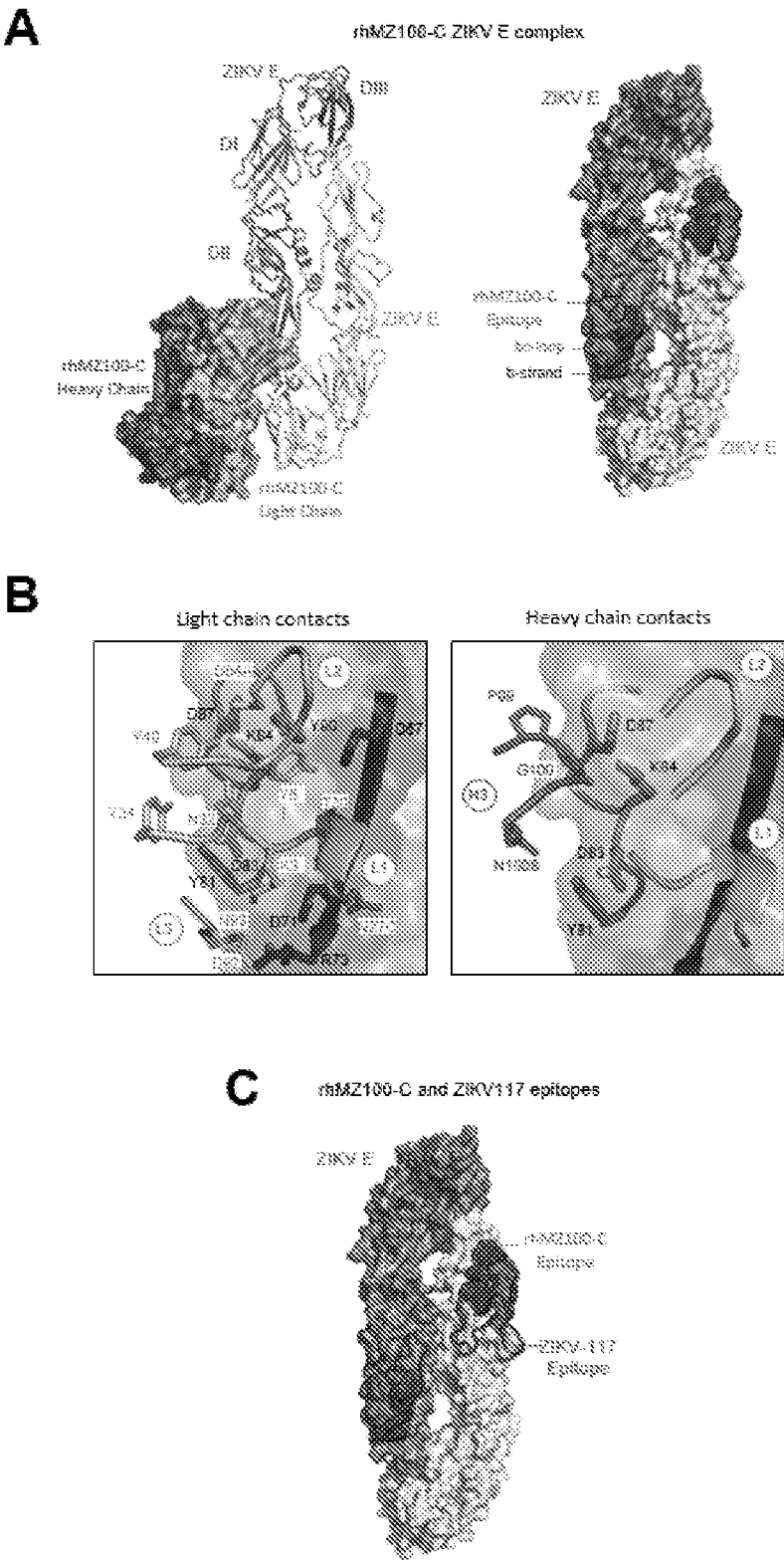


Figure 7

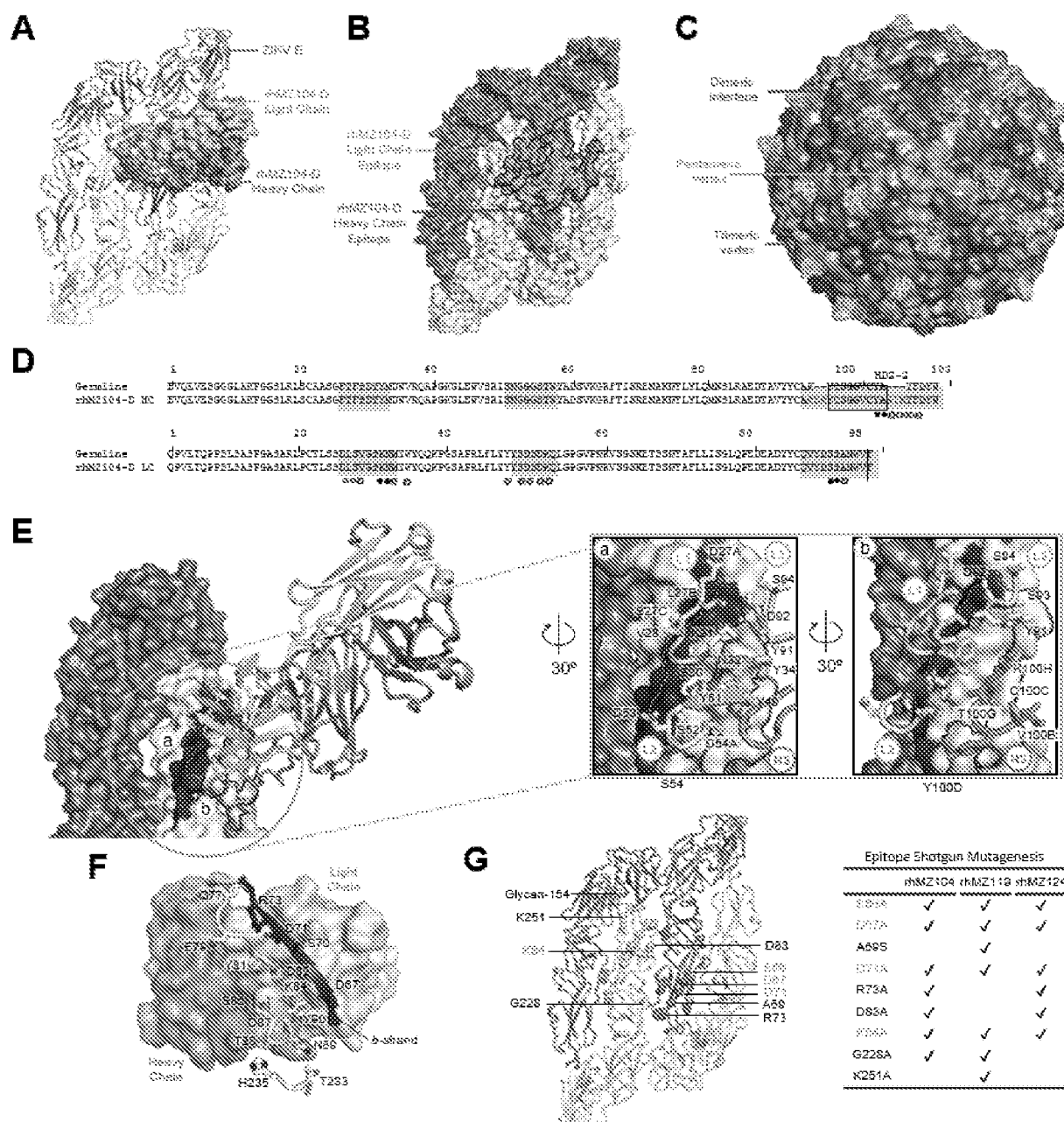


Figure 8

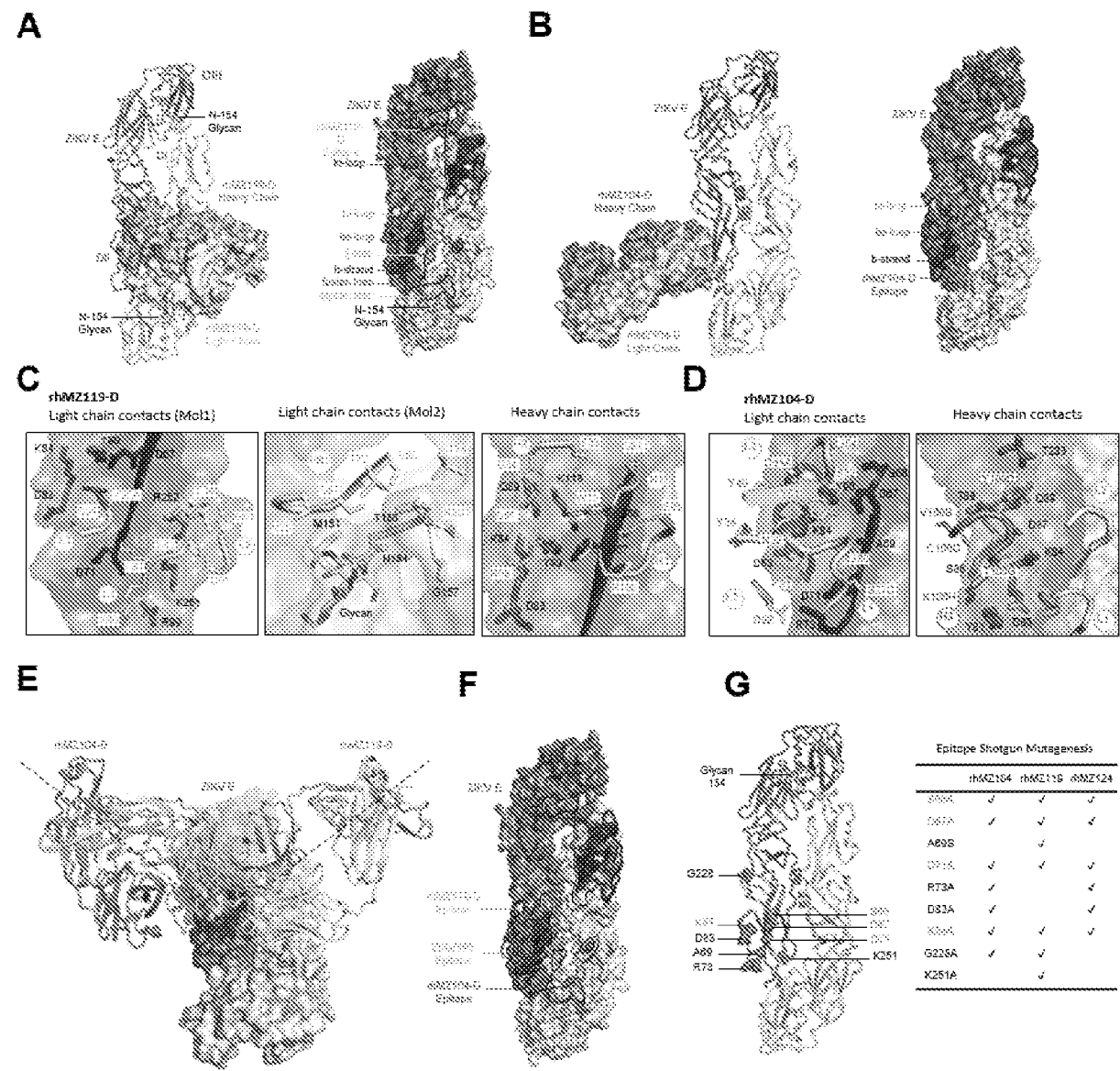
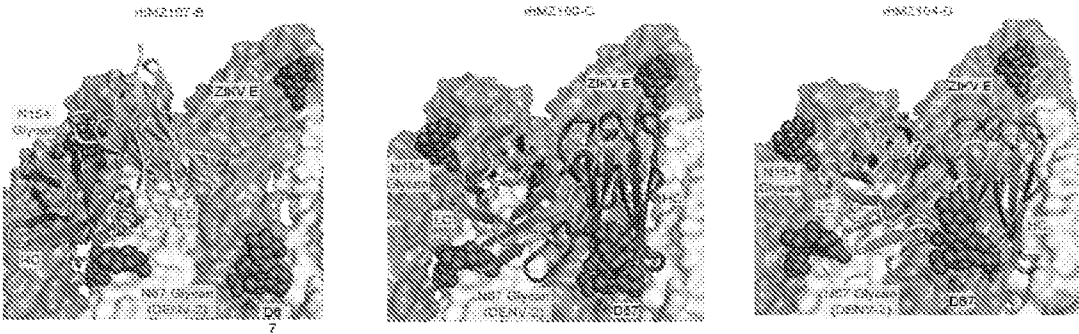
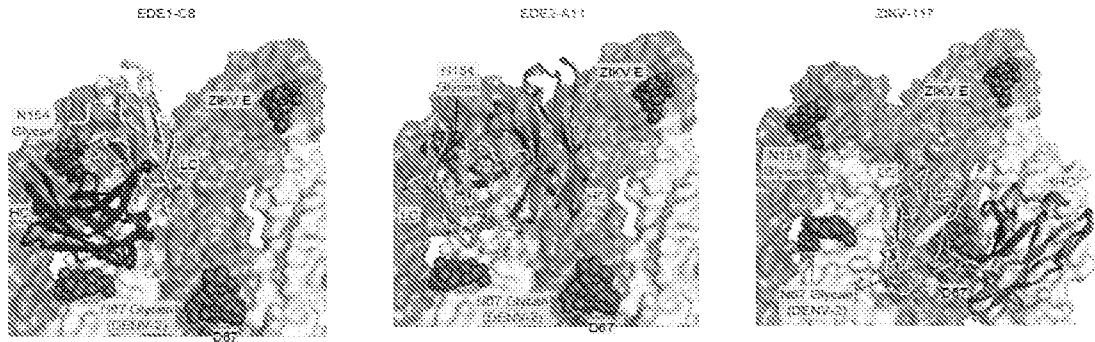


Figure 9

A



B



C

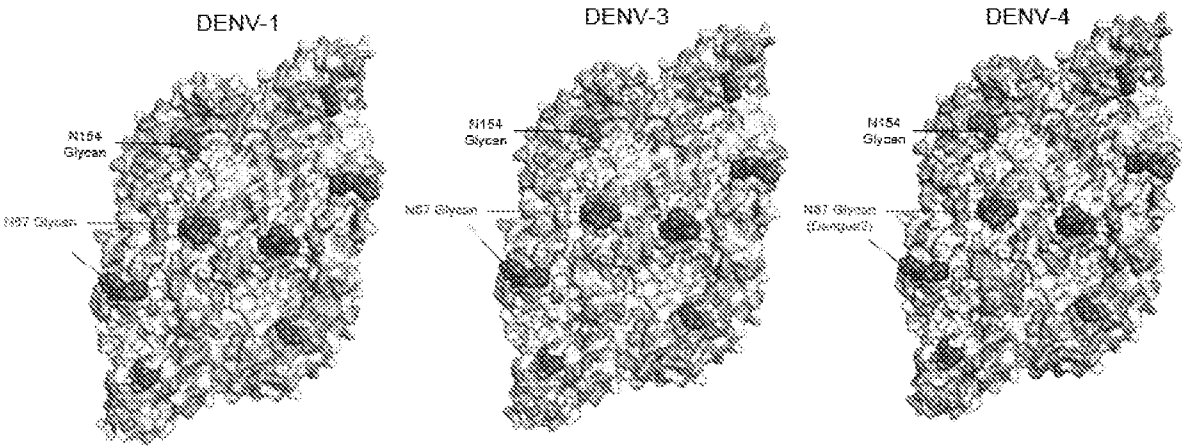


Figure 10

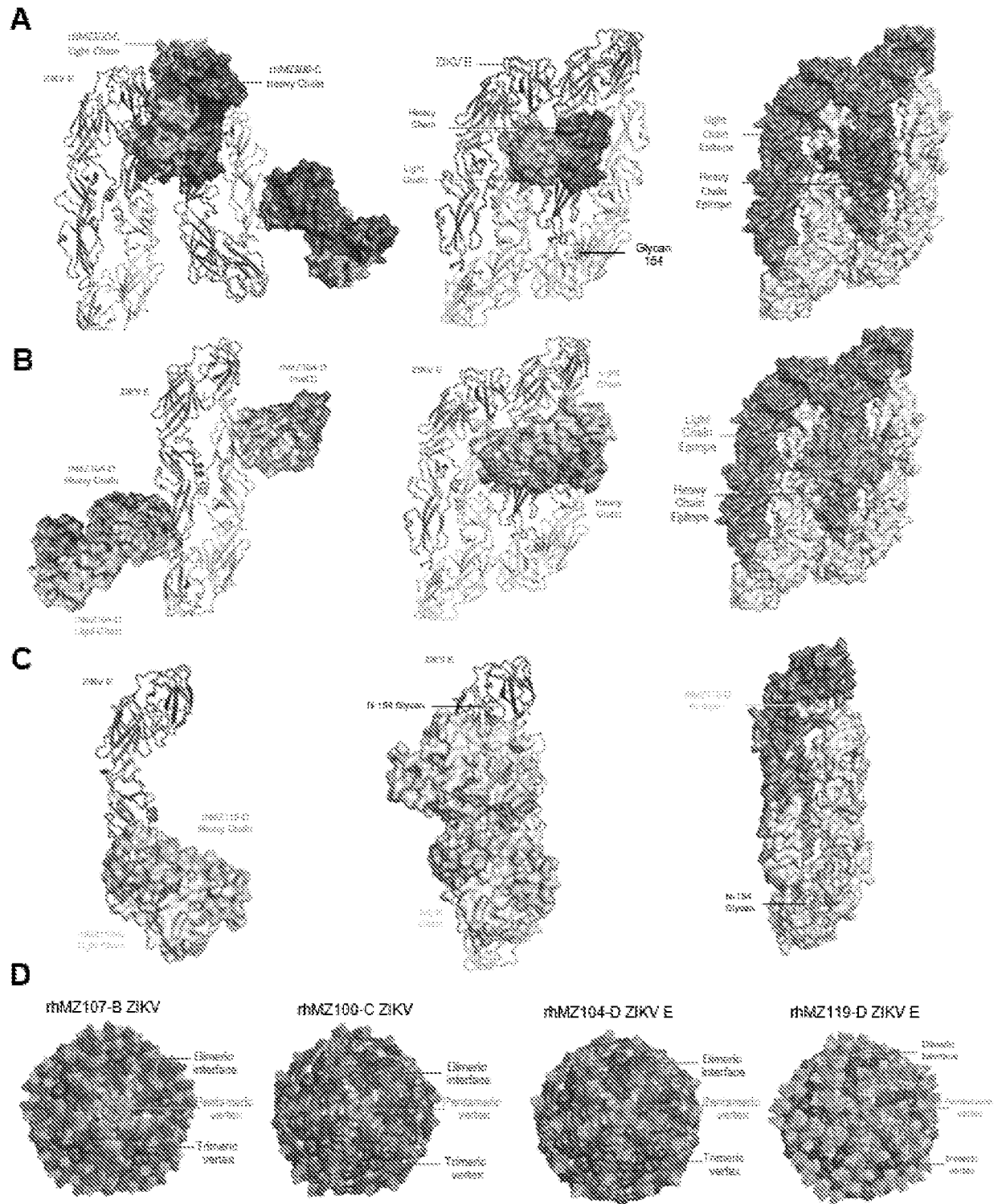


Figure 11

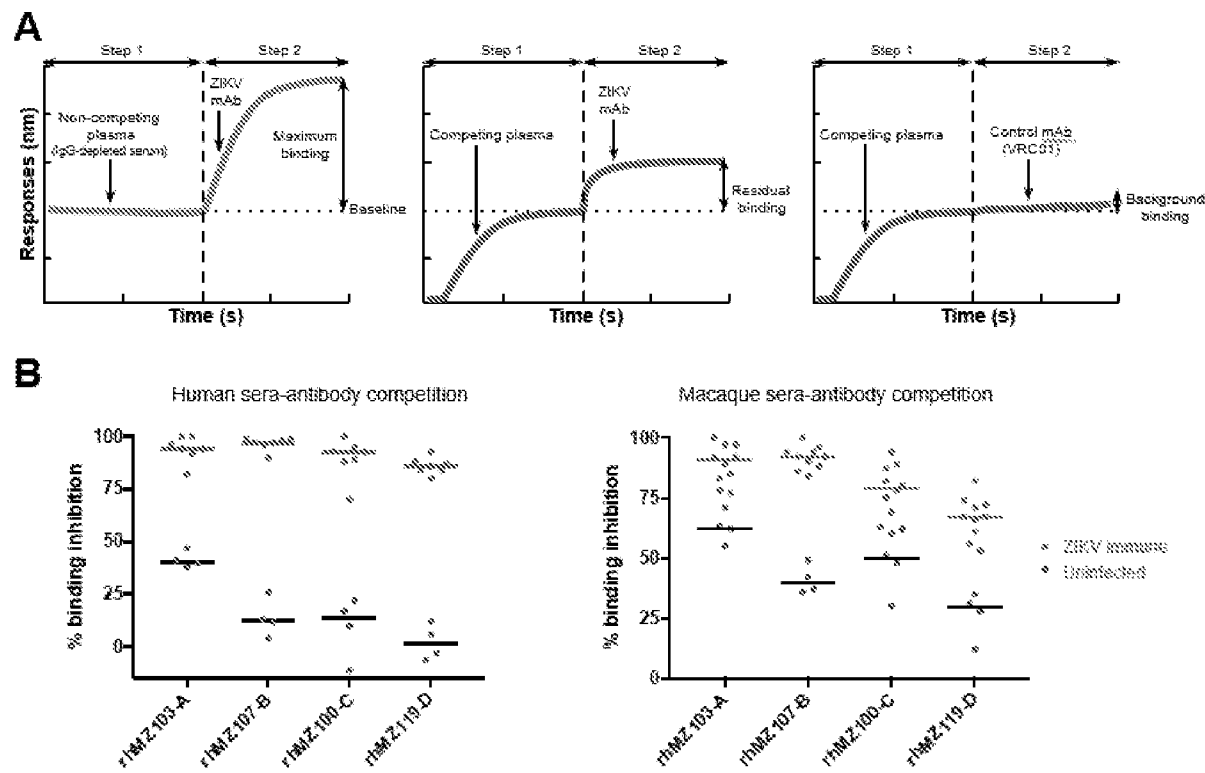


Figure 12

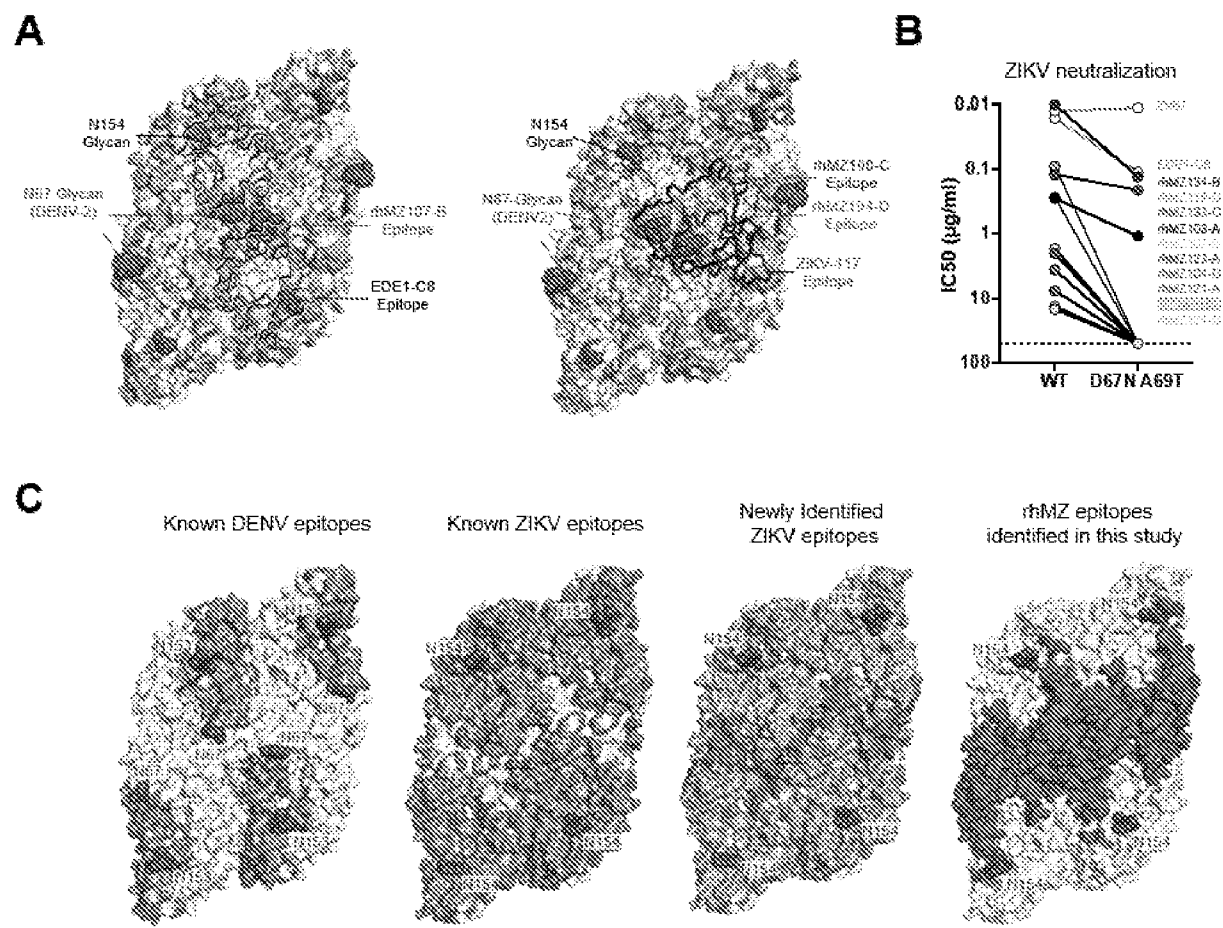


Figure 13

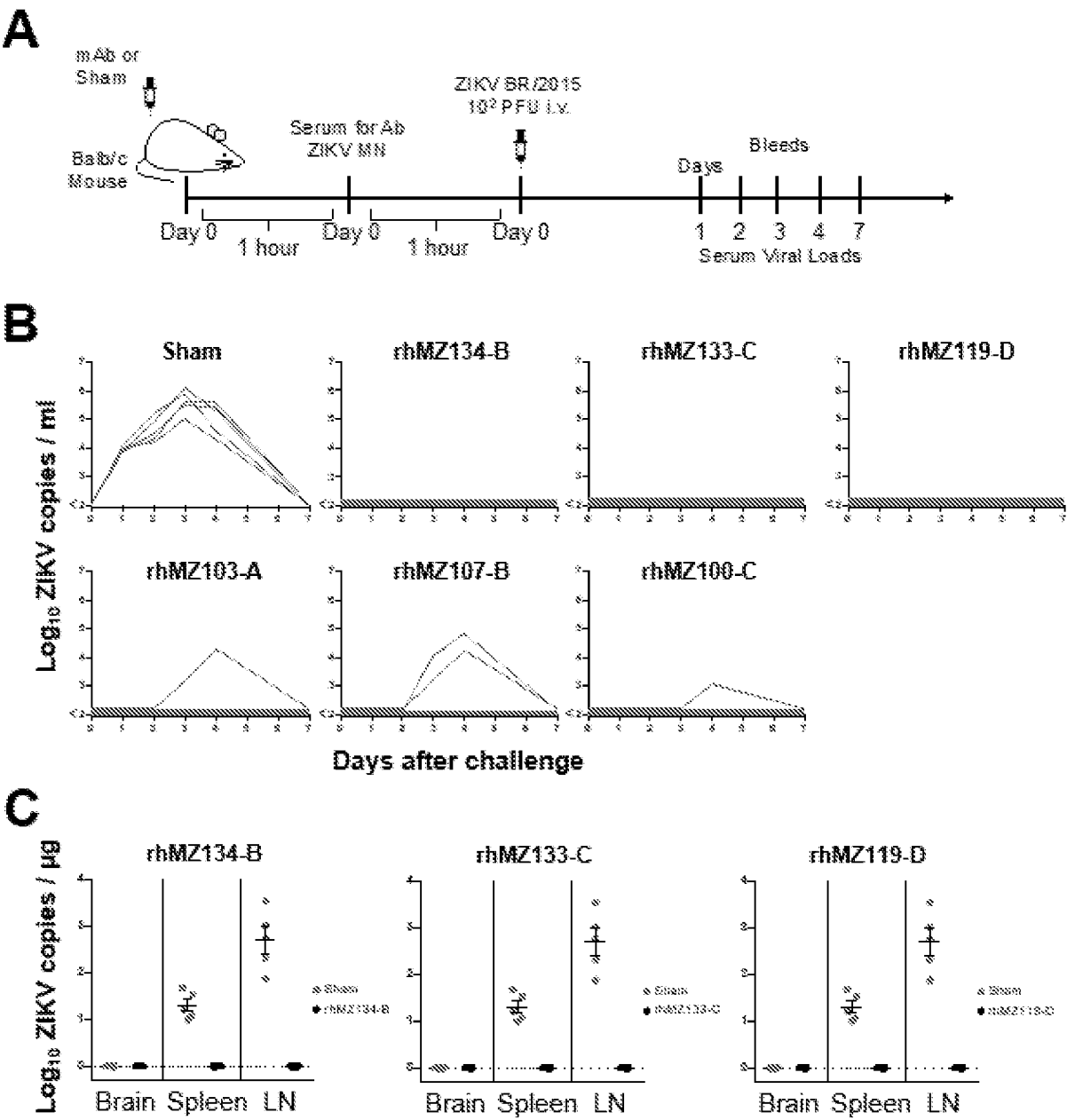


Figure 14

A

mAb ID	ZIKV E binding (BLI)	ZIKV E binding (ELISA)	Whole ZIKV binding (ELISA)	ZIKV Neutralization (MN50)	DENV-2 E binding (BLI)	Whole DENV-2 binding (ELISA)	Flavivirus Neutralization (MN50)
rhMZ100-C	++	+	+++	++	-	+	-
rhMZ101-B	+	-	++	+	-	+	-
rhMZ103-A	+	-	++	++	-	-	-
rhMZ104-D	++++	+++	+++	+	-	-	-
rhMZ105	+	-	+	-	-	-	-
rhMZ106	+	+	+	-	-	-	-
rhMZ107-B	++	-	+++	++	-	-	-
rhMZ113	+	-	+	-	-	-	-
rhMZ115	+	-	+	-	-	-	-
rhMZ118	+	-	+	-	+	+++	-
rhMZ119-D	++++	+	+++	++	-	-	-
rhMZ120	+	-	++	-	+	++	-
rhMZ121-A	+	-	+	+	-	-	-
rhMZ123-A	+	-	+	++	-	-	-
rhMZ124-D	++++	+	+++	++	-	-	-
rhMZ125	+	-	+	-	-	-	-
rhMZ129	+	-	+	-	-	-	NT
rhMZ130	+	+	+	-	-	-	-
rhMZ132	+	-	+	-	-	-	-
rhMZ133-C	+++	-	+++	+++	-	-	-
rhMZ134-B	++	-	++	++++	-	-	-
rhMZ136	+	-	+	-	-	-	-
rhMZ140	+	+	+	-	-	-	-
2A10G6	++++	++++	+++	NT	NT	+++	AI ^a
EDE1-C8	+++	+	+++	+++	++++	+++	DENV-1/-2/-3/-4
Z004	++++	NT	+++	++++	-	NT	DENV-1 ^{ss}

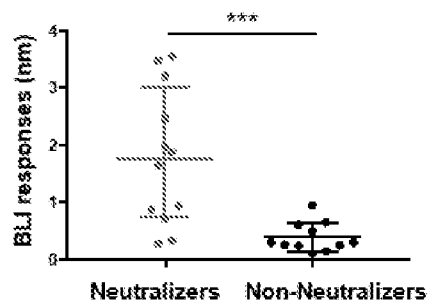
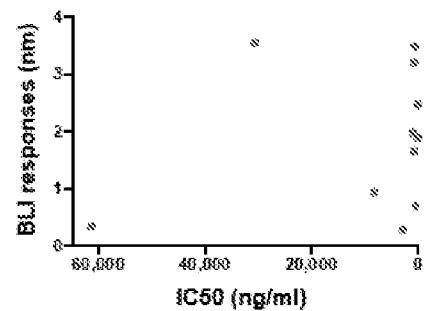
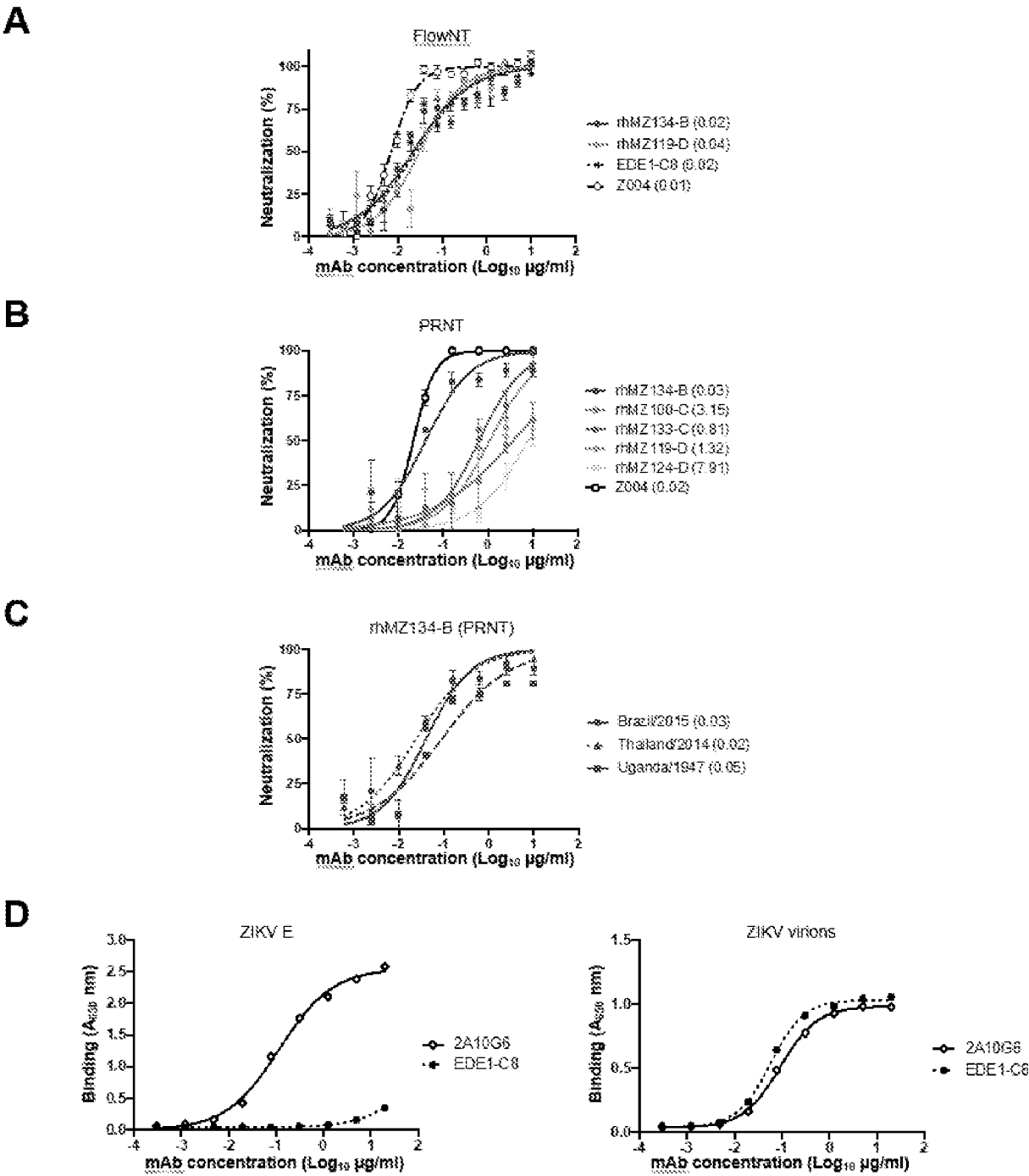
B**C**

Figure 15



A

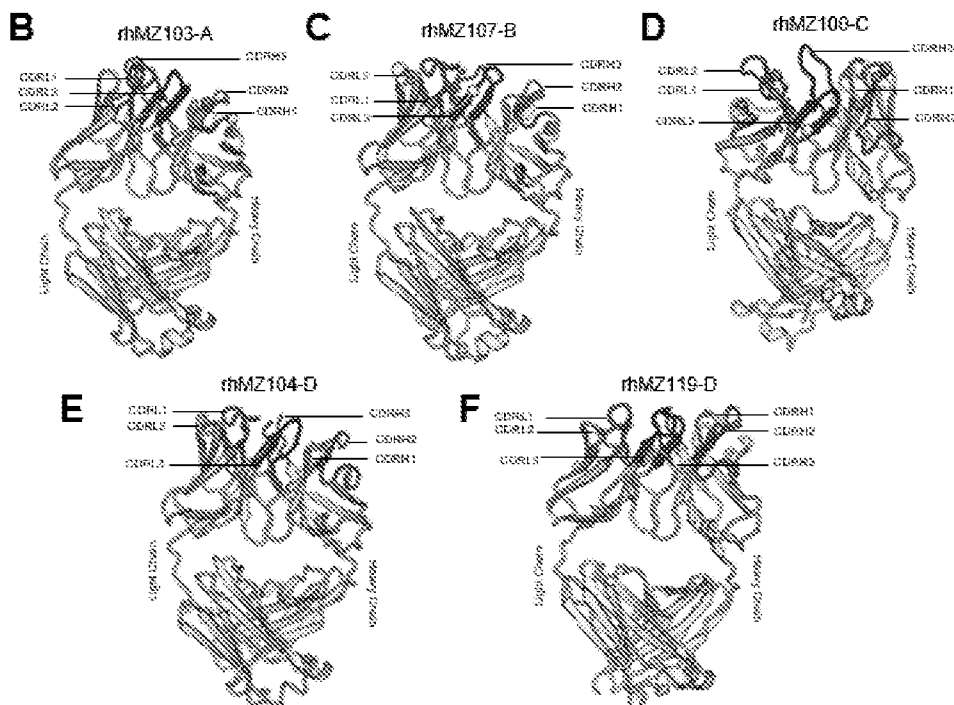


Figure 17

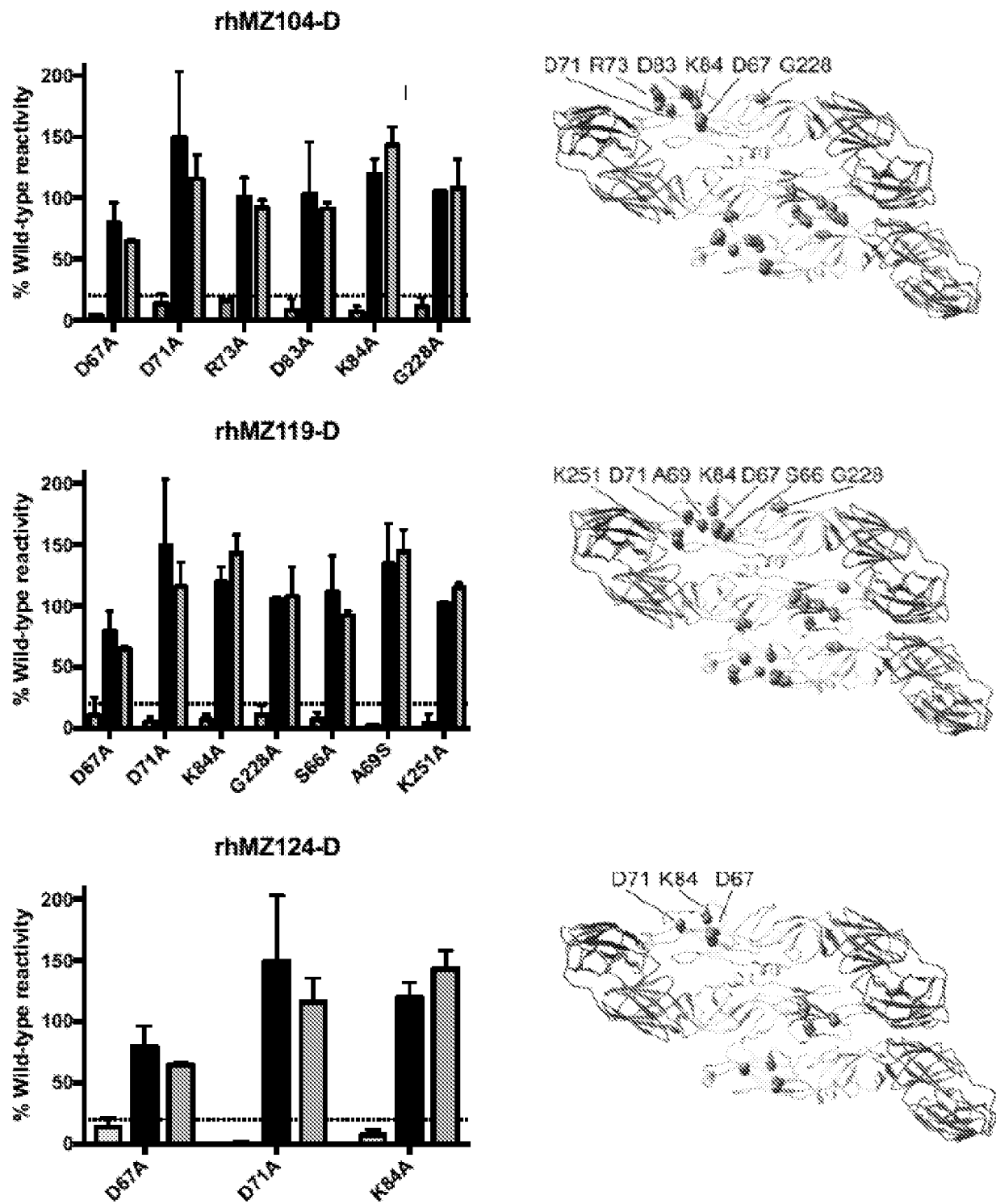


Figure 18

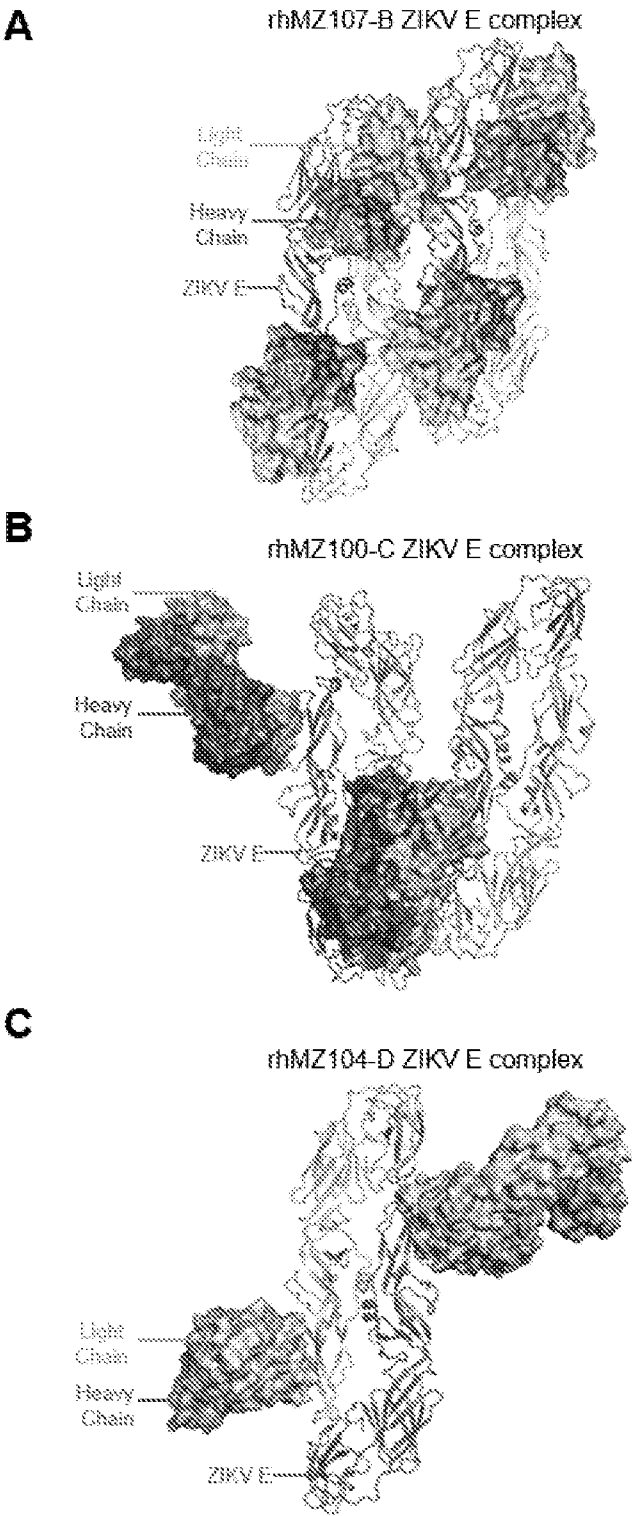


Figure 19

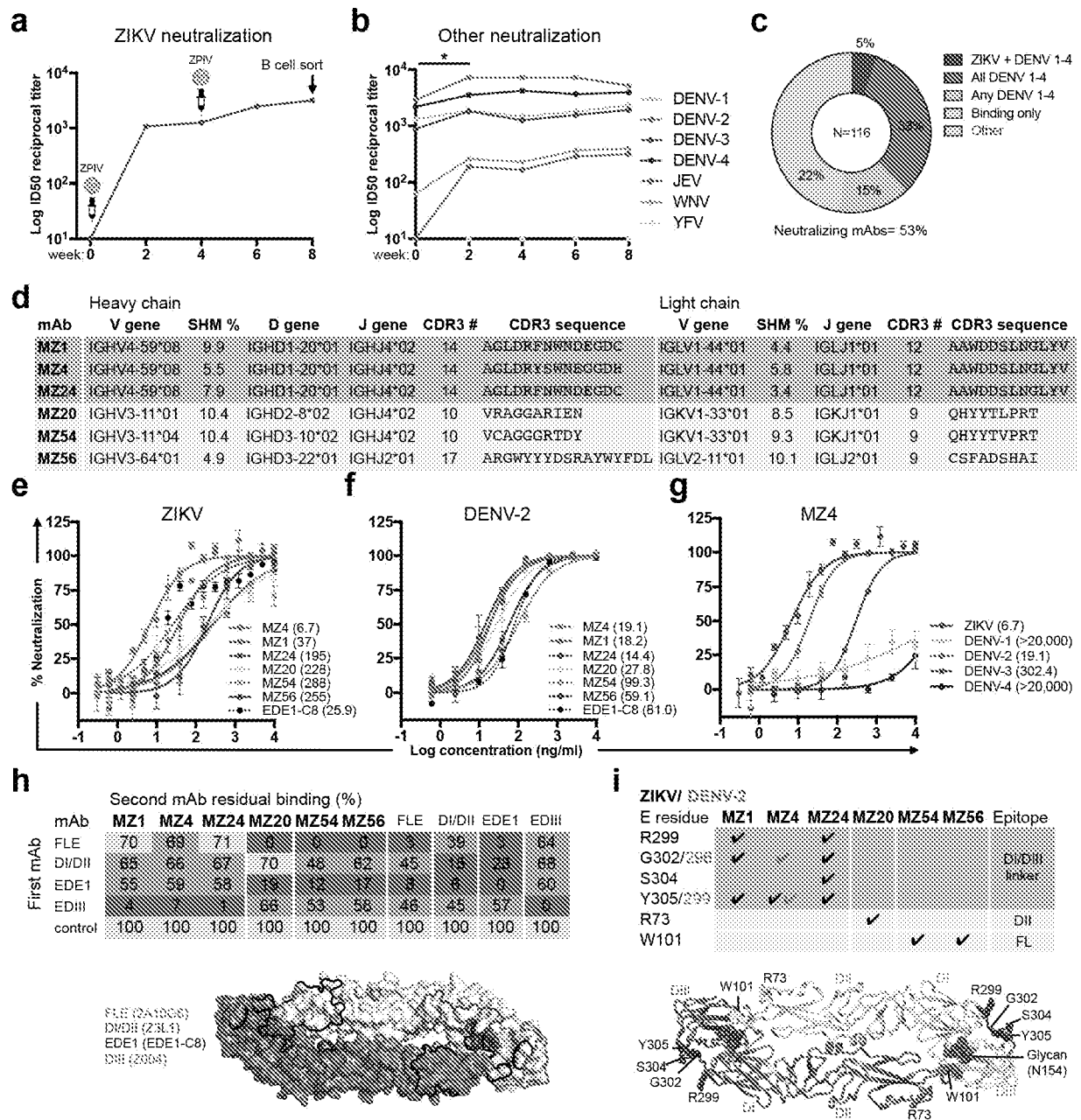


Figure 20

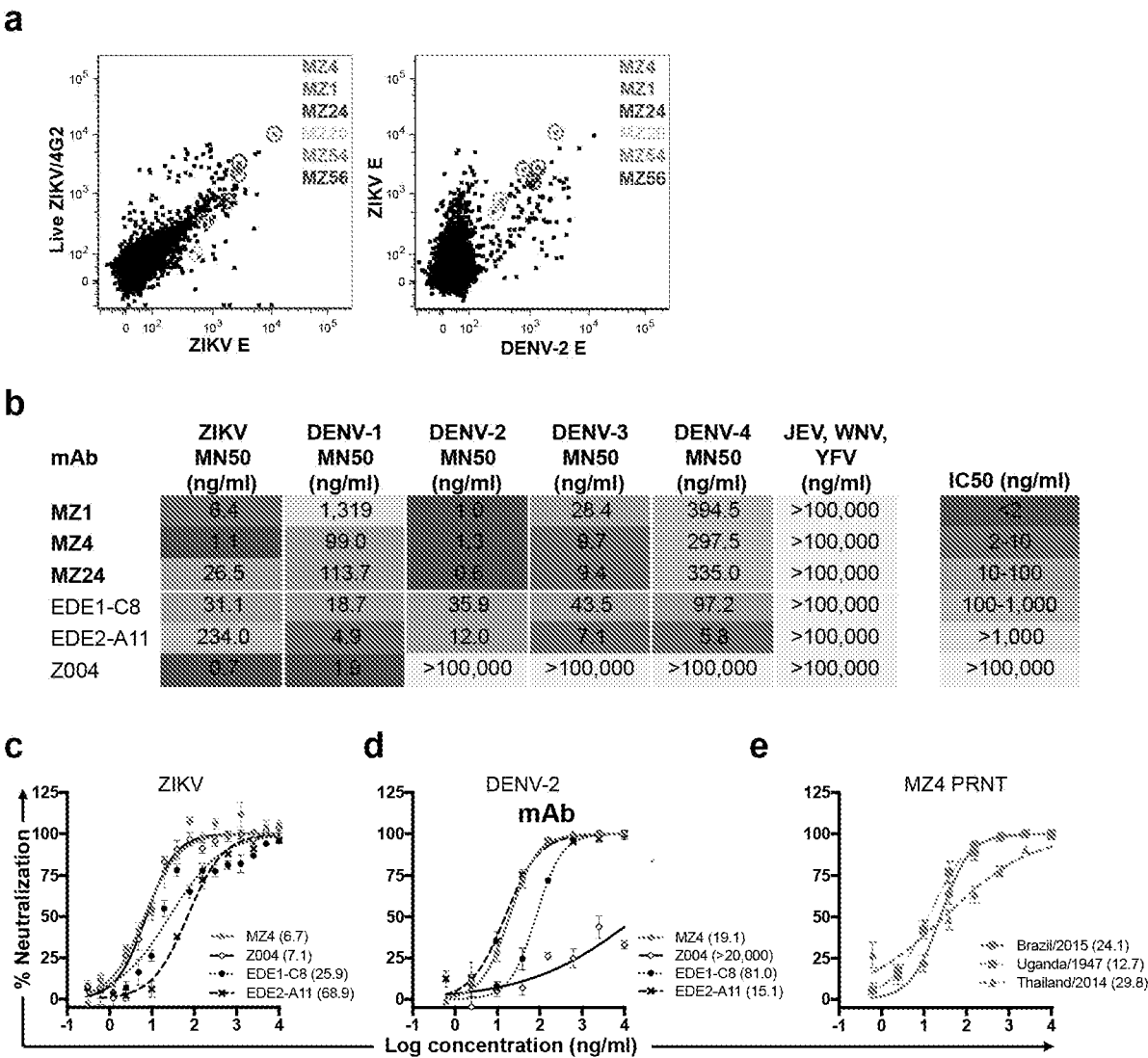


Figure 21

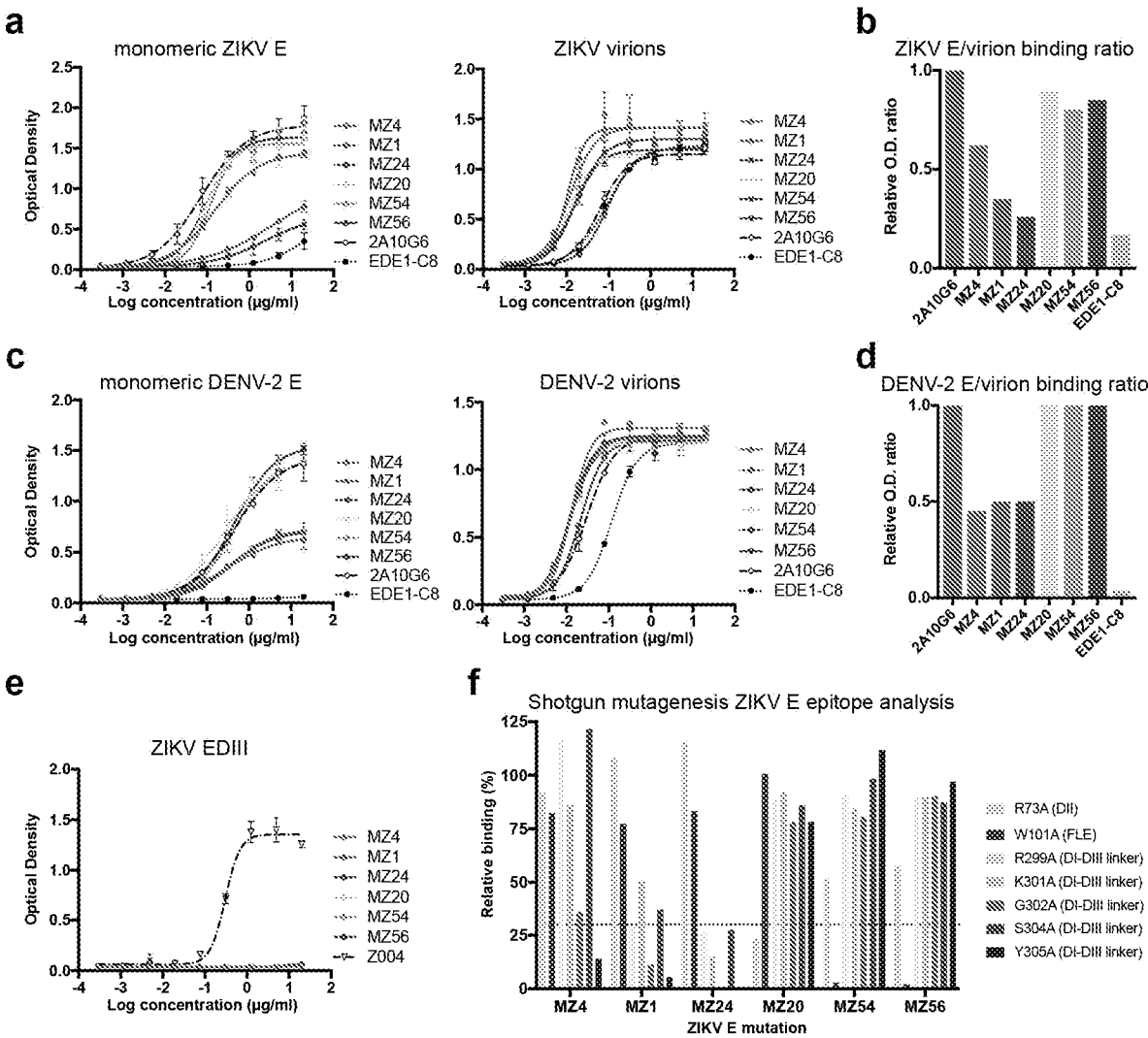


Figure 22

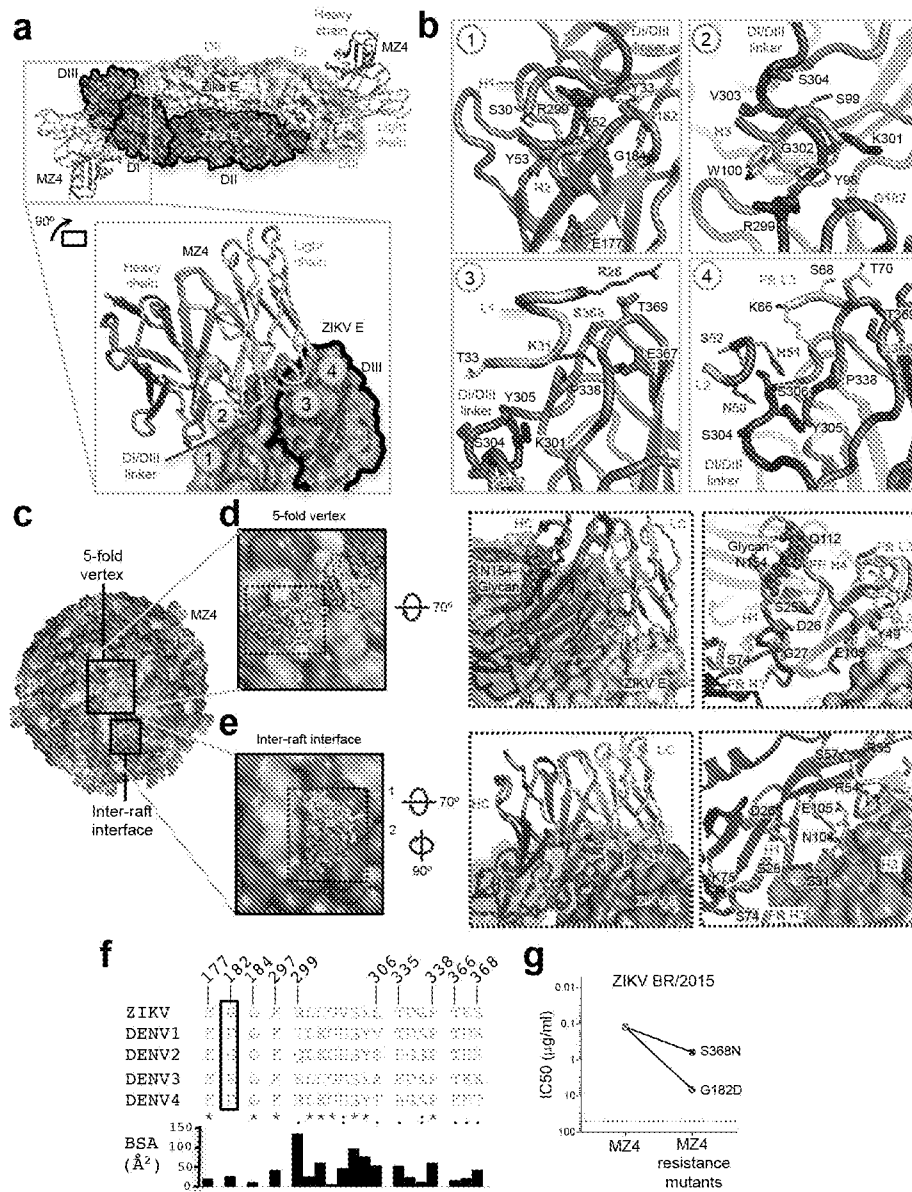


Figure 23

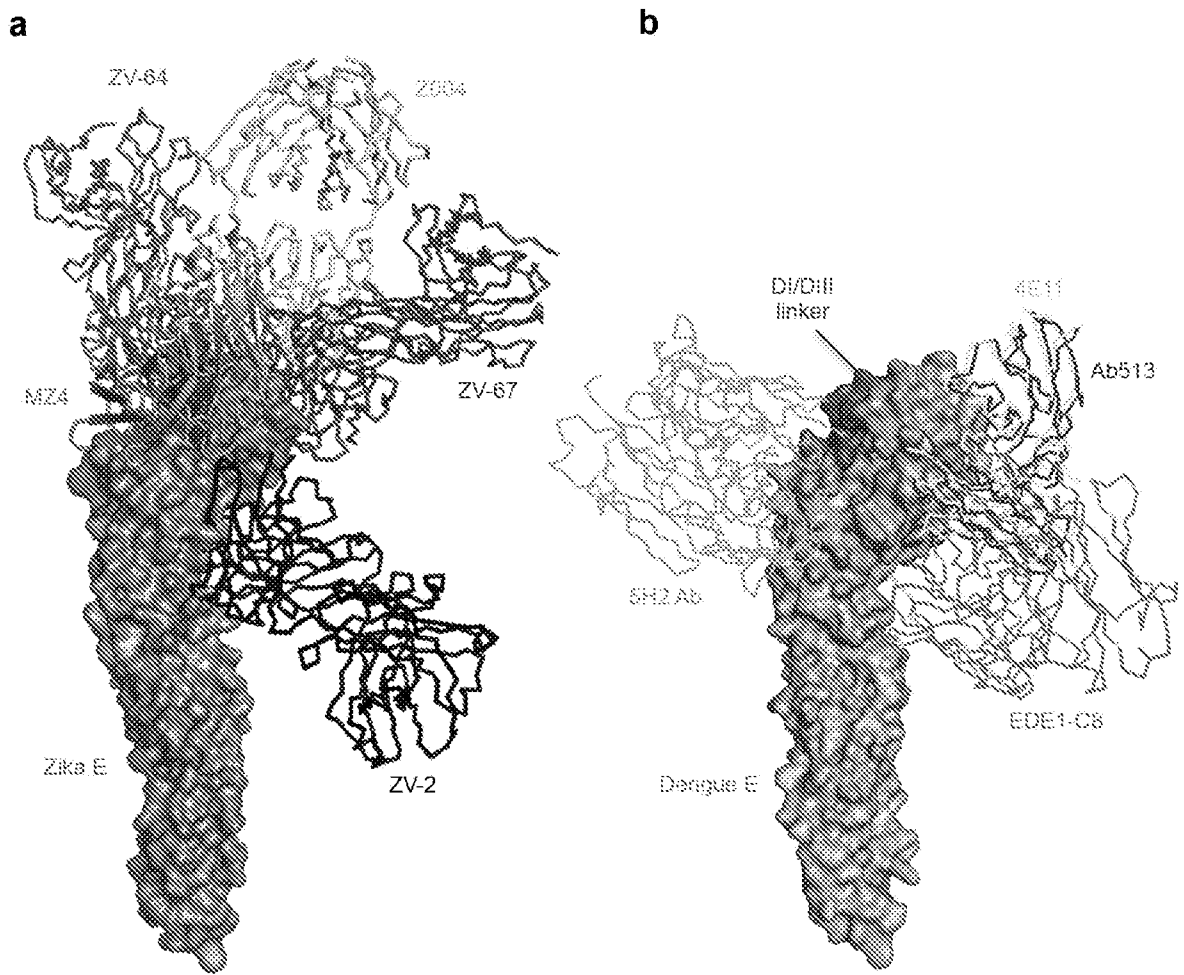


Figure 24

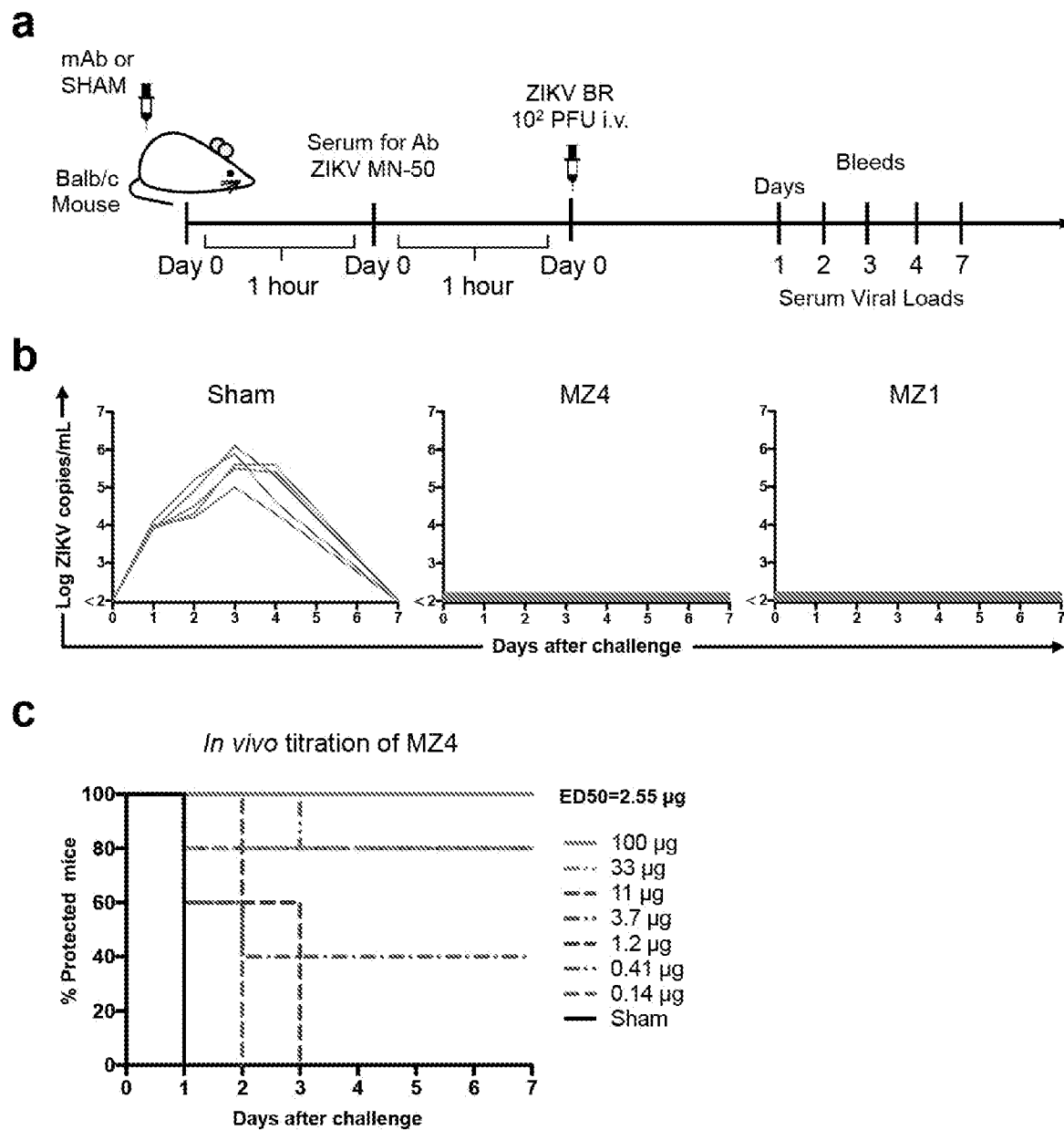


Figure 25

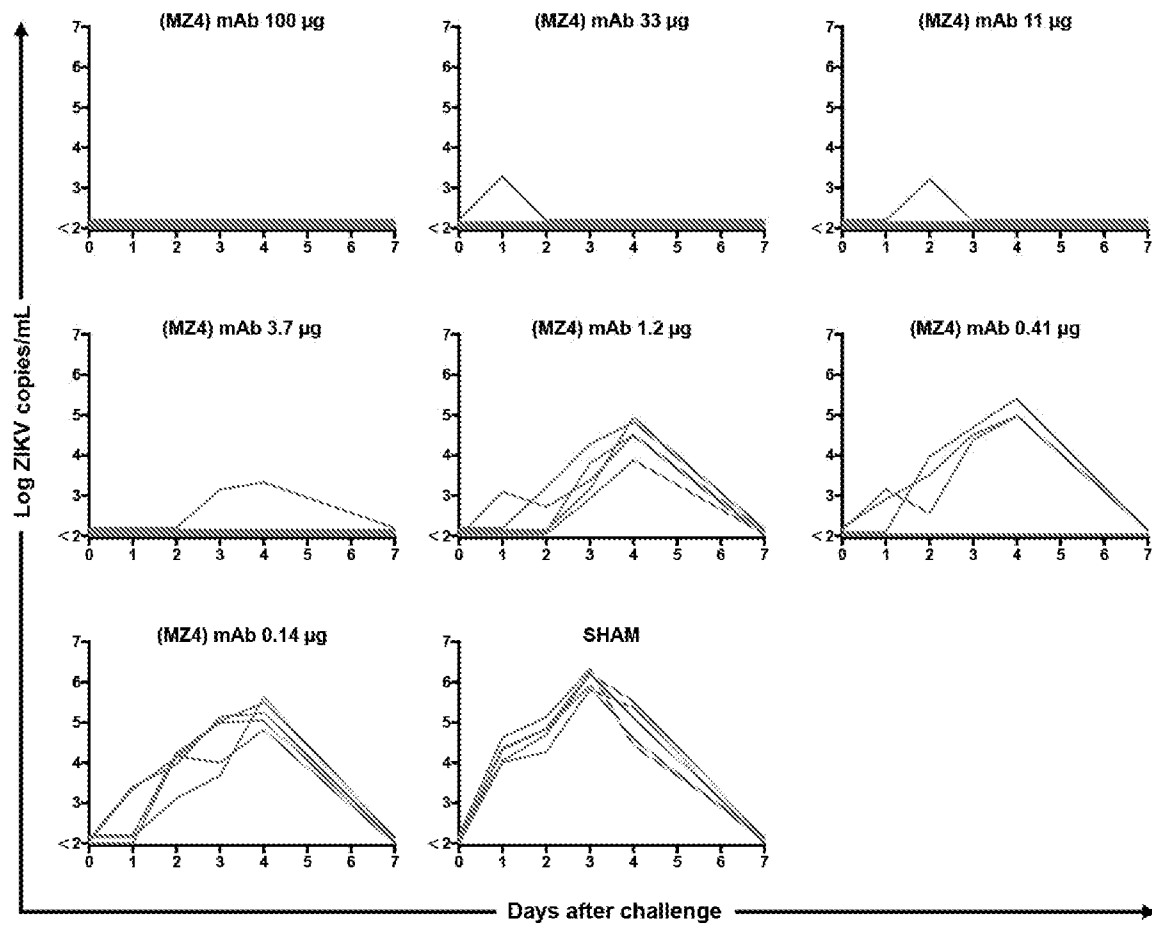


Figure 26

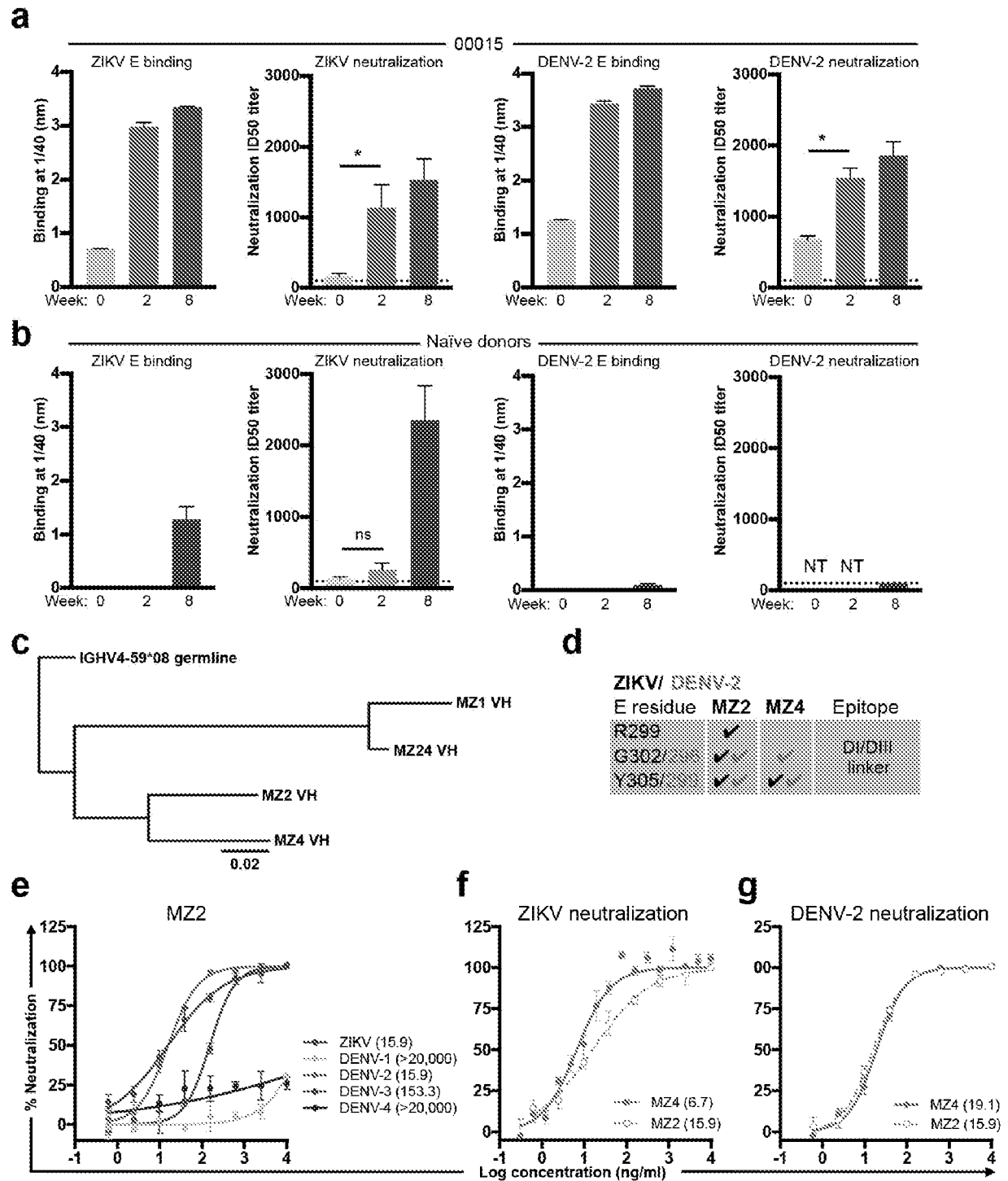


Figure 27

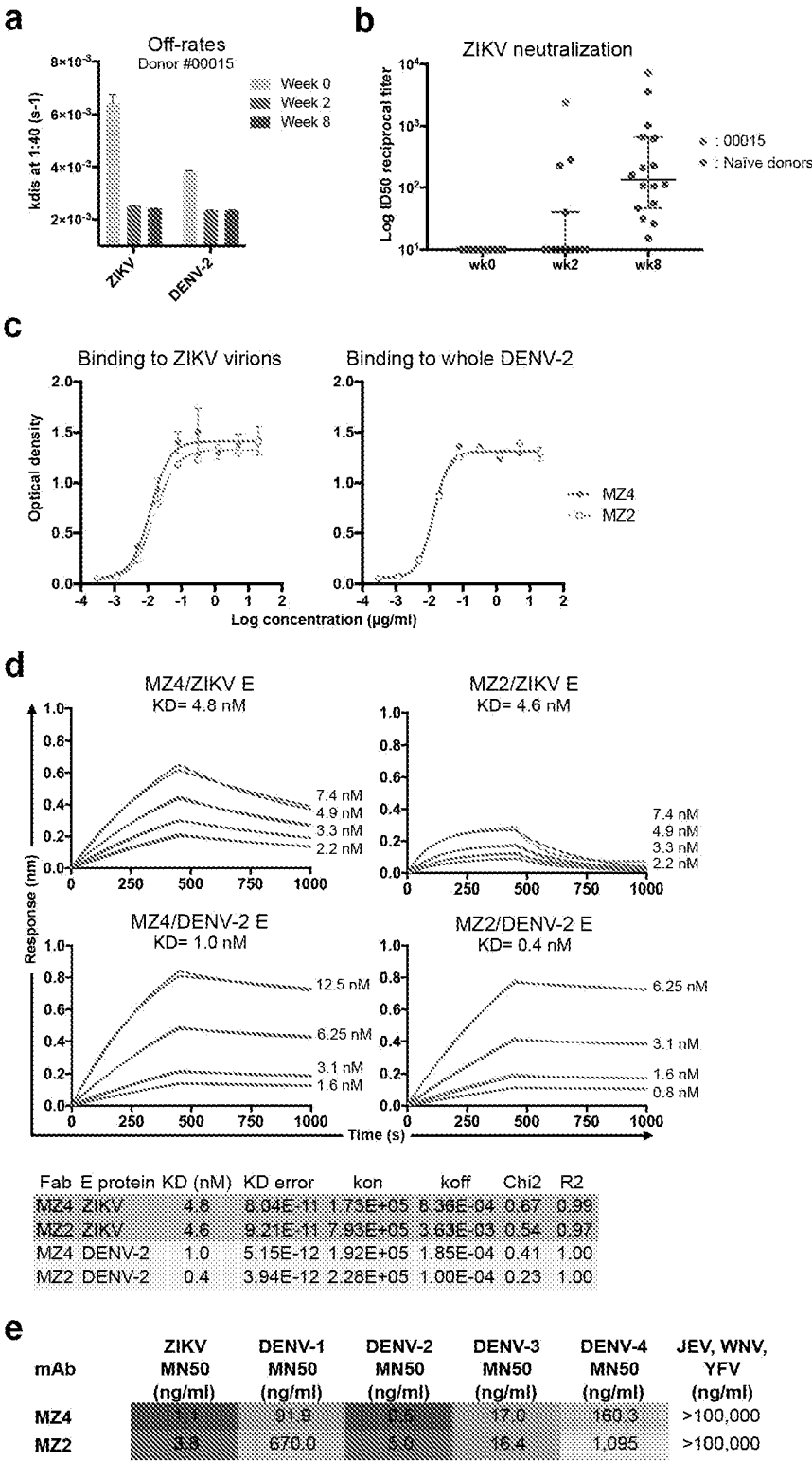
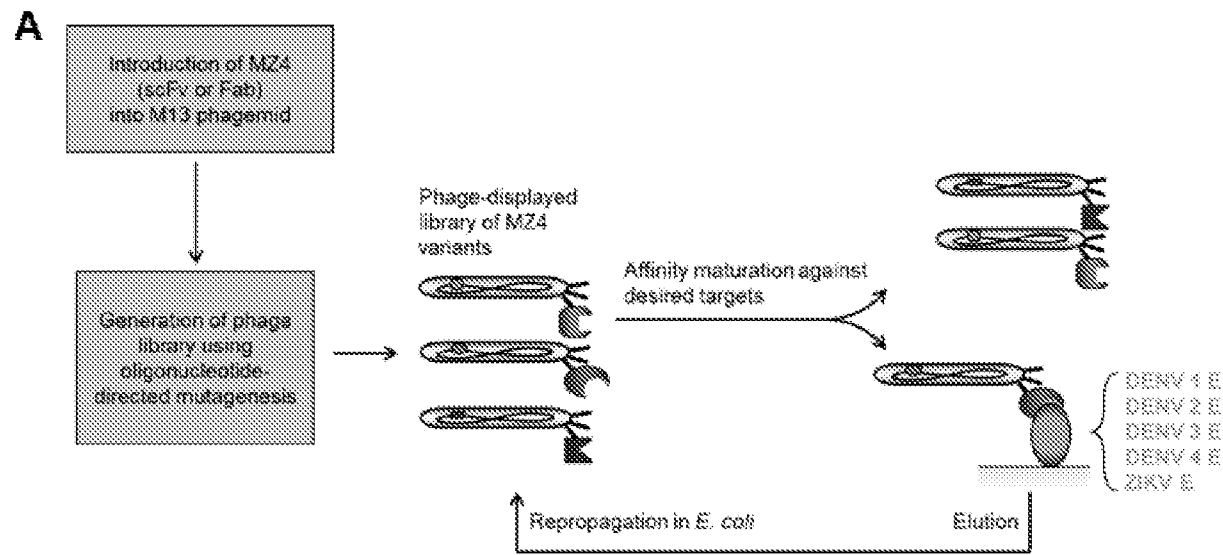


Figure 28



B

	All wt template	All Stop4 template
	Templ: Library: Displayed Protein? Proteins will be displayed whether or not they are library members, but majority will contain wild-type sequences	Templ: Library: Displayed Protein? Only library members will be displayed (wild-type is part of the mix)
Pros:	Most of the variants will be full length → less size-based amplification bias	No overrepresentation of WT in the selections
Cons:	WT will be overrepresented in the selection steps, could be harder to fish out variants	Truncated variants (still containing Stop 4 somewhere) may have a growth advantage

Figure 29

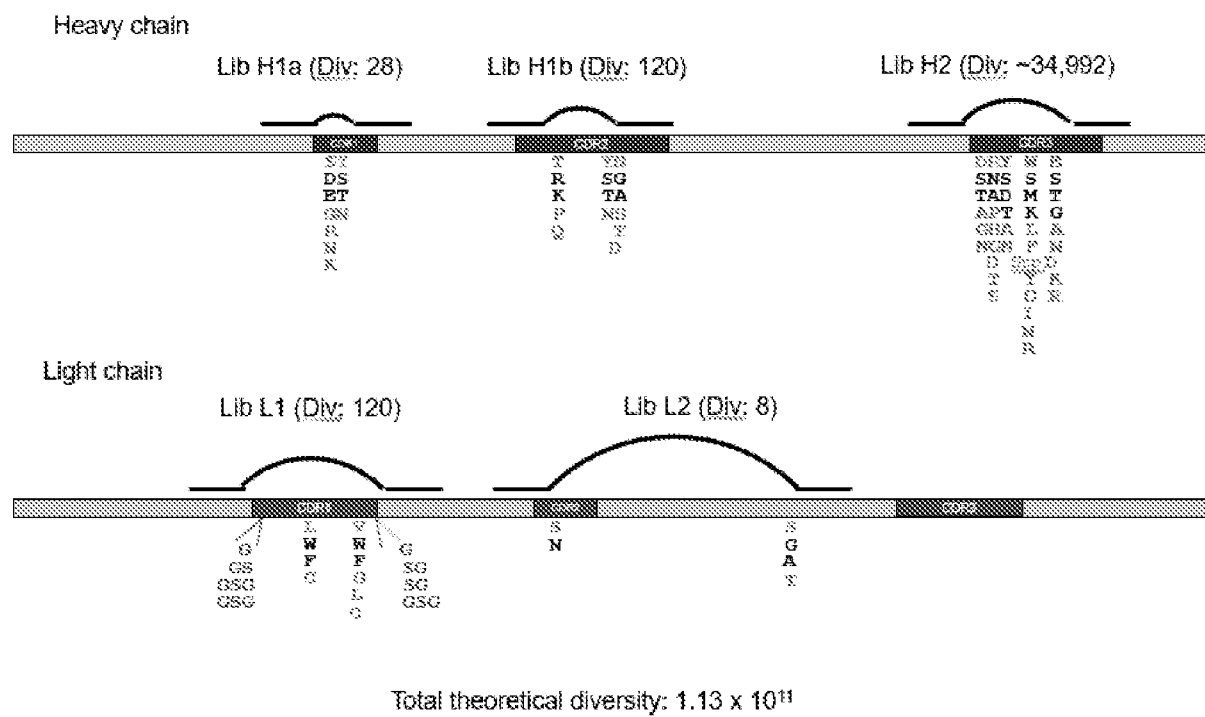


Figure 30

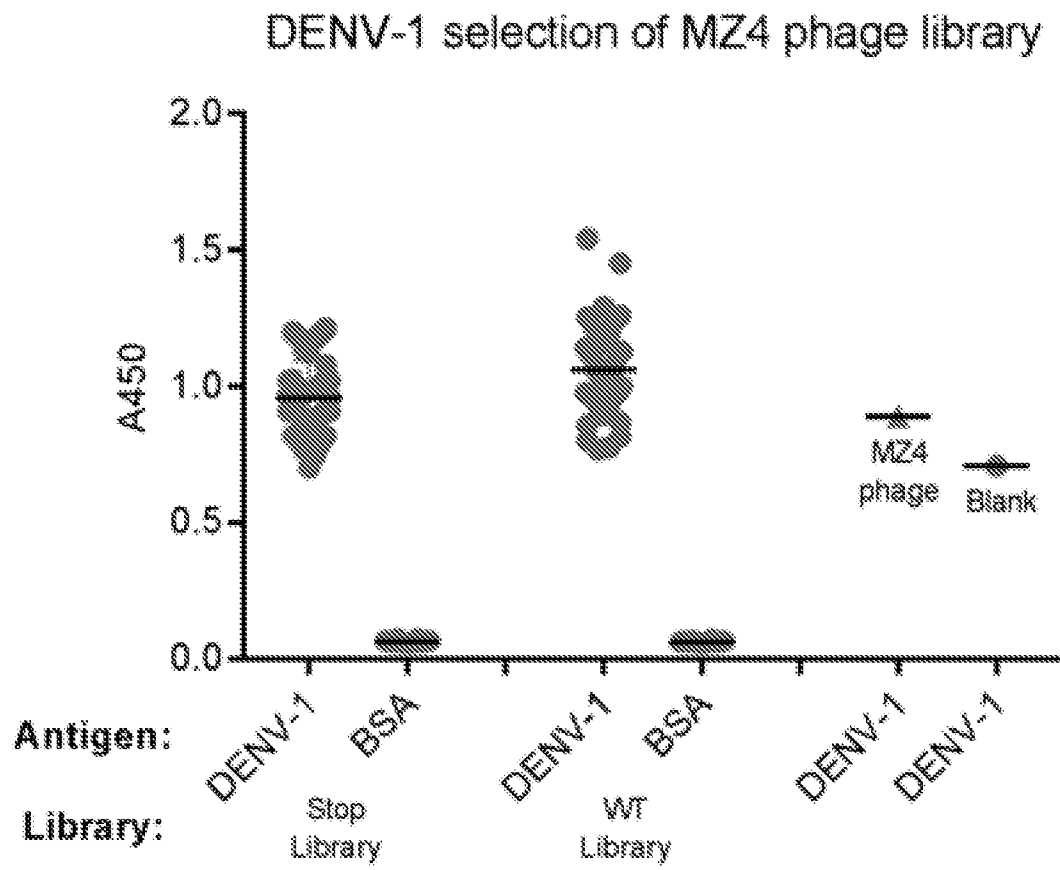


Figure 31 A-C

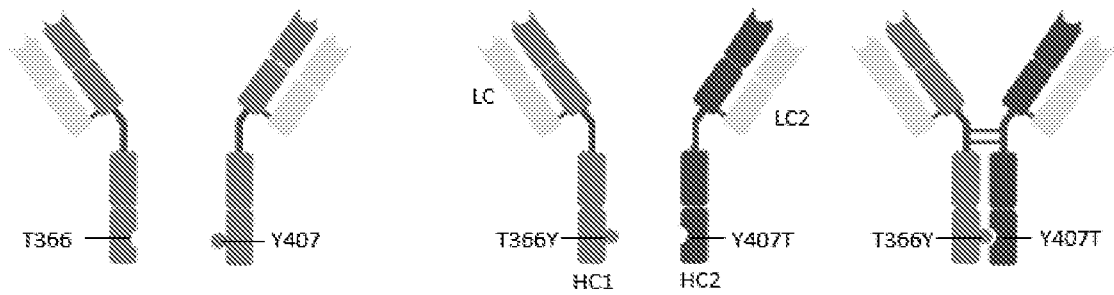
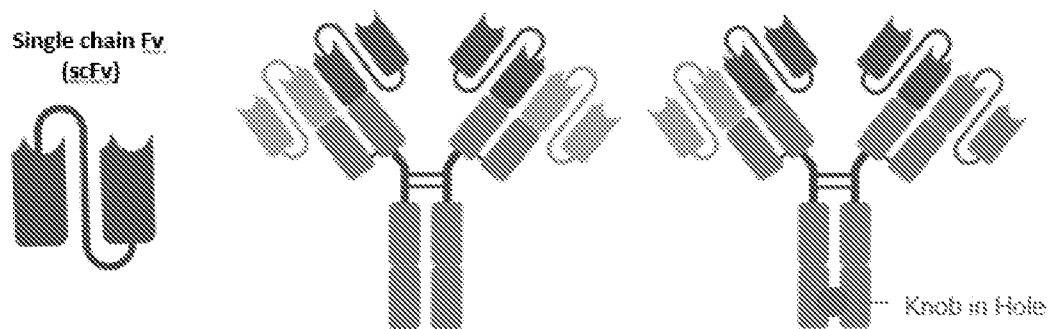
A**Knob in Hole (KIH) Approach****B****Variable Domain Approach****C****Hinge Insertion Approach**

Figure 31 D-E

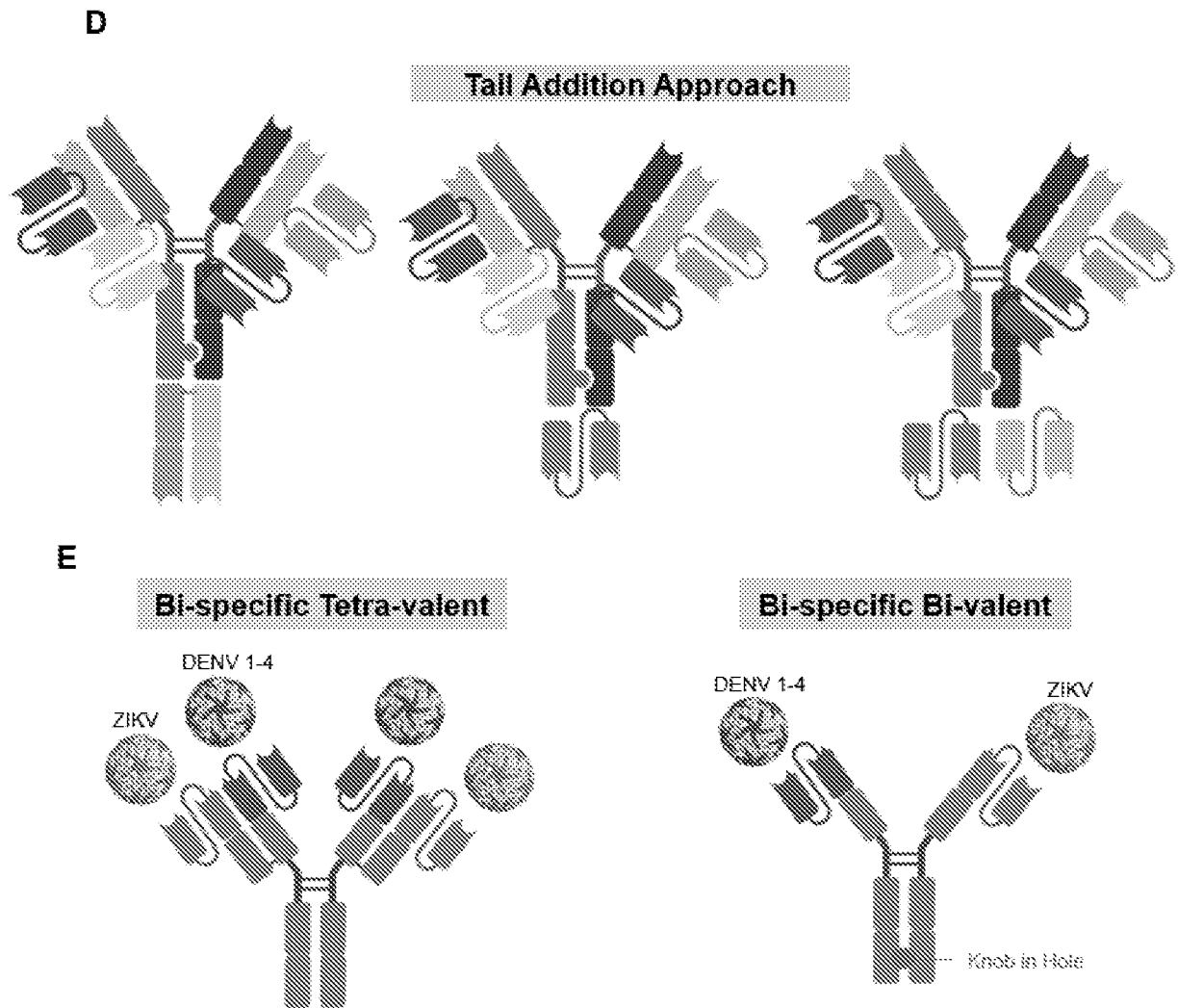


Figure 32

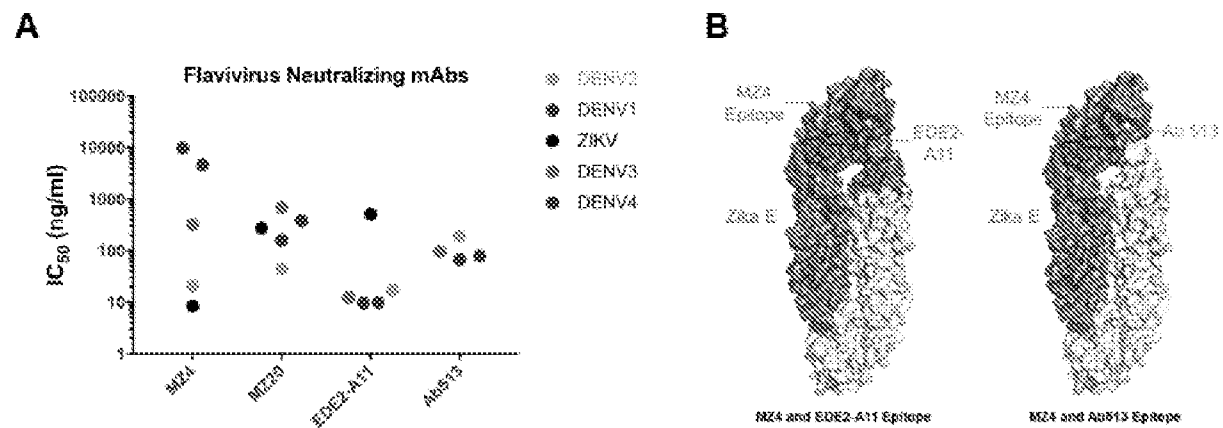


Figure 33

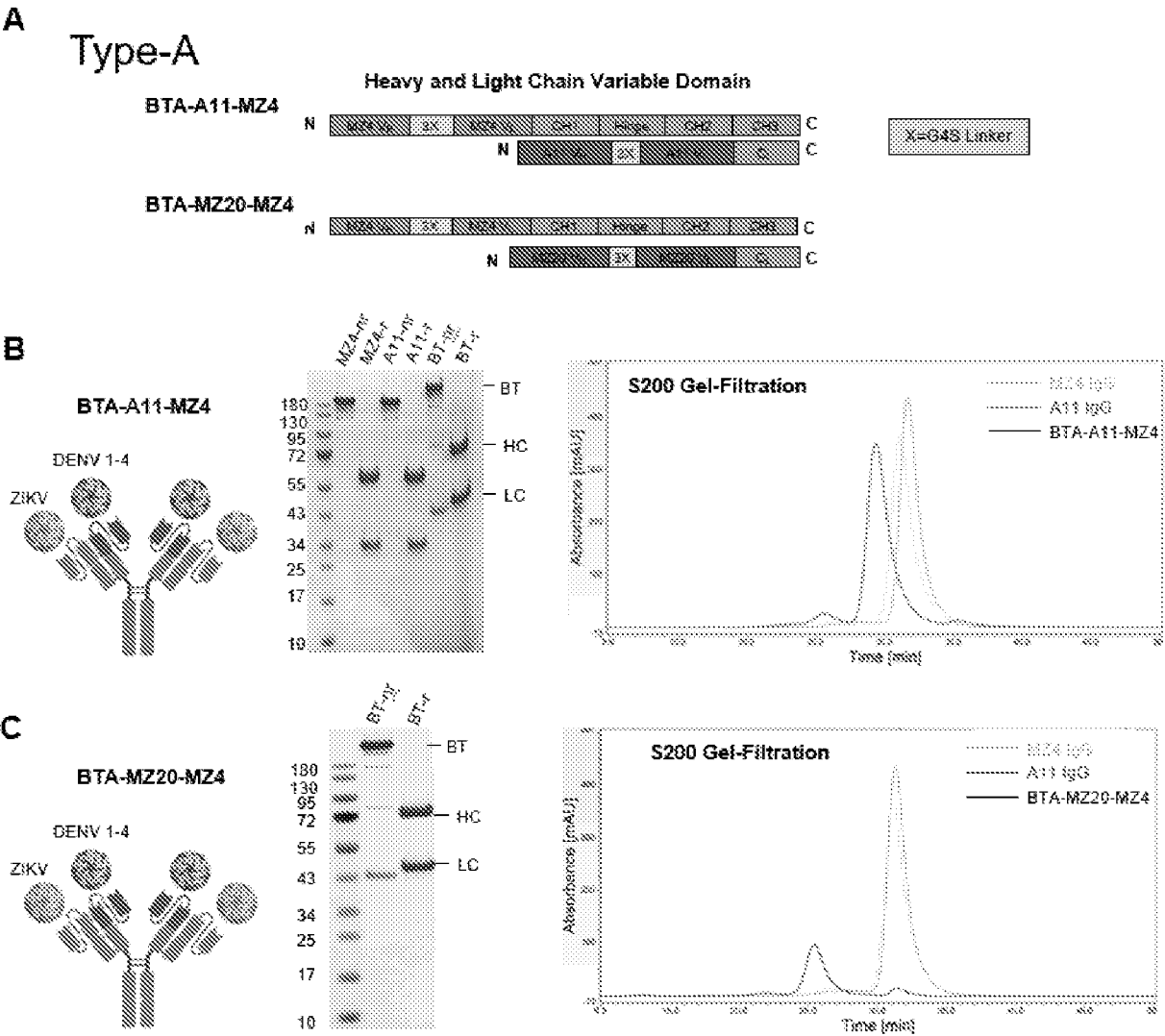


Figure 34

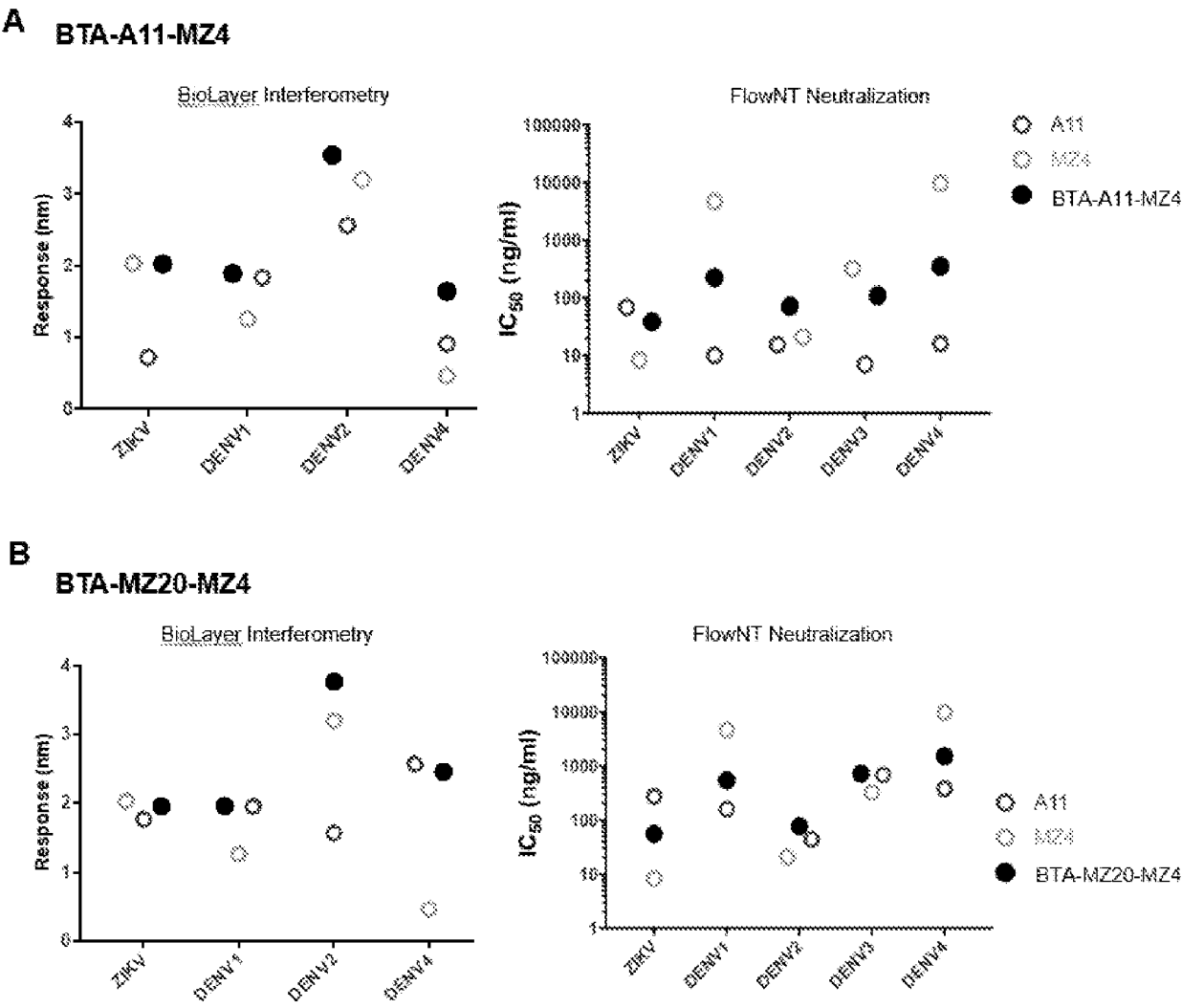
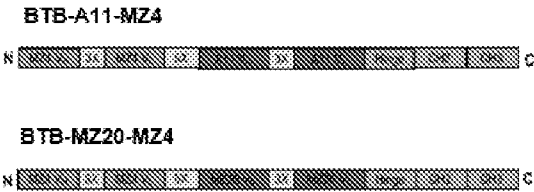
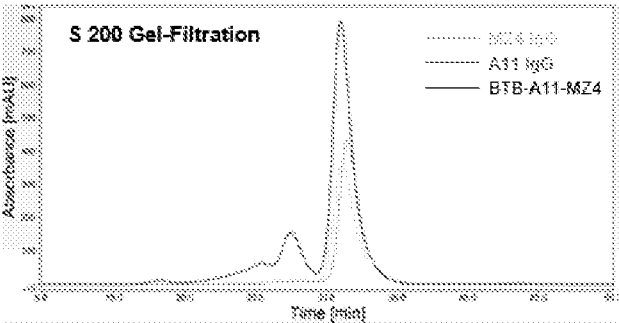
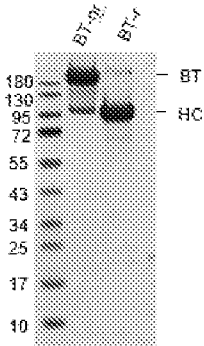
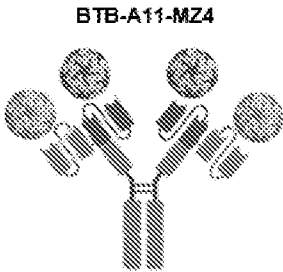


Figure 35

A Type-B



B



C

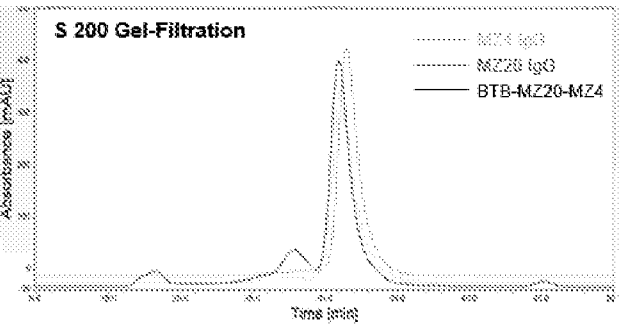
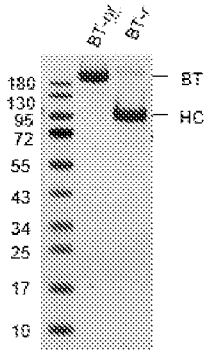
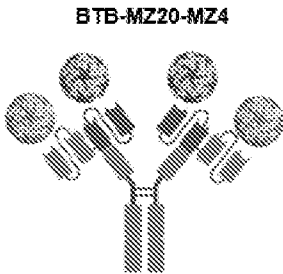
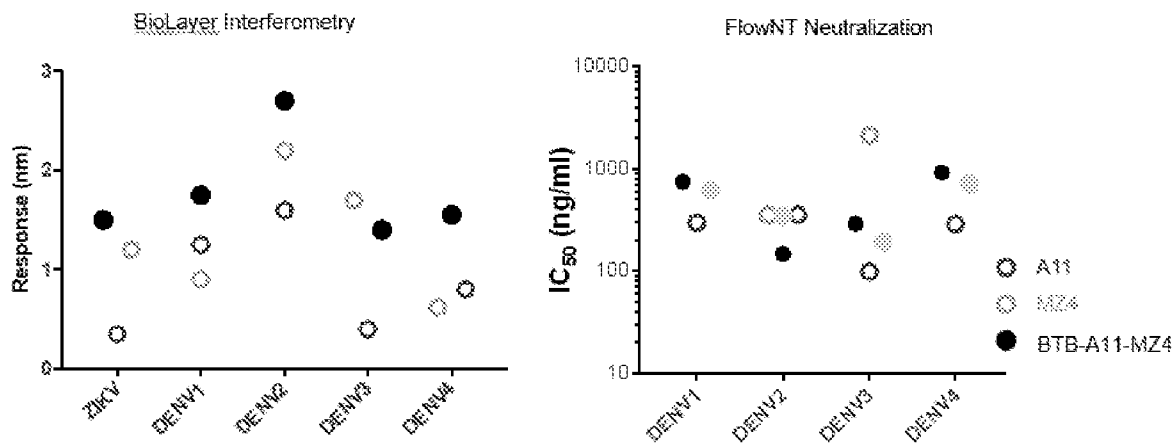


Figure 36

A BTB-A11-MZ4



B BTB-MZ20-MZ4

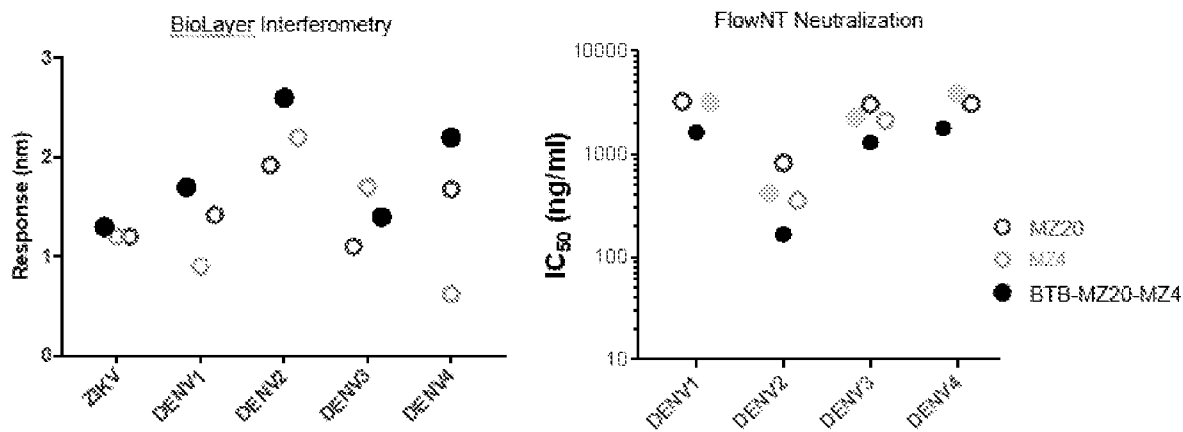


Figure 37

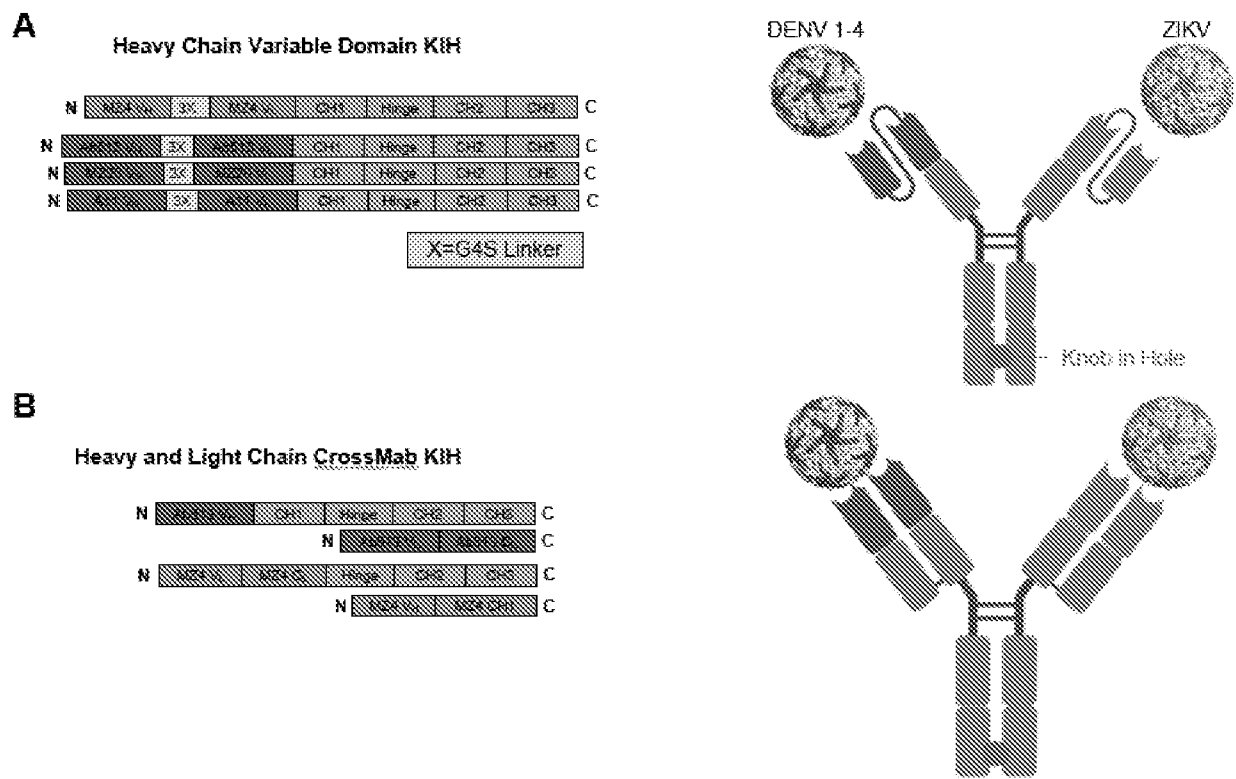


Figure 38

