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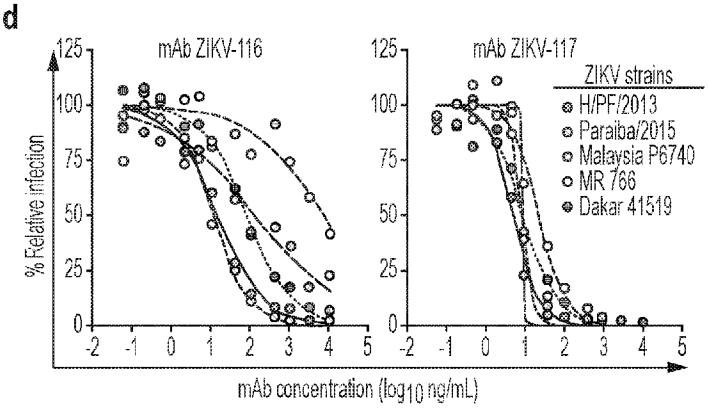

(57) Abstract: The present disclosure is directed to antibodies binding to and neutralizing Zika virus and methods for use thereof.

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DESCRIPTION

HUMAN ZIKA VIRUS ANTIBODIES AND METHODS OF USE THEREFOR

This application claims benefit of priority to U.S. Provisional Application Serial No. 62/461,260, filed November 2, 2016, the entire contents of which are hereby incorporated by reference.

BACKGROUND

1. Field of the Disclosure

The present disclosure relates generally to the fields of medicine, infectious disease, and immunology. More particular, the disclosure relates to human antibodies binding to Zika virus (ZIKV).

2. Background

ZIKV is an emerging mosquito-transmitted flavivirus that has become a global public health threat. Recent ZIKV epidemics in Micronesia, Brazil, other parts of South and Central America, and Mexico (Duffy *et al.*, 2009) are linked to Guillain-Barre syndrome in adults and microcephaly in newborn infants (Oehler *et al.*, 2014; Musso *et al.*, 2014) in the setting of infection during pregnancy (Araugo *et al.*, 2016; Gatherer & Kohl, 2016). As ZIKV is transmitted by *Aedes* species mosquitoes, which are global in distribution, countries in which these vectors are present could be sites for future epidemics. Despite the potential for causing disease in millions, specific treatments or vaccines for ZIKV are not available, leaving a considerable unmet need in the field.

SUMMARY

Thus, in accordance with the present disclosure, a method of detecting a Zika virus infection in a subject comprising (a) contacting a sample from said subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and (b) detecting Zika virus in said sample by binding of said antibody or antibody fragment to a Zika virus antigen in said sample. The sample may be a body fluid, and may be blood, sputum, tears, saliva, mucous or serum, semen, cervical or vaginal secretions, amniotic fluid, placental tissues, urine, exudate, transudate, tissue scrapings or feces. Detection may comprise ELISA, RIA, lateral flow assay or Western blot. The

method may further comprise performing steps (a) and (b) a second time and determining a change in Zika virus antigen levels as compared to the first assay.

The antibody or antibody fragment is encoded by clone-paired variable sequences as set forth in Table 1, may be encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1, or may be encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment.

Also provided is a method of treating a subject infected with Zika virus, or reducing the likelihood of infection of a subject at risk of contracting Zika virus, comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by clone-paired light and heavy chain variable sequences as set forth in Table 1, may be encoded by clone-paired light and heavy chain variable sequences having 95% identify to as set forth in Table 1, or may be encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired sequences from Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment, the antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to eliminate FcR interactions, such as a LALA mutation or a LS mutation, and the antibody may be a chimeric antibody or a bispecific antibody. The antibody or antibody fragment may be administered prior to infection or after infection. The subject may be a pregnant female, a sexually active female, or a female undergoing fertility treatments. Delivering may comprises antibody or antibody fragment administration, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment.

In another embodiment, there is provided is a monoclonal antibody, wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1, may be encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired sequences from Table 1, or may be encoded by light and heavy chain variable sequences having at least 95% identity to clone-paired sequences from Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment. The antibody may be is a chimeric antibody, or is bispecific antibody. The antibody may be an IgG or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to eliminate FcR interactions, such as a LALA or a LS mutation. The antibody or antibody fragment may further comprise a cell penetrating peptide and/or is an intrabody.

In further embodiment, there is provided a hybridoma or engineered cell encoding an antibody or antibody fragment wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1, may be encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired sequences from Table 1, or may be encoded by light and heavy chain variable sequences having at least 95% identity to clone-paired sequences from Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment. The antibody may be is a chimeric antibody, or is bispecific antibody. The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated

to eliminate FcR interactions, such as a LALA or a LS mutation. The antibody or antibody fragment may further comprise a cell penetrating peptide and/or is an intrabody.

In yet a further embodiment, there is provided a vaccine formulation comprising one or more antibodies or antibody fragments characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1, may be encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired sequences from Table 1, or may be encoded by light and heavy chain variable sequences having at least 95% identity to clone-paired sequences from Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment. The antibody may be is a chimeric antibody, or is bispecific antibody. The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to eliminate FcR interactions, such as a LALA or a LS mutation.

The vaccine formulation may comprise antibodies or antibody fragments that bind to E protein domain II. The vaccine formulation may comprise antibodies or antibody fragments that bind to E protein domain III, or to a quaternary epitope on the E protein dimer-dimer interface. The vaccine formulation may comprise antibodies or antibody fragments that do not cross react with dengue virus. The vaccine formulation may comprise antibodies or antibody fragments that neutralize Zika virus infections corresponding to African, Asian, and American lineages.

In still yet a further embodiment, there is provided a method of protecting the health of a placenta and/or fetus of a pregnant a subject infected with or at risk of infection with Zika virus comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively. The antibody or antibody fragment may be encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1, may be encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired sequences from Table 1, or may be encoded by light and heavy chain variable sequences having at least 95%

identity to clone-paired sequences from Table 1. The antibody or antibody fragment may comprise light and heavy chain variable sequences according to clone-paired sequences from Table 2, may comprise light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2, or may comprise antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2. The antibody fragment may be a recombinant scFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment. The antibody may be is a chimeric antibody, or is bispecific antibody. The antibody may be an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to eliminate FcR interactions, such as a LALA or a LS mutation. The antibody or antibody fragment may be administered prior to infection or after infection. The subject may be a pregnant female, a sexually active female, or a female undergoing fertility treatments. Delivering may comprise antibody or antibody fragment administration, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment. The antibody or antibody fragment may increase the size of the placenta as compared to an untreated control, and/or the antibody or antibody fragment reduces viral load and/or pathology of the fetus as compared to an untreated control.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." The word "about" means plus or minus 5% of the stated number.

It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein. Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIGS. 1A-D. Human antibody and B cell response to ZIKV infection. Serum samples from humans with a previous diagnosis of ZIKV infection were tested for **(FIG. 1A)** binding to ZIKV E protein in ELISA (with two technical replicates) and **(FIG. 1B)** neutralization of ZIKV in a FRNT assay (performed with at least two independent repeats in triplicate). Subjects 973 and 972 sera were tested from two separate time points with similar results – these data were combined). Subject 1001 had the highest endpoint titer in the binding assay and displayed potent neutralizing activity. Subject 657 was a control without history of exposure to ZIKV. **(FIG. 1C)** Supernatants of EBV-transformed B cell cultures from Subject 1001 were tested for binding to ZIKV E or DIII of ZIKV E or related flavivirus E proteins to assess the specificity of the immune response**.** The frequency of antigen-specific cells against each viral protein was determined with a threshold optical density (OD) of 1.5; with alternate lower OD thresholds of 1.0 or 0.5, the frequency was 0.69% or 0.97% for ZIKV E, respectively. **(FIG. 1D)** In four additional separate B cell transformation experiments, the frequency of B cells reactive with intact ZIKV E or E-FLM was determined.

FIG. 2A-E. Characterization of anti-ZIKV mAbs. **(FIG. 2A)** 29 mAbs were tested in binding, neutralization, and competition binding assays. The half-maximal binding concentration (EC_{50}) against ZIKV E and the IC_{50} (by focus reduction neutralization test) against H/PF/2013 strain for neutralizing antibodies (highlighted in blue) are shown. The mAbs are displayed in four groups (A, B, C, or D) based on a competition binding assay. The values are the percent of binding that occurred during competition compared to uncompeted binding, which was normalized to 100% and the range of competition is indicated by the box colors. Black filled boxes indicate strongly competing pairs (residual binding <30%), grey filled boxes indicate intermediate competition (residual binding 30-69%), and white filled boxes indicate non-competing pairs (residual binding $\geq$ 70%). The IC_{50} against H/PF/2013 strain for neutralizing antibodies is shown with active clones highlighted in blue. **(FIG. 2B)** A ribbon diagram of three protomers of ZIKV E (DI in red, DII in yellow and DIII in blue) is shown with critical residues highlighted as spheres from epitope mapping experiments for representative antibodies in each of the competition

binding groups. The colors of the critical residues correspond to the competition group designation as in FIG. 2A. The mutations in the E-FLM and DIII-LR mutants are indicated by black and silver spheres, respectively. **(FIG. 2C)** Representative mAbs from each competition binding group are listed with the domains and residues critical for binding. **(FIG. 2D)** Two mAbs were tested for neutralization of five strains of ZIKV. The concentrations at which 50% or 90% neutralization occurred are listed in **(FIG. 2E).** The neutralization data are pooled from at least three independent experiments performed in triplicate.

FIGS. 3A-F. Protective activity of ZIKV-117 in adult male and pregnant female mice. (FIG. 3A) Four to five week-old WT male mice were treated with 2 mg of anti-Ifnar1 mAb followed by subcutaneous inoculation with 10^3 FFU of mouse-adapted ZIKV-Dakar. Mice were treated with a single 100 μg or 250 μg dose of isotype control mAb (hCHK-152) or ZIKV-117 on D+1 or D+5 (n = 10 per group from two independent experiments), respectively. Significance was analyzed by the log-rank test (*, $P < 0.05$; **, $P < 0.01$). **(FIGS. 3B–C)** *Ifnar1*$^{-/-}$ female mice were mated with WT sires. At E5.5, dams were treated with 250 ug of either hCHK-152 isotype control mAb or ZIKV-117. Bars indicate the median values and reflect data pooled from four independent experiments. Significance for fetal survival and viral RNA was analyzed by chi-square **(FIG. 3B**; ****, $P < 0.0001$) and Mann-Whitney **(FIG. 3C**; *, $P < 0.05$) tests, respectively. **(FIGS. 3D–F)** WT female mice were mated with WT sires. At E5.5, dams were treated with anti-Ifnar1 mAb and one of the following: **(FIGS. 3D–E)** PBS, **(FIGS. 3D–F)** 250 μg of hCHK-152 isotype control mAb, **(FIGS. 3D–F)** 250 μg of ZIKV-117, or **(FIG. 3F)** 250 μg of ZIKV-117 LALA. At E6.5, dams were inoculated with 10^3 FFU of ZIKV-Dakar. **(FIGS. 3D, 3F)** Fetuses and placentas and **(FIG. 3E)** maternal brain and serum were harvested on E13.5 and viral RNA was measured by qRT-PCR. Bars indicate the median values of samples collected from three biological replicates **(FIG. 3D)**: n = 20 to 36; **(FIG. 3E)**: n = 5 to 9; **f**: n = 23 to 28). Significance was analyzed by ANOVA with a Dunn's multiple comparison test (*, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$; ****, $P < 0.0001$).

FIGS. 4A-E. Effect of ZIKV-117 treatment on the placenta and the fetus. (FIG. 4A) Cartoon depicting murine placental structures and zones. **(FIG. 4B-E)** Pregnant dams were treated with PBS, hCHK-152, or ZIKV-117 as described in **(FIG. 4D-F)** prior to infection with ZIKV-Dakar or mock-infected. **(FIG. 4B)** Hematoxylin and eosin staining of placenta at E13.5. Placental labyrinth zone is marked with a solid line. Low power

(scale bar = 1 mm) and high power (scale bar = 50 μm) images are presented in sequence. Black arrows indicate apoptotic trophoblasts in areas corresponding to regions of ZIKV infectivity (see panel **(FIG. 4D)**, below). **(FIG. 4C)** Measurements of thickness and indicated areas of placenta and fetus body size. Each symbol represents data from an individual placenta or fetus. Significance was analyzed by ANOVA with a Dunn's multiple comparison test (*, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$; ****, $P < 0.0001$, n.s.; not significant, $P > 0.05$). **(FIG. 4D)** *In situ* hybridization (ISH). Low power (scale bar = 500 μm) and high power (scale bar = 50 μm) images are presented in sequence. Black arrows indicate cells positive for ZIKV RNA in the junctional zone of the placenta. The images in panels are representative of several placentas from independent dams. **(FIG. 4E)**. Low (scale bar = 50 μm) and high (scale bar = 10 μm) power magnified images of immunofluorescence staining of placentas for vimentin (in green, which marks fetal capillary endothelium) from ZIKV-infected dams treated with PBS or ZIKV-117 or from uninfected pregnant animals. Nuclei are counter-stained blue with DAPI.

FIG. 5. Binding of human mAbs to Zika E protein, E DIII, or E fusion loop mutant (FLM). MAbs are organized by competition binding groups A to D.

FIGS. 6A-C. (FIG. 6A) High resolution epitope mapping of ZIKV mAbs. An alanine scanning mutation library for ZIKV envelope protein was constructed where each amino acid of prM/E was mutated individually to alanine (and alanine to serine) and expression constructs arrayed into 384-well plates, one mutation per well. Each clone in the ZIKV prM/E mutation library, expressed in HEK-293T cells, was tested for immunoreactivity with five mAbs from competition groups A-D, measured using an Intellicyt high-throughput flow cytometer. Shown here for each of the five mAbs is the reactivity with the ZIKV E protein mutants that identified the epitope residues for these mAbs. MAb reactivity for each alanine mutant are expressed as percent of the reactivity of mAb with wild-type ZIKV prM/E. Clones with reactivity <30% relative to WT ZIKV prM/E were identified as critical for mAb binding. Bars represent the mean and range of at least two replicate data points. Binding of Group B mAbs, ZIKV-116 and ZIKV-161, to **(FIG. 6B)** ZIKV E DIII WT or **(FIG. 6C)** DIII LR mutant was compared with mouse mAbs ZV-2 and ZV-54. Binding of ZIKV-116 and ZIKV-161 was decreased by mutations in DIII LR.

FIG. 7. Binding of human mAbs to permeabilized DENV-infected C6/36 cells. C6/36 cells were infected with DENV-1, DENV-2, DENV-3, DENV-4 or mock-infected. Cells were stained with the indicated anti-ZIKV mAbs, an isotype control (a humanized

antibody to chikungunya virus; hCHK-152), or a positive control (a cross-reactive antibody to DENV; chimeric human E60 [chE60]) and processed by flow cytometry. The data are representative of two independent experiments. The numbers in the box indicate the fraction of cells that stained positively.

FIG. 8. Detection of human IgG in placenta or fetal head tissues after treatment of dams with ZIKV-117 or PBS treated pregnant mice. As described in FIGS. 3A-F, WT female mice were mated with WT sires and monitored for pregnancy. At E5.5, dams were treated with anti-Ifnar1 mAb and PBS or 250 μg of ZIKV-117. One day later (E6.5), dams were infected with 10^3 FFU of ZIKV-Dakar. Fetuses and placentas (n = 4 each) were harvested on E13.5, homogenized, and tested for human IgG by ELISA. Human antibody in tissues was captured on ELISA plates coated with ZIKV E protein and detected using goat anti-human IgG (Fc-specific) antibody. The quantity of antibody was determined by comparison with a standard curve constructed using purified ZIKV-117 in a dilution series. Concentration of ZIKV-117 detected in treated or PBS mock-treated placenta or fetal head tissues, with standard curve. Four replicates were performed for each mouse tissue; results were averaged for each mouse. The graphs represent the mean ± SEM from 3 mice per group.

FIGS. 9A-B. Comparison of WT and LALA mutated antibodies. (FIG. 9A) Binding to recombinant human FcγR1. The functional abrogation of the binding of the LALA variant IgG was confirmed in an ELISA binding assay with recombinant human FcγRI. ZIKV-117 WT bound to FcγRI, whereas the ZIKV-117 LALA antibody did not. WT and LALA versions of another human mAb, CKV063, were used as controls. **(FIG. 9B)** Neutralization. ZIKV-117 WT and LALA antibodies exhibited equivalent neutralizing activity *in vitro* to each other and to the hybridoma-derived antibody.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

As discussed above, Zika virus (ZIKV) infection causes systemic and central nervous system pathology or disease, with congenital birth defects linked to infection during pregnancy (Coyne *et al.*, 2016). To develop candidate therapeutic agents against ZIKV, the inventors isolated a panel of human monoclonal antibodies (mAbs) from healthy subjects with prior ZIKV infection. A subset of mAbs recognized diverse epitopes on the envelope (E) protein and exhibited potent neutralizing activity. One of the most inhibitory mAbs, ZIKV-117, broadly neutralized infection of ZIKV strains corresponding to African, Asian, and American lineages. Epitope mapping studies revealed that ZIKV-117 recognized a quaternary

epitope on the E protein dimer-dimer interface. The inventors then evaluated the therapeutic efficacy of ZIKV-117 in pregnant or non-pregnant mice. In these models, mAb treatment markedly reduced tissue pathology, placental and fetal infection, and mortality. Thus, neutralizing human mAbs can protect against maternal-fetal transmission, infection and disease, and reveal important determinants for structure-based rational vaccine design efforts. These and other aspects of the disclosure are described in detail below.

I. Zika Virus

Zika virus (ZIKV) is a member of the virus family *Flaviviridae*. It is spread by daytime-active *Aedes* mosquitoes, such as *A. aegypti* and *A. albopictus*. Its name comes from the Zika Forest of Uganda, where the virus was first isolated in 1947. Zika virus is related to the dengue, yellow fever, Japanese encephalitis, and West Nile viruses. Since the 1950s, it has been known to occur within a narrow equatorial belt from Africa to Asia. From 2007 to 2016, the virus spread eastward, across the Pacific Ocean to the Americas, leading to the 2015–16 Zika virus epidemic.

The infection, known as Zika fever or Zika virus disease, often causes no or only mild symptoms, similar to a very mild form of dengue fever. While there is no specific treatment, paracetamol (acetaminophen) and rest may help with the symptoms. As of 2016, the illness cannot be prevented by medications or vaccines. Zika can also spread from a pregnant woman to her fetus. This can result in microcephaly, severe brain malformations, and other birth defects. Zika infections in adults may result rarely in Guillain–Barré syndrome.

In January 2016, the United States Centers for Disease Control and Prevention (CDC) issued travel guidance on affected countries, including the use of enhanced precautions, and guidelines for pregnant women including considering postponing travel. Other governments or health agencies also issued similar travel warnings, while Colombia, the Dominican Republic, Puerto Rico, Ecuador, El Salvador, and Jamaica advised women to postpone getting pregnant until more is known about the risks.

The Zika virus belongs to the *Flaviviridae* family and the *Flavivirus* genus, and is thus related to the dengue, yellow fever, Japanese encephalitis, and West Nile viruses. Like other flaviviruses, Zika virus is enveloped and icosahedral and has a nonsegmented, single-stranded, 10 kb positive-sense RNA genome. It is most closely related to the Spondweni virus and is one of the two known viruses in the Spondweni virus clade.

A positive-sense RNA genome can be directly translated into viral proteins. As in other flaviviruses, such as the similarly sized West Nile virus, the RNA genome encodes

seven nonstructural proteins and three structural proteins. One of the structural proteins encapsulates the virus. The RNA genome forms a nucleocapsid along with copies of the 12-kDa capsid protein. The nucleocapsid, in turn, is enveloped within a host-derived membrane modified with two viral glycoproteins. Viral genome replication depends on the synthesis of double sided RNA from the single stranded positive sense RNA (ssRNA(+)) genome followed by transcription and replication to provide viral mRNAs and new ssRNA(+) genomes.

There are two Zika lineages: the African lineage and the Asian lineage. Phylogenetic studies indicate that the virus spreading in the Americas is 89% identical to African genotypes, but is most closely related to the Asian strain that circulated in French Polynesia during the 2013–2014 outbreak.

The vertebrate hosts of the virus were primarily monkeys in a so-called enzootic mosquito-monkey-mosquito cycle, with only occasional transmission to humans. Before the current pandemic began in 2007, Zika "rarely caused recognized 'spillover' infections in humans, even in highly enzootic areas." Infrequently, however, other arboviruses have become established as a human disease and spread in a mosquito–human–mosquito cycle, like the yellow fever virus and the dengue fever virus (both flaviviruses), and the chikungunya virus (a togavirus). Though the reason for the pandemic is unknown, dengue, a related arbovirus that infects the same species of mosquito vectors, is known in particular to be intensified by urbanization and globalization. Zika is primarily spread by *Aedes aegypti* mosquitoes, and can also be transmitted through sexual contact or blood transfusions. The basic reproduction number (R_0, a measure of transmissibility) of Zika virus has been estimated to be between 1.4 and 6.6.

In 2015, news reports drew attention to the rapid spread of Zika in Latin America and the Caribbean. At that time, the Pan American Health Organization published a list of countries and territories that experienced "local Zika virus transmission" comprising Barbados, Bolivia, Brazil, Colombia, the Dominican Republic, Ecuador, El Salvador, French Guiana, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Martinique, Mexico, Panama, Paraguay, Puerto Rico, Saint Martin, Suriname, and Venezuela. By August 2016, more than 50 countries had experienced active (local) transmission of Zika virus.

Zika is primarily spread by the female *Aedes aegypti* mosquito, which is active mostly in the daytime, although researchers have found the virus in common Culex house mosquitoes as well. The mosquitos must feed on blood in order to lay eggs. The virus has also been isolated from a number of arboreal mosquito species in the *Aedes* genus, such as *A.*

africanus, *A. apicoargenteus*, *A. furcifer*, *A. hensilli*, *A. luteocephalus* and *A. vittatus*, with an extrinsic incubation period in mosquitoes of about 10 days.

The true extent of the vectors is still unknown. Zika has been detected in many more species of *Aedes*, along with *Anopheles coustani, Mansonia uniformis*, and *Culex perfuscus*, although this alone does not incriminate them as a vector.

Transmission by *A. albopictus*, the tiger mosquito, was reported from a 2007 urban outbreak in Gabon where it had newly invaded the country and become the primary vector for the concomitant chikungunya and dengue virus outbreaks. There is concern for autochthonous infections in urban areas of European countries infested by *A. albopictus* because the first two cases of laboratory-confirmed Zika infections imported into Italy were reported from viremic travelers returning from French Polynesia.

The potential societal risk of Zika can be delimited by the distribution of the mosquito species that transmit it. The global distribution of the most cited carrier of Zika, *A. aegypti*, is expanding due to global trade and travel. *A. aegypti* distribution is now the most extensive ever recorded – across all continents including North America and even the European periphery (Madeira, the Netherlands, and the northeastern Black Sea coast). A mosquito population capable of carrying Zika has been found in a Capitol Hill neighborhood of Washington, D.C., and genetic evidence suggests they survived at least four consecutive winters in the region. The study authors conclude that mosquitos are adapting for persistence in a northern climate. The Zika virus appears to be contagious via mosquitoes for around a week after infection. The virus is thought to be infectious for a longer period of time after infection (at least 2 weeks) when transmitted via semen.

Research into its ecological niche suggests that Zika may be influenced to a greater degree by changes in precipitation and temperature than Dengue, making it more likely to be confined to tropical areas. However, rising global temperatures would allow for the disease vector to expand their range further north, allowing Zika to follow.

Zika can be transmitted from men and women to their sexual partners. As of April 2016 sexual transmission of Zika has been documented in six countries – Argentina, Chile, France, Italy, New Zealand and the United States – during the 2015 outbreak.

In 2014, Zika capable of growth in lab culture was found in the semen of a man at least two weeks (and possibly up to 10 weeks) after he fell ill with Zika fever. In 2011 a study found that a U.S. biologist who had been bitten many times while studying mosquitoes in Senegal developed symptoms six days after returning home in August 2008, but not before having unprotected intercourse with his wife, who had not been outside the U.S. since 2008.

Both husband and wife were confirmed to have Zika antibodies, raising awareness of the possibility of sexual transmission. In early February 2016, the Dallas County Health and Human Services department reported that a man from Texas who had not travelled abroad had been infected after his male monogamous sexual partner had anal penetrative sex with him one day before and one day after onset of symptoms. As of February 2016, fourteen additional cases of possible sexual transmission have been under investigation, but it remained unknown whether women can transmit Zika to their sexual partners. At that time, the understanding of the "incidence and duration of shedding in the male genitourinary tract [was] limited to one case report." Therefore, the CDC interim guideline recommended against testing men for purposes of assessing the risk of sexual transmission.

In March 2016, the CDC updated its recommendations about length of precautions for couples, and advised that heterosexual couples with men who have confirmed Zika fever or symptoms of Zika should consider using condoms or not having penetrative sex (*i.e.*, vaginal intercourse, anal intercourse, or fellatio) for at least 6 months after symptoms begin. This includes men who live in—and men who traveled to—areas with Zika. Couples with men who traveled to an area with Zika, but did not develop symptoms of Zika, should consider using condoms or not having sex for at least 8 weeks after their return in order to minimize risk. Couples with men who live in an area with Zika, but have not developed symptoms, might consider using condoms or not having sex while there is active Zika transmission in the area. The Zika virus can spread from an infected mother to her fetus during pregnancy or at delivery.

As of April 2016, two cases of Zika transmission through blood transfusions have been reported globally, both from Brazil, after which the US Food and Drug Administration (FDA) recommended screening blood donors and deferring high-risk donors for 4 weeks. A potential risk had been suspected based on a blood-donor screening study during the French Polynesian Zika outbreak, in which 2.8% (42) of donors from November 2013 and February 2014 tested positive for Zika RNA and were all asymptomatic at the time of blood donation. Eleven of the positive donors reported symptoms of Zika fever after their donation, but only three of 34 samples grew in culture.

Zika virus replicates in the mosquito's midgut epithelial cells and then its salivary gland cells. After 5–10 days, the virus can be found in the mosquito's saliva. If the mosquito's saliva is inoculated into human skin, the virus can infect epidermal keratinocytes, skin fibroblasts in the skin and the Langerhans cells. The pathogenesis of the virus is

hypothesized to continue with a spread to lymph nodes and the bloodstream. Flaviviruses generally replicate in the cytoplasm, but Zika antigens have been found in infected cell nuclei.

Zika fever (also known as Zika virus disease) is an illness caused by the Zika virus. Most cases have no symptoms, but when present they are usually mild and can resemble dengue fever. Symptoms may include fever, red eyes, joint pain, headache, and a maculopapular rash. Symptoms generally last less than seven days. It has not caused any reported deaths during the initial infection. Infection during pregnancy causes microcephaly and other brain malformations in some babies. Infection in adults has been linked to Guillain–Barré syndrome (GBS). Diagnosis is by testing the blood, urine, or saliva for the presence of Zika virus RNA when the person is sick.

Prevention involves decreasing mosquito bites in areas where the disease occurs, and proper use of condoms. Efforts to prevent bites include the use of insect repellent, covering much of the body with clothing, mosquito nets, and getting rid of standing water where mosquitoes reproduce. There is no effective vaccine. Health officials recommended that women in areas affected by the 2015–16 Zika outbreak consider putting off pregnancy and that pregnant women not travel to these areas. While there is no specific treatment, paracetamol (acetaminophen) and rest may help with the symptoms. Admission to hospital is rarely necessary.

Effective vaccines have existed for several viruses of the flaviviridae family, namely yellow fever vaccine, Japanese encephalitis vaccine, and tick-borne encephalitis vaccine, since the 1930s, and dengue fever vaccine since the mid-2010s. World Health Organization (WHO) experts have suggested that the priority should be to develop inactivated vaccines and other non-live vaccines, which are safe to use in pregnant women and those of childbearing age.

As of March 2016, eighteen companies and institutions internationally were developing vaccines against Zika but a vaccine was unlikely to be widely available for about ten years. In June 2016 the FDA granted the first approval for a human clinical trial for a Zika vaccine.

The virus was first isolated in April 1947 from a rhesus macaque monkey that had been placed in a cage in the Zika Forest of Uganda, near Lake Victoria, by the scientists of the Yellow Fever Research Institute. A second isolation from the mosquito *A. africanus* followed at the same site in January 1948. When the monkey developed a fever, researchers isolated from its serum a "filterable transmissible agent" that was named Zika in 1948.

Zika had been known to infect humans from the results of serological surveys in Uganda and Nigeria, published in 1952: Among 84 people of all ages, 50 individuals had antibodies to Zika, and all above 40 years of age were immune. A 1952 research study conducted in India had shown a "significant number" of Indians tested for Zika had exhibited an immune response to the virus, suggesting it had long been widespread within human populations.

It was not until 1954 that the isolation of Zika from a human was published. This came as part of a 1952 outbreak investigation of jaundice suspected to be yellow fever. It was found in the blood of a 10-year-old Nigerian female with low-grade fever, headache, and evidence of malaria, but no jaundice, who recovered within three days. Blood was injected into the brain of laboratory mice, followed by up to 15 mice passages. The virus from mouse brains was then tested in neutralization tests using rhesus monkey sera specifically immune to Zika. In contrast, no virus was isolated from the blood of two infected adults with fever, jaundice, cough, diffuse joint pains in one and fever, headache, pain behind the eyes and in the joints. Infection was proven by a rise in Zika-specific serum antibodies.

From 1951 through 1983, evidence of human infection with Zika was reported from other African countries, such as the Central African Republic, Egypt, Gabon, Sierra Leone, Tanzania, and Uganda, as well as in parts of Asia including India, Indonesia, Malaysia, the Philippines, Thailand, Vietnam and Pakistan. From its discovery until 2007, there were only 14 confirmed human cases of Zika infection from Africa and Southeast Asia.

In April 2007, the first outbreak outside of Africa and Asia occurred on the island of Yap in the Federated States of Micronesia, characterized by rash, conjunctivitis, and arthralgia, which was initially thought to be dengue, chikungunya, or Ross River disease. Serum samples from patients in the acute phase of illness contained RNA of Zika. There were 49 confirmed cases, 59 unconfirmed cases, no hospitalizations, and no deaths. Between 2013 and 2014, further epidemics occurred in French Polynesia, Easter Island, the Cook Islands, and New Caledonia. On 22 March 2016 Reuters reported that Zika was isolated from a 2014 blood sample of an elderly man in Chittagong in Bangladesh as part of a retrospective study.

As of early 2016, a widespread outbreak of Zika was ongoing, primarily in the Americas. The outbreak began in April 2015 in Brazil, and has spread to other countries in South America, Central America, North America, and the Caribbean. The Zika virus reached Singapore and Malaysia in Aug 2016. In January 2016, the WHO said the virus was likely to spread throughout most of the Americas by the end of the year; and in February 2016, the WHO declared the cluster of microcephaly and Guillain–Barré syndrome cases reported in

Brazil – strongly suspected to be associated with the Zika outbreak – a Public Health Emergency of International Concern. It is estimated that 1.5 million people have been infected by Zika in Brazil, with over 3,500 cases of microcephaly reported between October 2015 and January 2016.

A number of countries have issued travel warnings, and the outbreak is expected to significantly impact the tourism industry. Several countries have taken the unusual step of advising their citizens to delay pregnancy until more is known about the virus and its impact on fetal development. With the 2016 Summer Olympic Games hosted in Rio de Janeiro, health officials worldwide have voiced concerns over a potential crisis, both in Brazil and when international athletes and tourists, who may be unknowingly infected, return home and possibly spread the virus. Some researchers speculate that only one or two tourists may be infected during the three week period, or approximately 3.2 infections per 100,000 tourists.

II. Monoclonal Antibodies and Production Thereof

A. General Methods

It will be understood that monoclonal antibodies binding to Zika virus will have several applications. These include the production of diagnostic kits for use in detecting and diagnosing Zika virus infection, as well as for treating the same. In these contexts, one may link such antibodies to diagnostic or therapeutic agents, use them as capture agents or competitors in competitive assays, or use them individually without additional agents being attached thereto. The antibodies may be mutated or modified, as discussed further below. Methods for preparing and characterizing antibodies are well known in the art (see, *e.g.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; U.S. Patent 4,196,265).

The methods for generating monoclonal antibodies (MAbs) generally begin along the same lines as those for preparing polyclonal antibodies. The first step for both these methods is immunization of an appropriate host or identification of subjects who are immune due to prior natural infection. As is well known in the art, a given composition for immunization may vary in its immunogenicity. It is often necessary therefore to boost the host immune system, as may be achieved by coupling a peptide or polypeptide immunogen to a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin or rabbit serum albumin can also be used as carriers. Means for conjugating a polypeptide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobencoyl-N-

hydroxysuccinimide ester, carbodiimyde and bis-biazotized benzidine. As also is well known in the art, the immunogenicity of a particular immunogen composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Exemplary and preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis)*, incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

In the case of human antibodies against natural pathogens, a suitable approach is to identify subjects that have been exposed to the pathogens, such as those who have been diagnosed as having contracted the disease, or those who have been vaccinated to generate protective immunity against the pathogen. Circulating anti-pathogen antibodies can be detected, and antibody producing B cells from the antibody-positive subject may then be obtained.

The amount of immunogen composition used in the production of polyclonal antibodies varies upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal). The production of polyclonal antibodies may be monitored by sampling blood of the immunized animal at various points following immunization. A second, booster injection, also may be given. The process of boosting and titering is repeated until a suitable titer is achieved. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored, and/or the animal can be used to generate MAbs.

Following immunization, somatic cells with the potential for producing antibodies, specifically B lymphocytes (B cells), are selected for use in the MAb generating protocol. These cells may be obtained from biopsied spleens or lymph nodes, or from circulating blood. The antibody-producing B lymphocytes from the immunized animal are then fused with cells of an immortal myeloma cell, generally one of the same species as the animal that was immunized or human or human/mouse chimeric cells. Myeloma cell lines suited for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render then incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). Any one of a number of myeloma cells may be used, as are known to those of skill in the art (Goding, pp. 65-66, 1986; Campbell, pp. 75-83, 1984).

Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in a 2:1 proportion,

though the proportion may vary from about 20:1 to about 1:1, respectively, in the presence of an agent or agents (chemical or electrical) that promote the fusion of cell membranes. Fusion methods using Sendai virus have been described by Kohler and Milstein (1975; 1976), and those using polyethylene glycol (PEG), such as 37% (v/v) PEG, by Gefter *et al.* (1977). The use of electrically induced fusion methods also is appropriate (Goding, pp. 71-74, 1986). Fusion procedures usually produce viable hybrids at low frequencies, about 1 x 10^{-6} to 1 x 10^{-8}. However, this does not pose a problem, as the viable, fused hybrids are differentiated from the parental, infused cells (particularly the infused myeloma cells that would normally continue to divide indefinitely) by culturing in a selective medium. The selective medium is generally one that contains an agent that blocks the *de novo* synthesis of nucleotides in the tissue culture media. Exemplary and preferred agents are aminopterin, methotrexate, and azaserine. Aminopterin and methotrexate block *de novo* synthesis of both purines and pyrimidines, whereas azaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the media is supplemented with hypoxanthine and thymidine as a source of nucleotides (HAT medium). Where azaserine is used, the media is supplemented with hypoxanthine. Ouabain is added if the B cell source is an Epstein Barr virus (EBV) transformed human B cell line, in order to eliminate EBV transformed lines that have not fused to the myeloma.

The preferred selection medium is HAT or HAT with ouabain. Only cells capable of operating nucleotide salvage pathways are able to survive in HAT medium. The myeloma cells are defective in key enzymes of the salvage pathway, *e.g.*, hypoxanthine phosphoribosyl transferase (HPRT), and they cannot survive. The B cells can operate this pathway, but they have a limited life span in culture and generally die within about two weeks. Therefore, the only cells that can survive in the selective media are those hybrids formed from myeloma and B cells. When the source of B cells used for fusion is a line of EBV-transformed B cells, as here, ouabain may also be used for drug selection of hybrids as EBV-transformed B cells are susceptible to drug killing, whereas the myeloma partner used is chosen to be ouabain resistant.

Culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays dot immunobinding assays, and the like. The selected hybridomas are then

serially diluted or single-cell sorted by flow cytometric sorting and cloned into individual antibody-producing cell lines, which clones can then be propagated indefinitely to provide mAbs. The cell lines may be exploited for MAb production in two basic ways. A sample of the hybridoma can be injected (often into the peritoneal cavity) into an animal (*e.g.*, a mouse). Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. When human hybridomas are used in this way, it is optimal to inject immunocompromised mice, such as SCID mice, to prevent tumor rejection. The injected animal develops tumors secreting the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can then be tapped to provide MAbs in high concentration. The individual cell lines could also be cultured *in vitro*, where the MAbs are naturally secreted into the culture medium from which they can be readily obtained in high concentrations. Alternatively, human hybridoma cells lines can be used *in vitro* to produce immunoglobulins in cell supernatant. The cell lines can be adapted for growth in serum-free medium to optimize the ability to recover human monoclonal immunoglobulins of high purity.

MAbs produced by either means may be further purified, if desired, using filtration, centrifugation and various chromatographic methods such as FPLC or affinity chromatography. Fragments of the monoclonal antibodies of the disclosure can be obtained from the purified monoclonal antibodies by methods which include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction. Alternatively, monoclonal antibody fragments encompassed by the present disclosure can be synthesized using an automated peptide synthesizer.

It also is contemplated that a molecular cloning approach may be used to generate monoclonals. For this, RNA can be isolated from the hybridoma line and the antibody genes obtained by RT-PCR and cloned into an immunoglobulin expression vector. Alternatively, combinatorial immunoglobulin phagemid libraries are prepared from RNA isolated from the cell lines and phagemids expressing appropriate antibodies are selected by panning using viral antigens. The advantages of this approach over conventional hybridoma techniques are that approximately 10^4 times as many antibodies can be produced and screened in a single round, and that new specificities are generated by H and L chain combination which further increases the chance of finding appropriate antibodies.

Other U.S. patents, each incorporated herein by reference, that teach the production of antibodies useful in the present disclosure include U.S. Patent 5,565,332, which describes the production of chimeric antibodies using a combinatorial approach; U.S. Patent 4,816,567

which describes recombinant immunoglobulin preparations; and U.S. Patent 4,867,973 which describes antibody-therapeutic agent conjugates.

B. Antibodies of the Present Disclosure

Antibodies according to the present disclosure may be defined, in the first instance, by their binding specificity. Those of skill in the art, by assessing the binding specificity/affinity of a given antibody using techniques well known to those of skill in the art, can determine whether such antibodies fall within the scope of the instant claims. In one aspect, there are provided monoclonal antibodies having clone-paired CDR's from the heavy and light chains as illustrated in Tables 3 and 4, respectively. Such antibodies may be produced by the clones discussed below in the Examples section using methods described herein.

In a second aspect, the antibodies may be defined by their variable sequence, which include additional "framework" regions. These are provided in Tables 1 and 2 that encode or represent full variable regions. Furthermore, the antibodies sequences may vary from these sequences, optionally using methods discussed in greater detail below. For example, nucleic acid sequences may vary from those set out above in that (a) the variable regions may be segregated away from the constant domains of the light and heavy chains, (b) the nucleic acids may vary from those set out above while not affecting the residues encoded thereby, (c) the nucleic acids may vary from those set out above by a given percentage, *e.g.*, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology, (d) the nucleic acids may vary from those set out above by virtue of the ability to hybridize under high stringency conditions, as exemplified by low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C, (e) the amino acids may vary from those set out above by a given percentage, *e.g.*, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology, or (f) the amino acids may vary from those set out above by permitting conservative substitutions (discussed below). Each of the foregoing applies to the nucleic acid sequences set forth as Table 1 and the amino acid sequences of Table 2.

C. Engineering of Antibody Sequences

In various embodiments, one may choose to engineer sequences of the identified antibodies for a variety of reasons, such as improved expression, improved cross-reactivity or diminished off-target binding. The following is a general discussion of relevant techniques for antibody engineering.

Hybridomas may be cultured, then cells lysed, and total RNA extracted. Random hexamers may be used with RT to generate cDNA copies of RNA, and then PCR performed using a multiplex mixture of PCR primers expected to amplify all human variable gene sequences. PCR product can be cloned into pGEM-T Easy vector, then sequenced by automated DNA sequencing using standard vector primers. Assay of binding and neutralization may be performed using antibodies collected from hybridoma supernatants and purified by FPLC, using Protein G columns.

Recombinant full length IgG antibodies were generated by subcloning heavy and light chain Fv DNAs from the cloning vector into an IgG plasmid vector, transfected into 293 Freestyle cells or CHO cells, and antibodies were collected an purified from the 293 or CHO cell supernatant.

The rapid availability of antibody produced in the same host cell and cell culture process as the final cGMP manufacturing process has the potential to reduce the duration of process development programs. Lonza has developed a generic method using pooled transfectants grown in CDACF medium, for the rapid production of small quantities (up to 50 g) of antibodies in CHO cells. Although slightly slower than a true transient system, the advantages include a higher product concentration and use of the same host and process as the production cell line. Example of growth and productivity of GS-CHO pools, expressing a model antibody, in a disposable bioreactor: in a disposable bag bioreactor culture (5 L working volume) operated in fed-batch mode, a harvest antibody concentration of 2 g/L was achieved within 9 weeks of transfection.

Antibody molecules will comprise fragments (such as F(ab'), $F(ab')_2$) that are produced, for example, by the proteolytic cleavage of the mAbs, or single-chain immunoglobulins producible, for example, via recombinant means. Such antibody derivatives are monovalent. In one embodiment, such fragments can be combined with one another, or with other antibody fragments or receptor ligands to form "chimeric" binding molecules. Significantly, such chimeric molecules may contain substituents capable of binding to different epitopes of the same molecule.

In related embodiments, the antibody is a derivative of the disclosed antibodies, *e.g.*, an antibody comprising the CDR sequences identical to those in the disclosed antibodies (*e.g.*, a chimeric, or CDR-grafted antibody). Alternatively, one may wish to make modifications, such as introducing conservative changes into an antibody molecule. In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally

understood in the art (Kyte and Doolittle, 1982). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

It also is understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: basic amino acids: arginine (+3.0), lysine (+3.0), and histidine (-0.5); acidic amino acids: aspartate (+3.0 ± 1), glutamate (+3.0 ± 1), asparagine (+0.2), and glutamine (+0.2); hydrophilic, nonionic amino acids: serine (+0.3), asparagine (+0.2), glutamine (+0.2), and threonine (-0.4), sulfur containing amino acids: cysteine (-1.0) and methionine (-1.3); hydrophobic, nonaromatic amino acids: valine (-1.5), leucine (-1.8), isoleucine (-1.8), proline (-0.5 ± 1), alanine (-0.5), and glycine (0); hydrophobic, aromatic amino acids: tryptophan (-3.4), phenylalanine (-2.5), and tyrosine (-2.3).

It is understood that an amino acid can be substituted for another having a similar hydrophilicity and produce a biologically or immunologically modified protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those that are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions generally are based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take into consideration the various foregoing characteristics are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

The present disclosure also contemplates isotype modification. By modifying the Fc region to have a different isotype, different functionalities can be achieved. For example, changing to IgG_1 can increase antibody dependent cell cytotoxicity, switching to class A can improve tissue distribution, and switching to class M can improve valency.

Beltramello *et al.* (2010) previously reported the modification of neutralizing mAbs, due to their tendency to enhance DV infection, by generating in which leucine residues at positions 1.3 and 1.2 of CH2 domain (according to the IMGT unique numbering for C-

domain) were substituted with alanine residues. This modification, also known as "LALA" mutation, abolishes antibody binding to FcγRI, FcγRII and FcγRIIIa, as described by Hessell *et al.* (2007). The variant and unmodified recombinant mAbs were compared for their capacity to neutralize and enhance infection by the four DENV serotypes. LALA variants retained the same neutralizing activity as unmodified mAbs, but were completely devoid of enhancing activity. LALA mutations of this nature are therefore contemplated in the context of the presently disclosed antibodies.

Fc variants mutagenesis are described in patent applications WO2010106180 and WO2012175751. These include M252Y/S254T/T256E (Fc-YTE), as well as M428L/N434S (Fc-LS).

Modified antibodies may be made by any technique known to those of skill in the art, including expression through standard molecular biological techniques, or the chemical synthesis of polypeptides. Methods for recombinant expression are addressed elsewhere in this document.

D. Single Chain Antibodies

A Single Chain Variable Fragment (scFv) is a fusion of the variable regions of the heavy and light chains of immunoglobulins, linked together with a short (usually serine, glycine) linker. This chimeric molecule retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of a linker peptide. This modification usually leaves the specificity unaltered. These molecules were created historically to facilitate phage display where it is highly convenient to express the antigen binding domain as a single peptide. Alternatively, scFv can be created directly from subcloned heavy and light chains derived from a hybridoma. Single chain variable fragments lack the constant Fc region found in complete antibody molecules, and thus, the common binding sites (*e.g.*, protein A/G) used to purify antibodies. These fragments can often be purified/immobilized using Protein L since Protein L interacts with the variable region of kappa light chains.

Flexible linkers generally are comprised of helix- and turn-promoting amino acid residues such as alaine, serine and glycine. However, other residues can function as well. Tang *et al.* (1996) used phage display as a means of rapidly selecting tailored linkers for single-chain antibodies (scFvs) from protein linker libraries. A random linker library was constructed in which the genes for the heavy and light chain variable domains were linked by a segment encoding an 18-amino acid polypeptide of variable composition. The scFv

repertoire (approx. 5×10^6 different members) was displayed on filamentous phage and subjected to affinity selection with hapten. The population of selected variants exhibited significant increases in binding activity but retained considerable sequence diversity. Screening 1054 individual variants subsequently yielded a catalytically active scFv that was produced efficiently in soluble form. Sequence analysis revealed a conserved proline in the linker two residues after the V_H C terminus and an abundance of arginines and prolines at other positions as the only common features of the selected tethers.

The recombinant antibodies of the present disclosure may also involve sequences or moieties that permit dimerization or multimerization of the receptors. Such sequences include those derived from IgA, which permit formation of multimers in conjunction with the J-chain. Another multimerization domain is the Gal4 dimerization domain. In other embodiments, the chains may be modified with agents such as biotin/avidin, which permit the combination of two antibodies.

In a separate embodiment, a single-chain antibody can be created by joining receptor light and heavy chains using a non-peptide linker or chemical unit. Generally, the light and heavy chains will be produced in distinct cells, purified, and subsequently linked together in an appropriate fashion (*i.e.*, the N-terminus of the heavy chain being attached to the C-terminus of the light chain via an appropriate chemical bridge).

Cross-linking reagents are used to form molecular bridges that tie functional groups of two different molecules, *e.g.*, a stablizing and coagulating agent. However, it is contemplated that dimers or multimers of the same analog or heteromeric complexes comprised of different analogs can be created. To link two different compounds in a step-wise manner, hetero-bifunctional cross-linkers can be used that eliminate unwanted homopolymer formation.

An exemplary hetero-bifunctional cross-linker contains two reactive groups: one reacting with primary amine group (*e.g.*, N-hydroxy succinimide) and the other reacting with a thiol group (*e.g.*, pyridyl disulfide, maleimides, halogens, *etc.*). Through the primary amine reactive group, the cross-linker may react with the lysine residue(s) of one protein (*e.g.*, the selected antibody or fragment) and through the thiol reactive group, the cross-linker, already tied up to the first protein, reacts with the cysteine residue (free sulfhydryl group) of the other protein (*e.g.*, the selective agent).

It is preferred that a cross-linker having reasonable stability in blood will be employed. Numerous types of disulfide-bond containing linkers are known that can be successfully employed to conjugate targeting and therapeutic/preventative agents. Linkers that contain a disulfide bond that is sterically hindered may prove to give greater stability *in vivo*,

preventing release of the targeting peptide prior to reaching the site of action. These linkers are thus one group of linking agents.

Another cross-linking reagent is SMPT, which is a bifunctional cross-linker containing a disulfide bond that is "sterically hindered" by an adjacent benzene ring and methyl groups. It is believed that steric hindrance of the disulfide bond serves a function of protecting the bond from attack by thiolate anions such as glutathione which can be present in tissues and blood, and thereby help in preventing decoupling of the conjugate prior to the delivery of the attached agent to the target site.

The SMPT cross-linking reagent, as with many other known cross-linking reagents, lends the ability to cross-link functional groups such as the SH of cysteine or primary amines (*e.g.*, the epsilon amino group of lysine). Another possible type of cross-linker includes the hetero-bifunctional photoreactive phenylazides containing a cleavable disulfide bond such as sulfosuccinimidyl-2-(p-azido salicylamido) ethyl-1,3'-dithiopropionate. The N-hydroxy-succinimidyl group reacts with primary amino groups and the phenylazide (upon photolysis) reacts non-selectively with any amino acid residue.

In addition to hindered cross-linkers, non-hindered linkers also can be employed in accordance herewith. Other useful cross-linkers, not considered to contain or generate a protected disulfide, include SATA, SPDP and 2-iminothiolane (Wawrzynczak & Thorpe, 1987). The use of such cross-linkers is well understood in the art. Another embodiment involves the use of flexible linkers.

U.S. Patent 4,680,338, describes bifunctional linkers useful for producing conjugates of ligands with amine-containing polymers and/or proteins, especially for forming antibody conjugates with chelators, drugs, enzymes, detectable labels and the like. U.S. Patents 5,141,648 and 5,563,250 disclose cleavable conjugates containing a labile bond that is cleavable under a variety of mild conditions. This linker is particularly useful in that the agent of interest may be bonded directly to the linker, with cleavage resulting in release of the active agent. Particular uses include adding a free amino or free sulfhydryl group to a protein, such as an antibody, or a drug.

U.S. Patent 5,856,456 provides peptide linkers for use in connecting polypeptide constituents to make fusion proteins, *e.g.*, single chain antibodies. The linker is up to about 50 amino acids in length, contains at least one occurrence of a charged amino acid (preferably arginine or lysine) followed by a proline, and is characterized by greater stability and reduced aggregation. U.S. Patent 5,880,270 discloses aminooxy-containing linkers useful in a variety of immunodiagnostic and separative techniques.

E. Intrabodies

In a particular embodiment, the antibody is a recombinant antibody that is suitable for action inside of a cell – such antibodies are known as "intrabodies." These antibodies may interfere with target function by a variety of mechanism, such as by altering intracellular protein trafficking, interfering with enzymatic function, and blocking protein-protein or protein-DNA interactions. In many ways, their structures mimic or parallel those of single chain and single domain antibodies, discussed above. Indeed, single-transcript/single-chain is an important feature that permits intracellular expression in a target cell, and also makes protein transit across cell membranes more feasible. However, additional features are required.

The two major issues impacting the implementation of intrabody therapeutic are delivery, including cell/tissue targeting, and stability. With respect to delivery, a variety of approaches have been employed, such as tissue-directed delivery, use of cell-type specific promoters, viral-based delivery and use of cell-permeability/membrane translocating peptides. With respect to the stability, the approach is generally to either screen by brute force, including methods that involve phage diplay and may include sequence maturation or development of consensus sequences, or more directed modifications such as insertion stabilizing sequences (*e.g.*, Fc regions, chaperone protein sequences, leucine zippers) and disulfide replacement/modification.

An additional feature that intrabodies may require is a signal for intracellular targeting. Vectors that can target intrabodies (or other proteins) to subcellular regions such as the cytoplasm, nucleus, mitochondria and ER have been designed and are commercially available (Invitrogen Corp.; Persic *et al.*, 1997).

By virtue of their ability to enter cells, intrabodies have additional uses that other types of antibodies may not achieve. In the case of the present antibodies, the ability to interact with the MUC1 cytoplasmic domain in a living cell may interfere with functions associated with the MUC1 CD, such as signaling functions (binding to other molecules) or oligomer formation. In particular, it is contemplated that such antibodies can be used to inhibit MUC1 dimer formation.

F. Purification

In certain embodiments, the antibodies of the present disclosure may be purified. The term "purified," as used herein, is intended to refer to a composition, isolatable from other

components, wherein the protein is purified to any degree relative to its naturally-obtainable state. A purified protein therefore also refers to a protein, free from the environment in which it may naturally occur. Where the term "substantially purified" is used, this designation will refer to a composition in which the protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the proteins in the composition.

Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the polypeptide from other proteins, the polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; isoelectric focusing. Other methods for protein purification include, precipitation with ammonium sulfate, PEG, antibodies and the like or by heat denaturation, followed by centrifugation; gel filtration, reverse phase, hydroxylapatite and affinity chromatography; and combinations of such and other techniques.

In purifying an antibody of the present disclosure, it may be desirable to express the polypeptide in a prokaryotic or eukaryotic expression system and extract the protein using denaturing conditions. The polypeptide may be purified from other cellular components using an affinity column, which binds to a tagged portion of the polypeptide. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

Commonly, complete antibodies are fractionated utilizing agents (*i.e.*, protein A) that bind the Fc portion of the antibody. Alternatively, antigens may be used to simultaneously purify and select appropriate antibodies. Such methods often utilize the selection agent bound to a support, such as a column, filter or bead. The antibodies is bound to a support, contaminants removed (*e.g.*, washed away), and the antibodies released by applying conditions (salt, heat, *etc.*).

Various methods for quantifying the degree of purification of the protein or peptide will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the amount of polypeptides within a fraction by SDS/PAGE analysis. Another method for assessing the

purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity. The actual units used to represent the amount of activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed protein or peptide exhibits a detectable activity.

It is known that the migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE (Capaldi *et al.*, 1977). It will therefore be appreciated that under differing electrophoresis conditions, the apparent molecular weights of purified or partially purified expression products may vary.

III. Active/Passive Immunization and Treatment/Prevention of Zika virus Infection

A. Formulation and Administration

The present disclosure provides pharmaceutical compositions comprising anti-Zika virus antibodies and antigens for generating the same. Such compositions comprise a prophylactically or therapeutically effective amount of an antibody or a fragment thereof, or a peptide immunogen, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a particular carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Other suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, *etc*. Examples of suitable pharmaceutical agents are

described in "Remington's Pharmaceutical Sciences." Such compositions will contain a prophylactically or therapeutically effective amount of the antibody or fragment thereof, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration, which can be oral, intravenous, intraarterial, intrabuccal, intranasal, nebulized, bronchial inhalation, or delivered by mechanical ventilation.

Active vaccines are also envisioned where antibodies like those disclosed are produced *in vivo* in a subject at risk of Zika virus infection. Such vaccines can be formulated for parenteral administration, *e.g.*, formulated for injection *via* the intradermal, intravenous, intramuscular, subcutaneous, or even intraperitoneal routes. Administration by intradermal and intramuscular routes are contemplated. The vaccine could alternatively be administered by a topical route directly to the mucosa, for example by nasal drops, inhalation, or by nebulizer. Pharmaceutically acceptable salts, include the acid salts and those which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

Passive transfer of antibodies, known as artificially acquired passive immunity, generally will involve the use of intravenous or intramuscular injections. The forms of antibody can be human or animal blood plasma or serum, as pooled human immunoglobulin for intravenous (IVIG) or intramuscular (IG) use, as high-titer human IVIG or IG from immunized or from donors recovering from disease, and as monoclonal antibodies (MAb). Such immunity generally lasts for only a short period of time, and there is also a potential risk for hypersensitivity reactions, and serum sickness, especially from gamma globulin of non-human origin. However, passive immunity provides immediate protection. The antibodies will be formulated in a carrier suitable for injection, *i.e.*, sterile and syringeable.

Generally, the ingredients of compositions of the disclosure are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile

water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compositions of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

IV. Antibody Conjugates

Antibodies of the present disclosure may be linked to at least one agent to form an antibody conjugate. In order to increase the efficacy of antibody molecules as diagnostic or therapeutic agents, it is conventional to link or covalently bind or complex at least one desired molecule or moiety. Such a molecule or moiety may be, but is not limited to, at least one effector or reporter molecule. Effector molecules comprise molecules having a desired activity, *e.g.*, cytotoxic activity. Non-limiting examples of effector molecules which have been attached to antibodies include toxins, anti-tumor agents, therapeutic enzymes, radionuclides, antiviral agents, chelating agents, cytokines, growth factors, and oligo- or polynucleotides. By contrast, a reporter molecule is defined as any moiety which may be detected using an assay. Non-limiting examples of reporter molecules which have been conjugated to antibodies include enzymes, radiolabels, haptens, fluorescent labels, phosphorescent molecules, chemiluminescent molecules, chromophores, photoaffinity molecules, colored particles or ligands, such as biotin.

Antibody conjugates are generally preferred for use as diagnostic agents. Antibody diagnostics generally fall within two classes, those for use in *in vitro* diagnostics, such as in a variety of immunoassays, and those for use *in vivo* diagnostic protocols, generally known as "antibody-directed imaging." Many appropriate imaging agents are known in the art, as are methods for their attachment to antibodies (see, for *e.g.*, U.S. Patents 5,021,236, 4,938,948, and 4,472,509). The imaging moieties used can be paramagnetic ions, radioactive isotopes, fluorochromes, NMR-detectable substances, and X-ray imaging agents.

In the case of paramagnetic ions, one might mention by way of example ions such as chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) and/or erbium (III), with gadolinium being particularly

preferred. Ions useful in other contexts, such as X-ray imaging, include but are not limited to lanthanum (III), gold (III), lead (II), and especially bismuth (III).

In the case of radioactive isotopes for therapeutic and/or diagnostic application, one might mention astatine^{211}, ^{14}carbon, ^{51}chromium, ^{36}chlorine, ^{57}cobalt, ^{58}cobalt, copper^{67}, ^{152}Eu, gallium^{67}, ^{3}hydrogen, iodine^{123}, iodine^{125}, iodine^{131}, indium^{111}, ^{59}iron, ^{32}phosphorus, rhenium^{186}, rhenium^{188}, ^{75}selenium, ^{35}sulphur, technicium^{99m} and/or yttrium^{90}. ^{125}I is often being preferred for use in certain embodiments, and technicium^{99m} and/or indium^{111} are also often preferred due to their low energy and suitability for long range detection. Radioactively labeled monoclonal antibodies of the present disclosure may be produced according to well-known methods in the art. For instance, monoclonal antibodies can be iodinated by contact with sodium and/or potassium iodide and a chemical oxidizing agent such as sodium hypochlorite, or an enzymatic oxidizing agent, such as lactoperoxidase. Monoclonal antibodies according to the disclosure may be labeled with technetium^{99m} by ligand exchange process, for example, by reducing pertechnate with stannous solution, chelating the reduced technetium onto a Sephadex column and applying the antibody to this column. Alternatively, direct labeling techniques may be used, *e.g.*, by incubating pertechnate, a reducing agent such as $SNCl_2$, a buffer solution such as sodium-potassium phthalate solution, and the antibody. Intermediary functional groups which are often used to bind radioisotopes which exist as metallic ions to antibody are diethylenetriaminepentaacetic acid (DTPA) or ethylene diaminetetracetic acid (EDTA).

Among the fluorescent labels contemplated for use as conjugates include Alexa 350, Alexa 430, AMCA, BODIPY 630/650, BODIPY 650/665, BODIPY-FL, BODIPY-R6G, BODIPY-TMR, BODIPY-TRX, Cascade Blue, Cy3, Cy5,6-FAM, Fluorescein Isothiocyanate, HEX, 6-JOE, Oregon Green 488, Oregon Green 500, Oregon Green 514, Pacific Blue, REG, Rhodamine Green, Rhodamine Red, Renographin, ROX, TAMRA, TET, Tetramethylrhodamine, and/or Texas Red.

Another type of antibody conjugates contemplated in the present disclosure are those intended primarily for use *in vitro*, where the antibody is linked to a secondary binding ligand and/or to an enzyme (an enzyme tag) that will generate a colored product upon contact with a chromogenic substrate. Examples of suitable enzymes include urease, alkaline phosphatase, (horseradish) hydrogen peroxidase or glucose oxidase. Preferred secondary binding ligands are biotin and avidin and streptavidin compounds. The use of such labels is well known to those of skill in the art and are described, for example, in U.S. Patents 3,817,837, 3,850,752, 3,939,350, 3,996,345, 4,277,437, 4,275,149 and 4,366,241.

Yet another known method of site-specific attachment of molecules to antibodies comprises the reaction of antibodies with hapten-based affinity labels. Essentially, hapten-based affinity labels react with amino acids in the antigen binding site, thereby destroying this site and blocking specific antigen reaction. However, this may not be advantageous since it results in loss of antigen binding by the antibody conjugate.

Molecules containing azido groups may also be used to form covalent bonds to proteins through reactive nitrene intermediates that are generated by low intensity ultraviolet light (Potter and Haley, 1983). In particular, 2- and 8-azido analogues of purine nucleotides have been used as site-directed photoprobes to identify nucleotide binding proteins in crude cell extracts (Owens & Haley, 1987; Atherton *et al.,* 1985). The 2- and 8-azido nucleotides have also been used to map nucleotide binding domains of purified proteins (Khatoon *et al.,* 1989; King *et al.,* 1989; Dholakia *et al.,* 1989) and may be used as antibody binding agents.

Several methods are known in the art for the attachment or conjugation of an antibody to its conjugate moiety. Some attachment methods involve the use of a metal chelate complex employing, for example, an organic chelating agent such a diethylenetriaminepentaacetic acid anhydride (DTPA); ethylenetriaminetetraacetic acid; N-chloro-p-toluenesulfonamide; and/or tetrachloro-3α-6α-diphenylglycouril-3 attached to the antibody (U.S. Patents 4,472,509 and 4,938,948). Monoclonal antibodies may also be reacted with an enzyme in the presence of a coupling agent such as glutaraldehyde or periodate. Conjugates with fluorescein markers are prepared in the presence of these coupling agents or by reaction with an isothiocyanate. In U.S. Patent 4,938,948, imaging of breast tumors is achieved using monoclonal antibodies and the detectable imaging moieties are bound to the antibody using linkers such as methyl-p-hydroxybenzimidate or N-succinimidyl-3-(4-hydroxyphenyl)propionate.

In other embodiments, derivatization of immunoglobulins by selectively introducing sulfhydryl groups in the Fc region of an immunoglobulin, using reaction conditions that do not alter the antibody combining site are contemplated. Antibody conjugates produced according to this methodology are disclosed to exhibit improved longevity, specificity and sensitivity (U.S. Patent 5,196,066, incorporated herein by reference). Site-specific attachment of effector or reporter molecules, wherein the reporter or effector molecule is conjugated to a carbohydrate residue in the Fc region have also been disclosed in the literature (O'Shannessy *et al.,* 1987). This approach has been reported to produce diagnostically and therapeutically promising antibodies which are currently in clinical evaluation.

V. Immunodetection Methods

In still further embodiments, the present disclosure concerns immunodetection methods for binding, purifying, removing, quantifying and otherwise generally detecting Zika virus and its associated antigens. While such methods can be applied in a traditional sense, another use will be in quality control and monitoring of vaccine and other virus stocks, where antibodies according to the present disclosure can be used to assess the amount or integrity (*i.e.*, long term stability) of H1 antigens in viruses. Alternatively, the methods may be used to screen various antibodies for appropriate/desired reactivity profiles.

Other immunodections methods include specific assays for determining the presence of Zika virus in a subject. A wide variety of assay formats are contemplated, but specifically those that would be used to detect Zika virus in a fluid obtained from a subject, such as saliva, blood, plasma, sputum, semen or urine. In particular, semen has been demonstrated as a viable sample for detecting Zika virus (Purpura *et al.*, 2016; Mansuy *et al.*, 2016; Barzon *et al.*, 2016; Gornet *et al.*, 2016; Duffy *et al.*, 2009; CDC, 2016; Halfon *et al.*, 2010; Elder *et al.* 2005). The assays may advantagenously for formatted for non-healthcare (home) use, including lateral flow assays (see below) analogous to home pregnancy tests. These assays may be packaged in the form of a kit with appropriate reagents and instructions to permit use by the subject of a family member.

Some immunodetection methods include enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoradiometric assay, fluoroimmunoassay, chemiluminescent assay, bioluminescent assay, and Western blot to mention a few. In particular, a competitive assay for the detection and quantitation of Zika virus antibodies directed to specific parasite epitopes in samples also is provided. The steps of various useful immunodetection methods have been described in the scientific literature, such as, *e.g.*, Doolittle and Ben-Zeev (1999), Gulbis and Galand (1993), De Jager *et al.* (1993), and Nakamura *et al.* (1987). In general, the immunobinding methods include obtaining a sample suspected of containing Zika virus, and contacting the sample with a first antibody in accordance with the present disclosure, as the case may be, under conditions effective to allow the formation of immunocomplexes.

These methods include methods for purifying Zika virus or related antigens from a sample. The antibody will preferably be linked to a solid support, such as in the form of a column matrix, and the sample suspected of containing the Zika virus or antigenic component will be applied to the immobilized antibody. The unwanted components will be washed from

the column, leaving the Zika virus antigen immunocomplexed to the immobilized antibody, which is then collected by removing the organism or antigen from the column.

The immunobinding methods also include methods for detecting and quantifying the amount of Zika virus or related components in a sample and the detection and quantification of any immune complexes formed during the binding process. Here, one would obtain a sample suspected of containing Zika virus or its antigens, and contact the sample with an antibody that binds Zika virus or components thereof, followed by detecting and quantifying the amount of immune complexes formed under the specific conditions. In terms of antigen detection, the biological sample analyzed may be any sample that is suspected of containing Zika virus or Zika virus antigen, such as a tissue section or specimen, a homogenized tissue extract, a biological fluid, including blood and serum, or a secretion, such as feces or urine.

Contacting the chosen biological sample with the antibody under effective conditions and for a period of time sufficient to allow the formation of immune complexes (primary immune complexes) is generally a matter of simply adding the antibody composition to the sample and incubating the mixture for a period of time long enough for the antibodies to form immune complexes with, *i.e.*, to bind to Zika virus or antigens present. After this time, the sample-antibody composition, such as a tissue section, ELISA plate, dot blot or Western blot, will generally be washed to remove any non-specifically bound antibody species, allowing only those antibodies specifically bound within the primary immune complexes to be detected.

In general, the detection of immunocomplex formation is well known in the art and may be achieved through the application of numerous approaches. These methods are generally based upon the detection of a label or marker, such as any of those radioactive, fluorescent, biological and enzymatic tags. Patents concerning the use of such labels include U.S. Patents 3,817,837, 3,850,752, 3,939,350, 3,996,345, 4,277,437, 4,275,149 and 4,366,241. Of course, one may find additional advantages through the use of a secondary binding ligand such as a second antibody and/or a biotin/avidin ligand binding arrangement, as is known in the art.

The antibody employed in the detection may itself be linked to a detectable label, wherein one would then simply detect this label, thereby allowing the amount of the primary immune complexes in the composition to be determined. Alternatively, the first antibody that becomes bound within the primary immune complexes may be detected by means of a second binding ligand that has binding affinity for the antibody. In these cases, the second binding ligand may be linked to a detectable label. The second binding ligand is itself often an

antibody, which may thus be termed a "secondary" antibody. The primary immune complexes are contacted with the labeled, secondary binding ligand, or antibody, under effective conditions and for a period of time sufficient to allow the formation of secondary immune complexes. The secondary immune complexes are then generally washed to remove any non-specifically bound labeled secondary antibodies or ligands, and the remaining label in the secondary immune complexes is then detected.

Further methods include the detection of primary immune complexes by a two-step approach. A second binding ligand, such as an antibody that has binding affinity for the antibody, is used to form secondary immune complexes, as described above. After washing, the secondary immune complexes are contacted with a third binding ligand or antibody that has binding affinity for the second antibody, again under effective conditions and for a period of time sufficient to allow the formation of immune complexes (tertiary immune complexes). The third ligand or antibody is linked to a detectable label, allowing detection of the tertiary immune complexes thus formed. This system may provide for signal amplification if this is desired.

One method of immunodetection uses two different antibodies. A first biotinylated antibody is used to detect the target antigen, and a second antibody is then used to detect the biotin attached to the complexed biotin. In that method, the sample to be tested is first incubated in a solution containing the first step antibody. If the target antigen is present, some of the antibody binds to the antigen to form a biotinylated antibody/antigen complex. The antibody/antigen complex is then amplified by incubation in successive solutions of streptavidin (or avidin), biotinylated DNA, and/or complementary biotinylated DNA, with each step adding additional biotin sites to the antibody/antigen complex. The amplification steps are repeated until a suitable level of amplification is achieved, at which point the sample is incubated in a solution containing the second step antibody against biotin. This second step antibody is labeled, as for example with an enzyme that can be used to detect the presence of the antibody/antigen complex by histoenzymology using a chromogen substrate. With suitable amplification, a conjugate can be produced which is macroscopically visible.

Another known method of immunodetection takes advantage of the immuno-PCR (Polymerase Chain Reaction) methodology. The PCR method is similar to the Cantor method up to the incubation with biotinylated DNA, however, instead of using multiple rounds of streptavidin and biotinylated DNA incubation, the DNA/biotin/streptavidin/antibody complex is washed out with a low pH or high salt buffer that releases the antibody. The resulting wash solution is then used to carry out a PCR reaction with suitable primers with appropriate

controls. At least in theory, the enormous amplification capability and specificity of PCR can be utilized to detect a single antigen molecule.

A. ELISAs

Immunoassays, in their most simple and direct sense, are binding assays. Certain preferred immunoassays are the various types of enzyme linked immunosorbent assays (ELISAs) and radioimmunoassays (RIA) known in the art. Immunohistochemical detection using tissue sections is also particularly useful. However, it will be readily appreciated that detection is not limited to such techniques, and western blotting, dot blotting, FACS analyses, and the like may also be used.

In one exemplary ELISA, the antibodies of the disclosure are immobilized onto a selected surface exhibiting protein affinity, such as a well in a polystyrene microtiter plate. Then, a test composition suspected of containing the Zika virus or Zika virus antigen is added to the wells. After binding and washing to remove non-specifically bound immune complexes, the bound antigen may be detected. Detection may be achieved by the addition of another anti-Zika virus antibody that is linked to a detectable label. This type of ELISA is a simple "sandwich ELISA." Detection may also be achieved by the addition of a second anti-Zika virus antibody, followed by the addition of a third antibody that has binding affinity for the second antibody, with the third antibody being linked to a detectable label.

In another exemplary ELISA, the samples suspected of containing the Zika virus or Zika virus antigen are immobilized onto the well surface and then contacted with the anti-Zika virus antibodies of the disclosure. After binding and washing to remove non-specifically bound immune complexes, the bound anti-Zika virus antibodies are detected. Where the initial anti-Zika virus antibodies are linked to a detectable label, the immune complexes may be detected directly. Again, the immune complexes may be detected using a second antibody that has binding affinity for the first anti-Zika virus antibody, with the second antibody being linked to a detectable label.

Irrespective of the format employed, ELISAs have certain features in common, such as coating, incubating and binding, washing to remove non-specifically bound species, and detecting the bound immune complexes. These are described below.

In coating a plate with either antigen or antibody, one will generally incubate the wells of the plate with a solution of the antigen or antibody, either overnight or for a specified period of hours. The wells of the plate will then be washed to remove incompletely adsorbed material. Any remaining available surfaces of the wells are then "coated" with a nonspecific

protein that is antigenically neutral with regard to the test antisera. These include bovine serum albumin (BSA), casein or solutions of milk powder. The coating allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific binding of antisera onto the surface.

In ELISAs, it is probably more customary to use a secondary or tertiary detection means rather than a direct procedure. Thus, after binding of a protein or antibody to the well, coating with a non-reactive material to reduce background, and washing to remove unbound material, the immobilizing surface is contacted with the biological sample to be tested under conditions effective to allow immune complex (antigen/antibody) formation. Detection of the immune complex then requires a labeled secondary binding ligand or antibody, and a secondary binding ligand or antibody in conjunction with a labeled tertiary antibody or a third binding ligand.

"Under conditions effective to allow immune complex (antigen/antibody) formation" means that the conditions preferably include diluting the antigens and/or antibodies with solutions such as BSA, bovine gamma globulin (BGG) or phosphate buffered saline (PBS)/Tween. These added agents also tend to assist in the reduction of nonspecific background.

The "suitable" conditions also mean that the incubation is at a temperature or for a period of time sufficient to allow effective binding. Incubation steps are typically from about 1 to 2 to 4 hours or so, at temperatures preferably on the order of 25°C to 27°C, or may be overnight at about 4°C or so.

Following all incubation steps in an ELISA, the contacted surface is washed so as to remove non-complexed material. A preferred washing procedure includes washing with a solution such as PBS/Tween, or borate buffer. Following the formation of specific immune complexes between the test sample and the originally bound material, and subsequent washing, the occurrence of even minute amounts of immune complexes may be determined.

To provide a detecting means, the second or third antibody will have an associated label to allow detection. Preferably, this will be an enzyme that will generate color development upon incubating with an appropriate chromogenic substrate. Thus, for example, one will desire to contact or incubate the first and second immune complex with a urease, glucose oxidase, alkaline phosphatase or hydrogen peroxidase-conjugated antibody for a period of time and under conditions that favor the development of further immune complex formation (*e.g.*, incubation for 2 hours at room temperature in a PBS-containing solution such as PBS-Tween).

After incubation with the labeled antibody, and subsequent to washing to remove unbound material, the amount of label is quantified, *e.g.*, by incubation with a chromogenic substrate such as urea, or bromocresol purple, or 2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid (ABTS), or H_2O_2, in the case of peroxidase as the enzyme label. Quantification is then achieved by measuring the degree of color generated, *e.g.*, using a visible spectra spectrophotometer.

In another embodiment, the present disclosure contemplates the use of competitive formats. This is particularly useful in the detection of Zika virus antibodies in sample. In competition based assays, an unknown amount of analyte or antibody is determined by its ability to displace a known amount of labeled antibody or analyte. Thus, the quantifiable loss of a signal is an indication of the amount of unknown antibody or analyte in a sample.

Here, the inventors propose the use of labeled Zika virus monoclonal antibodies to determine the amount of Zika virus antibodies in a sample. The basic format would include contacting a known amount of Zika virus monoclonal antibody (linked to a detectable label) with Zika virus antigen or particle. The Zika virus antigen or organism is preferably attached to a support. After binding of the labeled monoclonal antibody to the support, the sample is added and incubated under conditions permitting any unlabeled antibody in the sample to compete with, and hence displace, the labeled monoclonal antibody. By measuring either the lost label or the label remaining (and subtracting that from the original amount of bound label), one can determine how much non-labeled antibody is bound to the support, and thus how much antibody was present in the sample.

B. Western Blot

The Western blot (alternatively, protein immunoblot) is an analytical technique used to detect specific proteins in a given sample of tissue homogenate or extract. It uses gel electrophoresis to separate native or denatured proteins by the length of the polypeptide (denaturing conditions) or by the 3-D structure of the protein (native/ non-denaturing conditions). The proteins are then transferred to a membrane (typically nitrocellulose or PVDF), where they are probed (detected) using antibodies specific to the target protein.

Samples may be taken from whole tissue or from cell culture. In most cases, solid tissues are first broken down mechanically using a blender (for larger sample volumes), using a homogenizer (smaller volumes), or by sonication. Cells may also be broken open by one of the above mechanical methods. However, it should be noted that bacteria, virus or environmental samples can be the source of protein and thus Western blotting is not restricted

to cellular studies only. Assorted detergents, salts, and buffers may be employed to encourage lysis of cells and to solubilize proteins. Protease and phosphatase inhibitors are often added to prevent the digestion of the sample by its own enzymes. Tissue preparation is often done at cold temperatures to avoid protein denaturing.

The proteins of the sample are separated using gel electrophoresis. Separation of proteins may be by isoelectric point (pI), molecular weight, electric charge, or a combination of these factors. The nature of the separation depends on the treatment of the sample and the nature of the gel. This is a very useful way to determine a protein. It is also possible to use a two-dimensional (2-D) gel which spreads the proteins from a single sample out in two dimensions. Proteins are separated according to isoelectric point (pH at which they have neutral net charge) in the first dimension, and according to their molecular weight in the second dimension.

In order to make the proteins accessible to antibody detection, they are moved from within the gel onto a membrane made of nitrocellulose or polyvinylidene difluoride (PVDF). The membrane is placed on top of the gel, and a stack of filter papers placed on top of that. The entire stack is placed in a buffer solution which moves up the paper by capillary action, bringing the proteins with it. Another method for transferring the proteins is called electroblotting and uses an electric current to pull proteins from the gel into the PVDF or nitrocellulose membrane. The proteins move from within the gel onto the membrane while maintaining the organization they had within the gel. As a result of this blotting process, the proteins are exposed on a thin surface layer for detection (see below). Both varieties of membrane are chosen for their non-specific protein binding properties (*i.e.*, binds all proteins equally well). Protein binding is based upon hydrophobic interactions, as well as charged interactions between the membrane and protein. Nitrocellulose membranes are cheaper than PVDF, but are far more fragile and do not stand up well to repeated probings. The uniformity and overall effectiveness of transfer of protein from the gel to the membrane can be checked by staining the membrane with Coomassie Brilliant Blue or Ponceau S dyes. Once transferred, proteins are detected using labeled primary antibodies, or unlabeled primary antibodies followed by indirect detection using labeled protein A or secondary labeled antibodies binding to the Fc region of the primary antibodies.

C. Lateral Flow Assays

Lateral flow assays, also known as lateral flow immunochromatographic assays, are simple devices intended to detect the presence (or absence) of a target analyte in sample

(matrix) without the need for specialized and costly equipment, though many lab based applications exist that are supported by reading equipment. Typically, these tests are used as low resources medical diagnostics, either for home testing, point of care testing, or laboratory use. A widely spread and well known application is the home pregnancy test.

The technology is based on a series of capillary beds, such as pieces of porous paper or sintered polymer. Each of these elements has the capacity to transport fluid (*e.g.*, urine) spontaneously. The first element (the sample pad) acts as a sponge and holds an excess of sample fluid. Once soaked, the fluid migrates to the second element (conjugate pad) in which the manufacturer has stored the so-called conjugate, a dried format of bio-active particles (see below) in a salt-sugar matrix that contains everything to guarantee an optimized chemical reaction between the target molecule (*e.g.*, an antigen) and its chemical partner (*e.g.*, antibody) that has been immobilized on the particle's surface. While the sample fluid dissolves the salt-sugar matrix, it also dissolves the particles and in one combined transport action the sample and conjugate mix while flowing through the porous structure. In this way, the analyte binds to the particles while migrating further through the third capillary bed. This material has one or more areas (often called stripes) where a third molecule has been immobilized by the manufacturer. By the time the sample-conjugate mix reaches these strips, analyte has been bound on the particle and the third 'capture' molecule binds the complex. After a while, when more and more fluid has passed the stripes, particles accumulate and the stripe-area changes color. Typically there are at least two stripes: one (the control) that captures any particle and thereby shows that reaction conditions and technology worked fine, the second contains a specific capture molecule and only captures those particles onto which an analyte molecule has been immobilized. After passing these reaction zones, the fluid enters the final porous material – the wick – that simply acts as a waste container. Lateral Flow Tests can operate as either competitive or sandwich assays. Lateral flow assays are disclosed in U.S. Patent 6,485,982.

D. Immunohistochemistry

The antibodies of the present disclosure may also be used in conjunction with both fresh-frozen and/or formalin-fixed, paraffin-embedded tissue blocks prepared for study by immunohistochemistry (IHC). The method of preparing tissue blocks from these particulate specimens has been successfully used in previous IHC studies of various prognostic factors, and is well known to those of skill in the art (Brown *et al.*, 1990; Abbondanzo *et al.*, 1990; Allred *et al.*, 1990).

Briefly, frozen-sections may be prepared by rehydrating 50 ng of frozen "pulverized" tissue at room temperature in phosphate buffered saline (PBS) in small plastic capsules; pelleting the particles by centrifugation; resuspending them in a viscous embedding medium (OCT); inverting the capsule and/or pelleting again by centrifugation; snap-freezing in -70°C isopentane; cutting the plastic capsule and/or removing the frozen cylinder of tissue; securing the tissue cylinder on a cryostat microtome chuck; and/or cutting 25-50 serial sections from the capsule. Alternatively, whole frozen tissue samples may be used for serial section cuttings.

Permanent-sections may be prepared by a similar method involving rehydration of the 50 mg sample in a plastic microfuge tube; pelleting; resuspending in 10% formalin for 4 hours fixation; washing/pelleting; resuspending in warm 2.5% agar; pelleting; cooling in ice water to harden the agar; removing the tissue/agar block from the tube; infiltrating and/or embedding the block in paraffin; and/or cutting up to 50 serial permanent sections. Again, whole tissue samples may be substituted.

E. Immunodetection Kits

In still further embodiments, the present disclosure concerns immunodetection kits for use with the immunodetection methods described above. As the antibodies may be used to detect Zika virus or Zika virus antigens, the antibodies may be included in the kit. The immunodetection kits will thus comprise, in suitable container means, a first antibody that binds to Zika virus or Zika virus antigen, and optionally an immunodetection reagent.

In certain embodiments, the Zika virus antibody may be pre-bound to a solid support, such as a column matrix and/or well of a microtitre plate. The immunodetection reagents of the kit may take any one of a variety of forms, including those detectable labels that are associated with or linked to the given antibody. Detectable labels that are associated with or attached to a secondary binding ligand are also contemplated. Exemplary secondary ligands are those secondary antibodies that have binding affinity for the first antibody.

Further suitable immunodetection reagents for use in the present kits include the two-component reagent that comprises a secondary antibody that has binding affinity for the first antibody, along with a third antibody that has binding affinity for the second antibody, the third antibody being linked to a detectable label. As noted above, a number of exemplary labels are known in the art and all such labels may be employed in connection with the present disclosure.

The kits may further comprise a suitably aliquoted composition of the Zika virus or Zika virus antigens, whether labeled or unlabeled, as may be used to prepare a standard curve for a detection assay. The kits may contain antibody-label conjugates either in fully conjugated form, in the form of intermediates, or as separate moieties to be conjugated by the user of the kit. The components of the kits may be packaged either in aqueous media or in lyophilized form.

The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which the antibody may be placed, or preferably, suitably aliquoted. The kits of the present disclosure will also typically include a means for containing the antibody, antigen, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

VI. Examples

The following examples are included to demonstrate preferred embodiments. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventors to function well in the practice of embodiments, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

Example 1 - Materials and Methods

Research subjects. The inventors studied eight subjects in the U.S. with prior or recent ZIKV infection (Table 5). The studies were approved by the Institutional Review Board of Vanderbilt University Medical Center; samples were obtained after informed consent was obtained by the Vanderbilt Clinical Trials Center. Two subjects (972 and 973) were infected with an African lineage strain in 2008 (one subject while working in Senegal,

the second acquired the infection by sexual transmission from the first, as previously reported (Foy *et al.*, 2011). The other six subjects were infected during the current outbreak of an Asian lineage strain, following exposure in Brazil, Mexico, or Haiti.

Generation and quantification of human B cell lines secreting ZIKV E protein specific antibodies. Peripheral blood mononuclear cells (PBMCs) from heparinized blood were isolated with Ficoll-Histopaque by density gradient centrifugation. The cells either were used immediately or cryopreserved in the vapor phase of liquid nitrogen until use. Ten million PBMCs were cultured in 384-well plates (Nunc) using culture medium (ClonaCell-HY Medium A, StemCell Technologies) supplemented with 8 µg/ml of the TLR agonist CpG (phosphorothioate-modified oligodeoxynucleotide ZOEZOEZZZZZOEEZOEZZZT, Invitrogen), 3 µg/ml of Chk2 inhibitor (Sigma), 1 µg/ml of cyclosporine A (Sigma), and clarified supernatants from cultures of B95.8 cells (ATCC) containing Epstein-Barr virus (EBV). After 7 days, cells from each 384-well culture plate were expanded into four 96-well culture plates (Falcon) using ClonaCell-HY Medium A containing 8 µg/ml of CpG, 3 µg/ml of Chk2 inhibitor, and 10^7 irradiated heterologous human PBMCs (Nashville Red Cross) and cultured for an additional 4 days. Supernatants were screened in ELISA (described below) for reactivity with various ZIKV E proteins, which are described below.

The minimal frequency of ZIKV E-reactive B cells was estimated based on the number of wells with E protein-reactive supernatants compared with the total number of lymphoblastoid cell line colonies in the transformation plates [calculation: E-reactive B cell frequency = (number of wells with E-reactive supernatants) divided by [number of LCL colonies in the plate) x 100].

Protein expression and purification. The ectodomains of ZIKV E (H/PF/2013; GenBank Accession KJ776791) and the fusion-loop mutant E-FLM (containing four mutations: T76A, Q77G, W101R, L107R) were expressed transiently in Expi293F cells and purified as described previously (Zhao *et al.*, 2016). ZIKV DIII (residues 299–407 of strain H/PF/2013), WNV-DIII (residues 296–405 of strain New York 1999) and DENV2-DIII (residues 299-410 of strain 16681) were expressed in BL21 (DE3) as inclusion bodies and refolded *in vitro* (Nelson *et al.*, 2014). Briefly, inclusion bodies were denatured and refolded by gradual dilution into a refolding buffer (400 mM L-arginine, 100 mM Tris [pH 8.3], 2 mM EDTA, 5 and 0.5 mM reduced and oxidized glutathione) at 4°C. Refolded proteins were purified by size-exclusion chromatography using a Superdex 75, 16/60 (GE Healthcare).

Generation of human hybridomas. Cells from wells with transformed B cells containing supernatants that exhibited reactivity to ZIKV E protein were fused with

HMMA2.5 myeloma cells (kind gift from L. Cavacini) using an established electrofusion technique (Yu *et al.*, 2008). After fusion, hybridomas were suspended in a selection medium containing 100 μM hypoxanthine, 0.4 μM aminopterin, 16 μM thymidine (HAT Media Supplement, Sigma), and 7 μg/ml ouabain (Sigma) and cultured in 384-well plates for 18 days before screening hybridomas for antibody production by ELISA. After fusion with HMMA2.5 myeloma cells, hybridomas producing ZIKV E-specific antibodies were cloned biologically by single-cell fluorescence-activated cell sorting. Hybridomas were expanded in post-fusion medium (ClonaCell-HY Medium E, STEMCELL Technologies) until 50% confluent in 75-cm^2 flasks (Corning).

For antibody production, cells from one 75-cm^2 flask were collected with a cell scraper and expanded to four 225-cm^2 flasks (Corning) in serum-free medium (Hybridoma-SFM, Life Technologies). After 21 days, supernatants were clarified by centrifugation and filtered using 0.45-μm pore size filter devices. HiTrap Protein G or HiTrap MabSelectSure columns (GE Healthcare Life Sciences) were used to purify antibodies from filtered supernatants.

Sequence analysis of antibody variable region genes. Total cellular RNA was extracted from pelleted cells from hybridoma clones, and an RT-PCR reaction was performed using mixtures of primers designed to amplify all heavy-chain or light-chain antibody variable regions (Nelson *et al.*, 2014). The generated PCR products were purified using AMPure XP magnetic beads (Beckman Coulter) and sequenced directly using an ABI3700 automated DNA sequencer. The variable region sequences of the heavy and light chains were analyzed using the IMGT/V-Quest program (Brochet *et al.*, 2008; Guidicell & Lefranc, 2011).

ELISA and half-maximal effective concentration (EC_{50}) binding analysis. Wells of microtiter plates were coated with purified, recombinant ectodomain of ZIKV E, DIII, DIII LRM (DIII containing A310E and T335K mutations in the lateral ridge of DIII) or DIII of related flaviviruses DENV2 or WNV and incubated at 4°C overnight. In ELISA studies with purified mAbs, the inventors used recombinant ZIKV E protein ectodomain with His_6 tag produced in Sf9 insect cells (Meridian Life Sciences R01635). Plates were blocked with 5% skim milk in PBS-T for 1 hr. B cell culture supernatants or purified antibodies were added to the wells and incubated for 1 hr at ambient temperature. The bound antibodies were detected using goat anti-human IgG (γ-specific) conjugated with alkaline phosphatase (Southern Biotech) and pNPP disodium salt hexahydrate substrate (Sigma). In ELISAs that assessed binding of mAbs to DIII and DIII LRM, the inventors used previously described murine mAbs ZV-2 and ZV-54 (Zhao *et al.*, 2016) as controls. A goat anti-mouse IgG conjugated

with alkaline phosphatase (Southern Biotech) was used for detection of these antibodies. Color development was monitored at 405 nm in a spectrophotometer (Biotek). For determining half-maximal effective concentration binding (EC_{50}), microtiter plates were coated with ZIKV E or E-FLM that eliminated interaction of fusion-loop specific antibodies. Purified antibodies were diluted serially and applied to the plates. Bound antibodies were detected as above. A non-linear regression analysis was performed on the resulting curves using Prism (GraphPad) to calculate EC_{50} values.

ELISA for detection of human antibodies in murine tissues. Fetal head and placental tissues were collected at E13.5 from groups treated with ZIKV-117 or PBS (as a negative control), homogenized in PBS (250 µl) and stored at -20°C. ELISA plates were coated with ZIKV E protein, and thawed, clarified tissue homogenates were applied undiluted in triplicate. Bound antibodies were detected using goat anti-human IgG (Fc-specific) antibody conjugated with alkaline phosphatase. The quantity of antibody was determined by comparison with a standard curve constructed using purified ZIKV-117 in a dilution series.

Biolayer interferometry competition binding assay. His_6-tagged ZIKV E protein was immobilized on anti-His coated biosensor tips (Pall) for 2 min on an Octet Red biosensor instrument. After measuring the baseline signal in kinetics buffer (PBS, 0.01% BSA, and 0.002% Tween 20) for 1 min, biosensor tips were immersed into the wells containing first antibody at a concentration of 10 µg/ml for 7 min. Biosensors then were immersed into wells containing a second mAb at a concentration of 10 µg/ml for 7 min. The signal obtained for binding of the second antibody in the presence of the first antibody was expressed as a percent of the uncompeted binding of the second antibody that was derived independently. The antibodies were considered competing if the presence of first antibody reduced the signal of the second antibody to less than 30% of its maximal binding and non-competing if the signal was greater than 70%. A level of 30 -70% was considered intermediate competition.

Shotgun mutagenesis epitope mapping. Epitope mapping was performed by shotgun mutagenesis essentially as described previously (Davidson & Doranz, 2014. A ZIKV prM/E protein expression construct (strain ZikaSPH2015) was subjected to high-throughput alanine scanning mutagenesis to generate a comprehensive mutation library. Each residue within prM/E was changed to alanine, with alanine codons mutated to serine. In total, 672 ZIKV prM/E mutants were generated (100% coverage), sequence confirmed, and arrayed into 384-well plates. Each ZIKV prM/E mutant was transfected into HEK-293T cells and allowed to express for 22 h. Cells were fixed in 4% (v/v) paraformaldehyde (Electron Microscopy Sciences), and permeabilized with 0.1% (w/v) saponin (Sigma-Aldrich) in PBS

plus calcium and magnesium (PBS++). Cells were incubated with purified mAbs diluted in PBS++, 10% normal goat serum (NGS) (Sigma), and 0.1% saponin. Primary antibody screening concentrations were determined using an independent immunofluorescence titration curve against WT ZIKV prM/E to ensure that signals were within the linear range of detection. Antibodies were detected using 3.75 µg/ml of AlexaFluor488-conjugated secondary antibody (Jackson ImmunoResearch Laboratories) in 10% NGS/0.1% saponin. Cells were washed three times with PBS++/0.1% saponin followed by two washes in PBS. Mean cellular fluorescence was detected using a high-throughput flow cytometer (HTFC, Intellicyt). Antibody reactivity against each mutant prM/E clone was calculated relative to WT prM/E protein reactivity by subtracting the signal from mock-transfected controls and normalizing to the signal from WT 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 antibodies. This counter-screen strategy facilitates the exclusion of prM/E mutants that are locally misfolded or have an expression defect.

Vertebrate animal studies ethics statement. This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocols were approved by the Institutional Animal Care and Use Committee at the Washington University School of Medicine (Assurance number A3381-01). Inoculations were performed under anesthesia induced and maintained with ketamine hydrochloride and xylazine, and all efforts were made to minimize animal suffering.

Virnses and cells. ZIKV strain H/PF/2013 (French Polynesia, 2013) was obtained from X. de Lamballerie (Aix Marseille Université). ZIKV Brazil Paraiba 2015 was provided by S. Whitehead (Bethesda) and originally obtained from P.F.C. Vasconcelos (Instituto Evandro Cargas). ZIKV MR 766 (Uganda, 1947), Malaysia P6740 (1966), and Dakar 41519 (Senegal, 1982) were provided by the World Reference Center or Emerging Viruses and Arboviruses (R. Tesh, University of Texas Medical Branch). Nicaraguan DENV strains (DENV-1 1254-4, DENV-2 172-08, DENV-3 N2845-09, and DENV-4 N703-99) were provided generously by E. Harris (University of California, Berkeley). Virus stocks were propagated in C6/36 *Aedes albopictus* cells (DENV) or Vero cells (ZIKV). ZIKV Dakar 41519 (ZIKV-Dakar) was passaged twice *in vivo* in *Rag1*$^{-/-}$ mice (M. Gorman and M. Diamond, unpublished data) to create a mouse-adapted strain. Virus stocks were titrated by focus-forming assay (FFA) on Vero cells.

Neutralization assays. Serial dilutions of mAbs were incubated with 10^2 FFU of different ZIKV strains (MR 766, Dakar 41519, Malaysia P6740, H/PF/2013, or Brazil Paraiba 2015) for 1 hr at 37 °C. The mAb-virus complexes were added to Vero cell monolayers in 96-well plates for 90 min at 37°C. Subsequently, cells were overlaid with 1% (w/v) methylcellulose in MEM supplemented with 4% heat-inactivated FBS. Plates were fixed 40 h later with 1% PFA in PBS for 1 hr at room temperature. The plates were incubated sequentially with 500 ng/ml mouse anti-ZIKV (ZV-16, E. Fernandez and M. Diamond, unpublished) and HRP-conjugated goat anti-mouse IgG in PBS supplemented with 0.1% (w/v) saponin (Sigma) and 0.1% BSA. ZIKV-infected cell foci were visualized using TrueBlue peroxidase substrate (KPL) and quantitated on an ImmunoSpot 5.0.37 macroanalyzer (Cellular Technologies).

MAb binding to ZIKV- or DENV-infected cells. C6/36 *Aedes albopictus* cells were inoculated with a MOI 0.01 of ZIKV (H/PF/2013) or different DENV serotypes (Nicaraguan strains DENV-1 1254-4, DENV-2 172-08, DENV-3 N2845-09, DENV-4 N703-99). At 120 hr post infection, cells were fixed with 4% PFA diluted in PBS for 20 min at room temperature and permeabilized with HBSS supplemented with 10 mM HEPES, 0.1% saponin and 0.025% NaN_3 for 10 min at room temperature. Fifty-thousand cells were transferred to U-bottom plates and incubated for 30 min at 4°C with 5 µg/ml of anti-ZIKV human mAbs or negative (hCHK-152)[12]; or positive (hE60) (Williams *et al.*, 2013) isotype controls. After washing, cells were incubated with Alexa Fluor 647-conjugated goat anti-human IgG (Invitrogen) at 1:500, fixed in 1% PFA in PBS, processed on MACSQuant Analyzed (Miltenyi Biotec), and analyzed using FlowJo software (Tree Star).

Recombinant antibody expression and purification. Total RNA was extracted from hybridoma cells and genes encoding the VH and VL domains were amplified in RT-PCR using IgExp primers (Thornburg *et al.*, 2016). The PCR products were directly cloned into antibody expression vectors containing the constant domains of WT gamma1 chain, LALA mutant (a leucine (L) to alanine (A) substitution at positions 234 and 235) gamma1 chain for the VH domains, and WT kappa chain for the VL domain in an isothermal amplification reaction (Gibson reaction) (Gibson *et al.*, 2009). Plasmids encoding the heavy and light chain were transfected into 293F cells and full length recombinant IgG was secreted into transfected cell supernatants. Supernatants were collected and IgG purified using Protein G chromatography and eluted into PBS. The functional abrogation of the binding of the LALA variant IgG was confirmed in an ELISA binding assay with recombinant human FcγRI. The

binding of ZIKV-117 WT or LALA antibody to FcγRI was evaluated, in comparison with the binding pattern of control antibodies (human mAb CKV063 (Fong *et al.*, 2014) LALA mutated IgG).

Adult mouse lethal protection experiments. C57BL/6J male mice (4 to 5 week-old, Jackson Laboratories) were inoculated with 10^3 FFU of mouse-adapted ZIKV Dakar by subcutaneous route in the footpad. One-day prior to infection, mice were treated with 2 mg anti-Ifnar1 mAb (MAR1-5A3, Leinco Technologies) by intraperitoneal injection. ZIKV-specific human mAb (ZIKV-117) or an isotype control (hCHK-152) was administered as a single dose at day +1 (100 μg) or day +5 (250 μg) after infection via an intraperitoneal route. Animals were monitored for 21 days.

Pregnant mouse protection experiments. WT C57BL/6J mice were bred in a specific pathogen-free facility at Washington University School of Medicine. WT dams, prophylaxis studies. WT female and male mice were mated; at embryonic days E5.5, dams were treated with a single 250 μg dose of ZIKV mAb or isotype control by intraperitoneal injection as well as a 1 mg injection of anti-Ifnar1 (MAR1-5A3). At E6.5, mice were inoculated with 10^3 FFU of mouse-adapted ZIKV Dakar 41519 by subcutaneous injection in the footpad. At E7.5, dams received a second 1 mg dose of anti-Ifnar1 via an intraperitoneal route. WT dams, therapy. WT female and male mice were mated; at embryonic days E5.5, dams were treated with a 1 mg injection of anti-Ifnar1 (MAR1-5A3). At E6.5, mice were inoculated with mouse-adapted 10^3 FFU of ZIKV Dakar 41519 by subcutaneous injection in the footpad. At E7.5, dams received a second 1 mg dose of anti-Ifnar1 as well as a single 250 μg dose of ZIKV mAb or isotype control via an intraperitoneal route. All animals were sacrificed at E13.5, and placentas, fetuses and maternal tissues were harvested. Fetus size was measured as the crown-rump length x occipito-frontal diameter of the head.

Measurement of viral burden. ZIKV-infected tissues were weighed and homogenized with stainless steel beads in a Bullet Blender instrument (Next Advance) in 200 μL of PBS. Samples were clarified by centrifugation (2,000 x *g* for 10 min). All homogenized tissues from infected animals were stored at -20°C. Tissue samples and serum from ZIKV-infected mice were extracted with RNeasy 96 Kit (tissues) or Viral RNA Mini Kit (serum) (Qiagen). ZIKV RNA levels were determined by TaqMan one-step quantitative reverse transcriptase PCR (qRT-PCR) on an ABI7500 Fast Instrument using published primers and conditions (Lanciotti *et al.*, 2008). Viral burden was expressed on a $\log_{10}$ scale as

viral RNA equivalents per g or ml after comparison with a standard curve produced using serial 10-fold dilutions of ZIKV RNA.

Viral RNA *in situ* hybridization (ISH). RNA ISH was performed with RNAscope 2.5 (Advanced Cell Diagnostics) according to the manufacturer's instructions. PFA-fixed paraffin embedded placental sections were deparaffinized by incubation for 60 min at 60°C. Endogenous peroxidases were quenched with H_2O_2 for 10 min at room temperature. Slides were boiled for 15 min in RNAscope Target Retrieval Reagents and incubated for 30 min in RNAscope Protease Plus before probe hybridization. The probe targeting ZIKV RNA was designed and synthesized by Advanced Cell Diagnostics (catalog no. 467771). Negative (targeting bacterial gene *dapB*) control probes were also obtained from Advanced Cell Diagnostics (catalog no. 310043). Tissues were counterstained with Gill's hematoxylin and visualized with standard bright- field microscopy.

Histology and immunohistochemistry. Harvested placentas were fixed in 10% neutral buffered formalin at room temperature and embedded in paraffin. At least three placentas from different litters with the indicated treatments were sectioned and stained with hematoxylin and eosin to assess morphology. Surface area and thickness of placenta and different layers were measured using Image J software. For immunofluorescence staining on mouse placentas, deparaffinized tissues were blocked in blocking buffer (1% BSA, 0.3% Triton, 1× PBS) for 2 hr and incubated with anti-vimentin antibody (1:500, rabbit, Abcam ab92547). Secondary antibody conjugated with Alexa 488 (1:500 in PBS) was applied for 1 h at room temperature. Samples were counterstained with DAPI (4′6′-diamidino-2-phenilindole, 1:1,000 dilution).

Statistical analysis. All virological data were analyzed with GraphPad Prism software. Kaplan-Meier survival curves were analyzed by the log rank test, and viremia was compared using an ANOVA with a multiple comparisons test. A P value of < 0.05 indicated statistically significant differences.

Example 2 – Results

The inventors sought to isolate neutralizing human mAbs with broad specificity against all ZIKV strains. To do this, they initially tested the serological response of human survivors who had been infected with African or Asian lineage strain ZIKV in diverse geographic locations. Serum from each subject contained antibodies that reacted by ELISA with ZIKV E protein and neutralized infection of a contemporary Asian isolate (H/PF/2013)

from French Polynesia (FIGS. 1A-B). The inventors studied the B cells of Subject 1001 in detail. The frequency of B cells secreting antibodies to ZIKV E protein in the peripheral blood of Subject 1001 was 0.61% (FIG. 1C), when a threshold for detection of binding [absorbance at 405 nm (A_{405})] of 1.5 was used. They also tested the reactivity of antibodies with domain III (DIII) of the E protein from ZIKV, or the related dengue (DENV) or West Nile (WNV) viruses. Most of the ZIKV E reactive antibodies did not bind to DIII, and of those binding to DIII, most were ZIKV-specific (FIG. 1C). In a replicate of the assay performed with another aliquot of cells from the same subject (FIG. 1D), the frequency of ZIKV E-reactive B cell was 0.36%. Comparative binding to a WT ZIKV E or mutant (E-FLM) protein lacking the conserved fusion loop epitope in DII showed immunodominance (binding ~ 70% of mAbs) of the fusion loop.

The inventors obtained 32 stable cloned hybridomas secreting antibodies that bound to ZIKV E protein from the cells of three donors (mAb ZIKV-195 from Subject 1011, mAbs ZIKV-204 and ZIKV-216 from Subject 973, and the remaining 29 mAbs from Subject 1001). All except one mAb belonged to IgG1 isotype, with an equal distribution of light chain isotypes (FIG. 2A). Sanger sequencing of cDNA of the antibody variable gene regions revealed that each mAb represented an independently derived clone. The inventors determined the half maximal effective concentration for binding (EC_{50}) to ZIKV E protein (FIGS. 2A and FIG. 5); most of the mAbs bound to E protein at low concentrations, with EC_{50} values generally below 100 ng/ml. Six of the 32 mAbs exhibited neutralizing activity, with $FRNT_{50}$ values in the range of 0.9 to 420 ng/ml. They next determined how many antigenic sites were recognized by members of the panel using quantitative competition binding to ZIKV E protein. The inventors identified four major competition groups (designated A, B, C or D). Antibodies belonging to the largest group, Group A with 24 members, were directed against the fusion loop in DII as determined from the disparate binding patterns to E, DIII, or to E-FLM (FIG. 5). This group of fusion loop specific mAbs had a single neutralizing clone (ZIKV-88), with moderate potency. Group B mAbs (ZIKV-116 and ZIKV-161) neutralized ZIKV infection and bound to E, DIII, and E-FLM. Group C mAbs (ZIKV-19 and ZIKV-190) bound to E and E-FLM weakly, but did not potently neutralize infection. Group D mAbs ZIKV-195 and ZIKV-216 neutralized with moderate potency and were similar in binding to both E and E-FLM. The most potently inhibitory Group D mAb, ZIKV-117, bound to both E and E-FLM weakly. One antibody (ZIKV-216) competed with members of both Groups C and D and neutralized with moderate potency.

The inventors mapped the epitopes of representative mAbs from each competition group using a complete shotgun mutagenesis library (Davidson & Doranz, 2014) of ZIKV prM/E (Brazil Paraiba 2015 strain) protein variants in which each residue was changed individually to alanine (FIG. 2B and FIGS. 6A-C). Loss-of-binding analysis confirmed that Group A mAbs bound to the fusion loop in DII, whereas Group B mAbs bound DIII. Group B mAb ZIKV-116 bound an epitope involving residue K394 in the lateral ridge of DIII, which was confirmed in an ELISA showing reduced binding to a DIII protein with mutations A310E and T335K in the DIII lateral ridge [DIII-LR] (Zhao *et al.*, 2016). The non-neutralizing clones comprising Group C mAbs bound DII, and the group D neutralizing mAbs bound to a unique epitope in DII not described previously for the closely related DENV (Screaton *et al.*, 2015). The position of the residues affecting binding of ZIKV-117 suggests that on the virion this mAb may bind DII across two distinct dimers (at the "dimer-dimer" interface, FIG. 2C). The inventors were unable to isolate virus neutralization escape mutant viruses for ZIKV-117 despite six passages in cell culture under mAb selection pressure.

Of the 32 mAbs, six (ZIKV-88, ZIKV-116, ZIKV-161, ZIKV-195, ZIKV-216, and ZIKV-117) showed significant (< 1 μg/ml) neutralizing activity *in vitro* against ZIKV French Polynesia strain H/PF/2013. The $FRNT_{50}$ values for the mAbs were as follows: Group A mAb ZIKV-88 (420 ng/ml), Group B mAbs ZIKV-116 (16 ng/ml) and ZIKV-161 (0.9 ng/ml), Group C/D mAb ZIKV-216 (16 ng/ml) and Group D mAbs ZIKV-195 (346 ng/ml) and ZIKV-117 (5 ng/ml). The inventors assessed whether Group B mAb ZIKV-116 and Group D mAb ZIKV-117 could inhibit diverse ZIKV strains encompassing the African, Asian, and American lineages. ZIKV-117 neutralized potently all ZIKV strains tested including two African (MR 766 and Dakar 41519), two Asian (Malaysia P6740 and H/PF/2013), and an American (Brazil Paraiba 2015) strain with $FRNT_{50}$ values of 5 to 25 ng/ml (FIGS. 2D-E). In comparison, ZIKV-116 inhibited four of the five strains efficiently, but lost activity against MR 766, the original African strain (FIGS 2D-E). As recent studies have suggested that cross-reactive ZIKV-specific mAbs can enhance DENV infection *in vivo* (Stettler *et al.*, 2016), the inventors tested whether these two ZIKV neutralizing mAbs could bind to DENV-infected C6/36 cells. ZIKV-117 showed a type-specific pattern of binding as it failed to stain permeabilized cells infected with DENV-1, DENV-2, DENV-3, or DENV-4 or bind to purified WNV E protein (FIG. 7 and data not shown). In comparison, ZIKV-116 bound to cells infected with DENV1, DENV2, or DENV4, but did not bind to DENV2 DIII or WNV DIII in ELISA.

Recently, *in vivo* models of ZIKV pathogenesis and antibody prophylaxis have been reported[8,10,11] in mice deficient in type I IFN signaling. To determine whether ZIKV-117 had therapeutic activity, the inventors treated 4 to 5 week-old WT male C57BL/6 mice at day -1 with anti-Ifnar1 mAb, and then inoculated animals with 10^3 FFU of ZIKV-Dakar, an African strain that is pathogenic in mice. Subsequently, animals were treated with a single dose of ZIKV-117 or isotype control (hCHK-152) (Pal *et al.*, 2013), on day +1 (100 μg; 6.7 mg/kg) or day +5 (250 μg; 16.7 mg/kg). Animals treated with the non-binding isotype control (hCHK-152) developed significant lethality compared to those receiving ZIKV-117 (FIG. 3A), which were protected even when administered only a single dose five days after virus inoculation.

The inventors and others have demonstrated intrauterine growth restriction, placental injury, and fetal demise following ZIKV infection of pregnant mice with deficiencies in type I IFN signaling (Mysorekar *et al.*, 2016; Miner *et al.*, 2016; Yockey *et al., 2016).* To assess the protective ability of ZIKV-117 during fetal development, WT pregnant dams were treated at day -1 (embryo day (E)5.5) with an anti-Ifnar1 mAb. At the same time, these animals were administered vehicle control (PBS), 250 μg isotype control hCHK-152, or 250 μg ZIKV-117 as prophylaxis. One day later, dams were infected subcutaneously with 10^3 FFU of ZIKV-Dakar. Fetuses at E13.5 from anti-Ifnar1 mAb treated dams given PBS or hCHK-152 showed high levels (*e.g.*, ~10^5 to 10^7 FFU equivalents/g) of viral RNA in the placenta and fetal brain (FIG. 3B). In comparison, mice treated with ZIKV-117 had markedly reduced levels of virus in the placenta and fetal brain (*e.g.*, ~10^0 to 10^3 FFU equivalents/g) (FIG. 3B). This phenotype was associated with transport of antibody across the maternal-fetal placental barrier such that levels (816 ± 53 ng/ml for the placenta and 1,675 ± 203 ng/ml for the fetal head) of human ZIKV E-specific IgG were detected (FIG. 8). It should be noted that the levels of neonatal Fc receptor (FcRn) in the placenta of mice are lower than other mammalian species (Kim *et al.*, 2009), thus reduced levels of transport of maternal or exogenous IgG into the fetus is expected (Pentsuk & van der Laan, 2009). Although this factor could underestimate the therapeutic effect of exogenous anti-ZIKV IgG or maternal antibodies, the inventors achieved levels in placenta and fetal head that still were orders of magnitude above the $FRNT_{50}$ value for ZIKV-117. Dams treated with ZIKV-117 also had lower levels of viral RNA in the maternal brain and serum (FIG. 3C).

Antibody-dependent enhancement (ADE) of infection of the closely related DENV is due to cross-reactive antibodies that fail to neutralize heterologous serotype infection and instead facilitate uptake and infection of FcγR-expressing myeloid cells (Morens, 1994).

Because flavivirus antibodies can promote ADE in cell culture (Dejnirattisai *et al.*, 2016; Charles and Christofferson, 2016) with unknown consequences *in vivo*, the inventors evaluated the protective efficacy of a recombinant form of ZIKV-117 IgG containing a leucine (L) to alanine (A) substitution at positions 234 and 235 (LALA) (Hessell *et al.*, 2007), which lacked efficient binding to FcγR, retained interactions with FcRn (Hessell *et al.*, 2007), and neutralized ZIKV *in vitro* equivalently compared to the parent mAb (FIGS. 9A-B). The LALA variant of ZIKV-117 showed similar protective activity against infection of the placenta and fetus relative to the parent mAb (FIG. 3D). As the majority of the protection conferred by ZIKV-117 in the pregnancy model likely is due to neutralization and not Fc effector functions, LALA variants could be used without a loss in potency or risk of ADE from a future infection with a heterologous flavivirus such as DENV.

The inventors next assessed the post-exposure efficacy of ZIKV-117 during pregnancy. Mice treated with anti-Ifnar1 mAb at E5.5 were infected with 10^3 FFU of ZIKV-Dakar at E6.5 and then given a single dose of PBS, 250 μg of hCHK-152, or 250 μg of ZIKV-117 at E7.5. Compared to PBS or isotype control mAb treatment, administration of ZIKV-117 resulted in markedly reduced viral burden in the dams, the placenta, and fetus when measured at E13.5 (FIGS. 3E-F).

The inventors also evaluated the consequences of ZIKV-117 administration on pathology in the placenta and fetus. The reduction in viral load mediated by ZIKV-117 was associated with decreased destruction of the placenta (as judged by labyrinth layer and overall placenta area), less trophoblast cell death, and increased body size of the fetus (FIGS. 4A-C) compared to fetuses of PBS- or hCHK-152-treated dams. When administered as prophylaxis, ZIKV-117 fully protected against ZIKV-induced placental insufficiency and intrauterine growth restriction, as the placental area and fetal size from infected dams treated with anti-ZIKV mAb were similar to that of uninfected placentas[14]. *In situ* hybridization revealed an almost complete absence of viral RNA in the junctional zone and decidua of the placenta in animals treated with ZIKV-117 compared to staining observed in PBS or hCHK-152-treated controls (FIG. 4D). The inventors also observed vascular damage associated with ZIKV infection of the placenta (Miner *et al.*, 2016), characterized as diminished vimentin staining of fetal endothelial cells, which was rescued by ZIKV-117 to levels similar to those in uninfected placentas (FIG. 4E). The histopathological data suggests that ZIKV-117 treatment can reduce the ability of ZIKV to cross the fetal endothelial cell barrier, and thereby prevent vertical transmission and improve placental health and fetal outcome.

TABLE 1 – NUCLEOTIDE SEQUENCES FOR ANTIBODY VARIABLE REGIONS

Clone	Variable Sequence Region	SEQ ID NO:
ZIKV-2 heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCCTCAGTACTTTTGCTATGCACT GGGTCCGCCAGGCTCCAGGCAAGGGGCTGCAGTGGGTGGCTGTTACATCATA TGATGGAAGCAGTAAATTCTACGCAGACTCCGTGGAGGGCCGATTCACCATCT CCAGAGACACGTCCAAGAACACGTTGTATCTGCAAATGACCAGCCTGACAGCT GAGGACACGGCTGTGTATTTCTGTGCGAGAGGCTTCGGCGGTAGTGGTGATT ACTACGTAGGGGGATTTGATATCTGGGGCCAAGGGACCCTGGTCACCGTCTCC TCA	1
ZIKV-2 light	CAGTCTGCCCTGACTCAGCCTCGCTCAGTGTCCGGGTCTCCTGGACAGTCAGTC ACCATCTCCTGCACTGGAACCAGCAGTGATGTTGGTGGTTATGACTATGTCTCC TGGTACCAACAGCACCCAACCGAAGCCCCCAAACTCATCATTCATGATGTCAAT AAGCGGCCCTCAGGGGTCCCTGATCGCTTCTCTGGCTCCAAGTCTGGCAACAC GGCCTCCCTGACCATCTCTGGGCTCCAGGCTGAAGATGAGGCTGATTATTACT GCTGCTCTTTTGCAGGCAGCCACAGTTTTGTTTTATTCGGCGGAGGGACCAGG CTGACCGTCCTA	2
ZIKV-8 heavy	CAGGTGCAGCTGGTGGAGTCCGGGGGACGCGTGGTCCAGCCTGGGAGGTCCC TGAGGCTCTCCTGTGCGGCCTCTGGATTCACCTTCAGTAATTATGCCATGCACT GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCTGTTATCTCATA CGATGGAAGTTATACACATTACACAGAGGCCGTGAGGGACCGATTCACCATCT CCAGAGACAATTCCAAGAGGACGGTGTGGCTGGAAATGAACAGTCTGAGAGT CGACGACACGGCTATGTATTACTGTGCGAGAGATGCGCTTGGATACTATGATA ATTCTGATTATACTTCTTGGGGCCTGGGGACCCTGGTCACCGTCTCCTCA	3
ZIKV-8 light	GAAATTGTGTTGACGCAGTCTCCAGACACCCTGTCTTTGGCTCCAGGGGAAAG AGCCACCCTCTCGTGCAGGGCCGGGCAGACTATTACCAGCAGCCACTTAGCCT GGTACCGGCTAAAACCCGGCCAGGCTCCCAGACTCATCATCTATGATGCATCC AGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAC AGTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTATATTACT GTCAGCAGTATGCTACCCCACCGTGGACGTTCGGCCAAGGGACCAAGGTGGA GATCAAA	4
ZIKV-12 heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCC CTGAGACTCTCCTGTGTAGCCTCTGGATTCACCTTCAGCGATTATGCTATGCAC TGGGTCCGCCAGGCTCCAGGGAAGGGACTGGAATATGTTTCAACCATTAATAG TAATGGGGGTAGCACATTTTATGCGGACTCTGTGCAGGACAGATTCACCATCT CCAGAGACAATTCCAAGAACACGCTTTATCTTCAAATGGACAGCCTGAGACCT GAGGACATGGCTGTCTATTATTGTGTGAGTCTGTACAACTATCCAGTCTTAGAC TACTGGGGCCTGGGAACCCTGGTCACCGTCTCCTCA	5

ZIKV-12 light	CAGGCTGTGGTGACTCAGGAGACCTCACTGACTGTGTCCCCAGGAGGGACAG TCACTCTCACCTGTGGCTCCAGCACTGGAGCTGTCACCAGTGGTCATTATCCCT ACTGGTTCCAGCAGAAGCCTGGCCAAGCGCCCAGGACACTGATTTATCATACA AACAACAAACACTCCTGGACACCTGCCCGGTTCTCAGGCTCCCTCCTTGGGGG CAAAGCTGCCCTGACCCTTTCGGGTGCGCACCCCGACGATGAAGCTGAATACT ACTGTTTGATCTTGTATCCTGATGCTCGCGTCTTTGGCGGAGGGACCAAGCTG ACCGTCCTA	6
ZIKV-15 heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGGGGTCC CTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGCACCCATGACATGCAC TGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCACTTATACGGT TTGGTGGCAAAGATATATACTATGCAGACTCCGTGGAGGGCCGATTCACCGTC TCCAGAGACAATTCCATGAACACGCTCTATCTGCAACTGAGCGGCCTGAGAGC TGATGACACGGCTCTGTACTACTGTGCGAAAGGCGCCCGATTCTATGATTCTAA TGGTTTCCCCGTTTACGCTGAATACTTCGAACACTGGGGCCAGGGCACCCTGG TCACCGTCTCCTCA	7
ZIKV-15 light	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCGAGTCAGGACATCAGCAATTTTTTAGCCTGGTAT CAGCAGAGACCAGGGAAAGTTCCTAAACTCTTGATCTATGCTGCATCCACCTTG CAATCTGGGGTCCCATCTCGGTTCAGTGGCAGTGGGTCTGGGACAGATTTCAC TCTCACCATCAGCAGCGTGCAGCCTGAAGATGTTGCAACTTATTTCTGTCAAAA GTATAACAATGCCCCGCTCACATTCGGCGGAGGGACCAAGGTAGAGATCAAA	8
ZIKV-19 heavy	CAGGTGCAGCTGGTGCAGTCAGGGCCTGAGGTGAAGAAGCCGGGGTCCTCG GTGAAGGTCTCCTGCAAGGCTTCTGGAGTCAGCTTCAACACCTATGAGATCAG CTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAAGGATCATC CCTATCTTTGCTACACCAACCTACGCACTGAAGTTCCAGGGCAGAGTCACGATT ACCACGGACGAATCCACGACCACAGGTTACATGGAGCTGAGCAGCCTGAGAT CTGAGGACACGGCCGTGTATTACTGTGCGGGAAGGCCCTATGGTCCGGGGAG TTGGTTGCCCCTGGACGTCTGGGGCCAAGGGACCCTGGTCACCGTCTCCTCA	9
ZIKV-19 light	GACATTGTGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCGAGTCAGGGCATTAGCTCTTATTTGGCCTGGTAT CAGCAAAAACCAGGGAAACTTCCTAAGCTCCTGATCTATGCTGCATCCACTTTG CAATCAGGGGTCCCATCTCGGTTCAGCGGCAGTGGATCTGGGACAGATTTCAC TCTCACCATCAGCAGCCTGCAGCCTGAAGACGTTGCAGCTTATTACTGTCAAAA GTATGACAGTGCCCCATTCACTTTCGGCCCTGGGACCAAAGTGGATCTCAAA	10
ZIKV-27 heavy	CAGGTGCAGCTGGTGCAGTCTGGACCTGAGGTGAAGAAACCTGGGGCCTCAG TGAAGGTCTCCTGCAAGGCTTCTGGTTTCACCTCTATGAATTATGGTATCAGCT GGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGATAATCGC TTACAATGGAAACACAAACTATGCACAGAAGTTCCAGGGCAGAGTCTCCATGA CCATAGACACATCCACGACCACTGCCTACATGGAACTGAGGAGCCTGAGATCT GACGACACGGCCGTATATTACTGTGCGAGCCGAATAGAAGTGGCTGATACGG TCTACGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA	11

ZIKV-27 light	GAAATTGTGTTGACTCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAG AGCCACCCTCTCGTGCAGGGCCAGTCAGACTACTAGCAGCAGCTTCTTAGCCT GGTACCAGCAGAAGCCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCC AACAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAG ACTTCACTCTCACTATCAGCAAACTGGAGCCTGAAGATTTTGCGGTCTATTACT GTCAGCAGTATGACAGCTCACCTCCGGGATTCACTTTCGGCCCTGGGACCAAA GTGGATATCAAA	12
ZIKV-33 heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGCTGTGGTCCAGCCTGGGAGGTCCC TGAGACTCTCCTGTGCAGCCTCTGGATTAAGTTTCAGTGACTATGCTATCCACT GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTAATTGCACA TGATGGAAGGAATAAATATTATGCCGACTCCGTGATGGGCCGAGTCGCCATCT CCGGAGACAATTCCAAGAACACGGTGTATCTGCAAATGAGCAGCCTGAGAGC TGAAGACACGGCCACTTATTACTGTGCGAGAGGGTTTTACCATGATAAAACTG GTTCCTACTGGTACTTCGATCTCTGGGGCCGTGGCACCCTGGTCACCGTCTCCT CA	13
ZIKV-33 light	CAGTCTGTGTTGACTCAGCCGCCCTCAGTGTTTGCGGCCGCAGGACAGAGGGT CACCATCTCCTGCTCTGGAAGCAGCTCCAACATTGGGGATAATTATGTATCCTG GTACCAGCAGTTCCCAGGAACAGCCCCCAAAATCCTCATTTACGAGAATGATA AGCGAGCCTCAGGGATTCCTGACCGATTCTCTGGCTCCAAGTCTGGCACGTCA GCCACCCTGGGCATCACCGGACTCCGGACTGGGGACGAGGCCGATTATTTCTG CGGAACATGGGATAGCAGCCTGACTACAGCGGTTTTCGGCGGAGGGACCAAG TTGACCGTCCTA	14
ZIKV-46 heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTAAAGCCTGGGGGGTCG CTTAGACTCTCCTGTGCAACCTCCGGATTCAGTGTCACTAACGCCTGGATGAGC TGGGTCCGCCAGGCTCCAGGGAGGGGGCTGGAGTGGGTTGGCCGTATTAAAA ACAAAGCTGATGATTGGACAACAGACTACGCTGCACCCGTGAGAGGCAGATT CACCATCTCAAGAGATGATTCTAAAGACACCGTGTATCTGCAAATGAACAGCC TGAAAAGCGAGGACACAGCCCTTTATTACTGTAGTACTTATTATTATGATAGTA GTGGTCATTTTGTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA	15
ZIKV-46 light	GAAATTGTGTTGACTCAGTCTCCAGCCACCCTGTCTTTGTCTGCAGGGGACAGA GCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCATCTACTTACTTTGGTAC CAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAAGAG GGCCACTGGCATCCCAGCCAGGTTCAGTGGCAGTGGGTCGGGGACAGACTTC ACTCTCACCATCACCAGCCTAGAGCCTGAAGATTTTGCAGTTTATTACTGTCTTC AGCGTGGCATCTGGCCATCGTTCGGCCAAGGGACCAAGGTGGAAATCAAA	16

ZIKV-47 heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCACTTCCTATGCTTTTCACT GGGTCCGCCAGGCTCCAGGCAAGGGCCTGGAGTGGGTGGCAGTTATTTCATA TGATGGAAGCCAAAAATTCTACGCAGACTCTGTGATGGACCGCTTCACCATCTC CAGAGACAGTTCCAAGAACACGCAGTATCTACAAATGGACAGCCTGAGACCTG AGGACACGGCTGTGTATTACTGTGCGACCAAGGGGCAGTCCCAGATTCCTGTT ACCGCTGAATACTTCGAACATTGGGGCCGGGGCACCCTGGTCACCGTCTCCTC A	17
ZIKV-47 light	CAGTCTGTGCTGACGCAGCCGCCCTCAGTGTCTGGGGCCCCAGGGCAGAGGG TCACCATCTCCTGCACTGGCAGCAGCTCCAACATCGGGGCAGGTTATGATGTG CACTGGTACCAGCAGCTTCCAGGAACAGCCCCCAAACTCGTCATCTTTGCTAAC AGCAATCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGACAC CTCAGCCTCCCTGGCCATCACTGGGCTCCAGGCTGAGGATGAGGCTGATTATT ACTGCCAGTCCTATGACAGCAGCCTGAGTCGTTATGTGGTATTCGGCGGAGGG ACCCAGGTGACCGTCCTA	18
ZIKV-48 heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCGATGCTATGCAC TGGGTCCGCCAGGTTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTACATCAT ATGATGGAAGTAATAAATACTACGCAGACTCCGTGGAGGGCCGATTCACCATC TCCAGGGACAATTCCAAGAACACGCTGTTTCTTGAAATGACCAGCCTGAGAGT TGAGGACTCGGCTATATATTACTGTGCGAGAGGGTTTACGGTGATCCATGCTT TTGATATCTGGGGCCTAGGGACCCTGGTCACCGTCTCCTCA	19
ZIKV-48 light	CAGTCTGTGCTGACTCAGCCTCGCTCAGTGTCCGGGTCTCTTGGACAGTCAGTC TCCATCTCCTGCACTGGAACCAGCAGTGATGTTGGGGGATATAACTATGTCTCC TGGTACCTACAACACCCAGGCAAAGCCCCCAAACTCATCATTTATGATGTCAGT AAGCGGCCCTCAGGAGTCCCTAGTCGCTTCTCTGGCTCCAAGTCTGGCAACAC GGCCTCCCTGACCATCTCTGGGCTCCAGGCTGAGGATGAGGCTGATTATTCCT GCTCCTCATATGCAGGCACCTTTGTGGTCTTCGGCGGAGGGACCAAGCTGACC GTCCTA	20
ZIKV-49 heavy	CAGGTGCAGCTGGTGGAGTCGGGCCCAGGACTGCTGAAGCCTTCGGAGACCC TGTCCCTCACCTGCGCTGTCTCTGGTGGCTCCATCAACAGTAGTAGTTTCCACT GGGGCTGGATCCGCCAGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGGCTA TCTATTATACTGGGAGCACCTACTACAACCCGTCCCTCAAGAGTCCAGTCACCG TTTCAGTGGACACGTCCAAGAACCAGTTCTCCCTGGAGCTGAGCTCTGTGACC GCCGCGGACACGGCCGTCTATTTCTGTGCGAGAGTGGTTGCTACAGTTACTAC GAGACGGGGGCTGGGGTCTTTTGATATCTGGGGCCAAGGGACCCTGGTCACC GTCTCCTCA	21

ZIKV-49 light	GACATCGTGATGACCCAGTCTCCATCTTCCGTGTCGGCATTTGTAGGAGACAG AGTCACCATCACTTGTCGGGCGAGTCAGGGTATTAGCAACTGGTTAGCCTGGT ATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATTTATGCTGCATCCAGT TTGCAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGATTT CACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTACTATTGTCA ACAGGCTAACAGTTTCCCGTGGACGTTCGGCCAAGGGACCAAGGTGGAAATC AAA	22
ZIKV-50 heavy	GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGAGGGTCC CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTGACCCCTACATGGAC TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTGGCCGTGTTCGAA ACAAACCTAACAGTTACACCACAGAATACGCCGCGTCGGTGACAGGCAGGTTC ACCATCTCAAGAGATGATTTAAAGAACTCAGTGTATCTGCAAATGAACAGCCT GAAAACCGAGGACACGGCCGTGTATTTTTGTGTTAGAGTGGCCCTTCCAAAGG CTTTTGATGTCTGGGGCCAAGGGACCCTGGTCACCGTCTCCTCA	23
ZIKV-50 light	GACATCCAGATGACCCAGTCTCCACCCTCCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCAGGCGAGTCAGGACATTAGTATCTATTTAAATTGGTTT CAGCACAAACCAGGGAAAGCCCCTAAGCTCCTGATCTACGATGCTTCCAATTT GGAAACAGGGGTCCCATCAAGGTTCAGTGGAAGTGGATCTGGGACAGACTTT TCTTTCACCATCAGCGGCCTGCAGCCTGAGGACGTTGCATCATATTACTGTCTA CAGTATGATAATCCCCCCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAA	24
ZIKV-55 heavy	CAGGTGCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAG TGAAGGTCTCCTGTAAGGCCTCTGGTTACACCTTTACCAGTTTTGGTATCAGCT GGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATGGAATAGCG CTTGGATCAGTGCACACAATGGCAACGCAGTCTATGGAAAGAAGTTCCAGGG CAGAGTCGCCATGACCATAGACACGTCCACGAGCACAGCCTACTTGGACGTGA GGAGCCTGAGATCTGACGACACGGCCGTCTATTACTGTGCGAGAGTCGGAGG ATGGCAACAGATTCCCTACTTTGACTTCTGGGGCCAGGGAACCCTGGTCACCG TCTCCTCA	25
ZIKV-55 light	GACATTGTGATGACCCAGTCTCCATCCTTCCTGTTTGCTTCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCCAGTGAGGGCCCTGACAGTTATTTAGCCTGGTA TCAGCAAAAGCCAGGGAAAGCCCCTAACCTCCTGATCTATGCTGCTTCCACTTT GCAAAGTGGGGTCCCATCACGGTTCAGCGGCAGTGGATCTGGGACAGAATTC ACTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAA CACCTTAATGGTTACCCTTCGTTCGGCCAAGGGACACGACTGGAAATTAAA	26
ZIKV-70 heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGGGGTCC CTGAGACTCTCCTGTGTAGGCTCTGGACTCACCCTCAGTTCCTATGCTATGCAC TGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATCTCAT CTGATGGAAGCAATCGATACTACGCGGACTCCGTGGAGGACCGATTCACCATC TCTAGAGACAATTCCAAGAACATACTGTACCTACAAATGAACACCCTGAGACCT GACGACACGGCTTTTTATTACTGTGCGAGAGGTTACTACTTTGATGATAGTGGT TCTTACTGGTACTTCGATCTCTGGGGCCGTGGCACCCTGGTCACCGTCTCCTCA	27

ZIKV-70 light	CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGT CACCATCTCCTGCTCTGGAAGCAGCTCCAACATTGGGAATAATTATGTATCCTG GTACCAGCAACTCCCAGGAGCAGCCCCCAGAGTCCTCATTTATGAGGATAGTA AGCGACCCTCAGGGATTCCTGACCGATTCTCTGGCTCCAAGTCTGGCACGTCA GCCACCCTGGCCATCACCGGACTCCAGACTGGGGACGAGGCCGATTATTACTG CGCAACATGGGATGGCGGGCTGAGTGTTATTTTCGGCGGAGGGACCCAGGTG ACCGTCCTA	28
ZIKV-71 heavy	CAGGTTCAGCTGGTGCAGTCTGGGGGCGAGGTGAAGAAGCCTGGGGCCTCAG TGAAGGTCTCCTGCAAGGCTTCTGGTTACAGCTTCATTAACTATGGAATCAGTT GGGTGCGGCAGGCCCCTGGACAAGGGCTTGAGTGGATGGGATATATTATCCC TTACAATGGGGACACGAGCTATGCACAGCAATTCCAGGGCAGAGTCACCATG GCCGCAGACACATCCGCGACAACAGTTTTCATGGAAGTGGGGAGCCTGAGAT TAGACGACACGGCCGTATACTACTGTGCGAGAGCAATAGTGGGGGAAACTGT GACAGGCTATGTCTATGGTATGGACGTCTGGGGCCAAGGGACCCTGGTCACC GTCTCCTCA	29
ZIKV-71 light	GACATCCAGTTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCCAGTCAGGGCATTGACATTTTTTTGGCCTGGTAT CAGCAAAAGCCAGGGAAAGCCCCTAACCTCCTGATCTATTCTGCATCCACTTTG CAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACATTTTTCAC TCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAATA TCTTAATACTTCCCCATGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA	30
ZIKV-78 heavy	GACATCCAGTTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCCAGTCAGGGCATTGACATTTTTTTGGCCTGGTAT CAGCAAAAGCCAGGGAAAGCCCCTAACCTCCTGATCTATTCTGCATCCACTTTG CAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACATTTTTCAC TCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAATA TCTTAATACTTCCCCATGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA	31
ZIKV-78 light	CAGGCTGTGGTGACTCAGGAGCCCTCACTGACCGTGTCCCCAGGAGGGACAG TCACTTTCACCTGTGCCTCCAGCACTGGAGCTGTCACCAGTGGTCATTATCCCT ACTGGTTCCAGCAGAAACCTGGCCAAGCCCCCAGGACACTGATTTATCATTCTT CCAAGAAACACTCCTGGACTCCTGACCGGTTCTCAGGCTCCCTCCTTGGGGGC AAAGCTGCCCTGACGCTTTCGGGGGCGCAGCCTGAAGATGAGGCTGAGTATT ACTGCTTACTCTCTTATAGTGGTGGTCGGCCGGTGTTCGGCGGAGGGACCCAG GTGACCGTCCTA	32

ZIKV-81 heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC CTGAGACTCTCCTGTGCAGCCTCTGGATTCATCTTCAGTAACTTTGCTATGCACT GGGTCCGCCAGGCCCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATTTCATA TGATGGAAGCAATACATACTATTCAGACTCCGTGGAGGGCCGATTCACCATCT CCAGAGACAATTCCAAGAACATGCTGTTCTTGGAAGTGAACACCCTGAGAACT GAGGACACTGCTGTATATTACTGTGCGATCGGAGGGGGGCCCCCCGATTTTTT GGCCGCGCCTTTCAACGCTGAAGTCTTGCAGCACTGGGGCCAGGGCACCCTGG TCACCGTCTCCTCA	33
ZIKV-81 light	CAGTCTGTGCTGACGCAGCCGCCCTCAGTCTCTGGGGCCCCAGGGCAGAGGG TCACCATCTCCTGCACTGGGAGCAGTTCCAACATCGGGGCCGGTTATGATATA CATTGGTACCAGCAGCTTCCAGGAACAGCCCCCAAACTCCTCATCTATGGTAAC ACCAACCGGCCCTCAGGGGTCCCGGACCGATTCTCTGGCTCCAAGTCTGGCAC CTCAGGCTCCCTGGCCATCACTGGGCTCCAGGCTGAGGATGAGGCTGATTATT TCTGCCAGTCGTATGACACCGGCCTGAGTGTGGTATTCGGCGGAGGGACCCA GGTGACCGTCCTA	34
ZIKV-82 heavy	Not determined	35
ZIKV-82 light	GACATCCAGATGACCCAGTCTCCATCTTCCGTGTCTGCGTCTGTGGGGGACAC AGTCACCATCACTTGTCGGGCGAGTCAGGATATCACTTACGTGTTAACCTGGTA TCAGCAGAAACCAGGGAAAGCCCCTAAACTCCTGATCTATGCTGCAGCCAATT TGGCAAGTGGGGTCCCGTCAAGGTTCAGCGGCAGTGGATCTGGGACACATTT CACTCTCACCATCCGCAGCCTGCAGCCTGAAGATTTTGCAACTTACTATTGTCA ACAGGCTAACAGTTTCCCCTGGACGTTCGGCCAAGGGACCAAGGTGGACATCA AA	36
ZIKV-86 heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTGATTATGCCATGCAC TGGGTCCGCCAGGCTCCAGGCAAGGGACTGGAGTGGGTCGCAGTTATATCAT ATGATATAAATACAAAATATTATGCAGAGTCCGTGGAGGGGCGATTTTCCATC TCCAGAGACGATTCCATAAACACCGTTTATCTACAAATGAACAGCCTGAGACCT GACGACACGGCTGTCTATTTCTGTGCGAGAGATGTCTATGGCGGGGGGGTTCC CTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCC	37
ZIKV-86 light	GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCCAGTCAGAGTGTTAGTGACTGGTTGGCCTGGTA TCAGCAGAAACCAGGGAAAGCCCCTAAACTCCTGATCTATAAGGCGTCTACTT TAGAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGAGTT CACTCTCACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTATTACTGCCA CCAGTATAGTTTTTATTGGACGTTCGGCCAAGGGACCAAGGTGGATATCAAA	38

ZIKV-88 heavy	CAGGTGCAGCTGCTTGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCC TGAGACTCTCCTGTGTAGCCTCTGGACTCACCTTCAGTACCTCTGCCATGCACT GGGTCCGCCAGGCTCCAGGCAAGGGACTGGAGTGGGTGGCAGTTATCTCATA TGATGGGAGCTATAAATTCTACGCAGATTCCGTGAGGGACCAATTCACCATCT CCAGAGACAATTCCAAGACCACGCTGTATTTGCAAATGGACGGCCTGACACCT GAGGACACGGCTGTATATTACTGTGCGAGAGGTTACAACGACGACAGTAGTG GGTCTTACTGGTATTTCGATCTCTGGGGCCGTGGCACCCTGGTCACCGTCTCCT CA	39
ZIKV-88 light	CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGT CACCATGTCCTGCTCTGGAAGCAGCTCCAACATTGGGAGTAATTTCGTTTCCTG GTACCAGCAACTCCCAGGAACAGCCCCCAAGGTCCTCATTTTTGACAATAATCA GCGACCCTCAGGGATTCCTGACCGATTCTCCGGCTCCAAGTCTGGCACGTCAG CCACCCTGGCCATCACCGGACTCCAGCCTGGGGACGAGGCCGTTTATCATTGC GGAACATGGGATAGCAGCCTGACCTTCGCGGTCTTCGGCGGAGGGACCAAGC TGACCGTCCTA	40
ZIKV-116 heavy	GAGGTGAAGCTGGTGGAGTCTGGGAGAGGCCTAGTTCGGCCTGGGGGGTCC CTGAGACTCTCTTGTGCAGCCTCTGGATTCACCTTTAGCAACTATGCCATGAGC TGGGTCCGCCAGGGTCCAGGGATGGGACTGGAGTGGGTCTCAACGATCACTG CCGATAGTGATAGCAAATATTACGTGGACTCTGTGAAGGGCCGGTTCACCATC TCCAGAGACAATTCCAAGGACACATTATTTCTACACATGACCAGCCTGAGAGC CGAAGACACGGCCGTTTACTACTGTGCGAAAGATCGCCTCTCTCGGGGGGTCG GGGAGTTATATGACTCGTGGGGCCAGGGAACCCTGGTCACCGTCTCTTCA	41
ZIKV-116 light	GACATACAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCCAGCCAGAGTATTGATGTCTGGTTGGCCTGGTA TCAGCAGAAGCCAGGGAAAGCCCCTAAACTCCTGATGTATAAGACGTCTACTT TACAAACTGGGGTCCCATCAAGATTCAGCGGCAGTGGATCTGGGACAGAATTC ACTCTCACCATCAGCAGCCTGCAGACTGATGATTTTGCAACTTATTACTGCCAA AAGTACGATAGTTATCCGTGGACGTTCGGCCCAGGGACCAAGGTGGAAATCA AA	42
ZIKV-117 heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCGGCCTGGGGGGTCC CTCAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAAAAACTATGGCATCCAC TGGGTCCGCCAGGCTCCAGGCAAGGGGCCGGAGTGGGTGGCATTTGTACGGT ATGATGGAAATAACAAGTACTATGCAGACTCCGTGAAGGGCCGATTCACCATC TCCAGAGACAATGCCAAGAACACGCTGTCTCTGCAAATGAACAGCCTGAGAGT TGAAGACACGGCTGTCTATTTCTGTGCGAGGGATCCTGAAACTTTCGGGGGGT TTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA	43

ZIKV-117 light	GAAACAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAG AGGCACCCTCTCCTGCAGGGCCAGTGAGAGTGTTAGCAGCAACTTGGCCTGGT ACCAGCAGAAACCTGGCAAGGCTCCCCGGCTCCTCATCTATGGTGCATCCACC AGGGCCACTGGTATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGAGT TCACTCTCACCATCAGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCA GCAGTATTATTACTCGCCTCGAACGTTCGGCCAAGGGACCAAGGTGGAAGTCA AA	44
ZIKV-146 heavy	GAGGTGCAGCTGGTGGAGTCCGGGGGAGGCTTGGTCCAGCCTGGGGGGTCC CTGAGACTCTCCTGTGTCGCCTCTGGATTTACGTTCAGTGATTATGCTATGCAC TGGGTCCGCCAGGCTCCAGGGAAGGGACTGGAATATGTTTCAACCATTAATAG TAATGGGGGTAACACTTTTTATGCGAACTCTGTGGAGGACAGATTCACCATCTC CAGAGACAATTCCAAGAGCACGCTTTATCTTCAACTGGACAGCCTGAGACTTG AGGACACGGCTGTCTATTATTGTGTGAGTCTGTGGAACTATCCAGTCTTAGACT ACTGGGGCCTGGGAACCCTGGTCACCGTCTCCTCA	45
ZIKV-146 light	CAGGCTGTGGTGACTCAGGAGCCCTCACTGACTGTGTCCCCAGGAGGGACAG TCACTCTCACCTGTGGCTCCAGCACTGGAGCTGTCACCAGTGGTCATTATCCCT ACTGGTTCCAGCAGAAGCCTGGCCAAGCGCCCAGGACACTGATTTATCATACA AACAGCAAACACTCCTGGACACCTGCCCGGTTCTCAGGCTCCCTCCTGGGGGG CAAAGCTGCCCTGACCCTTTCGGGTGCGCAGCCCGAGGATGAGGCTGAATATT ACTGCTTGCTCTTGTATCCTGATGCTCGGGTATTCGGCGGAGGGACCAGGCTG ACCGTCCTA	46
ZIKV-158 heavy	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC CTGCGACTCTCCTGTACTTCCTCTGGGTTCACCTTCAATACCTATCCTATGCACT GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGCTGGCAACTATCTCATA TGTTGAAACTGATAAGTACTACACAGACTCCGTGCAGGGCCGATTCACCGTCT CCAGAGACAACTCGAAGAACACGCTTTATCTGCAAATGAACAGCCTGAGCGTT GAGGACACGGCTGTCTATTACTGTGCGAGAGGGTGGGCGGTGACTACGTCCC ATGCTTTTGATGTTTGGGGCCAAGGGACCCTGGTCACCGTCTCCTCA	47
ZIKV-158 light	CAGTCTGCCCTGACTCAGCCTCGCTCAGTGTCCGGGTCTCCTGGACAGTCAGTC ACCATCTCCTGCACTGGAACCAGCAGTGATGTTGGTGGTTATGACTTTGTCTCC TGGTACCAAGAGCACCCAGGCAAAGCCCCCAAACTCATGATTTATGATGTCAC TAAGAGGCCCTCAGGGGTCCCTGATCGCTTCTCTGGCTCCAAGTCTGGCAACA CGGCCTCCCTGACCATCTCTGGGCTCCAGGCTGAGGATGAGGCTGATTATTATT GTTGCTCATATGCAGGCGGCTACACTTTCGTGGTCTTCGGCGGAGGGACCCAG GTGACCGTCCTA	48

ZIKV-165 heavy CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGGGGTCC CTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCACTAACTATGGCATGCAC TGGGTCCGCCAGGCTCCAGGCAAGGGCCTGGAGTGGGTGGCGTTTATACGGC CTGATGGAAATGATAAATACTATGCAGACTCCGTAAAGGGCCGATTCACCATC TCCAGAGACAATTCCAAGAACTCGCTCTATCTGCAAATGAACAGCCTGGGAGC TGAGGACACAGCTGTATATTATTGTGCGAAAGACTACTATCATACTACTGATGA TTATTGGGCTGAATTCTTCCAGCACTGGGGCCAGGGCACCCTGGTCACCGTCTC CTCA 49

ZIKV-165 light GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGA GTCACCATCACTTGCCGGGCCAGTCAGAGTATTAATAACTGGTTGGCCTGGTA TCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTAATCTATATGGCGTCCAACT TAGAAAGTGGGGTCCCATCACGGTTCAGCGGCAGTGGATCTGGGACAGAATT CACTCTCACCATCAGTAGCCTGCAGCCTGATGATTTTGCAAGTTATTACTGCCA ACACTATAATTTTTACCCCGGGTTCGGCCAAGGGACCAAGGTGGAAATCAAA 50

ZIKV-190 heavy GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGATACAGCCTGGGGGGTCC CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGC TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTG GTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATC TCCAGAGACAATTCCAAGAACACACTGTCTCTGCAAATGAACAGCCTGAGAGC CGAGGACACGGCCGTATATTACTGTGCGAGAGCCTTCTATTACGATTTTTGGAC CTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA 51

ZIKV-190 light CAGTCTGCCCTGACTCAGCCTCGCTCAGTGTCCGGGTCTCCTGGACAGTCAGTC ACCATCTCCTGCACTGGAACCAGCAGTGATGTTGGTGGTTATAACTATGTCTCC TGGTACCAACAGCACCCAGGCAAAGCCCCCAAACTCATGATTTATGCTGTCACT AAGCGGCCCTCAGGGGTCCCTGATCGCTTCTCTGGCTCCAAGTCTGGCAACAC GGCCTCCCTGACCATCTCTGGGCTCCAGGCTGAGGATGAGGCTGATTATTACT GCTGCTCATATGCAGGCAGCTACACTTATGTGGTATTCGGCGGAGGGACCAAG CTGACCGTCCTA 52

ZIKV-195 heavy CAGGTGCAGGTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTCTGCCATGCAC TGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAATGGGTGGCAGTTATATCAT ATGATGGAAGTAATAAATACTATGGAGACTCCGTGAAGGGCCGATTCACCATC TCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGCACAGCCTGAGAGC TGAGGACACGGCTGTTTATTACTGTGCGAAAGACCGAGATGCCTACAATACCG TCGGCTATTTTGCTTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCCTG GTCACCGTCTCCTCA 111

ZIKV-195 light CAGTCTGTGCTGACTCAGCCACCCTCGGTGTCTGAAGCCCCCAGGCAGAGGGT CACCATCTCCTGTTCTGGAAGCAGCTCCAACATCGGAAATAATGCTGTAAACTG GTACCAGCAGCTCCCAGGAAAGGCTCCCAAACTCCTCATCTATTATGATGATCT GCTGCCCTCAGGGGTCTCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCAGC CTCCCTGGCCATCAGTGGGCTCCAGTCTGAGGATGAGGCTGATTATTACTGTG CAGCATGGGATGACAGCCTGACTCGTTATGTCTTCGGAACTGGGACCAAGGTC ACCGTCCTA 112

ZIKV-204 heavy CAGGTCACCTTGAGGGAGTCTGGCCCTGCGCTGCTGAAACCCACACAGACCCT CACACTGACCTGCACCTTCTCTGGATTCTCACTCAGCACTAATGAAACGTGTGT GAGCTGGATCCGTCAGCCCCCAGGGAAGGCCCTGGAGTGGCTTGCGCTCATT GATTGGGATGATGATCAATACTCCAGCACATCTCTGGCGGCCAGGCTCACCGT CTCTAAGGACACCTCCAAAAACCAGGTGGTCCTCACAATGACCAACGTGGCCC CTGTGGACACAGCCACGTATTACTGTGCACTGACACGTCCTACGTTGACTGCCC AGAACGGGGACAAATATTACAACTACTACTACGGCATGGACGTCTGGGGCCA AGGGACCCTGGTCACCGTCTCCTCA 113

ZIKV-204 light GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAG AGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCATGTACTTAGCCT GGTATCAACAAAAACGTGGCCAGCCTCCCAGACTCCTCATCTATGGTACATTCA ACAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGA CTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTG TCAGCAGTATGGTAGCTCATCGTTCACTTTCGGCGGAGGGACCAAGGTGGAG ATCAAAC 114

ZIKV-216 heavy GAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGGGAAAAAGCCCGGTGAATCT CTGAAGATCTCTTGTAAGGGTTCTGGATACAATTTTTCCAACTACTGGATCGGC TGGGTGCGCCAGATGCCCGGGAAAGGCCTGGAGTGGATGGGCATCATCTATC CGGGTGACTCTGATACCAGATATAGCCCGTCCTTCCAAGGCCAGGTCACCATCT CAGCCGACAAGTCCATCAACACCGCCTATCTGCAGTGGAGAAGCCTGAGGGCC TCGGACTCCGCCATGTTTTATTGTGCGAGAGGGGTAATGATAACAACTCCTAAT CCTTACGACTGGTTCGACGCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTC A 115

ZIKV-216 light CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATC ACCATCTCCTGCACTGGAACCGGCAGTGACATTGGTACTTATGACTATGTCTCC TGGTACCAGCAACATCCAGGCAAAGCCCCCAAACTCATGATTTATGGTGTCACT AAGCGGCCCTCAGGGGTTTCTCATCGCTTCTCTGGCTCCAAGTCCGTCAACACG GCCTCCCTGACCATCTCTGGGCTCCAGGCTGAAGACGAGGCTGATTATTTCTGC AGTTCATATTCAACCAGCAGCACTTTTGTGGTATTCGGCGGAGGGACCAAGCT GACCGTCCTA 116

ZIKV-218 heavy CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCC CTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTGGCCATGCTTTGCAC TGGGTCCGCCAGGCTCCAGGCAAGGGTCTGCAGTGGGTGGCGGTGATATCAT CTGATGCTACCAGTAAGTTCTACGCAGACTCCGTGGAGGGCCGATTCAGCATC TCCAGAGACAACCCCAAAAACACACTGTTTCTGCAACTTGACAGCCTGGGACG TGAAGATTCGGGTATATATTACTGTGTGCTTGGTTTTACCAGCAGCTGGGACCT AACAGCCTACGCCTTTGACTATTGGGGCCAGGGAACCCTGGTCACCGTCTCCTC A 117

ZIKV-218 light CAGTCTGCCCTGACTCAGCCTCACTCAGTGTCCGGGTCTCCTAGACAGTCAGTC ACCATCTCCTGCGTTGGAACTAGCGATGATGTTGGTGCTTATAGCTCTGTCTCC TGGTACCAACAACACCCGGGCAAAGCCCCCAAGCTCCTGGTTTATGATGTCGC TGAGCGGCCCTCAGGGGTCCCTGATCGCTTCTCTGGCTCCAATTCTGGCAACAC GGCCTCCCTGACTATCTCTGGGCTCCAGTCTGACGATGAGGCAACATATTACTG CTGCGCATATGCCGGCACATATGTGGTATTCGGGGGAGGGAACAAGGTGACC GTCCTA 118

TABLE 2 – PROTEIN SEQUENCES FOR ANTIBODY VARIABLE REGIONS

Clone	Variable Sequence	SEQ ID NO:
ZIKV-2 heavy	QVQLVESGGGVVQPGRSLRLSCAASGFTLSTFAMHWVRQAPGKGLQWVAVTS YDGSSKFYADSVEGRFTISRDTSKNTLYLQMTSLTAEDTAVYFCARGFGGSGDYYV GGFDIWGQGTLVTVSS	53
ZIKV-2 light	QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYDYVSWYQQHPTEAPKLIIHDVNK RPSGVPDRFSGSKSGNTASLTISGLQAEDEADYYCCSFAGSHSFVLFGGGTRLTVL	54
ZIKV-8 heavy	QVQLVESGGRVVQPGRSLRLSCAASGFTFSNYAMHWVRQAPGKGLEWVAVISY DGSYTHYTEAVRDRFTISRDNSKRTVWLEMNSLRVDDTAMYYCARDALGYYDNS DYTSWGLGTLVTVSS	55
ZIKV-8 light	EIVLTQSPDTLSLAPGERATLSCRAGQTITSSHLAWYRLKPGQAPRLIIYDASSRAT GIPDRFSGSGSGTQFTLTISRLEPEDFAVYYCQQYATPPWTFGQGTKVEIK	56
ZIKV-12 heavy	EVQLVESGGGLVQPGGSLRLSCVASGFTFSDYAMHWVRQAPGKGLEYVSTINSN GGSTFYADSVQDRFTISRDNSKNTLYLQMDSLRPEDMAVYYCVSLYNYPVLDYW GLGTLVTVSS	57
ZIKV-12 light	QAVVTQETSLTVSPGGTVTLTCGSSTGAVTSGHYPYWFQQKPGQAPRTLIYHTN NKHSWTPARFSGSLLGGKAALTLSGAHPDDEAEYYCLILYPDARVFGGGTKLTVL	58
ZIKV-15 heavy	QVQLVESGGGVVQPGGSLRLSCAASGFTFSTHDMHWVRQAPGKGLEWVALIRF GGKDIYYADSVEGRFTVSRDNSMNTLYLQLSGLRADDTALYYCAKGARFYDSNGF PVYAEYFEHWGQGTLVTVSS	59
ZIKV-15 light	DIQMTQSPSSLSASVGDRVTITCRASQDISNFLAWYQQRPGKVPKLLIYAASTLQS GVPSRFSGSGSGTDFTLTISSVQPEDVATYFCQKYNNAPLTFGGGTKVEIK	60
ZIKV-19 heavy	QVQLVQSGPEVKKPGSSVKVSCKASGVSFNTYEISWVRQAPGQGLEWMGRIIPI FATPTYALKFQGRVTITTDESTTTGYMELSSLRSEDTAVYYCAGRPYGPGSWLPLD VWGQGTLVTVSS	61
ZIKV-19 light	DIVMTQSPSSLSASVGDRVTITCRASQGISSYLAWYQQKPGKLPKLLIYAASTLQS GVPSRFSGSGSGTDFTLTISSLQPEDVAAYYCQKYDSAPFTFGPGTKVDLK	62
ZIKV-27 heavy	QVQLVQSGPEVKKPGASVKVSCKASGFTSMNYGISWVRQAPGQGLEWMGWII AYNGNTNYAQKFQGRVSMTIDTSTTTAYMELRSLRSDDTAVYYCASRIEVADTVY DPWGQGTLVTVSS	63
ZIKV-27 light	EIVLTQSPGTLSLSPGERATLSCRASQTTSSSFLAWYQQKPGQAPRLLIYGASNRA TGIPDRFSGSGSGTDFTLTISKLEPEDFAVYYCQQYDSSPPGFTFGPGTKVDIK	64

ZIKV-33 heavy	QVQLVESGGAVVQPGRSLRLSCAASGLSFSDYAIHWVRQAPGKGLEWVAVIAH DGRNKYYADSVMGRVAISGDNSKNTVYLQMSSLRAEDTATYYCARGFYHDKTG SYWYFDLWGRGTLVTVSS	65
ZIKV-33 light	QSVLTQPPSVFAAAGQRVTISCSGSSSNIGDNYVSWYQQFPGTAPKILIYENDKR ASGIPDRFSGSKSGTSATLGITGLRTGDEADYFCGTWDSSLTTAVFGGGTKLTVL	66
ZIKV-46 heavy	EVQLVESGGGLVKPGGSLRLSCATSGFSVTNAWMSWVRQAPGRGLEWVGRIK NKADDWTTDYAAPVRGRFTISRDDSKDTVYLQMNSLKSEDTALYYCSTYYYDSSG HFVDYWGQGTLVTVSS	67
ZIKV-46 light	EIVLTQSPATLSLSAGDRATLSCRASQSVSIYLLWYQQKPGQAPRLLIYDASKRATG IPARFSGSGSGTDFTLTITSLEPEDFAVYYCLQRGIWPSFGQGTKVEIK	68
ZIKV-47 heavy	QVQLVESGGGVVQPGRSLRLSCAASGFTFTSYAFHWVRQAPGKGLEWVAVISY DGSQKFYADSVMDRFTISRDSSKNTQYLQMDSLRPEDTAVYYCATKGQSQIPVT AEYFEHWGRGTLVTVSS	69
ZIKV-47 light	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLVIFANSN RPSGVPDRFSGSKSDTSASLAITGLQAEDEADYYCQSYDSSLSRYVVFGGGTKVTV L	70
ZIKV-48 heavy	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSDAMHWVRQVPGKGLEWVAVTS YDGSNKYYADSVEGRFTISRDNSKNTLFLEMTSLRVEDSAIYYCARGFTVIHAFDI WGLGTLVTVSS	71
ZIKV-48 light	QSVLTQPRSVSGSLGQSVSISCTGTSSDVGGYNYVSWYLQHPGKAPKLIIYDVSKR PSGVPSRFSGSKSGNTASLTISGLQAEDEADYSCSSYAGTFVVFGGGTKLTVL	72
ZIKV-49 heavy	QVQLVESGPGLLKPSETLSLTCAVSGGSINSSSFHWGWIRQPPGKGLEWIGAIYYT GSTYYNPSLKSPVTVSVDTSKNQFSLELSSVTAADTAVYFCARVVATVTTRRGLGS FDIWGQGTLVTVSS	73
ZIKV-49 light	DIVMTQSPSSVSAFVGDRVTITCRASQGISNWLAWYQQKPGKAPKLLIYAASSLQ SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANSFPWTFGQGTKVEIK	74
ZIKV-50 heavy	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDPYMDWVRQAPGKGLEWVGRVR NKPNSYTTEYAASVTGRFTISRDDLKNSVYLQMNSLKTEDTAVYFCVRVALPKAF DVWGQGTLVTVSS	75
ZIKV-50 light	DIQMTQSPPSLSASVGDRVTITCQASQDISIYLNWFQHKPGKAPKLLIYDASNLET GVPSRFSGSGSGTDFSFTISGLQPEDVASYYCLQYDNPPTFGGGTKVEIK	76
ZIKV-70 heavy	QVQLVESGGGVVQPGGSLRLSCVGSGLTLSSYAMHWVRQAPGKGLEWVAVISS DGSNRYYADSVEDRFTISRDNSKNILYLQMNTLRPDDTAFYYCARGYYFDDSGSY WYFDLWGRGTLVTVSS	77
ZIKV-70 light	QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGAAPRVLIYEDSKR PSGIPDRFSGSKSGTSATLAITGLQTGDEADYYCATWDGGLSVIFGGGTQVTVL	78

ZIKV-71 heavy	QVQLVQSGGEVKKPGASVKVSCKASGYSFINYGISWVRQAPGQGLEWMGYIIPY NGDTSYAQQFQGRVTMAADTSATTVFMEVGSLRLDDTAVYYCARAIVGETVTG YVYGMDVWGQGTLVTVSS	79
ZIKV-71 light	DIQLTQSPSSLSASVGDRVTITCRASQGIDIFLAWYQQKPGKAPNLLIYSASTLQSG VPSRFSGSGSGTFFTLTISSLQPEDFATYYCQYLNTSPWTFGQGTKVEIK	80
ZIKV-78 heavy	EVQLVESGGGLVKPGGSLRLSCEASEFTFSDYAMTWVRQPPGKGLEWVSTISGS GGGTFYADSVEDRFTISRENSENTLFLQMDNLRVEDTATYFCAVLFNSNENSPYY DASVFDIWGQGTLVTVSS	81
ZIKV-78 light	QAVVTQEPSLTVSPGGTVTFTCASSTGAVTSGHYPYWFQQKPGQAPRTLIYHSSK KHSWTPDRFSGSLLGGKAALTLSGAQPEDEAEYYCLLSYSGGRPVFGGGTQVTVL	82
ZIKV-81 heavy	QVQLVESGGGVVQPGRSLRLSCAASGFIFSNFAMHWVRQAPGKGLEWVAVISY DGSNTYYSDSVEGRFTISRDNSKNMLFLEVNTLRTEDTAVYYCAIGGGPPDFLAAP FNAEVLQHWGQGTLVTVSS	83
ZIKV-81 light	QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDIHWYQQLPGTAPKLLIYGNTN RPSGVPDRFSGSKSGTSGSLAITGLQAEDEADYFCQSYDTGLSVVFGGGTQVTVL	84
ZIKV-82 heavy	Not determined	85
ZIKV-82 light	DIQMTQSPSSVSASVGDTVTITCRASQDITYVLTWYQQKPGKAPKLLIYAAANLAS GVPSRFSGSGSGTHFTLTIRSLQPEDFATYYCQQANSFPWTFGQGTKVDIK	86
ZIKV-86 heavy	QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYAMHWVRQAPGKGLEWVAVISY DINTKYYAESVEGRFSISRDDSINTVYLQMNSLRPDDTAVYFCARDVYGGGVPW GQGTLVTVSS	87
ZIKV-86 light	DIQMTQSPSTLSASVGDRVTITCRASQSVSDWLAWYQQKPGKAPKLLIYKASTLE SGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCHQYSFYWTFGQGTKVDIK	88
ZIKV-88 heavy	QVQLLESGGGVVQPGRSLRLSCVASGLTFSTSAMHWVRQAPGKGLEWVAVISY DGSYKFYADSVRDQFTISRDNSKTTLYLQMDGLTPEDTAVYYCARGYNDDSSGSY WYFDLWGRGTLVTVSS	89
ZIKV-88 light	QSVLTQPPSVSAAPGQKVTMSCSGSSSNIGSNFVSWYQQLPGTAPKVLIFDNNQ RPSGIPDRFSGSKSGTSATLAITGLQPGDEAVYHCGTWDSSLTFAVFGGGTKLTVL	90
ZIKV-116 heavy	EVKLVESGRGLVRPGGSLRLSCAASGFTFSNYAMSWVRQGPGMGLEWVSTITA DSDSKYYVDSVKGRFTISRDNSKDTLFLHMTSLRAEDTAVYYCAKDRLSRGVGELY DSWGQGTLVTVSQ	91
ZIKV-116 light	DIQMTQSPSTLSASVGDRVTITCRASQSIDVWLAWYQQKPGKAPKLLMYKTSTL QTGVPSRFSGSGSGTEFTLTISSLQTDDFATYYCQKYDSYPWTFGPGTKVEIK	92
ZIKV-117 heavy	QVQLVESGGGVVRPGGSLRLSCAASGFTFKNYGIHWVRQAPGKGPEWVAFVRY DGNNKYYADSVKGRFTISRDNAKNTLSLQMNSLRVEDTAVYFCARDPETFGGFD YWGQGTLVTVSS	93

ZIKV-117 light	ETVMTQSPATLSVSPGERGTLSCRASESVSSNLAWYQQKPGKAPRLLIYGASTRA TGIPDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYYSPRTFGQGTKVEVK	94
ZIKV-146 heavy	EVQLVESGGGLVQPGGSLRLSCVASGFTFSDYAMHWVRQAPGKGLEYVSTINSN GGNTFYANSVEDRFTISRDNSKSTLYLQLDSLRLEDTAVYYCVSLWNYPVLDYWG LGTLVTVSS	95
ZIKV-146 light	QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGHYPYWFQQKPGQAPRTLIYHTN SKHSWTPARFSGSLLGGKAALTLSGAQPEDEAEYYCLLLYPDARVFGGGTRLTVL	96
ZIKV-158 heavy	QVQLVESGGGVVQPGRSLRLSCTSSGFTFNTYPMHWVRQAPGKGLEWLATISY VETDKYYTDSVQGRFTVSRDNSKNTLYLQMNSLSVEDTAVYYCARGWAVTTSHA FDVWGQGTLVTVSS	97
ZIKV-158 light	QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYDFVSWYQEHPGKAPKLMIYDVT KRPSGVPDRFSGSKSGNTASLTISGLQAEDEADYYCCSYAGGYTFVVFGGGTQVT VL	98
ZIKV-165 heavy	QVQLVESGGGVVQPGGSLRLSCAASGFTFTNYGMHWVRQAPGKGLEWVAFIR PDGNDKYYADSVKGRFTISRDNSKNSLYLQMNSLGAEDTAVYYCAKDYYHTTDD YWAEFFQHWGQGTLVTVSS	99
ZIKV-165 light	DIQMTQSPSTLSASVGDRVTITCRASQSINNWLAWYQQKPGKAPKLLIYMASNL ESGVPSRFSGSGSGTEFTLTISSLQPDDFASYYCQHYNFYPGFGQGTKVEIK	100
ZIKV-190 heavy	EVQLVESGGGLIQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGS GGSTYYADSVKGRFTISRDNSKNTLSLQMNSLRAEDTAVYYCARAFYYDFWTFDY WGQGTLVTVSS	101
ZIKV-190 light	QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYAVT KRPSGVPDRFSGSKSGNTASLTISGLQAEDEADYYCCSYAGSYTYVVFGGGTKLTV L	102
ZIKV-195 heavy	QVQVVESGGGVVQPGRSLRLSCAASGFTFSSSAMHWVRQAPGKGLEWVAVISY DGSNKYYGDSVKGRFTISRDNSKNTLYLQMHSLRAEDTAVYYCAKDRDAYNTVG YFAYYYGMDVWGQGTLVTVSS	103
ZIKV-195 light	QSVLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVNWYQQLPGKAPKLLIYYDDLLP SGVSDRFSGSKSGTSASLAISGLQSEDEADYYCAAWDDSLTRYVFGTGTKVTVL	104
ZIKV-204 heavy	QVTLRESGPALLKPTQTLTLTCTFSGFSLSTNETCVSWIRQPPGKALEWLALIDWD DDQYSSTSLAARLTVSKDTSKNQVVLTMTNVAPVDTATYYCALTRPTLTAQNGD KYYNYYYGMDVWGQGTLVTVSS	105

ZIKV-204 light	EIVLTQSPGTLSLSPGERATLSCRASQSVSSMYLAWYQQKRGQPPRLLIYGTFNRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSSFTFGGGTKVEIK	106
ZIKV-216 heavy	EVQLVQSGAEGKKPGESLKISCKGSGYNFSNYWIGWVRQMPGKGLEWMGIIYP GDSDTRYSPSFQGQVTISADKSINTAYLQWRSLRASDSAMFYCARGVMITTPNPY DWFDAWGQGTLVTVSS	107
ZIKV-216 light	QSALTQPASVSGSPGQSITISCTGTGSDIGTYDYVSWYQQHPGKAPKLMIYGVTK RPSGVSHRFSGSKSVNTASLTISGLQAEDEADYFCSSYSTSSTFVVFGGGTKLTVL	108
ZIKV-218 heavy	QVQLVESGGGVVQPGRSLRLSCAASGFTFSGHALHWVRQAPGKGLQWVAVISS DATSKFYADSVEGRFSISRDNPKNTLFLQLDSLGREDSGIYYCVLGFTSSWDLTAYA FDYWGQGTLVTVSS	109
ZIKV-218 light	QSALTQPHSVSGSPRQSVTISCVGTSDDVGAYSSVSWYQQHPGKAPKLLVYDVA ERPSGVPDRFSGSNSGNTASLTISGLQSDDEATYYCCAYAGTYVVFGGGNKVTVL	110

TABLE 3 – CDR HEAVY CHAIN SEQUENCES			
Antibody	**CDRH1 (SEQ ID NO:)**	**CDRH2 (SEQID NO:)**	**CDRH3 (SEQ ID NO:)**
ZIKV-2	GFTLSTFA (174)	TSYDGSSK (175)	ARGFGGSGDYYVGGFDI (176)
ZIKV-8	GFTFSNYA (177)	ISYDGSYT (178)	ARDALGYYDNSDYTS (179)
ZIKV-12	GFTFSDYA	INSNGGST	VSLYNYPVLDY
ZIKV-12	(125)	(126)	(127)
ZIKV-15	GFTFSTHD (128)	IRFGGKDI (129)	AKGARFYDSNGFPVYAEYFEH (130)
ZIKV-19	GVSFNTYE (131)	IIPIFATP (132)	AGRPYGPGSWLPLDV (133)
ZIKV-27	GFTSMNYG (134)	IIAYNGNT (135)	ASRIEVADTVYDP (136)
ZIKV-33	GLSFSDYA (137)	IAHDGRNK (138)	ARGFYHDKTGSYWYFDL (139)
ZIKV-46	GFSVTNAW (140)	IKNKADDWTT (141)	STYYYDSSGHFVDY (142)
ZIKV-47	GFTFTSYA (143)	ISYDGSQK (144)	ATKGQSQIPVTAEYFEH (145)
ZIKV-48	GFTFSSDA (146)	TSYDGSNK (147)	ARGFTVIHAFDI (148)
ZIKV-49	GGSINSSSFH (149)	IYYTGST (150)	ARVVATVTTRRGLGSFDI (151)
ZIKV-50	GFTFSDPY (152)	VRNKPNSYTT (153)	VRVALPKAFDV (154)
ZIKV-55	GYTFTSFG (155)	NSAWISAH (156)	VYYCARVGGWQQIPYFDF (157)
ZIKV-70	GLTLSSYA (158)	ISSDGSNR (159)	ARGYYFDDSGSYWYFDL (160)
ZIKV-71	GYSFINYG (161)	IIPYNGDT (162)	ARAIVGETVTGYVYGMDV (163)
ZIKV-78	EFTFSDYA (164)	ISGSGGGT (165)	AVLFNSNENSPYYDASVFDI (166)
ZIKV-81	GFIFSNFA (167)	ISYDGSNT (168)	AIGGGPPDFLAAPFNAEVLQH (169)
ZIKV-82	Not determined	Not determined	Not determined
ZIKV-86	GFTFSDYA (171)	ISYDINTK (172)	ARDVYGGGVP (173)

	(180)	(181)	(182)
ZIKV-15	GFTFSTHD (183)	IRFGGKDI (184)	AKGARFYDSNGFPVYAEYFEH (185)
ZIKV-19	GVSFNTYE (186)	IIPIFATP (187)	AGRPYGPGSWLPLDV (188)
ZIKV-27	GFTSMNYG (189)	IIAYNGNT (190)	ASRIEVADTVYDP (191)
ZIKV-33	GLSFSDYA (192)	IAHDGRNK (193)	ARGFYHDKTGSYWYFDL (194)
ZIKV-46	GFSVTNAW (195)	IKNKADDWTT (196)	STYYYDSSGHFVDY (197)
ZIKV-47	GFTFTSYA (198)	ISYDGSQK (199)	ATKGQSQIPVTAEYFEH (200)
ZIKV-48	GFTFSSDA (201)	TSYDGSNK (202)	ARGFTVIHAFDI (203)
ZIKV-49	GGSINSSSFH (204)	IYYTGST (205)	ARVVATVTTRRGLGSFDI (206)
ZIKV-50	GFTFSDPY (207)	VRNKPNSYTT (208)	VRVALPKAFDV (209)
ZIKV-55	GYTFTSFG (210)	NSAWISAH (211)	VYYCARVGGWQQIPYFDF (212)
ZIKV-70	GLTLSSYA (213)	ISSDGSNR (214)	ARGYYFDDSGSYWYFDL (215)
ZIKV-71	GYSFINYG (216)	IIPYNGDT (217)	ARAIVGETVTGYVYGMDV (218)
ZIKV-78	EFTFSDYA (219)	ISGSGGGT (220)	AVLFNSNENSPYYDASVFDI (221)
ZIKV-81	GFIFSNFA (222)	ISYDGSNT (223)	AIGGGPPDFLAAPFNAEVLQH (224)
ZIKV-82	Not determined	Not determined	Not determined
ZIKV-86	GFTFSDYA (225)	ISYDINTK (226)	ARDVYGGGVP (227)

TABLE 4 – CDR LIGHT CHAIN SEQUENCES			
Antibody	**CDRH1 (SEQ ID NO:)**	**CDRH2**	**CDRH3 (SEQ ID NO:)**
ZIKV-2	SSDVGGYDY (228)	DVN	CSFAGSHSFVL (229)
ZIKV-8	QTITSSH (230)	DAS	QQYATPPWT (231)
ZIKV-12	TGAVTSGHY (232)	HTN	LILYPDARV (233)
ZIKV-15	QDISNF (234)	AAS	QKYNNAPLT (235)
ZIKV-19	QGISSY (236)	AAS	QKYDSAPFT (237)
ZIKV-27	QTTSSSF (238)	GAS	QQYDSSPPGFT (239)
ZIKV-33	SSNIGDNY (240)	END	GTWDSSLTTAV (241)
ZIKV-46	QSVSIY (242)	DAS	LQRGIWPS (243)
ZIKV-47	SSNIGAGYD (244)	ANS	QSYDSSLSRYVV (245)
ZIKV-48	SSDVGGYNY (246)	DVS	SSYAGTFVV (247)
ZIKV-49	QGISNW (248)	AAS	QQANSFPWT (249)
ZIKV-50	QDISIY (250)	DAS	LQYDNPPT (251)
ZIKV-55	EGPDSY (252)	AAS	QHLNGYPS (253)
ZIKV-70	SSNIGNNY (254)	EDS	ATWDGGLSVI (255)
ZIKV-71	QGIDIF (256)	SAS	QYLNTSPWT (257)
ZIKV-78	TGAVTSGHY (258)	HSS	LLSYSGGRPV (259)
ZIKV-81	SSNIGAGYD (260)	GNT	QSYDTGLSVV (261)
ZIKV-82	QDITYV (262)	AAA	QQANSFPWT (263)
ZIKV-86	QSVSDW (264)	KAS	HQYSFYWT (265)

TABLE 5 – Research Subjects with Time and Place of Infection

ZIKV strain lineage	Subject	Year infected	Country in which infection occurred
African	972	2008	Senegal
	973	2008	Sexual transmission from Subject 972*
Asian	1001	2015	Brazil
	1002	2016	Mexico
	1010	2016	Haiti
	1011	2016	Haiti
	1012	2016	Haiti
	1016	2016	Haiti

*Case was reported previously:

Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, Lanciotti RS, Tesh RB. Probable non-vector-borne transmission of Zika virus, Colorado, USA. *Emerg Infect Dis.* 2011;17:880-2.

Example 3 – Discussion

These studies reveal a number of features of humoral immunity to ZIKV. First, following infection, a subset of human B cells encode mAbs that neutralize ZIKV *in vitro* with high potency, the most potent with FRNT50 values <10 ng/ml. Second, the human B cell response is directed against multiple antigenic sites on ZIKV E protein, predominantly against the fusion loop in DII, and other structural features in DII and DIII, results that agree with a recent study10. The most inhibitory antibodies recognized antigenic sites in DIII (lateral ridge) and in DII at a unique site not reported to be targeted by DENV antibodies, located at the dimer-dimer interface of the E protein. The most potent neutralizing antibodies exhibited a breadth of inhibitory activity against strains from Africa, Asia, and the Americas. Treatment of ZIKV-infected male mice with mAb ZIKV-117 showed strong post-exposure

therapeutic activity *in vivo*. Even a single ZIKV-117 dose given five days after infection protected against lethal ZIKV infection, a timeline that was similar to the most protective antibodies reported against other flaviviruses (Oliphant *et al.*, 2005). Prophylaxis or post-exposure therapy of pregnant mice with ZIKV-117 reduced infection in the mothers, and in placental and fetal tissues. To the inventors' knowledge, this is the first evidence showing that an antiviral agent can prevent or control ZIKV infection in pregnancy. Accordingly, ZIKV-117 or human antibodies with similar profiles, could be developed as a preventive or treatment measure during pregnancy for at-risk humans. By defining key epitopes on the E protein associated with antibody-mediated protection, these studies also inform vaccine efforts to design new epitope-based immunogens that elicit highly protective antibody responses against ZIKV.

* * * * * * * * * * * * * * * * *

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this disclosure have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the disclosure. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the disclosure as defined by the appended claims.

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The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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WHAT IS CLAIMED IS:

1. A method of detecting a Zika virus infection in a subject comprising:

   (a) contacting a sample from said subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively; and

   (b) detecting Zika virus in said sample by binding of said antibody or antibody fragment to a Zika virus antigen in said sample.

2. The method of claim 1, wherein said sample is a body fluid.

3. The method of claims 1-2, wherein said sample is blood, sputum, tears, saliva, mucous or serum, semen, cervical or vaginal secretions, amniotic fluid, placental tissues, urine, exudate, transudate, tissue scrapings or feces.

4. The method of claims 1-3, wherein detection comprises ELISA, RIA, lateral flow assay or Western blot.

5. The method of claims 1-4, further comprising performing steps (a) and (b) a second time and determining a change in Zika virus antigen levels as compared to the first assay.

6. The method of claims 1-5, wherein the antibody or antibody fragment is encoded by clone-paired variable sequences as set forth in Table 1.

7. The method of claims 1-5, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired variable sequences as set forth in Table 1.

8. The method of claims 1-5, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 95% identity to clone-paired sequences as set forth in Table 1.

9. The method of claims 1-5, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.

10. The method of claims 1-5, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.

11. The method of claims 1-5, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.

12. The method of claims 1-11, wherein the antibody fragment is a recombinant ScFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment.

13. A method of treating a subject infected with Zika virus, or reducing the likelihood of infection of a subject at risk of contracting Zika virus, comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.

14. The method of claim 13, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences as set forth in Table 1.

15. The method of claim 13-14, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences having 95% identify to as set forth in Table 1.

16. The method of claim 13-14, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired sequences from Table 1.

17. The method of claim 13, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.

18. The method of claim 13, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.

19. The method of claim 13, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.

20. The method of claims 13-19, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment.

21. The method of claims 13-20, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to eliminate FcR interactions, such as a LALA mutation or a LS mutation.

22. The method of claims 13-19, wherein said antibody is a chimeric antibody or a bispecific antibody.

23. The method of claim 13-22, wherein said antibody or antibody fragment is administered prior to infection or after infection.

24. The method of claim 13-23, wherein said subject is a pregnant female, a sexually active female, or a female undergoing fertility treatments.

25. The method of claim 13-24, wherein delivering comprises antibody or antibody fragment administration, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment.

26. A monoclonal antibody, wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.

27. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1.

28. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired sequences from Table 1.

29. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 95% identity to clone-paired sequences from Table 1.

30. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.

31. The monoclonal antibody of claim 26, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.

32. The monoclonal antibody of claims 26-31, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment.

33. The monoclonal antibody of claims 26-31, wherein said antibody is a chimeric antibody, or is bispecific antibody.

34. The monoclonal antibody of claim 26-33, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to eliminate FcR interactions, such as a LALA mutation or a LS mutation.

35. The monoclonal antibody of claim 26-34, wherein said antibody or antibody fragment further comprises a cell penetrating peptide and/or is an intrabody.

36. A hybridoma or engineered cell encoding an antibody or antibody fragment wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.

37. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1.

38. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired variable sequences from Table 1.

39. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 95% identity to clone-paired variable sequences from Table 1.

40. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.

41. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired variable sequences from Table 2.

42. The hybridoma or engineered cell of claim 36, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.

43. The hybridoma or engineered cell of claims 36-42, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment.

44. The hybridoma or engineered cell of claim 36-43, wherein said antibody is a chimeric antibody or a bispecific antibody.

45. The hybridoma or engineered cell of claim 36-43, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to eliminate FcR interactions, such as a LALA mutation or a LS mutation.

46. The hybridoma or engineered cell of claim 36-45, wherein said antibody or antibody fragment further comprises a cell penetrating peptide and/or is an intrabody.

47. A vaccine formulation comprising one or more antibodies or antibody fragments characterized by clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.

48. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments is encoded by light and heavy chain variable sequences according to clone-paired sequences from Table 1.

49. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments is encoded by light and heavy chain variable sequences having at least 70%, 80%, or 90% identity to clone-paired sequences from Table 1.

50. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments is encoded by light and heavy chain variable sequences having at least 95% identity to clone-paired sequences from Table 1.

51. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.

52. The vaccine formulation of claim 47, wherein at least one of said antibodies or antibody fragments comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.

53. The vaccine formulation of claims 47-52, wherein at least one of said antibody fragments is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment.

54. The vaccine formulation of claims 47-52, wherein at least one of said antibodies is a chimeric antibody, or is bispecific antibody.

55. The vaccine formulation of claims 47-54, wherein at least one of said antibodies is an IgG, or is a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to eliminate FcR interactions, such as a LALA mutation or a LS mutation.

56. The vaccine formulation of claims 47-55, wherein at least one of said antibodies or antibody fragments further comprises a cell penetrating peptide and/or is an intrabody.

57. The vaccine formulation of claims 47-56, wherein said formulation comprises antibodies or antibody fragments that bind to E protein domain II.

58. The vaccine formulation of claim 57, wherein said formulation comprises antibodies or antibody fragments that bind to E protein domain III or to a quaternary epitope on the E protein dimer-dimer interface.

59. The vaccine formulation of claims 47-58, wherein said formulation comprises antibodies or antibody fragments that do not cross react with dengue virus.

60. The vaccine formulation of claim 47-59, wherein said formulation comprises antibodies or antibody fragments that neutralize Zika virus infections corresponding to African, Asian, and American lineages.

61. A method of protecting the health of a placenta and/or fetus of a pregnant a subject infected with or at risk of infection with Zika virus comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences from Tables 3 and 4, respectively.

62. The method of claim 61, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences as set forth in Table 1.

63. The method of claim 61-62, the antibody or antibody fragment is encoded by clone-paired light and heavy chain variable sequences having 95% identify to as set forth in Table 1.

64. The method of claim 61-62, wherein said antibody or antibody fragment is encoded by light and heavy chain variable sequences having 70%, 80%, or 90% identity to clone-paired sequences from Table 1.

65. The method of claim 61, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences according to clone-paired sequences from Table 2.

66. The method of claim 61, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 70%, 80% or 90% identity to clone-paired sequences from Table 2.

67. The method of claim 61, wherein said antibody or antibody fragment comprises light and heavy chain variable sequences having 95% identity to clone-paired sequences from Table 2.

68. The method of claims 61-67, wherein the antibody fragment is a recombinant scFv (single chain fragment variable) antibody, Fab fragment, $F(ab')_2$ fragment, or Fv fragment.

69. The method of claims 61-68, wherein said antibody is an IgG, or a recombinant IgG antibody or antibody fragment comprising an Fc portion mutated to eliminate FcR interactions, such as a LALA mutation.

70. The method of claims 61-67 wherein said antibody is a chimeric antibody or a bispecific antibody.

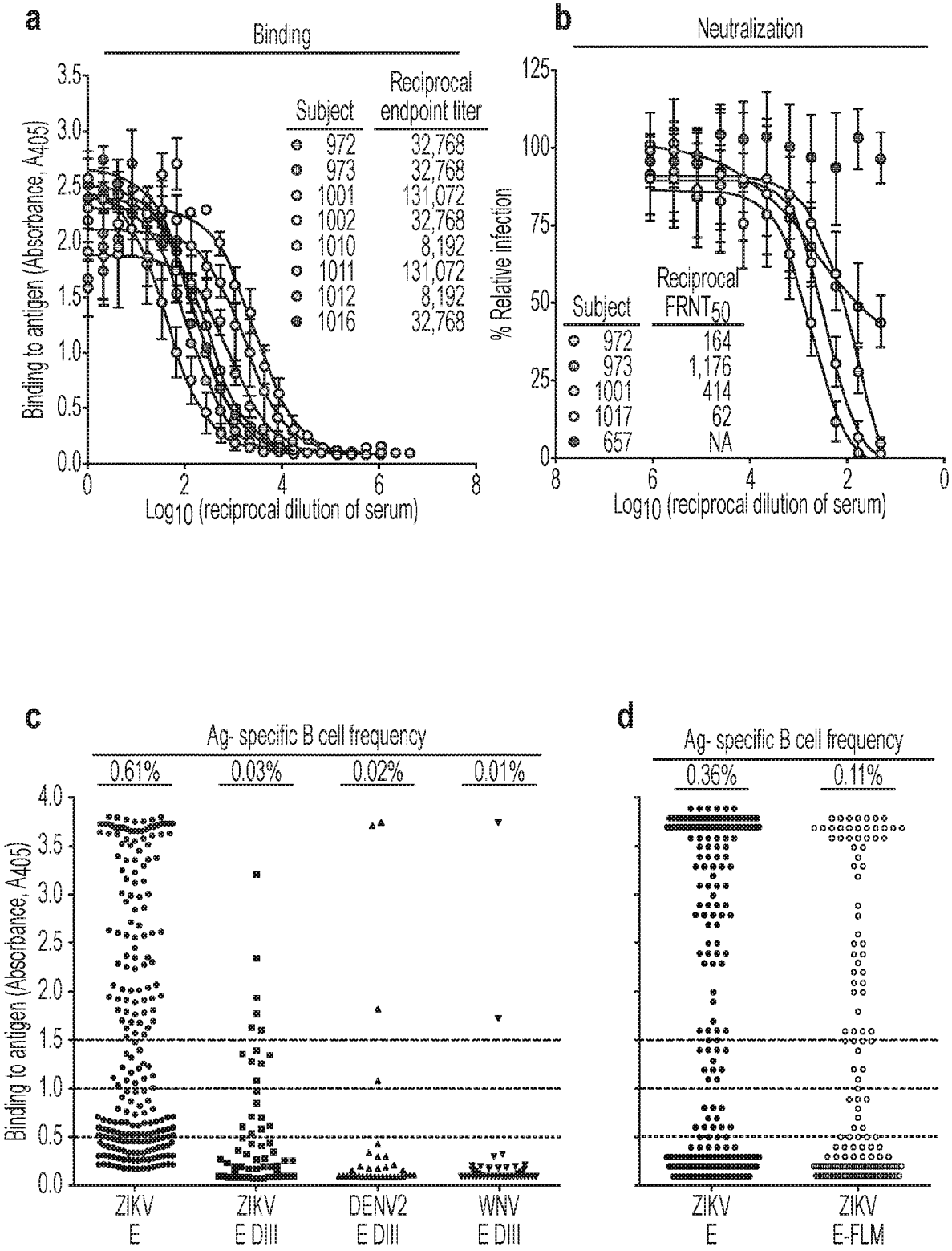
71. The method of claim 61-70, wherein said antibody or antibody fragment is administered prior to infection or after infection.

72. The method of claim 61-71, wherein said subject is a pregnant female, a sexually active female, or a female undergoing fertility treatments.

73. The method of claim 61-72, wherein delivering comprises antibody or antibody fragment administration, or genetic delivery with an RNA or DNA sequence or vector encoding the antibody or antibody fragment.

74. The method of claim 61, wherein the antibody or antibody fragment increases the size of the placenta as compared to an untreated control.

75. The method of claim 61, wherein the antibody or antibody fragment reduces viral load and/or pathology of the fetus as compared to an untreated control.


a
Binding
Binding to antigen (Absorbance, A405)
Log10 (reciprocal dilution of serum)
Subject
Reciprocal endpoint titer
972 32,768
973 32,768
1001 131,072
1002 32,768
1010 8,192
1011 131,072
1012 8,192
1016 32,768
b
Neutralization
% Relative infection
Log10 (reciprocal dilution of serum)
Subject
Reciprocal FRNT50
972 164
973 1,176
1001 414
1017 62
657 NA
c
Ag- specific B cell frequency
0.61%
0.03%
0.02%
0.01%
Binding to antigen (Absorbance, A405)
ZIKV E
ZIKV E DIII
DENV2 E DIII
WNV E DIII
d
Ag- specific B cell frequency
0.36%
0.11%
ZIKV E
ZIKV E-FLM

FIGS. 1A-D

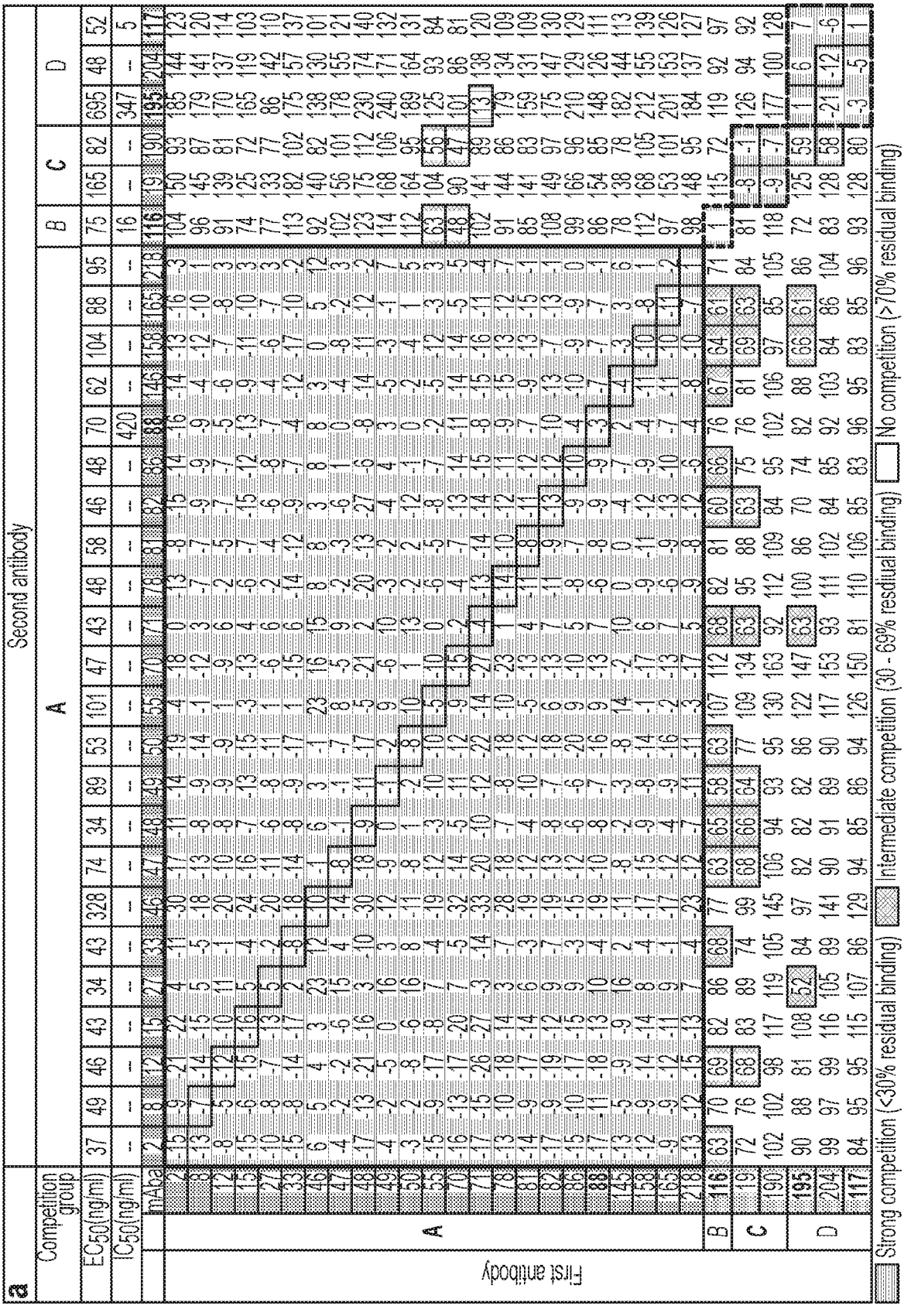
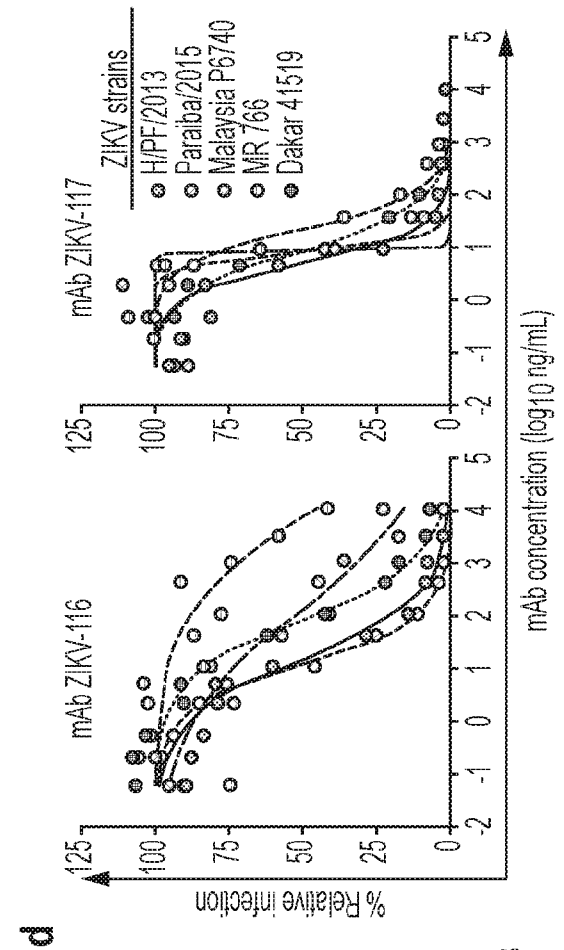

a
Second antibody
Competition group
A
B
C
D
EC50(ng/ml)
IC50(ng/ml)
mAbs
First antibody
Strong competition (<30% residual binding)
Intermediate competition (30 - 69% residual binding)
No competition (>70% residual binding)

FIG. 2A



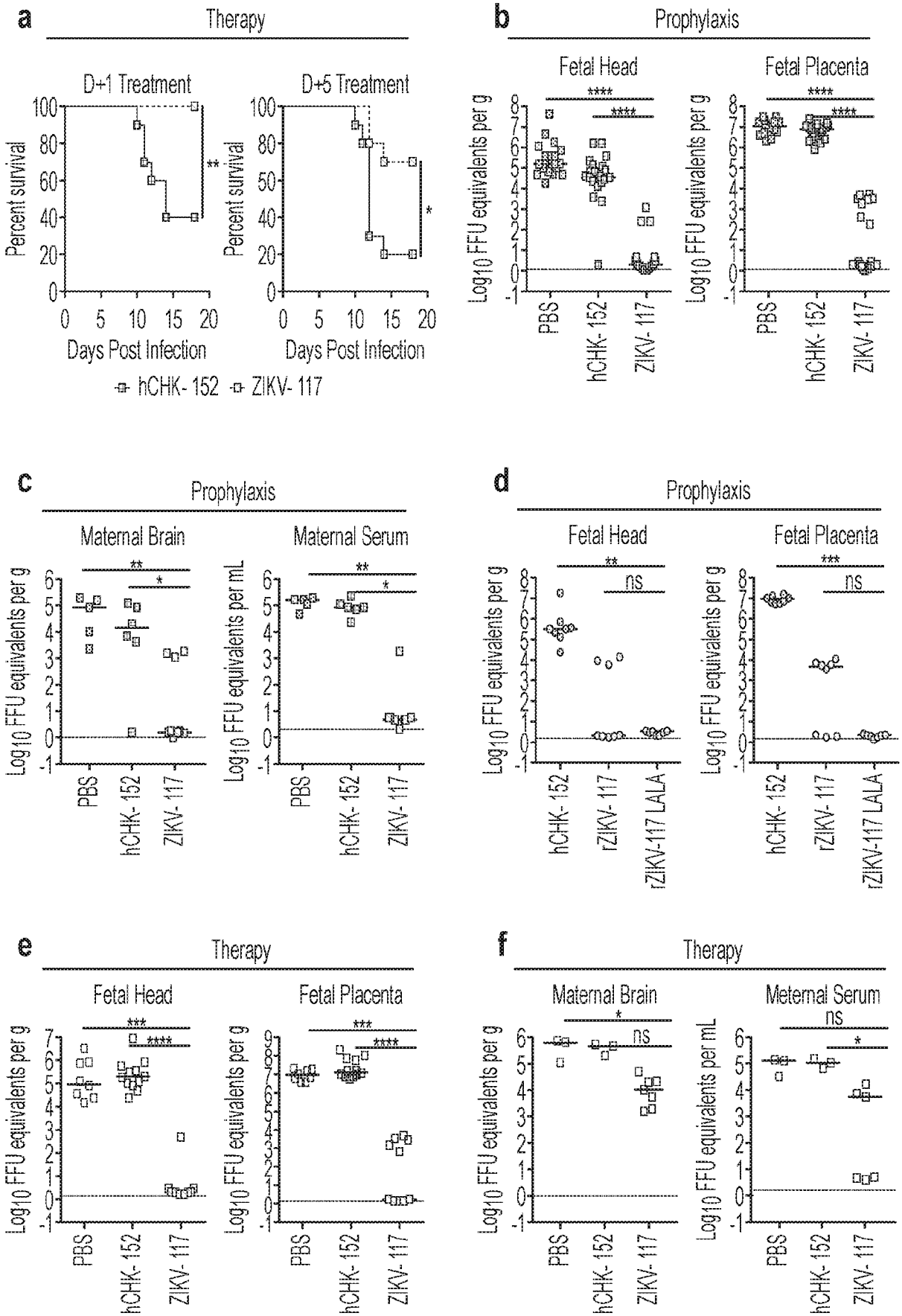
c

mAb	Competition group	Domain	Critical residues
ZIKV-12	A	FL	W101, F108
ZIKV-15	A	FL	W101
ZIKV-116	B	DIII	T309, E393, K394
ZIKV-19	C	DII	W217, F218, D220, P222
ZIKV-117	D	DII	D67, Q89, K118

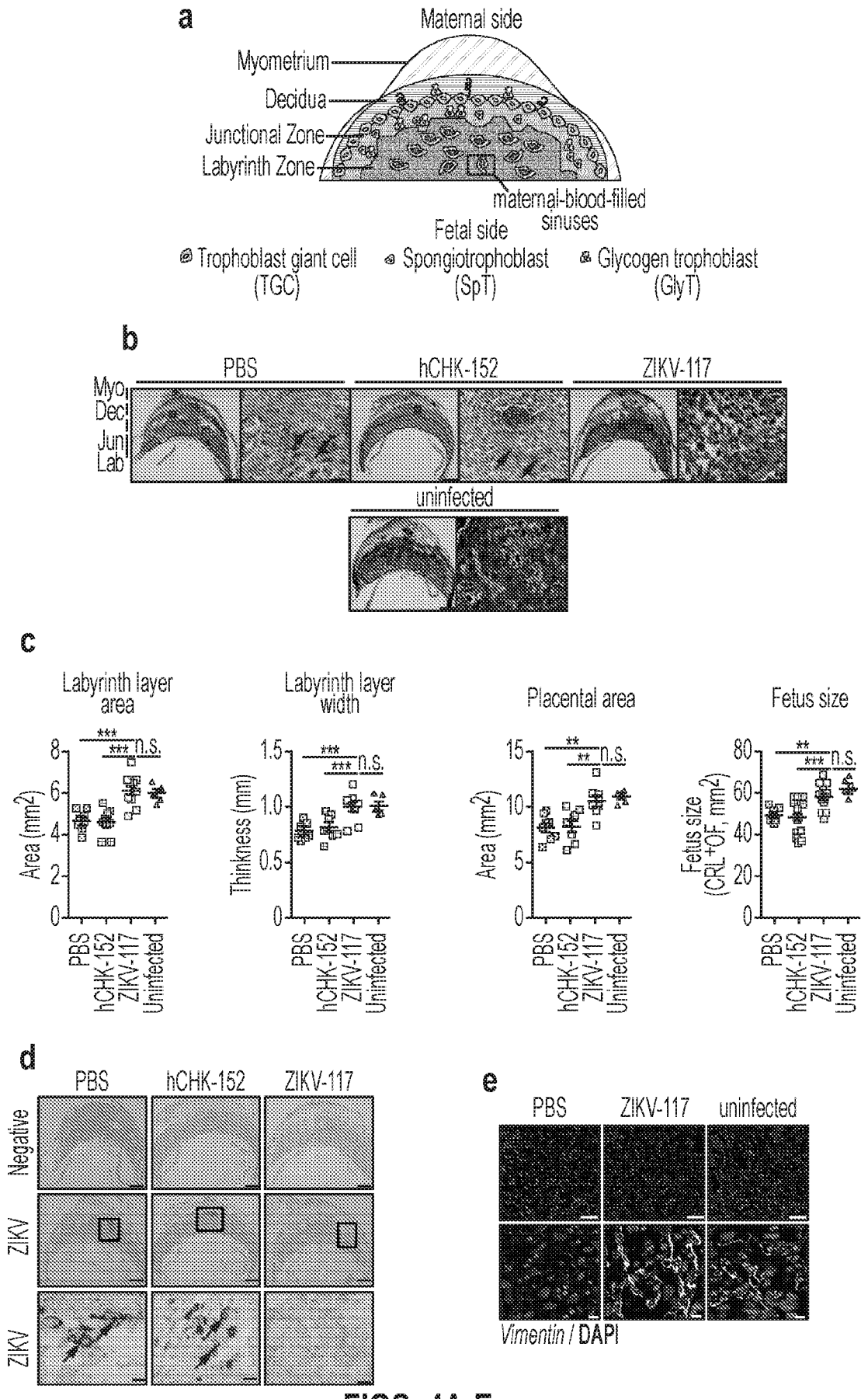
e

Virus strain	mAb ZIKV-116		mAb ZIKV-117	
	IC_{30}	IC_{30}	IC_{30}	IC_{30}
H/PF/2013	14.7	310.3	5.4	26.7
Paraiba/2015	11.6	114.0	8.9	18.8
Malaysia P6740	131.5	35,440.3	9.8	11.2
MR 766	6,798.0	753,425.8	24.9	139.4
Dakar 41519	81.0	1,608.0	9.8	82.0

FIGS. 2B-E


a
Therapy
D+1 Treatment
D+5 Treatment
Percent survival
Days Post Infection
hCHK-152
ZIKV-117
b
Prophylaxis
Fetal Head
Fetal Placenta
Log10 FFU equivalents per g
PBS
hCHK-152
ZIKV-117
c
Prophylaxis
Maternal Brain
Maternal Serum
Log10 FFU equivalents per g
Log10 FFU equivalents per mL
d
Prophylaxis
Fetal Head
Fetal Placenta
hCHK-152
rZIKV-117
rZIKV-117 LALA
ns
e
Therapy
Fetal Head
Fetal Placenta
f
Therapy
Maternal Brain
Meternal Serum

FIGS. 3A-F


a
Maternal side
Myometrium
Decidua
Junctional Zone
Labyrinth Zone
maternal-blood-filled sinuses
Fetal side
Trophoblast giant cell (TGC)
Spongiotrophoblast (SpT)
Glycogen trophoblast (GlyT)
b
PBS
hCHK-152
ZIKV-117
Myo
Dec
Jun
Lab
uninfected
c
Labyrinth layer area
Labyrinth layer width
Placental area
Fetus size
Area (mm2)
Thinkness (mm)
Area (mm2)
Fetus size (CRL+OF, mm2)
***
**
n.s.
PBS
hCHK-152
ZIKV-117
Uninfected
d
PBS
hCHK-152
ZIKV-117
Negative
ZIKV
ZIKV
e
PBS
ZIKV-117
uninfected
Vimentin / DAPI

FIGS. 4A-E

5/11

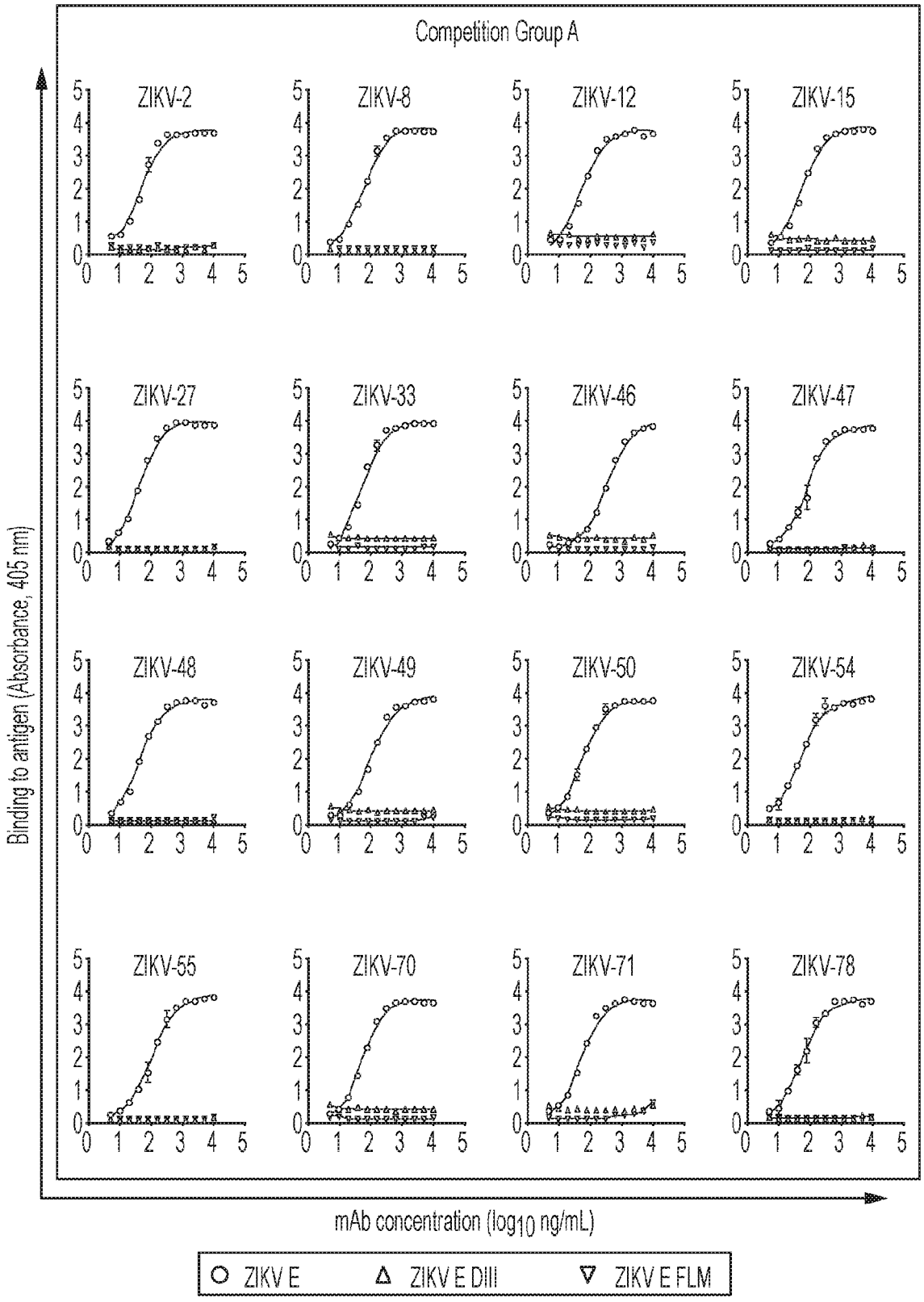

Competition Group A
ZIKV-2
ZIKV-8
ZIKV-12
ZIKV-15
ZIKV-27
ZIKV-33
ZIKV-46
ZIKV-47
ZIKV-48
ZIKV-49
ZIKV-50
ZIKV-54
ZIKV-55
ZIKV-70
ZIKV-71
ZIKV-78
Binding to antigen (Absorbance, 405 nm)
mAb concentration (log10 ng/mL)
ZIKV E
ZIKV E DIII
ZIKV E FLM

FIG. 5

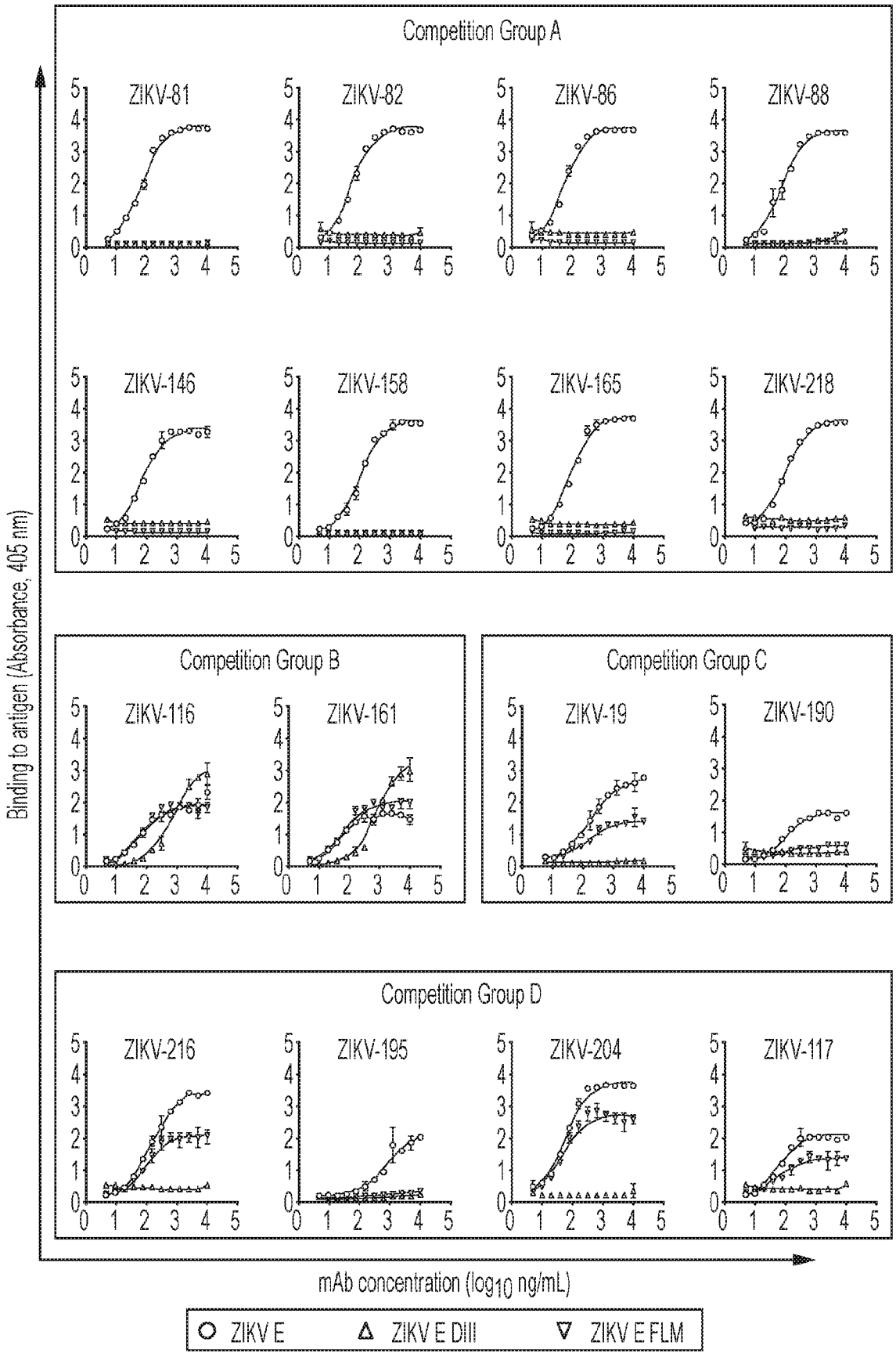
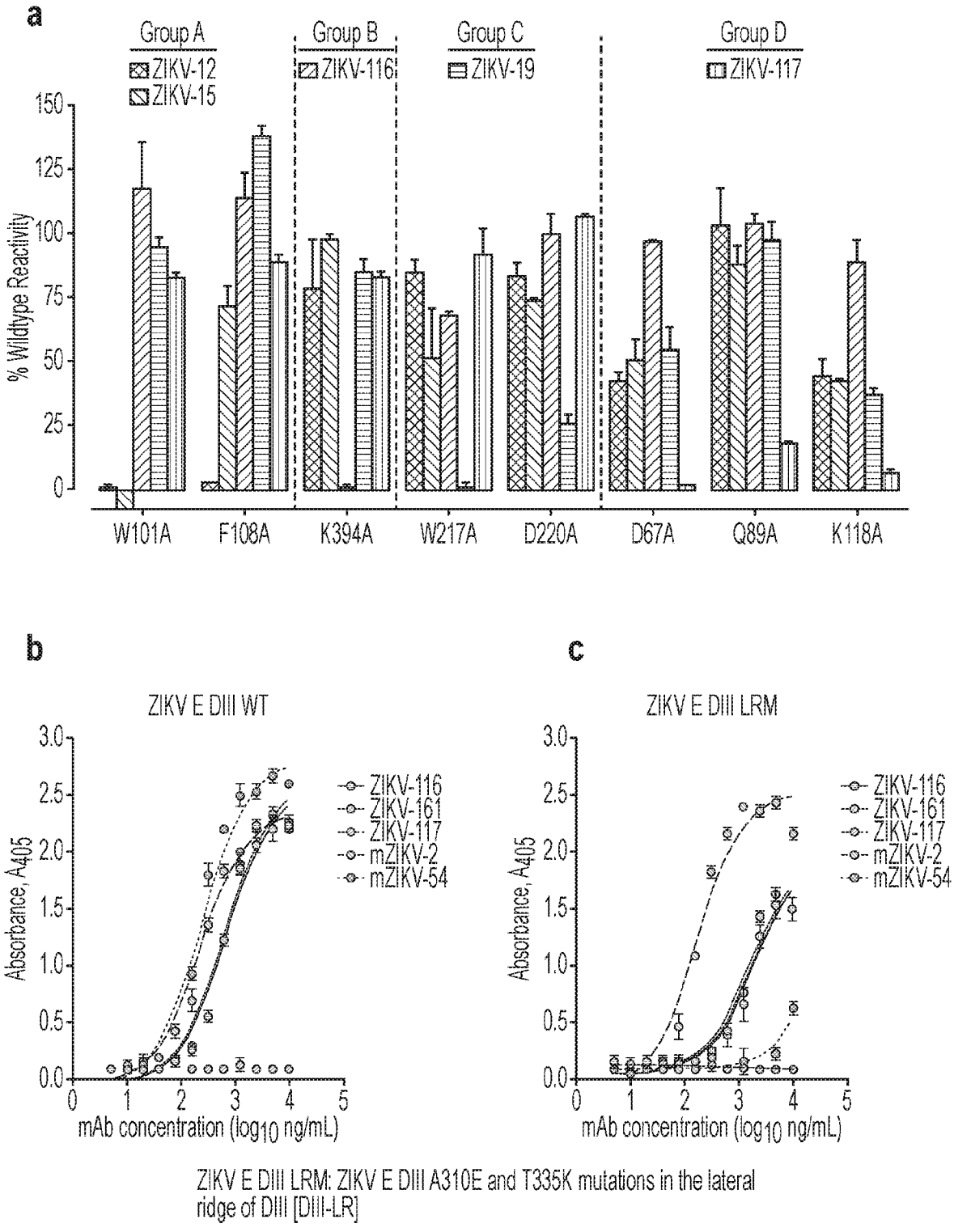


FIG. 5 continued



ZIKV E DIII LRM: ZIKV E DIII A310E and T335K mutations in the lateral ridge of DIII [DIII-LR]

FIGS. 6A-C

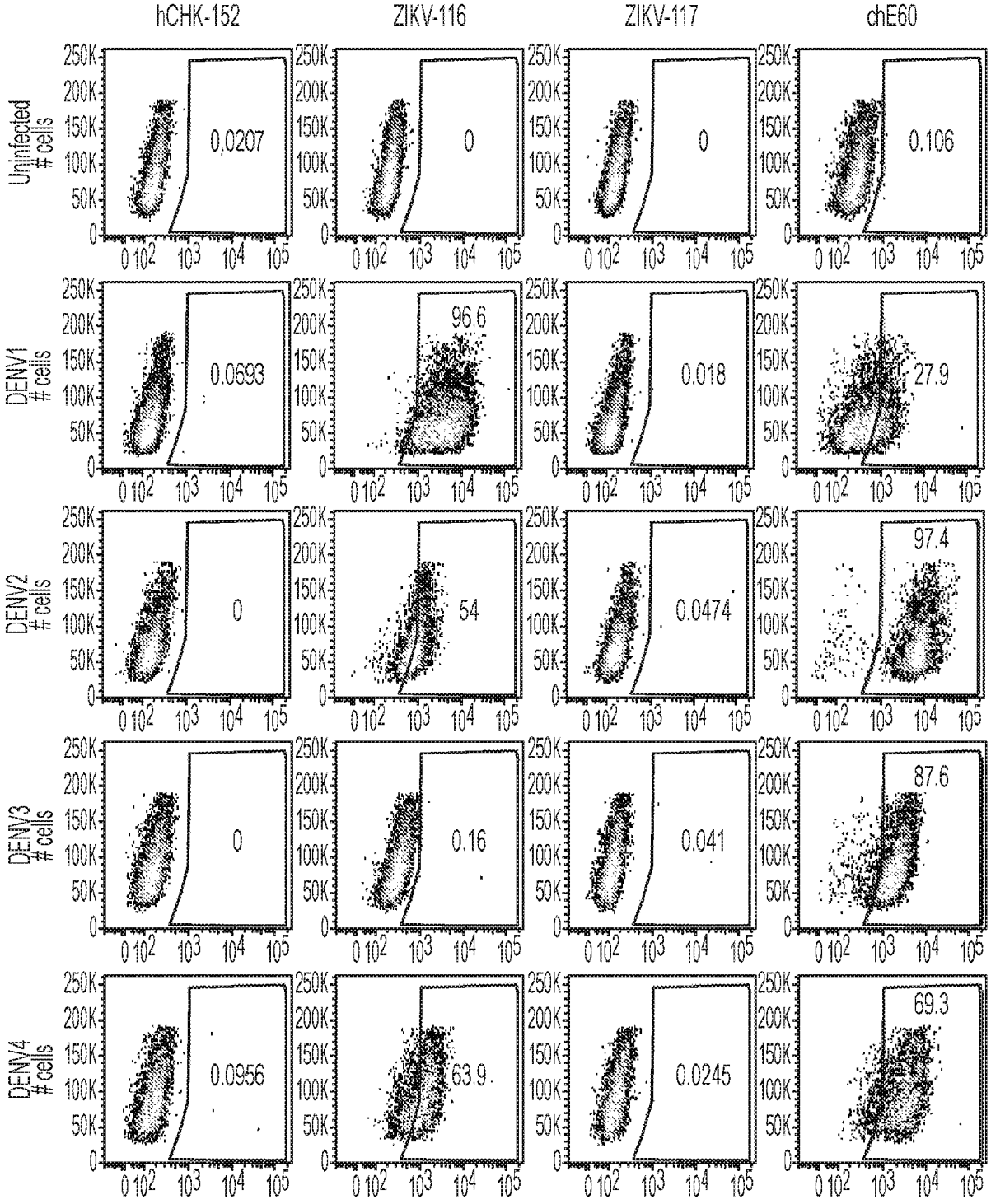

hCHK-152
ZIKV-116
ZIKV-117
chE60
Uninfected # cells
DENV1 # cells
DENV2 # cells
DENV3 # cells
DENV4 # cells
0.0207
0
0
0.106
0.0693
96.6
0.018
27.9
0
54
0.0474
97.4
0
0.16
0.041
87.6
0.0956
63.9
0.0245
69.3

FIG. 7

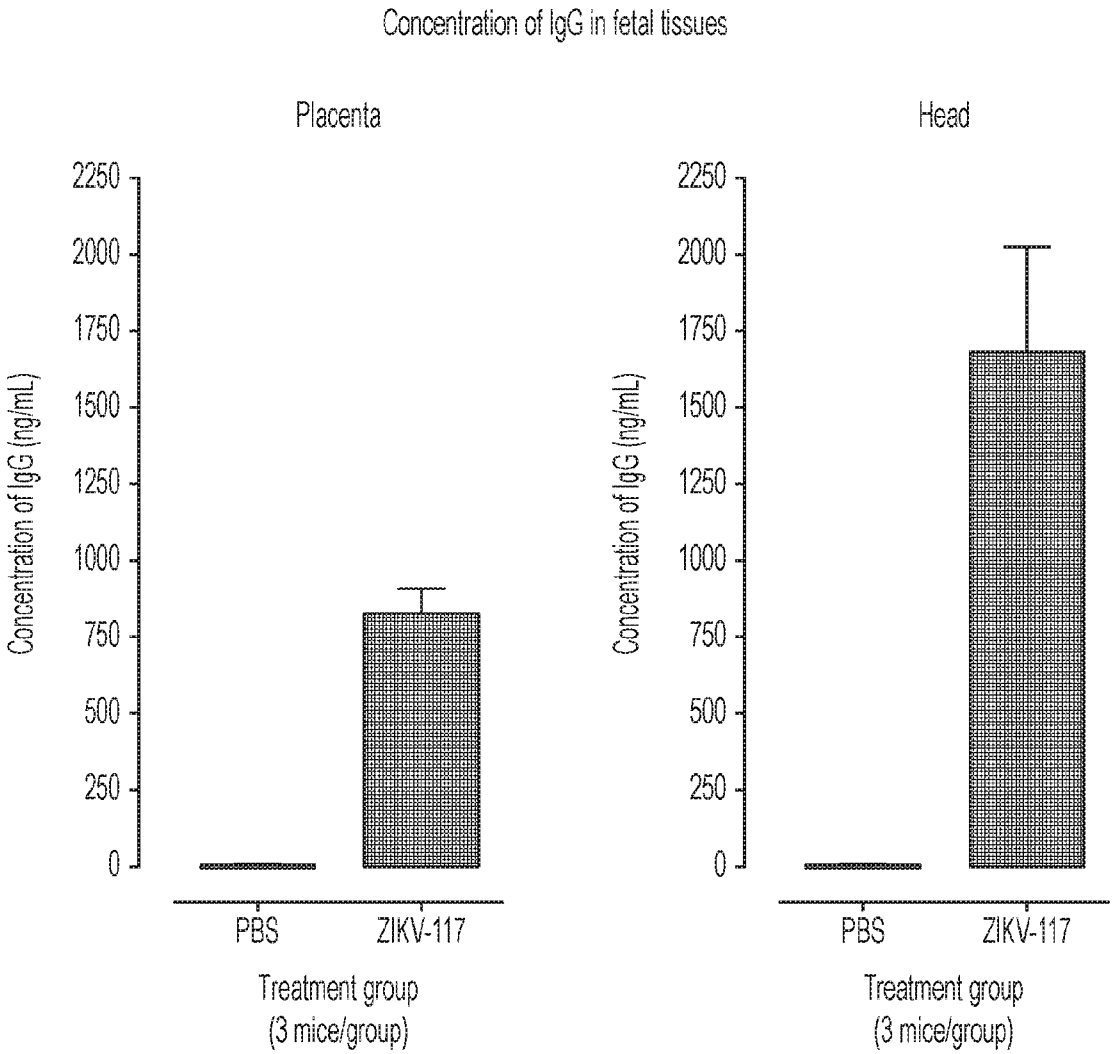

Concentration of IgG in fetal tissues
Placenta
Head
Concentration of IgG (ng/mL)
2250
2000
1750
1500
1250
1000
750
500
250
0
PBS
ZIKV-117
Treatment group
(3 mice/group)

FIG. 8

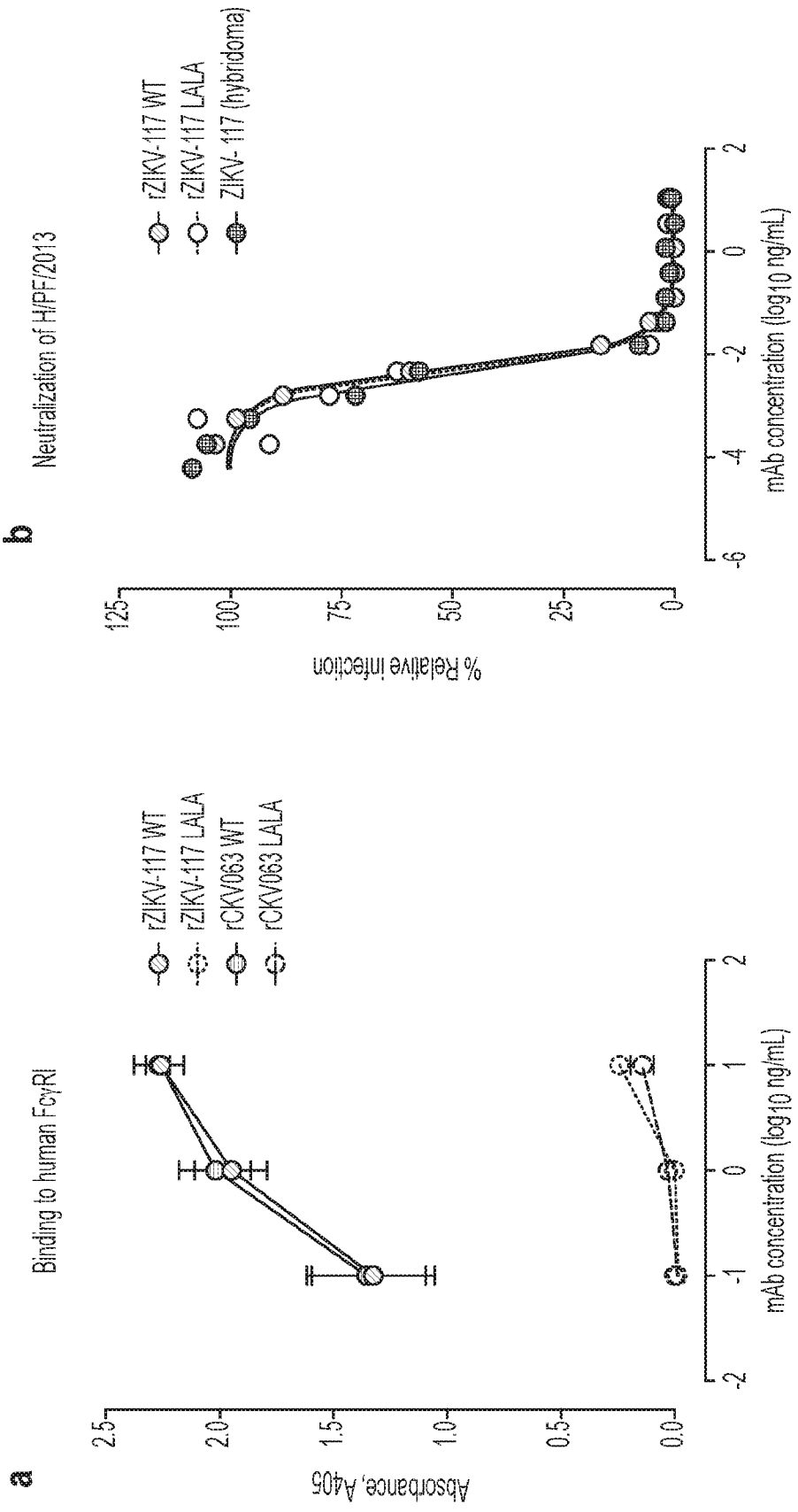

a
Binding to human FcγRI
rZIKV-117 WT
rZIKV-117 LALA
rCKV063 WT
rCKV063 LALA
Absorbance, A405
2.5
2.0
1.5
1.0
0.5
0.0
-2
-1
0
1
2
mAb concentration (log10 ng/mL)
b
Neutralization of H/PF/2013
rZIKV-117 WT
rZIKV-117 LALA
ZIKV- 117 (hybridoma)
% Relative infection
125
100
75
50
25
0
-6
-4
-2
0
2
mAb concentration (log10 ng/mL)

FIG. 9A-B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/59531

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/42; C07K 16/08, 16/10; G01N 33/487, 33/50, 33/53, 33/563, 33/569, 33/577 (2018.01)
CPC - A61K 39/42; C07K 16/08, 16/1081; G01N 33/487, 33/5005, 33/5091, 33/53, 33/563, 33/56983, 33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2010/093335 A1 (NANYANG TECHNOLOGICAL UNIVERSITY) 19 August 2010; abstract; page 2, 4th paragraph; page 3, 5th paragraph; page 4, 1st and 2nd paragraphs; page 5, 5th and 6th paragraphs; page 9, 9th paragraph; page 15, 5th paragraph; page 23, 2nd paragraph; page 36, 5th paragraph; page 41, 6th paragraph; page 43, 1st paragraph; page 47, 5th paragraph; page 68, 5th paragraph	1-2, 13-14, 17-19, 26-31, 36-42, 47-52, 61-62, 65-67, 74-75
A	(SHUKLA, S et al.) Rapid Detection Strategies for the Global Threat of Zika Virus: Current State, New Hypotheses, and Limitations. Frontiers in Microbiology. 24 October 2016, Vol. 7, Article 1685; pages 1-15; page 10, 1st column, 3rd paragraph, and 2nd column, 1st paragraph; Figure 3; DOI: 10.3389/fmicb.2016.01685	1-2
A	(PAULI, NT et al.) Staphylococcus aureus infection induces protein A-mediated immune evasion in humans. The Journal of Experimental Medicine. 17 November 2014, Epub 27 October 2014, Vol. 211, No. 12; pages 2331-2339; page 2332, 1st column, 1st paragraph; DOI: 10.1084/jem.20141404.	1-2, 13-14, 17-19, 26-31, 36-42, 47-52
A	US 2011/0014211 A1 (AZUMA, M et al.) January 20, 2011; paragraph [0031]	1-2, 13-14, 17-19, 61-62, 65-67, 74-75
A	(ANDREWS, SF et al.) Immune history profoundly affects broadly protective B cell responses to influenza. Science Translational Medicine. 2 December 2015, Vol. 7, No. 316; pages 1-12; page 9, 2nd column, 3rd paragraph; DOI: 10.1126/scitranslmed.aad0522	26-31

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 2018 (05.02.2018)	21 FEB 2018
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/59531

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/59531

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 3-12, 15-16, 20-25, 32-35, 43-46, 53-60, 63-64, 68-73
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
-***-Please see supplemental page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Groups I+, Claims 1-2, 13-14, 17-19, 26-31, 36-42, 47-52, 61-62, 65-67, 74-75; SEQ ID NO: 1 (heavy chain nucleotide sequence); SEQ ID NO: 2 (light chain nucleotide sequence); SEQ ID NO: 53 (heavy chain protein sequence); SEQ ID NO: 54 (light chain protein sequence); SEQ ID NO: 119 (HCDR1), SEQ ID NO: 120 (HCDR2), SEQ ID NO: 121 (HCDR3), SEQ ID NO: 228 (LCDR1), DVN (LCDR2), SEQ ID NO: 229 (LCDR3)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/59531

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2015/0086555 A1 (THERACLONE SCIENCES, INC.) 26 March 2015; paragraphs [0020], [0094], [0150], [0153], [0574]	26-31, 36-42, 47-52
A	US 2011/0300156 A1 (VERPLOEGEN, S et al.) 8 December 2011; abstract; paragraphs [0042], [0308], [0318], [0429], [0452], [0525]	36-42, 47-52
A	(MARTINEZ, ME) Preventing Zika Virus Infection during Pregnancy Using a Seasonal Window of Opportunity for Conception. PLoS Biology. 28 July 2016, Vol. 14, No. 7; pages 1-11; abstract; page 2, 2nd paragraph; page 3, 2nd paragraph; DOI: 10.1371/journal.pbio.1002520	61-62, 65-67, 74-75

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/59531

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-2, 13-14, 17-19, 26-31, 36-42, 47-52, 61-62, 65-67, 74-75; SEQ ID NO: 1 (heavy chain nucleotide sequence); SEQ ID NO: 2 (light chain nucleotide sequence); SEQ ID NO: 53 (heavy chain protein sequence); SEQ ID NO: 54 (light chain protein sequence); SEQ ID NO: 119 (HCDR1), SEQ ID NO: 120 (HCDR2), SEQ ID NO: 121 (HCDR3), SEQ ID NO: 228 (LCDR1), DVN (LCDR2), SEQ ID NO: 229 (LCDR3) are directed toward an antibodies binding to and neutralizing Zika virus and methods for use thereof.

The antibody will be searched to the extent that it encompasses SEQ ID NO: 1 (heavy chain nucleotide sequence); SEQ ID NO: 2 (light chain nucleotide sequence); SEQ ID NO: 53 (heavy chain protein sequence); SEQ ID NO: 54 (light chain protein sequence); SEQ ID NO: 119 (HCDR1), SEQ ID NO: 120 (HCDR2), SEQ ID NO: 121 (HCDR3), SEQ ID NO: 228 (LCDR1), DVN (LCDR2), SEQ ID NO: 229 (LCDR3). Applicant is invited to elect additional pair(s) of antibody heavy and light chain sequence(s), with specified SEQ ID NO: for each, and corresponding CDR sequence(s) for each, to be searched. Additional antibody sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 2 (in-part), 13 (in-part), 14 (in-part), 17 (in-part), 18 (in-part), 19 (in-part), 26 (in-part), 27 (in-part), 28 (in-part), 29 (in-part), 30 (in-part), 31 (in-part), 36 (in-part), 37 (in-part), 38 (in-part), 39 (in-part), 40 (in-part), 41 (in-part), 42 (in-part), 47 (in-part), 48 (in-part), 49 (in-part), 50 (in-part), 51 (in-part), 52 (in-part), 61 (in-part), 62 (in-part), 65 (in-part), 66 (in-part), 67 (in-part), 74 (in-part), and 75 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (heavy chain nucleotide sequence); SEQ ID NO: 2 (light chain nucleotide sequence); SEQ ID NO: 53 (heavy chain protein sequence); SEQ ID NO: 54 (light chain protein sequence); SEQ ID NO: 119 (HCDR1), SEQ ID NO: 120 (HCDR2), SEQ ID NO: 121 (HCDR3), SEQ ID NO: 228 (LCDR1), DVN (LCDR2), SEQ ID NO: 229 (LCDR3). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be an antibody encompassing SEQ ID NO: 3 (heavy chain nucleotide sequence); SEQ ID NO: 4 (light chain nucleotide sequence); SEQ ID NO: 55 (heavy chain protein sequence); SEQ ID NO: 55 (light chain protein sequence); SEQ ID NO: 122 (HCDR1), SEQ ID NO: 123 (HCDR2), SEQ ID NO: 124 (HCDR3), SEQ ID NO: 230 (LCDR1), DAS (LCDR2), SEQ ID NO: 231 (LCDR3).

No technical features are shared between the antibody sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a method of detecting a Zika virus infection in a subject comprising: (a) contacting a sample from said subject with an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences; and (b) detecting Zika virus in said sample by binding of said antibody or antibody fragment to a Zika virus antigen in said sample; a method of treating a subject infected with Zika virus, or reducing the likelihood of infection of a subject at risk of contracting Zika virus, comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences; a monoclonal antibody, wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences; a hybridoma or engineered cell encoding an antibody or antibody fragment wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences; a vaccine formulation comprising one or more antibodies or antibody fragments characterized by clone-paired heavy and light chain CDR sequences; a method of protecting the health of a placenta and/ or fetus of a pregnant a subject infected with or at risk of infection with Zika virus comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences; these shared technical features are previously shared by WO 2010/093335 A1 (NANYANG TECHNOLOGICAL UNIVERSITY) (hereinafter 'Nanyang') in view of the publication entitled 'Preventing Zika Virus Infection during Pregnancy Using a Seasonal Window of Opportunity for Conception' by Matinez, M.E. (hereinafter 'Martinez').

Nanyang discloses a method of detecting a Zika virus infection in a subject (a method of detecting a flaviviral infections including Zika virus infection in a subject; page 2, fourth paragraph; page 5, fifth paragraph; page 47, fifth paragraph) comprising: (a) contacting a sample from said subject with an antibody or antibody fragment (comprising: (a) contacting a sample from said subject with an antibody or antibody fragment; page 5, fifth paragraph) having clone-paired heavy and light chain CDR sequences (having 9F12 (clone-paired) heavy and light chain CDR sequences; page 9, ninth paragraph; page 23, second paragraph); and (b) detecting Zika virus in said sample by binding of said antibody or antibody fragment to a Zika virus antigen in said sample ((b) detecting Zika virus in said sample by binding of said antibody or antibody fragment to a Zika virus antigen in said sample; page 5, fifth paragraph; page 47, fifth paragraph); a method of treating a subject infected with Zika virus (a method of treating a subject infected with Zika virus; page 5, sixth paragraph; page 47, fifth paragraph), comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences (comprising delivering to said subject an antibody or antibody fragment having 9F12 (clone-paired) heavy and light chain CDR sequences; page 5, sixth paragraph; page 9, ninth paragraph; page 23, second paragraph; page 47, fifth paragraph); a monoclonal antibody (page 9, ninth paragraph), wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences (9F12 antibody is characterized by clone-paired heavy and light chain CDR sequences; page 9, ninth paragraph; page 23, second paragraph); a hybridoma (page 61, fifth paragraph) encoding an antibody or antibody fragment wherein the antibody or antibody fragment is characterized by clone-paired heavy and light chain CDR sequences (encoding an antibody wherein the antibody is characterized by clone-paired heavy and light chain CDR sequences; page 9, ninth paragraph; page 23, second paragraph; page 61, fifth paragraph); a vaccine formulation (page 4, third paragraph; page 39, second paragraph) comprising one or more antibodies or antibody fragments characterized by clone-paired heavy and light chain CDR sequences (comprising one or more antibodies or antibody fragments characterized by clone-paired heavy and light chain CDR sequences; page 9, ninth paragraph; page 23, second paragraph); a method of preventing Zika virus (page 4, third paragraph), comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences (comprising delivering to said subject an antibody or antibody fragment having clone-paired heavy and light chain CDR sequences; page 4, third paragraph; page 9, ninth paragraph; page 23, second paragraph).

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from Previous Supplemental Box-***-

Nanyang does not disclose protecting the health of a placenta and/ or fetus of a pregnant a subject infected with or at risk of infection with Zika virus.

Martinez discloses protecting the health of a placenta and/ or fetus of a pregnant a subject infected with or at risk of infection with Zika virus (vaccines for preventing transmission to pregnant women; abstract; page 2, second paragraph; page 3, second paragraph).

It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the disclosure of Nanyang, to include protecting the health of a placenta and/ or fetus of a pregnant a subject infected with or at risk of infection with Zika virus, as disclosed by Martinez, in order to provide an effective method to prevent Zika infection and damage to fetuses.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Nanyang and Martinez references, unity of invention is lacking.

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摘要(译)

本公开涉及结合并中和寨卡病毒的抗体及其使用方法。