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(71) Demandeur/Applicant:
TYCHAN PTE. LTD., SG

(72) Inventeurs/Inventors:
BUDIGI, YADUNANDA, KUMAR, SG;
LEE, DESBBIE CHING PING, SG;
CHIONH, YOK HIAN, SG;
MCBEE, MEGAN EARLEY, SG

(74) Agent: SMART & BIGGAR LLP

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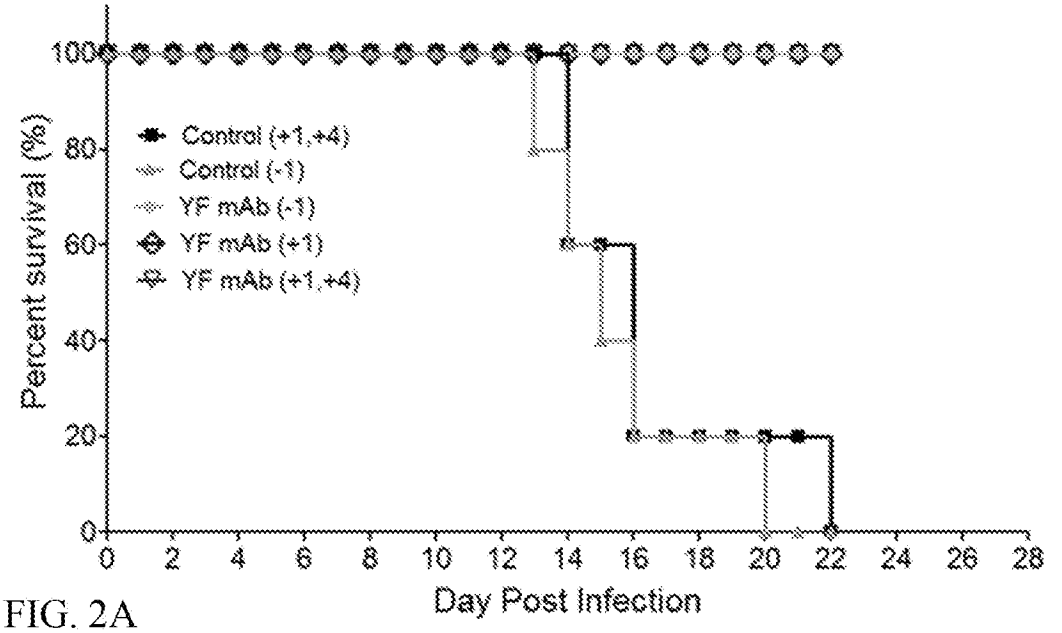

FIG. 2A

(57) Abrégé/Abstract:
The present disclosure is based, at least in part, on the discovery of antibodies that specifically bind YFV and/or neutralize the virus. Compositions and methods related to such antibodies or antigen-binding portions thereof are provided.

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(71) Applicant: **TYCHAN PTE. LTD.** [SG/SG]; 80 Robinson Road, #17-02, Singapore 068898 (SG).

(72) Inventor; and
(71) Applicant: **SASISEKHARAN, Ram** [US/US]; 4 Duval Way, Bedford, MA 01730 (US).

(72) Inventor: **BUDIGI, Yadunanda, Kumar**; 119 Sunset Way, #03-01, Clementi Park Condominium, Singapore 597151 (SG).

(74) Agent: **VATLAND, Janice, A.** et al.; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

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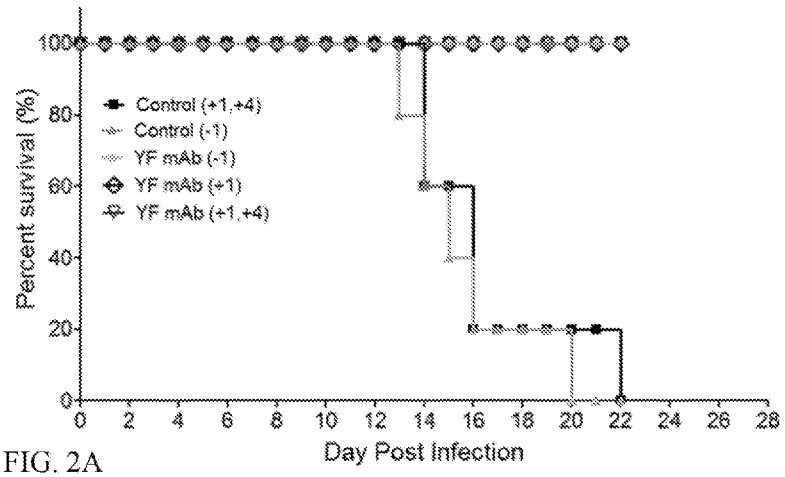

FIG. 2A

(57) **Abstract:** The present disclosure is based, at least in part, on the discovery of antibodies that specifically bind YFV and/or neutralize the virus. Compositions and methods related to such antibodies or antigen-binding portions thereof are provided.

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METHODS AND COMPOSITIONS FOR TREATING YELLOW FEVER

RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. §119 of United States Provisional Application 62/656,352, filed April 11, 2018, the entire contents of which are incorporated herein by reference.

BACKGROUND

Yellow fever is an acute viral haemorrhagic disease caused by the Yellow Fever Virus (YFV). In humans the primary vector for YFV transmission is the mosquito *Aedes aegypti*, which transmits several other viruses including Dengue and Zika Virus. YFV is endemic in tropical and subtropical areas of Africa and Central and South America. Infected individuals develop a wide range of symptoms from asymptomatic infection to acute viral hemorrhagic disease (10-15%), of which there is a 50% fatality rate. Since the 1930s, a live attenuated vaccine has been available on the market. However, a global shortage in supplies have hampered efforts to prevent and control YFV outbreaks. The most recent indication of this can be seen in Brazil, where the YFV has already infected over 700 people and claimed more than 200 lives since the outbreak began in 2017. Currently, there is no YFV therapy available to treat those who have not been vaccinated or in which the vaccine has not provided a protective effect. There is a need for YFV therapies.

SUMMARY OF THE INVENTION

The present disclosure is based, at least in part, on the discovery of antibodies that specifically bind YFV and/or neutralize the virus. For example, the antibodies, or antigen-binding portions thereof, can prevent YFV from infecting cells in some embodiments. In an embodiment, the antibodies, or antigen-binding portions thereof, of any one of the compositions or methods provided herein, specifically bind to engage the key epitope residues (N106, K93 and K104) that are the most solvent exposed and antibody-accessible on the E-protein. In an embodiment, the antibodies, or antigen-binding portions thereof, of any one of the compositions or methods provided herein, have energetically favorable paratope (CDR) interactions around these key epitope residues and/or are characterized by a threshold minimum binding energy.

Accordingly, one aspect of the present disclosure provides an antibody or antigen-binding portion thereof that specifically binds to the E-protein or an Envelope Protein

Domain II (E-DII) epitope of Yellow Fever Virus. In an embodiment of any one of the compositions or methods provided herein, the E-protein or E-DII epitope comprises an asparagine at position 106, a lysine at position 93, and a lysine at position 104 of Yellow Fever Virus E-protein.

In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion comprises the three CDRs of a heavy chain variable region (VH). In an embodiment of any one of the methods or compositions provided, the three CDRs are those found in any one of the VH sequences set forth herein, such as in Table 1 (e.g., SEQ ID. NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36). In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises the three CDRs of a light chain variable region (VL). In an embodiment of any one of the methods or compositions provided, the three CDRs are those found in any one of the VL sequences set forth herein, such as in Table 2 (SEQ ID. NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73). In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion comprises the three CDRs of any one of the VH sequences set forth herein, such as in Table 1 (e.g., SEQ ID. NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36) and the three CDRs of any one of the VL sequences set forth herein, such as in Table 2 (SEQ ID. NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73). In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises the three CDRs of the VH sequence and the three CDRs of the VL sequence of any one of the specific combinations of VH sequences and VL sequences as set forth in Table 3. Thus, in an embodiment of any one of the methods or compositions provided herein, the antigen-binding portion thereof is an antigen-binding portion of such an antibody.

Provided herein in one aspect is a nucleic acid encoding the three CDRs of any one of the VH sequences provided herein, such as provided directly above, and/or the three CDRs of any one of the VL sequences provided herein, such as provided directly above.

In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises any one of the VH amino acid sequences provided herein, such as set forth in any one of SEQ ID. NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and

36. In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises any one of the VL amino acid sequences provided herein, such as set forth in any one of SEQ ID. NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73. In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises any one of the VH amino acid sequences provided herein, such as set forth in any one of SEQ ID. NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36 and any one of the VL amino acid sequences provided herein, such as set forth in any one of SEQ ID. NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73. In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises any one specific combination of the combinations of VH amino acid sequences and VL amino acid sequences provided herein, such as set forth in Table 3.

Provided herein in one aspect is a nucleic acid encoding any one of the VH sequences provided herein, such as provided directly above, and/or any one of the VL sequences provided herein, such as provided directly above.

In an embodiment of any one of the methods or compositions provided herein, the antibody or antigen-binding portion thereof comprises three CDRs that have at least 90%, 95%, 96%, 97%, 98% or 99% identity to the three CDRs of any one of the VH sequences set forth herein, such as in Table 1 (e.g., SEQ ID. NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36). In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises three CDRs that have at least 90%, 95%, 96%, 97%, 98% or 99% identity to the three CDRs of any one of the VL sequences set forth herein, such as in Table 2 (SEQ ID. NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73). In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion comprises three CDRs that have at least 90%, 95%, 96%, 97%, 98% or 99% identity to any one of the VH sequences set forth herein, such as in Table 1 (e.g., SEQ ID. NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36) and three CDRs that have at least 90%, 95%, 96%, 97%, 98% or 99% identity to any one of the VL sequences set forth herein, such as in Table 2 (SEQ ID. NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56,

57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73). In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises three CDRs that have at least 90%, 95%, 96%, 97%, 98% or 99% identity to the VH sequence and three CDRs that have at least 90%, 95%, 96%, 97%, 98% or 99% identity to the VL sequence of any one of the specific combinations of VH sequences and VL sequences provided herein, such as set forth in Table 3. Thus, in an embodiment of any one of the methods or compositions provided herein, the antigen-binding portion thereof is an antigen-binding portion of such an antibody.

Provided herein in one aspect is a nucleic acid encoding the three CDRs of any one of the VH sequences provided herein, such as provided directly above, and/or the three CDRs of any one of the VL sequences provided herein, such as provided directly above.

In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises a VH amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to any one of the VH amino acid sequences provided herein, such as set forth in any one of SEQ ID. NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36. In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises a VL sequence that has at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to any one of the VL amino acid sequences provided herein, such as set forth in any one of SEQ ID. NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73. In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises a VH amino acid sequences that has at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to any one of the VH amino acid sequences provided herein, such as set forth in any one of SEQ ID. NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36 and a VL sequence that has at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to any one of the VL amino acid sequences provided herein, such as set forth in any one of SEQ ID. NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73. In an embodiment of any one of the methods or compositions provided, the antibody or antigen-binding portion thereof comprises a VH sequence and a VL sequence that each independently have at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the VH and VL sequences of any one specific

combination of the combinations of VH amino acid sequences and VL amino acid sequences provided herein, such as set forth in Table 3.

Provided herein in one aspect is a nucleic acid encoding the VH sequence as provided directly above and/or the VL sequences as provided directly above.

In an embodiment of any one of the methods or compositions provided herein, the antigen-binding portion thereof is an antigen-binding portion of any one of the antibodies provided herein.

Any one of the antibodies described herein can be a full-length antibody. The antibody or antigen-binding portion thereof can be human, humanized, or chimeric in an embodiment of any one of the methods or compositions provided herein. The antibody or antigen-binding portion thereof can be a single-chain antibody in an embodiment of any one of the methods or compositions provided herein. Any of the antibodies described herein can be either monoclonal or polyclonal. These two terms do not limit the source of an antibody or the manner in which it is made. The antibody or antigen-binding portion thereof can be a monoclonal antibody or antigen-binding portion thereof in an embodiment of any one of the methods or compositions provided herein. Further, the antigen-binding portion thereof can be an scFv, a F(ab')2 fragment, a dAb fragment, a Fab fragment, a Fab' fragment, an Fv, or a disulfide-linked Fv fragment in an embodiment of any one of the methods or compositions provided herein. The antibody or antigen-binding portion thereof may be a single domain antibody, a diabody, a multispecific antibody, a bispecific antibody, or a dual-specific antibody. The antibody or antigen-binding portions thereof may be an isolated antibody or antigen-binding portion thereof in an embodiment of any one of the methods or compositions provided herein.

Further disclosed are methods of treating Yellow Fever, or a disease or condition associated with Yellow Fever Virus, by administering a therapeutically effective amount of one or more antibodies or antigen-binding portions thereof that specifically bind YFV, to a subject in need of such treatment. In an embodiment of any one of the methods provided herein, the subject has not been vaccinated for the YFV or in which a YFV vaccination did not provide an adequate protective effect. In an embodiments of any one of the method or compositions provided herein, the antibodies or antigen-binding portions thereof are any one or more of the antibodies or antigen-binding portions thereof described herein. In an embodiment of any one of the methods or compositions provided herein, the antibody or antigen-binding portion thereof specifically binds to the E-protein or an E-DII epitope of YFV. In an embodiments of any one of the methods or composition provided herein, the

antibody or antigen-binding portion thereof specifically binds to residues 93, 104, and 106 of the E-protein or E-DII epitope of Yellow Fever Virus E-protein. In an embodiment of any one of the methods or compositions provided herein, the amount of the antibody or antigen-binding portion thereof is effective in reducing one or more symptoms of Yellow Fever in the subject. Any one of the anti-YFV antibodies or antigen-binding portions thereof may be administered systemically, *e.g.*, *via* an enteral route or via a parenteral route, in any one of the methods provided herein.

The subject to be treated in any one of the methods described herein can be a patient (*e.g.*, a human patient) who has or is suspected of having Yellow Fever, or a disease or condition associated with Yellow Fever Virus. In an embodiment of any one of the methods provided herein, the subject is a human patient who has or is suspected of having acute viral hemorrhagic disease.

Also provided herein in some aspects are (a) pharmaceutical compositions for use in treating Yellow Fever or a disease or condition associated with Yellow Fever Virus (e.g., acute viral hemorrhagic disease) in a subject, the pharmaceutical composition comprising any one or more of the antibodies or antigen-binding portions thereof described herein and a pharmaceutically acceptable carrier; and (b) uses of the just-described antibodies or antigen-binding portions thereof in medicaments and/or in the manufacturing of a medicament for treatment of Yellow Fever or a disease or condition associated with Yellow Fever Virus in a subject.

Also provided herein in some aspects are methods for producing the antibodies or antigen-binding portions thereof, nucleic acids encoding any one of the antibodies or antigen-binding portions thereof, vectors that can comprise any one or more of the nucleic acids provided herein, and related host cells.

In one aspect the method for producing the antibodies or antigen-binding portions thereof is any one of the methods described herein. In one embodiment, the method comprises considering the distinct domain proximal structural regions present on the viral assembly (e.g. inter-chain interfaces near the icosahedral axes of symmetry) and selecting promising Fv scaffolds. Conventional epitope prediction methods use domain structures to predict epitope surface regions embedded within the domain region. However, neutralizing flaviviral antibodies can recognize quaternary epitope surfaces (spanning two or more E protein chains). Thus, traditional methods do not incorporate valuable information.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 shows an example an in vivo study design of efficacy of engineered mAbs against YF-17D-204 in a mouse model of infection. Efficacy of designed mAbs was tested in a lethal model of Yellow fever infection in AG129 mice. Protective efficacy of mAbs was tested in prophylaxis or as therapy.

Figs. 2A-2B shows in vivo efficacy of designed mAb against Yellow Fever Virus. Fig. 2A shows the survival curve of YF-17D infected AG129 mice treated with mAb at a dose of 10mg/kg as prophylaxis (-1) or as Therapy (+1 and +1,+4). Fig. 2B shows blood viral titers of yellow fever virus in treated animals compared with control groups at Days 4 and 6 post infection. The administration of mAb resulted in complete protection compared to control groups (Fig. 2A). Administration of mAb also led to greater than 2 Log10 reduction in viremia at days 4 and 6 post infection (Fig. 2B).

DETAILED DESCRIPTION

The following description is merely intended to illustrate various embodiments of the invention. As such, specific embodiments discussed herein are not to be construed as limitations to the scope of the invention. It will be apparent to one skilled in the art that various changes or equivalents may be made without departing from the scope of the invention.

Various aspects of the disclosure relate to antibodies, antigen-binding portions thereof, and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors and host cells for making such antibodies and fragments. Provided herein are antibodies and/or antigen-binding portions thereof that specifically bind Yellow Fever Virus (YFV) and/or neutralize the virus. In some embodiments, the antibody or antigen-binding portion thereof binds to an E-DII epitope of the Yellow Fever Virus and prevents the virus from infecting cells. In some embodiments, the antibody or antigen-binding portion thereof binds to an E-DII epitope comprising an asparagine at position 106, a lysine at position 93 and/or a lysine at position 104 of Yellow Fever Virus E-protein. The antibodies and antigen-binding portions, as provided herein, in some embodiments, are used to treat or prevent Yellow Fever Virus infection in a subject.

An antibody (interchangeably used in plural form), as used herein, broadly refers to an immunoglobulin (Ig) molecule or any functional mutant, variant, or derivation thereof. It is desired that functional mutants, variants, and derivations thereof, as well as antigen-binding portions, retain the essential epitope binding features of an Ig molecule.

Antibodies are capable of specific binding to a target through at least one antigen

recognition site, located in the variable region of the immunoglobulin molecule. Generally, an intact or full-length antibody comprises two heavy chains and two light chains. Each heavy chain contains a heavy chain variable region (V_H) and a first, second and third constant regions (C_H1, C_H2 and C_H3). Each light chain contains a light chain variable region (V_L) and a constant region (CL). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FRl, CDRl, FR2, CDR2, FR3, CDR3, FR4. A full-length antibody can be an antibody of any class, such as IgD, IgE, IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "antigen-binding portion refer to a portion or region of an intact or full-length antibody molecule that can bind specifically to a target. Preferably, antigen-binding portions provided herein retain the ability to specifically bind to YFV. An antigen-binding portion may comprise the heavy chain variable region (VH), the light chain variable region (VL), or both. Each of the VH and VL typically contains three complementarity determining regions CDR1, CDR2, and CDR3.

Examples of antigen-binding portions include, but are not limited to: (1) an Fab fragment, which can be a monovalent fragment having a VL- CL chain and a VH-CH chain; (2) an $F(ab')_2$ fragment, which can be a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region, i.e. a dimer of Fab; (3) an Fv fragment having the VL and VH domains of a single arm of an antibody; (4) a single chain Fv (scFv), which can be a single polypeptide chain composed of a VH domain and a VL domain through a peptide linker; (5) a $(scFv)_2$, which can comprise two VH domains linked by a peptide linker and two VL domains, which are associated with the two VH domains via disulfide bridges; 6) a Fd fragment consisting of the VH and CHI domains; (7) a dAb fragment, which comprises a single variable domain; and (8) an isolated complementarity determining region (CDR).

Furthermore, although the two domains of the Fv fragment, VL and VH, can be coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2: 1121-1123). Diabodies are also encompassed within the term "antigen-binding portion".

The term "human antibody" refers to antibodies having variable and constant regions corresponding substantially to, or derived from, antibodies obtained from human subjects, e.g., encoded by human germline immunoglobulin sequences or variants thereof. The human antibodies described herein may include one or more amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). Such mutations may present in one or more of the CDRs, particularly CDR3, or in one or more of the framework regions. In some embodiments, the human antibodies may have at least one, two, three, four, five, or more positions replaced with an amino acid residue that is not encoded by the human germline immunoglobulin sequence. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom H. R., (1997) TIB Tech. 15:62-70; Azzazy H., and Highsmith W. E., (2002) Clin. Biochem. 35:425-445; Gavilondo J. V., and Larrick J. W. (2002) BioTechniques 29: 128-145; Hoogenboom H., and Chames P. (2000) Immunology Today 21:371-378),

antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann S-A., and Green L. L. (2002) Current Opinion in Biotechnology 13:593-597; Little M. et al (2000) Immunology Today 21:364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions as defined above. In certain embodiments, however, such recombinant human antibodies may be subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies may be sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

Some embodiments of the disclosure provide fully human antibodies capable of binding the E-DII epitope of Yellow Fever Virus. In some embodiments, the E-DII epitope comprises an asparagine at position 106, a lysine at position 93, and a lysine at position 104 of Yellow Fever Virus E-protein.

The protein sequences for the various VH and VL regions are displayed in Table 1 and Table 2 respectively.

Table 1. Protein sequence for variable heavy chains

>VH.01; SEQ ID NO: 1
EVKLVESGGGLVKPGGSLKLSCAASGFTFTNYAMSWVRQTPEKRLEWVASISSGHTPYYPDSVKGRFTISRDNA RNILFLQMSSLRSEDTAMYYCARGDYYGSVYSAMDYWGQGTSVTVSS
>VH.11; SEQ ID NO: 2
EVQLVESGGGLVQPGRSLRLSCAASGFTFTNYAMSWVRQAPGKGLEWVSSISSGHTPYYPDSVKGRFTISRDNA KKSLYLQMNSLRAEDTALYYCARGDYYGSVYSAMDYWGQGTTVTVSS
>VH.21; SEQ ID NO: 3
EVQLVESGGGLVQPGRSLRLSCAASGFTFTDYYMSWVRQAPGKGLEWVSSISSGHTPYYPDSVKDRFTISRDNA KKSLYLQMNSLRAEDTALYYCARGDYYGTVYSAMDYWGQGTTVTVSS
>VH.31; SEQ ID NO: 4
EVQLVESGGGLVQPGRSLRLSCAASGYAFTNYAMSWVRQAPGKGLEWVSSISSGHTPYYPDTVKGRFTISRDN AKKSLYLQMNSLRAEDTALYYCARGDYYGSSYSAMDYWGQGTTVTVSS
>VH.3.11; SEQ ID NO: 5
EVQLVESGGGLVQPGRSLRLSCAASGYAFTNYGVNWVRQAPGKGLEWVSSISSGGSTYYPDSVKGRFTISRDNA KKSLYLQMNSLRAEDTALYYCARHDYYGSSY-AMDYWGQGTTVTVSS
>VH.3.21; SEQ ID NO: 6
EVQLVESGGGLVQPGRSLRLSCAASGYAFTNYGVNWVRQAPGKGLEWVSSISSGGSTYYPDSVKGRFTISRDNA KKSLYLQMNSLRAEDTALYYCARSHYYGSSYDAMDYWGQGTTVTVSS
>VH.41; SEQ ID NO: 7
EVQLVESGAEVKKPGSSVKVSCKASGFTFTNYAMSWVRQAPGQGPEWMGSISSGHTPYYPDSVKGGRVTITA

DDFAGTVYMELSSLRSEDTAMYYCRGDYYGSVYSAMDYWGKGTTVTVSS
>VH.51; SEQ ID NO: 8
EVQLVESGAEVKKPGSSVKVSCKASGFTFTNYAMSWVRQAPGQGPEWMGSISSGHTPYYPDSVKGRVTITAD DFAGTVYMELSSLRSEDTAMYYCARGDYYGSVYSAMDYWGKGTTVTVSS
>VH.61; SEQ ID NO: 9
QVQLVQSGAEVKKPGASVKVSCKAGFTFTNYAMSWVRQAPEQGLEWMGSISSGHTPYYPDSVKGRVTMTAD TSTNTAYMELRSLRSDDTAVYYCARGDYYGSVYSAMDYWGQGTLVTVSS
>VH.71; SEQ ID NO: 10
EVQLVESGGGLVQPGGSLRLSCSASGFTFTNYAMSWVRQAPGKGLEYVSSISSGHTPYYPDSVKGRFTISRDNSK NTLYFEMNSLRPEDTAVYYCVRGDYYGSVYSAMDYWGQGTTVTVSS
>VH.1; SEQ ID NO: 11
ASVLSEVQLQQSGPELVKPGASVKLSCKTSENTFTEYTMHWVKQSHGKSLEWIGGIDPNNGGTNYNQKFKGK ATLTVDKSSNTAYMELRSLTSEDSAVYYCGRRDYYALDYWGQGTSVTVAS
>VH.2; SEQ ID NO: 12
QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKLRPGQGFEWIGDINPNNGGPSYNEKFKRKATLTV DTSSSTAYMQLSSLTSEDSAVYYCTIDDGYRFGYWGQGTLVTVSA
>VH.3; SEQ ID NO: 13
QVQLQQSGSELMKPGASVQISCKATGYTFSDYWIEWVKQRPGHGLEWIGDILCGTGRTRYNEKLKAMATFTA DTSSNTAFMQLSSLTSEDSAVYYCARSASYGDYADYWGHGTTLTVSS
>VH.4; SEQ ID NO: 14
AQLQQSGTGLARPGASVKLSCKASGYTFTSYGISWVTQRAGQGLEWIGVIYPRSGNTYYNEKFRGKATLTADKS SSSAYMELRGLTAEDSAVYFCARENYGSVYWGQGTTLTVSS
>VH.5; SEQ ID NO: 15
EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHWVKQRPEQGLEWIGRIDPANGYSKYDPKFQGKATITAD TSSNAAYLQLSSLTSEDTAVYFCARDYEGFAYWGQGTLVTVSS
>VH.6; SEQ ID NO: 16
EVQLVESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVIWYDGSKTYYGDSVKGRFTISK DNSKKMVNLQMDSLGVEDTAFYYCARGIAGGWAFWGIDLWGQGTLVTVSS
>VH.7; SEQ ID NO: 17
EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMHWVKQRPEQGLEWIGRIDPANGYSKYDPKFQGKATITAD TSSNAAYLQLSSLTSEDTAVYFCARDYEGFAYWGQGTLVTVSS
>VH.8; SEQ ID NO: 18
EVQLVQSGAEVRKPGASTKVSCKASGYTFTHYYMHWVRQAPGQGLEWMGIINPSGGSTTYAQKLQGRVTMT RDTSTSTVYMELSSLRSEDTAVYYCARDWGSNYVWGSYPKYWGQGTLVTVSS
>VH.9; SEQ ID NO: 19
EVQLVESGGGLVQPGRSLRLSCAASGFTFNDYAMHWVRQAPGKGLEWVSTISWNSGSIGYADSVKGRFTISR DNAKKSLYLQMNSLRAEDTALYYCAKDIQYGNYYYGMDVWGQGTTVTVSS
>VH.10; SEQ ID NO: 20
DVQLVEPGAELVQPGASVKMSCKASGYTFSSYWINWEKQRPGKGLEWIGNIYPGSGTVNYDDKFKSKATLTID TSSNTAYMQLSSLTSEDSAVYYCTRGGSHAMDYWGQGTSVTVSS
>VH.11; SEQ ID NO: 21
EVQLVESGGGLVRPGGSLRLSCAASGFSYSNHWMHWVRQAPGKGLVWVSRINSDGSTRNYADFVKGRFTISR DNAENTLYLEMNSLTADDTAVYYCVRDGVRFYYDSTGYYPDSFFKYGMDVWGQGTTVTVSS
>VH.12; SEQ ID NO: 22
EVQLVESGGGLVRPGGSLRLSCAASGFSYSNHWMHWVRQAPGKGLVWVSRINSDGSTRNYADFVKGRFTISR DNAENTLYLEMNSLTADDTAVYYCVRDGVRFYYDSTGYYPDSFFKYGMDVWGQGTTVTVSS
>VH.13; SEQ ID NO: 23
EVKLVESGGGLVLPGGSLRLSCATSGFTFTDYYMTWVRQPPGKALEWLGFIGNKANDYTTEYSASVKGRFTISR DDSQSILYLQMSTLRAEDRATYYCATVYGNYPYFDVWGAGTTVAVSS
>VH.14; SEQ ID NO: 24

EVQLVESGAEVKKPGSSVKVSCKASGGTFNNYAISWVRQAPGQGLEWMGGIIPIFGGANYAQKFQGRVTITAD RSTSTVYMELSGLRSEDTAVYYCARRPQSIFDWNFDLWGRGTLVTVSSAGTKGPS
>VH.15; SEQ ID NO: 25
EVQLVESGGGLVQPGGSLKLSCAASGFTFSSHWMHWVRQAPGKGLVWVSRTNSDGSSTSYADSVKGRFMIS RDNSKNTVYLHMNGLRAEDTAVYFCARDGVRYYYDSTGYYPDNFFQYGLDVWGQGTTVTVSSA
>VH.16; SEQ ID NO: 26
EVQLQQSGPELVKPGASVKISCKASGYTFTDYNMHWVKQSHGKSLEWVGYTYPYNGGIGYNQKFKSKATLTLD NSSRTAYMELRSLTSEDSAVYYCVRRGYRYDGAHFDYWGQGTTLTVSS
>VH.17; SEQ ID NO: 27
DVQLVESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTLHYADTVKGRFTISRD NPKNTLFLQMTSLRSEDTAMYYCARWGNYPHYAMDYWGQGTSVTVSS
>VH.18; SEQ ID NO: 28
QVQLVQSGAEVKKPGASVKVSCKAGFNIKDVYMSWVRQAPEQGLEWMGRIDPENGDTKYDPKLQGRVTMT ADTSTNTAYMELRSLRSDDTAVYYCARGWEGFAYWGQGTLVTVSS
>VH.19; SEQ ID NO: 29
EVQLVESGGGLVQPGGSLRLSCSASGFTFSTYSMHWVRQAPGKGLEYVSAITGEGDSAFYADSVKGRFTISRDN SKNTLYFEMNSLRPEDTAVYYCVGGYSNFYYYYTMDVWGQGTTVTVSSG
>VH.20; SEQ ID NO: 30
EVQLVESGGGLVRPGGSLRLSCAASGFSYSNHWMHWVRQAPGKGLVWVSRINSDGSTRNYADFVKGRFTISR DNAENTLYLEMNSLTADDTAVYYCVRDGVRFYYDSTGYYPDSFFKYGMDVWGQGTTVTVSS
>VH.21; SEQ ID NO: 31
DVQLVESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTLHYADTVKGRFTISRD NPKNTLFLQMTSLRSEDTAMYYCARWGNYPHYAMDYWGQGTSVTVSS
>VH.22; SEQ ID NO: 32
QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLT ADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSA
VH.23; SEQ ID NO: 33
QVQLVQSGAEVKKPGSSVKVSCKASGYAFSYSWINWVRQAPGQGLEWMGRIFPGDGDTDYNGKFKGRVTIT ADKSTSTAYMELSSLRSEDTAVYYCARNVFDGYWLVYWGQGTLVTVSS
>VH.24; SEQ ID NO: 34
EVQLVESGAEVKKPGSSVKVSCKASGGPFRSYAISWVRQAPGQGPEWMGGIIPIFGTTKYAPKFQGRVTITADD FAGTVYMELSSLRSEDTAMYYCAKHMGYQVRETMDVWGKGTTVTVSS
>VH.25; SEQ ID NO: 35
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLEWVSSIYPSGGITFYADTVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARIKLGTVTTVDYWGQGTLVTVSS
>VH.26; SEQ ID NO: 36
QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWWDDKKHYNPSLKDRLTISKD TSKNQVVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTTVTVSS

Table 2. Protein sequence for variable light chains

>VL.0; SEQ ID NO: 37
DIRMTQSPSSMYASLGERVTVTCKASQDINSYLSWLQQKPGKSPKTLIYRANRLFDGVPSRFSGSGSGQDYSLTIS SLEYEDMGIFYCLQYDEFPFTFGSGTKLEIK
>VL.111; SEQ ID NO: 38
DIRMTQSPSSLSASVGDRVTITCKASQDINSYLSWLQQKPGKSPKTLIYRANRLFDGVPSRFSGSGSGTDFTLTISS LQPEDFAIYYCLQYDEFPFTFGSGTKVEIK
>VL.1.11; SEQ ID NO: 39
DIRMTQSPSSLSASVGDRVTITCKASQDINSYLNWLQQKPGKSPKTLIYRVNRLVDGVPSRFSGSGSGTDFTLTIS SLQPEDFAIYYCLQYDEFPYTFGSGTKVEIK

>VL.1.21; SEQ ID NO: 40
DIRMTQSPSSLSASVGDRVTITCKASQDIKSYLSWLQQKPGKSPKTLIYRVNRLVDGVPSRFSGSGSGTDFTLTISS LQPEDFAIYYCLQYDEFPYTFGSGTKVEIK
>VL.1.31; SEQ ID NO: 41
DIRMTQSPSSLSASVGDRVTITCKASQDINSYLNWLQQKPGKSPKTLIYRVNRLVDGVPSRFSGSGSGTDFTLTIS SLQPEDFAIYYCLHYDEFPYTFGSGTKVEIK
>VL.1.41; SEQ ID NO: 42
DIRMTQSPSSLSASVGDRVTITCKASQDIKSYLSWLQQKPGKSPKTLIYRVNRLVDGVPSRFSGSGSGTDFTLTISS LQPEDFAIYYCLHYDEFPYTFGSGTKVEIK
>VL.211; SEQ ID NO: 43
DIRMTQSPSSLSASVGDRVTITCRASQDINSYLSWLQQKPGKSPKTLIYRANRLMIGVPSRFSGSGSGTDFTLTISS LQPEDFAIYYCLQYDDFPLTFGSGTKVEIK
>VL.311; SEQ ID NO: 44
DIRMTQSPSSLSASVGDRVTITCKASQDINSFLTWLQQKPGKSPKTLIYRANRVFDGVPSRFSGSGSGTDFTLTISS LQPEDFAIYYCLQYDDFPLTFGSGTKVEIK
>VL.411; SEQ ID NO: 45
QSVLTQPPSVSAAPGQKVTISCKASQDINSYLSWYQQLPGTAPKLLIYRANRLFDGIPDRFSGSKSGTSATLGITGL QTGDEANYYCLQYDEFPFTFGGGTKLTVL
>VL.511; SEQ ID NO: 46
DIVMTQSPASLAVSLGQRATISCKASQDINSYLSWYQQKPGQPPKLLIYRANRLFDGVPDRFSGSGSGTDFTLTIS SLQAEDVAVYYCLQYDEFPFTFGQGTKLEIKR
>VL.611; SEQ ID NO: 47
EIVLTQSPATLSLSPGERATLSCKASQDINSYLSWYQHKPGQAPRLLIYRANRLFDGVPARFSGSRSGTDFTLTISTL EPEDFAVYYCQLQYDEFPFTFGQGTKVEIK
>VL.1; SEQ ID NO: 48
DIVMTQSQKFMSTSVGDRVSITCKASQHVGSAVAWYQQKPGQSPTLLIHSASNRYTGVPDRFTGSGSGTDFTLT ISNIQSEDLADYFCQQYNSYPTFGGGTKLEIK
>VL.2; SEQ ID NO: 49
DIQMTQSPASLSASVGETVTITCRASGNIHNYLAWYQQKQGKSPQLLVYNAKTLADGVPSRFSASGSGTQYSLKI NSLQPEDFGSYYCQHFWSTPRTFGGGTKLEIKR
>VL.3; SEQ ID NO: 50
DIVMTQSHKFMSTSVGDRVSITCKASQDVSTAVAWYQQKPGQSPKLLISWASTRHTGVPDRFTGSGSGTDYTLT ISSVQAEDLALYYCQQHYTTPLTFGAGTKLELK
>VL.4; SEQ ID NO: 51
DIVMTQSQKFMSTSVGDRVSITCKASQNVGTAVAWYQQKPGQSPKLLIYSASNRYTGVPDRFTGSGSGTDFTLT ISNMQSEDLADYFCQQFSSYPYTFGGGTKLEIK
>VL.5; SEQ ID NO: 52
DIVLTQSPASLAVSLGQRATISCRASESVVRYGNSFMHWYQQKPGQPPKLLIYRASSLESGIPTRFSGSGSRTDFTL TINPVEADDVATYYCQQTNVDPWAFGGGTKLEIK
>VL.6; SEQ ID NO: 53
DVVMTQSPGTLSLSPGERATLSCRASQNVYSYLGWYQHKPGRSPRLLIFGVTSRATGVPDRFSGSGSGTDFTLTIS RLEPEDFAVYYCQQYAGSAYTFGQGTKVEIKR
>VL.7; SEQ ID NO: 54
DIVLTQSPASLAVSLGQRATISCRASESVVRYGNSFMHWYQQKPGQPPKLLIYRASSLESGIPTRFSGSGSRTDFTL TINPVEADDVATYYCQQTNVDPWAFGGGTKLEIK
>VL.8; SEQ ID NO: 55
QSVLTQPSSVSGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYGNNQRPSGVPDRFSGSKSGTSASLAI SGLQSEDEADYYCAAWDDSLNGPVFGGGTKLTVLG
>VL.9; SEQ ID NO: 56
DIQMTQSPSSLSASVGDRVTITCRTSQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSL

QPEDFATYYCQQSYSTPRTFGQGTKVEIK
>VL.10; SEQ ID NO: 57
DIVMTQSQKFMSTSVGDRVSITCKASQNVRTSVAWYQQKPGQSPKALIYLASNRHTGVPDRFTGSGSGTDFTLT ISNVQSEDLADYFCLQHWTYPYTFGGGTKLEIK
>VL.11; SEQ ID NO: 58
GASQSVLTQPVSVSGSPGQSITISCTGTSSNADTYNLVSWYQQRPGKAPKLMIYEGTKRPSGVSNRFSASKSATA ASLTISGLQPEDEADYYCCSYATSRTLVFGGGTKLTVV
>VL.12; SEQ ID NO: 59
GASQSVLTQPVSVSGSPGQSITISCTGTSSNADTYNLVSWYQQRPGKAPKLMIYEGTKRPSGVSNRFSASKSATA ASLTISGLQPEDEADYYCCSYATSRTLVFGGGTKLTVVAA
>VL.13; SEQ ID NO: 60
QIVLTQSPAIMSASPGEKVTMTCSASSSVSYMHWYQQKSGTSPKIWIYESSKLASGVPVRFSGSGSGTSYSLTISS MEAEDVATYYCQQWSSHPHPLTFGAGTKLELK
>VL.14; SEQ ID NO: 61
QSVLTQPPSASGTPGQRVTISCSGSSSNVGSNYVYWYQQLPGTAPKLLIYRNNRRPSGVPDRFSGSKSGTSASLAI SGLRSEDEADYYCATWDDSLSGLVFGGGTKLTVLGQPKA
>VL.15; SEQ ID NO: 62
RSQSALTQPASVSGSPGQSITISCTGISSDVETYNLVSWYEQHPGKAPKLIIYEASKRPSGVSNRFSGSKSGNTASLA ISGLQAEDEADYYCCSYAGGKSLVFGGGTRLTVLGQP
>VL.16; SEQ ID NO: 63
DIKMTQSPSSMYASLGERVTITCKASQGINSDLSWFQQKPGKSPKTLIYRANRLVDGVPSRFSGSGSGQDYSLTIS SLEYEDMGIYYCLQYDEFPLTFGAGTKLELK
>VL.17; SEQ ID NO: 64
NIVMTQSPKSMSMSVGERVTLTCKASENVGTYVSWYQQKPEQSPKLLIYGASNRYTGVPDRFTGSGSATDFTLTI SSVQAEDLADYHCGQSYSTPYTFGGGTKLEIK
>VL.18; SEQ ID NO: 65
DIVMTQSPASLAVSLGQRATISCRASENVDKYGNSFMHWYQQKPGQPPKLLIYRASELQWGVPDRFSGSGSGT DFTLTISSLQAEDVAVYYCQRSNEVPWTFGQGTKLEIKRTVAHHHHHH
>VL.19; SEQ ID NO: 66
SEIVLTQSPATLSLSPGERATLSCRASQSISTFLAWYQHKPGQAPRLLIYDASTRATGVPARFSGSRSGTDFTLTISTL EPEDFAVYYCQQRYNWPPYTFGQGTKVEIK
>VL.20; SEQ ID NO: 67
QSVLTQPVSVSGSPGQSITISCTGTSSNADTYNLVSWYQQRPGKAPKLMIYEGTKRPSGVSNRFSASKSATAASLT ISGLQPEDEADYYCCSYATSRTLVFGGGTKLTVVGQP
>VL.21; SEQ ID NO: 68
NIVMTQSPKSMSMSVGERVTLTCKASENVVTYVSWYQQKPEQSPKLLIYGASNRYTGVPDRFTGSGSATDFTLTI SSVQAEDLADYHCGQGYSYPYTFGGGTKLEIK
>VL.22; SEQ ID NO: 69
QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGSGTSYSLTISRV EAEDAATYYCQQWTSNPPTFGGGTKLEIK
>VL.23; SEQ ID NO: 70
DIVMTQTPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLVSGVPDRFSGSGSGTDFT LKISRVEAEDVGVYYCAQNLELPYTFGGGTKVEIK
>VL.24; SEQ ID NO: 71
QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNDYVSWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSKSGTSATLGIT GLQTGDEANYYCATWDRRPTAYVVFGGGTKLTVL
>VL.25; SEQ ID NO: 72
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVSNRFSGSKSGNTAS LTISGLQAEDEADYYCSSYTSSSTRVFGTGTKVTVL
>VL.26; SEQ ID NO: 73

DIQMTQSPSTLSASVGDRVTITCSASSRVGYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTEFTLTISSL
QPDDFATYYCFQGSGYPFTFGGGTKVEIK

An "isolated" substance means that it has been altered by the hand of man from the natural state. If an "isolated" substance presents in nature, it has been changed or removed from its original environment, or both. For example, a polypeptide naturally present in a living subject is not "isolated" but the polypeptide is isolated if it has been substantially separated from the coexisting materials of its natural state and/or exists in a substantially pure state.

The term "specifically binds" or "specifically binding" refers to a non-random binding reaction between two molecules, such as the binding of the antibody or antigen-binding portion thereof to an epitope of the antigen. An antibody or antigen-binding portion thereof that "specifically binds" to a target or an epitope is a term well understood in the art, and methods to determine such specific binding are also well known in the art. A molecule is said to exhibit "specific binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen or an epitope than it does with alternative targets/epitopes. An antibody or antigen-binding portion thereof "specifically binds" to a target antigen if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules.

An "epitope" is a region of an antigen that is bound by an antibody. The term includes any polypeptide determinant capable of specific binding to an immunoglobulin. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics.

As used herein, the term "neutralizing" refers to neutralization of an activity, such as a biological activity, of a target protein (e.g., Yellow Fever Virus E-protein). In one embodiment, a neutralizing antibody binds to the E-DII epitope of Yellow Fever Virus E-protein and results in inhibition of a biological activity of Yellow Fever Virus and/or prevents the virus from infecting cells.

Subjects may be human, but also include other mammals, particularly those mammals useful as laboratory models for human disease, *e.g.* mouse, rat, rabbit, dog, *etc.*

The term "treat", "treatment" or "treating" refers to an action, application or therapy, wherein a subject, including a human being, is subjected to medical aid with the purpose of improving the subject's condition, directly or indirectly. Particularly, the term refers to reducing incidence, or alleviating one or more symptoms, eliminating recurrence, preventing recurrence, preventing incidence, improving one or more symptoms, and/or improving prognosis or a combination thereof in some embodiments. The skilled artisan would understand that treatment does not necessarily result in the complete absence or removal of symptoms. For example, with respect to Yellow Fever Virus, "treatment" or "treating" may refer to reducing the severity or duration of acute viral haemorrhagic disease caused by Yellow Fever.

An "effective amount" or an "effective dose" or a "therapeutically effective amount" in connection with administration of a pharmacological agent, as used herein, refers to an amount of a drug or pharmaceutical agent (e.g., an antibody or antigen-binding fragment or portion thereof, or a composition comprising the same) which, as compared to a corresponding subject who has not received such amount, results in an intended pharmacological result, or an effect in treatment, healing, prevention, or amelioration of a disease or disease symptom, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder, or any symptom thereof. The effective amount or dose of a pharmacological agent may vary depending on the particular active ingredient employed, the mode of administration, and/or the age, size, and condition of the subject to be treated.

The present disclosure also provides compositions (e.g., pharmaceutical compositions) comprising an antibody or antigen-binding portion thereof that binds specifically to the E-DII epitope of Yellow Fever Virus E-protein. In some embodiments, the E-DII epitope comprises an asparagine at position 106, a lysine at position 93, and a lysine at position 104 of Yellow Fever Virus E-protein. Compositions can be prepared from any one of the antibodies or antigen-binding portions described herein. The antibodies or antigen-binding portions thereof, as well as the encoding nucleic acids or nucleic acid sets, vectors comprising such, or host cells comprising the vectors, as described herein can be mixed with a pharmaceutically acceptable carrier (excipient), such as to form a pharmaceutical composition for use in treating a target disease. Pharmaceutically acceptable excipients (carriers) including buffers, are well known in the art. See, e.g., Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover.

The pharmaceutical compositions can comprise pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formulations or aqueous solutions.

(Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations used, and may comprise buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™ (polysorbate), PLURONICS™ (poloxamers) or polyethylene glycol (PEG).

The phrase "pharmaceutically acceptable", as used in connection with compositions of the present disclosure, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a subject. Preferably, as used herein, 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 mammals, and more particularly in humans. "Acceptable" means that the carrier is compatible with the active ingredient of the composition (*e.g.*, the nucleic acids, vectors, cells, or therapeutic antibodies) and does not negatively affect the subject to which the composition(s) are administered. Any of the pharmaceutical compositions to be used in the present methods can comprise pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formations or aqueous solutions.

Pharmaceutically acceptable carriers, including buffers, are well known in the art, and may comprise phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; amino acids; hydrophobic polymers; monosaccharides; disaccharides; and other carbohydrates; metal complexes; and/or non-ionic surfactants. See, *e.g.* Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover.

A pharmaceutical composition can be presented in unit dosage form and can be prepared by any suitable method, many of which are well-known. Such methods can include the step of bringing an anti-YFV antibody into association with a carrier that constitutes one or more accessory ingredients.

Compositions provided herein can be a sterile aqueous preparation, which preferably in some embodiments is isotonic with the blood of the recipient. This aqueous preparation can be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile aqueous preparations also can be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution.

The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Moreover, vectors may be capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" or "expression vectors".

The term "recombinant host cell" or "host cell", as used herein, is intended to refer to a cell into which exogenous DNA has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell, but, to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

EXAMPLES

Anti-YFV monoclonal antibodies are designed using structure-guided analysis of the YFV envelope protein epitopes, focusing on the envelope domain II fusion loop. Mutationally constrained epitope(s) on the surface of the yellow fever virus are identified. Appropriate antibody scaffolds that satisfy the epitope-paratope constraints are then identified, and the antibodies to target and neutralize the virus are engineered.

Anti-YFV antibodies are cloned, expressed and purified using known methods, and screened for expression and biophysical properties. The in silico designed antibodies are cloned in mammalian expression vectors and purified for further analysis. Both expression as well as various analytical parameters indicative of purity and stability are assessed. The

potency of the antibodies against yellow fever virus is evaluated using in vitro and in vivo models of infection.

In vitro neutralization potency of engineered antibodies are assessed against the YF17D-204 vaccine strain of the yellow fever virus, using a plaque reduction neutralization test (PRNT). For selected antibodies, additional evaluation of potency are conducted using murine models of YFV infection.

Rational Design of Anti-YF mAbs

Promising anti-YFV monoclonal antibodies must neutralize a broad range of strains and target regions that are associated with robust protection. Epitopes play a critical role in the efficacy of a therapeutic antibody. In YFV infection, the envelope (E) protein is the predominant target of neutralizing antibodies. In order to define promising epitopes, a structure-guided analysis of the whole YFV assembly was conducted to identify spatially clustered, solvent accessible and sequence-conserved residues. This was done by mapping the sequence conservation scores (computed from an alignment of YFV envelope protein sequences) onto the homology model of a whole YFV E-protein assembly. Residues that have sequence conservation greater than 70% and solvent accessible surface area > 40% were considered as putative epitope residues. From this analysis, the region proximal to the domain II (E-DII) hydrophobic fusion loop appeared highly accessible and conserved across YFV strains.

In order to engineer antibodies against the E-DII epitope, a structure-guided search for Fv scaffolds that recognize highly homologous epitope surfaces was conducted. Promising scaffolds were docked against the epitope (using the software ZRANK) and rank-ordered based on shape complementarity (> 0.6), buried surface area (1,000 Sq. A°) and potential to make contacts with the critical E-DII epitope residues (N106, K93 and K104 of E-protein; numbering correspond to primary sequence of 17D YF vaccine strain). Then, the CDR loops of the top ranking scaffolds were redesigned through Rosetta Antibody Design to make optimal contacts with the epitope. The engineered antibodies were screened for in vitro neutralization of YFV.

Synthetic DNA sequences encoding the VH and VL domains provided in Tables 1 and 2 were cloned in frame with an IgG1 constant region, into mammalian expression vector and various combinations of the heavy and light chains combined into at least one hundred and ten YFV antibodies. Examples of such combinations are shown in **Table 3** below.

Table 3. YF antibody combinations

	pVL.0	pVL.1	pVL.1.1	pVL.1.2	pVL.1.3	pVL.1.4	pVL.2	pVL.3	pVL.4	pVL.5	pVL.6
pVH.0	1	2					3	4			
pVH.1	5	6					7	8			
pVH.2	9	10					11	12			
pVH.3	13	14					15	16			
pVH.3.1			22	23	24	25					
pVH.3.2			26	27	28	29					
pVH.4									18		
pVH.5									19		
pVH.6										20	
pVH.7											21

Illustrative Examples of Expression Levels and Biophysical Properties

A subset of YFV antibodies were purified from larger scale transfections in Expi293 cells. Proteins from cell culture supernatants were purified using the HiTrap Protein A column. Following dialysis, the protein concentration was estimated by nanodrop using theoretical extinction coefficients. The yields of various antibodies are summarized in Table 4.

Table 4. Yield of YF mAbs expressed in Expi293 cells

mAb	Expression mg/L
1	34.4
2	171
3	65.8

Additionally, purity and stability of various antibodies were determined by (1) UV spectrometry, (2) size exclusion chromatography, (3) microfluidic capillary electrophoresis and (4) thermal shift dye disassociation assay, respectively. Illustrative examples of such analytical biophysical parameters are summarized in Table 5 below.

Table 5. Purity and Stability of Select YF antibodies

			1	2
Abs maxima 1 (nm)			228	228
Abs maxima 2 (nm)			278	279
E1% (g/100 mL)			14.2	13.8
HMW-1 (%)			0.16	0
HMW-2 (%)			0.68	0.62
Monomer (%)			99.04	98.99
LMW (%)			0.12	0.39
Reduced	LC	Size (kDa)	26.3	26.1
		% of total	30.6	30.5
	HC	Size (kDa)	58.5	58.4
		% of total	68.7	69.5
Intact		Size (kDa)	154.7	154.8
		% of total	88.0	87.0
Melting temperature T_m (°C)			69.0	69.1

Additional characterization of the glycoforms of various antibodies were determined by procainamide-label assisted LC-FLD-MS. Illustrative examples of glycosylation profiles observed are below (Table 6).

Table 6. Glycosylation Profiles

Glycan	1 (%)	2 (%)
Man5	0.8 ± 0.01	1.2 ± 0.07
G0	0.3 ± 0.00	0.5 ± 0.03
G0F	42.9 ± 0.05	51.6 ± 1.27
G1	0.1 ± 0.00	0.2 ± 0.02
G1F	29.4 ± 0.14	25.2 ± 0.12
G1'F	15.8 ± 0.04	15.3 ± 0.70
G2F	10.5 ± 0.12	6.0 ± 0.32
A1F	0.2 ± 0.00	0.1 ± 0.01

Illustrative Examples of Neutralization Potency of Engineered Antibodies Against YF-17D

Plaque Reduction Neutralization Test (PRNT) was conducted to determine the neutralization efficacy of the YFV antibodies against the YFV (17D-204). Vero cells were infected with either virus alone, no virus or virus pre-incubated with various dilutions of YFV antibodies. Plates were incubated for 7 days, fixed and stained with crystal violet to visualize plaque formations. Neutralization curves were generated using the Prism software (**Figure 1**) and the 50% effective concentration (EC50) values were calculated by nonlinear regression using a variable slope (**Table 6**). The neutralization efficacy of an antibody is directly proportional to plaque reduction and the potency is depicted as concentration at which 50% of the virus particles are neutralized. The antibodies were highly potent in neutralizing the virus and illustrative examples are shown below in **Table 7**.

Table.7. Summary of EC50 values for YF mAbs

mAb	EC50 (µg/ml)	STDEV
1	0.012	0.006
2	0.007	0.002
3	0.011	0.010

Illustrative Examples of In Vivo Efficacy

Figure 1 provides an illustrative example of in vivo efficacy of engineered antibodies against YF-17D-204 in a mouse model of infection. Efficacy of designed antibodies was tested in a lethal model of Yellow fever infection in AG129 mice. Protective efficacy of antibodies was tested in prophylaxis or as therapy as indicated in Figure 1.

An illustrative example shown in Figure 2, administration of antibody resulted in complete protection compared to control groups (Figure 2A). Administration of antibody also led to greater than 2 Log_{10} reduction in viremia at days 4 and 6 post infection (Figure 2B).

What Is Claimed Is:

CLAIMS

1. An antibody or antigen-binding portion thereof, wherein said antibody or antigen-binding portion thereof specifically binds to an E-DII epitope of Yellow Fever Virus, and wherein the E-DII epitope comprises an asparagine at position 106, a lysine at position 93, and a lysine at position 104 of Yellow Fever Virus E-protein.

2. The antibody or antigen-binding portion thereof of claim 1, wherein the antibody or antigen-binding portion thereof comprises three CDRs of a heavy chain variable region (VH), wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID. NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36.

3. The antibody or antigen-binding portion thereof of claim 1 or 2, wherein the antibody or antigen-binding portion thereof comprises three CDRs of a light chain variable region (VL), wherein the VL comprises an amino acid sequence selected from the group consisting of SEQ ID. NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73.

4. The antibody or antigen-binding portion thereof of any one of the preceding claims, wherein the antibody or antigen-binding portion thereof comprises a VH, wherein the VH comprises an amino acid sequence selected from the group consisting of SEQ ID. NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36.

5. The antibody or antigen-binding portion thereof of any of claims 1-4, wherein the antibody or antigen-binding portion thereof comprises a VL, wherein the VL comprises an amino acid sequence selected from the group consisting of SEQ ID. NOs: 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, and 73.

6. The antibody or antigen-binding portion thereof of any of the preceding claims,

wherein the antibody or antigen-binding portion thereof is a human antibody or antigen-binding portion thereof, a monoclonal antibody or antigen-binding portion thereof, a chimeric antibody or antigen-binding portion thereof, or a humanized antibody or antigen-binding portion thereof.

7. The antibody or antigen-binding portion thereof of any of the preceding claims, wherein the antibody or antigen-binding portion thereof is a single chain antibody or antigen-binding portion thereof, a F(ab') 2 fragment, a dAb fragment, a Fab fragment, or a Fv fragment.

8. The antibody or antigen-binding portion thereof of any of the preceding claims, wherein the antibody or antigen-binding portion thereof is an isolated antibody or antigen-binding portion thereof.

9. A composition comprising an antibody or antigen-binding portion thereof of any of the preceding claims and a carrier.

10. The composition of claim 9, wherein the composition is a pharmaceutical composition that comprises a pharmaceutically acceptable carrier.

11. An antibody or antigen-binding portion thereof for use in medicament, wherein the antibody or antigen-binding portion thereof is any of claims 1-8.

12. A method for treating Yellow Fever, or a disease or condition associated with Yellow Fever Virus, comprising administering to a subject in need thereof a therapeutically effective amount of an antibody or antigen-binding portion thereof of any of claims 1-8.

13. Use of the antibody or antigen-binding portion thereof of any of claims 1-8 in the manufacture of a medicament for treating a disease or condition associated with Yellow Fever Virus.

14. A nucleic acid comprising a nucleotide sequence encoding an antibody heavy chain variable region (VH), an antibody light chain variable region (VL) or both, wherein the VH and VL is set forth in any of claims 2-7.

15. A vector comprising the nucleic acid of claim 14.

16. The vector of claim 15, wherein the vector is an expression vector.

17. A host cell comprising the nucleic acid of claim 14 or the vector of claims 15 or 16.

18. A method for producing an antibody or antigen-binding portion thereof that binds to Yellow Fever Virus E-protein, comprising:

culturing the host cell of claim 17 under conditions allowing for expression of the antibody.

19. The method of claim 18, further comprising harvesting the antibody or antigen-binding portion thereof.

20. The method of claim 18 or 19, wherein the antibody or antigen-binding portion thereof specifically binds to the E-DII epitope of Yellow Fever Virus E-protein.

21. The method of claim 20, wherein the E-DII epitope comprises residues 93, 104, and 106 of Yellow Fever Virus E-protein.

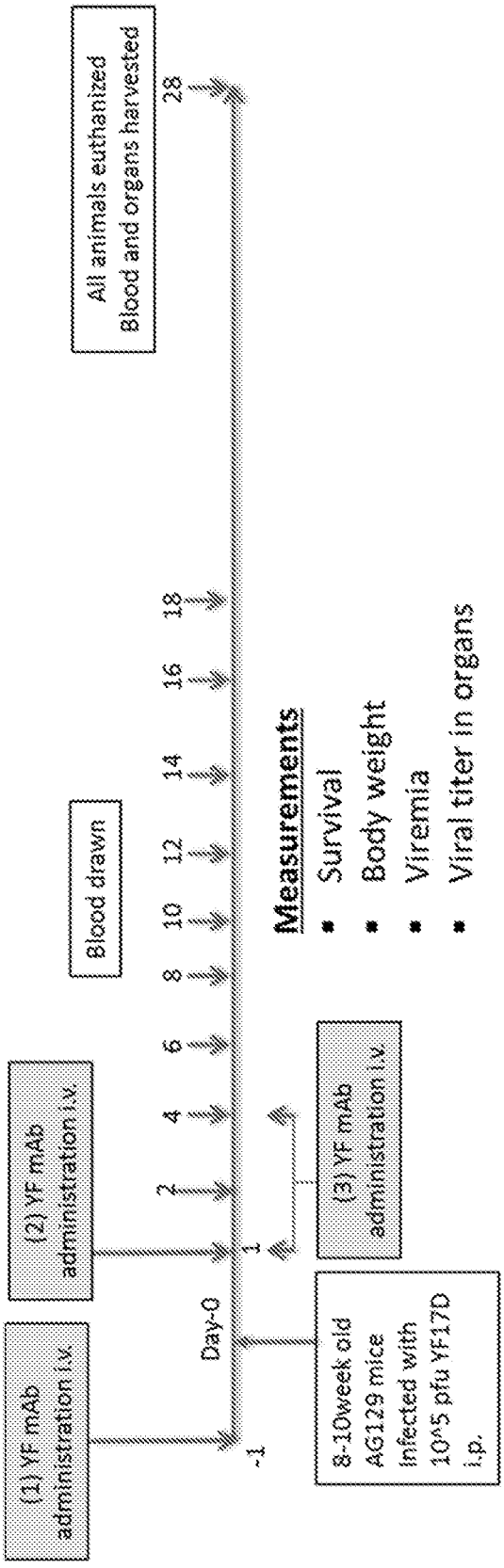
All animals euthanized
Blood and organs harvested
28
18
16
14
12
10
8
6
4
2
1
-1
Day-0
Blood drawn
(1) YF mAb administration i.v.
(2) YF mAb administration i.v.
(3) YF mAb administration i.v.
8-10week old AG129 mice infected with 10^5 pfu YF17D i.p.
Measurements
Survival
Body weight
Viremia
Viral titer in organs

FIG. 1

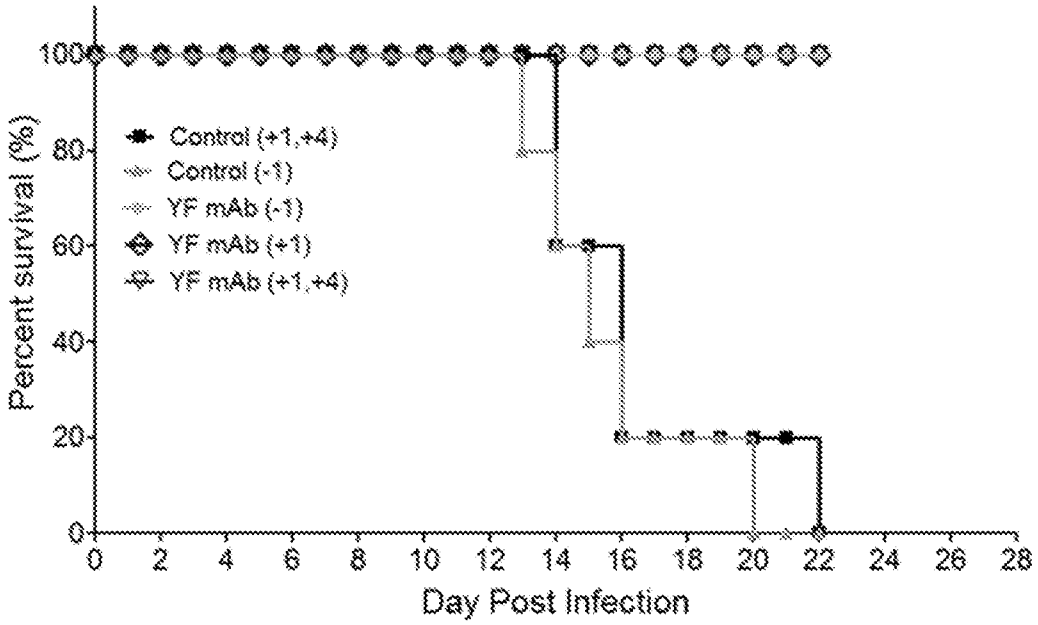
Percent survival (%)
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Day Post Infection
Control (+1,+4)
Control (-1)
YF mAb (-1)
YF mAb (+1)
YF mAb (+1,+4)

FIG. 2A

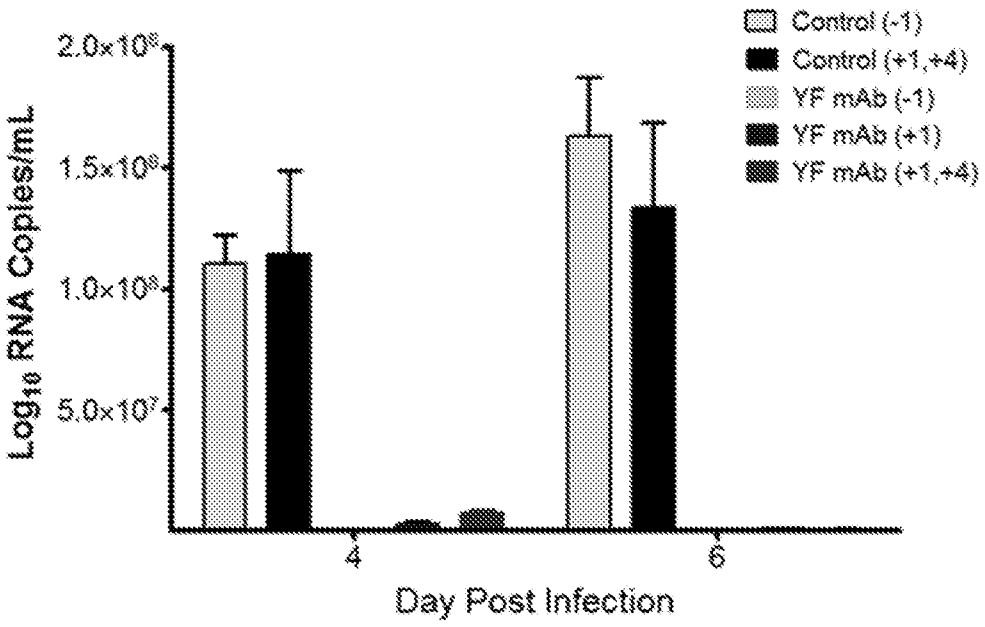
Log10 RNA Copies/mL
2.0x10^8
1.5x10^8
1.0x10^8
5.0x10^7
4
6
Day Post Infection
Control (-1)
Control (+1,+4)
YF mAb (-1)
YF mAb (+1)
YF mAb (+1,+4)

FIG. 2B

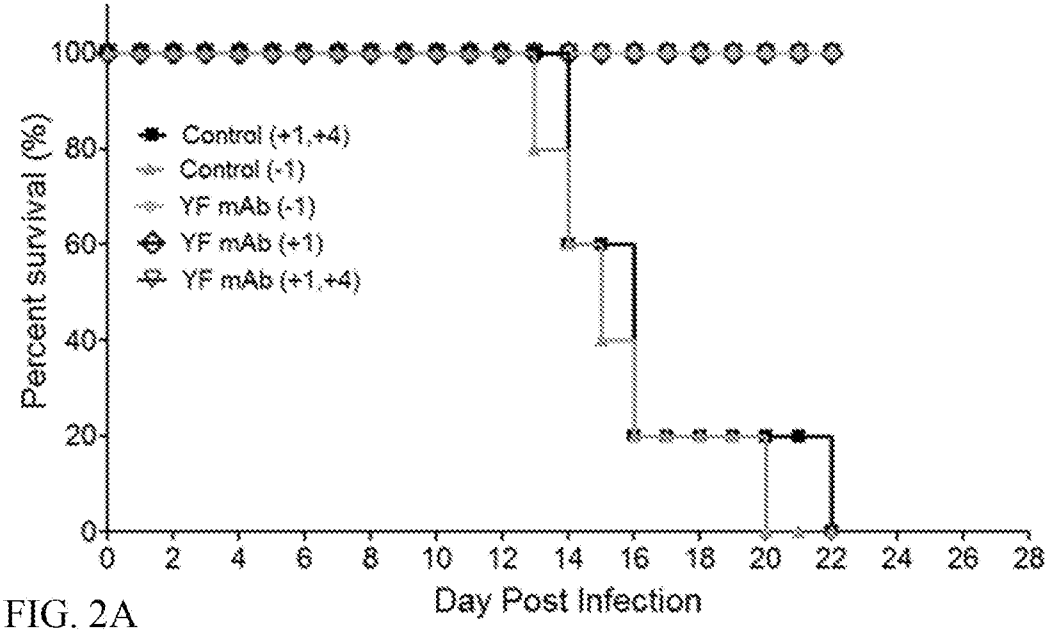
Percent survival (%)
100
80
60
40
20
0
Control (+1,+4)
Control (-1)
YF mAb (-1)
YF mAb (+1)
YF mAb (+1,+4)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Day Post Infection

FIG. 2A