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(71) Applicant: UNIVERSITY OF MIAMI [US/US]; Office of Technology Transfer, 1951 Nw 7th Avenue-3rd Floor, Suite 310, Miami, FL 33136-1112 (US).

(72) Inventors: STRBO, Natasa; c/o University of Miami, 1951 NW 7th Avenue-3rd Floor, Suite 310, Miami, FL (US). ROMERO, Laura; c/o University of Miami, 1951 NW 7th Avenue-3rd Floor, Suite 310, Miami, FL (US).

(74) Agent: ALTIERI, Stephen, L. et al.; Morgan, Lewis & Bockius LLP, 1111 Pennsylvania Avenue, NW, Washington, DC 20004 (US).

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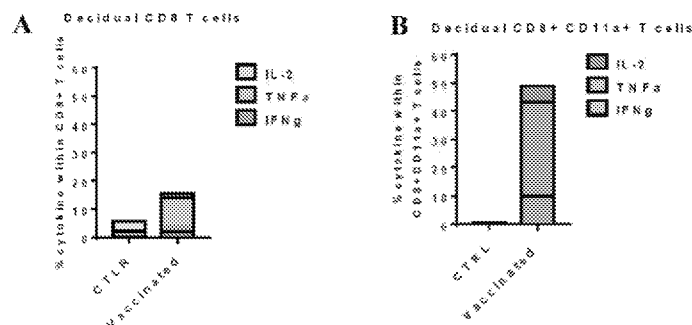
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FIGURE 14

Gp96-Ig-ZIKVEnv vaccine induced ZIKV-Env-specific CD8⁺ T lymphocyte responses, as assessed by multiparameter intracellular cytokine staining (ICS) assays



(57) Abstract: The present invention provides an expression vector, host cells, methods and kits for the treatment or prevention of a flavivirus infection in a subject.

VECTORS AND VACCINE CELLS FOR IMMUNITY AGAINST ZIKA VIRUS**FIELD**

[0001] The present invention relates, in part, to compositions and methods useful for immune modulation in connection with, for example, infection by the Zika virus.

RELATED APPLICATIONS

[0002] This application claims priority to and the benefit of US Provisional Patent Application No. 62/406,506, which was filed on October 11, 2016, the contents of which are incorporated herein by reference in their entirety.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0003] The contents of the text file named "HTB-ZOL-029PCUMIP-114_Sequence Listing_ST25", which was created on October 3, 2017 and is 294 KB in size, are hereby incorporated herein by reference in their entirety.

BACKGROUND

[0004] The Zika virus (ZIKV) is a member of the virus family *Flaviviridae*. It is spread by, among others daytime-active *Aedes* mosquitoes, such as *A. aegypti* and *A. albopictus*.

[0005] Although in most instances ZIKV infection results in a self-limiting febrile illness associated with rash and conjunctivitis, severe neurological phenotypes can occur, including Guillain-Barre syndrome and meningoencephalitis (Carteaux et al. NEJM 374: 1595-1596 (2016); Oehler et al., Eurosurveillance 19(9), 06 March 2014). Infection in pregnant women is of major concern, as it is linked to catastrophic fetal abnormalities including microcephaly, spontaneous abortion, and intrauterine growth restriction (IUGR) due to placental insufficiency (Brasil et al., NEJM doi:10.1056/NEJMoa1602412 (4 March 2016)). Currently, effective ZIKV vaccines do not exist or target only adults without impacting an existing fetus in a pregnant woman.

[0006] Accordingly, there remains an urgent need for ZIKV vaccines that could prevent and/or mitigate ZIKV infections and which will not only protect adults from Zika infection but also pregnant women and their embryos and fetuses.

SUMMARY

[0007] Accordingly, in various aspects, the present invention relates to compositions and methods that provide vaccine protection from flavivirus infection, *e.g.*, Zika infection. Importantly, in various aspects, the compositions and methods protect adult subjects, inclusive of pregnant female adult subjects and their fetuses.

[0008] In various embodiments, the present compositions and methods protect a fetus from flavivirus infection, *e.g.*, Zika infection, and the catastrophic fetal abnormalities associated therewith, by providing placental protection that is mediated by a chaperone-, *e.g.* gp96-, based vaccine that induces Zika antigen specific cytotoxic T cell (CTL) responses in the placenta and/or decidua.

[0009] In various embodiments, the present invention provides an expression vector system comprising (i) a nucleic acid encoding a fusion protein comprising a chaperone protein and an immunoglobulin, or a fragment thereof, and (ii) a nucleic acid encoding a flavivirus protein, or an antigenic portion thereof, wherein each nucleic acid is operably linked to a promoter. In exemplary embodiments, the flavivirus protein is a Zika virus (ZIKV) protein. Accordingly, the present invention provides an expression vector system comprising (i) a nucleic acid encoding a fusion protein comprising a chaperone protein and an immunoglobulin, or a fragment thereof, (i) a nucleic acid encoding a ZIKV protein, or an antigenic portion thereof, wherein each nucleic acid is operably linked to a promoter. Related host cells comprising the expression vector system of the present invention are provided herein.

[0010] The present invention also provides a composition comprising an expression vector system, a host cell, or a population of cells, as presently disclosed herein, and an excipient, carrier, or diluent. In exemplary aspects, the composition is a pharmaceutical composition.

[0011] Additionally, provided by the present invention is a kit comprising an expression vector system, a host cell, a population of cells, or a composition, as presently disclosed herein.

[0012] The present invention further provides a method of eliciting an immune response against a flavivirus in a subject, comprising administering to the subject the expression vector of the present invention or a population of cells transfected with the expression vector, in an amount effective to elicit an immune response against flavivirus in the subject. In exemplary embodiments, the flavivirus is a Zika virus. Accordingly, the present invention further

provides a method of eliciting an immune response against ZIKV in a subject, comprising administering to the subject the expression vector of the present invention or a population of cells transfected with the expression vector, in an amount effective to elicit an immune response against ZIKV in the subject.

[0013] The present inventions furthermore provide a method of treating or preventing a flavivirus infection in a subject, comprising administering to the subject the expression vector of the present invention or a population of cells transfected with the expression vector, in an amount effective to treat or prevent the flavivirus infection. In exemplary embodiments, the flavivirus infection is a ZIKV infection. Accordingly, the present inventions furthermore provide a method of treating or preventing a Zika virus (ZIKV) infection in a subject, comprising administering to the subject the expression vector of the present invention or a population of cells transfected with the expression vector, in an amount effective to treat or prevent the ZIKV infection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0100] Figure 1 is a schematic illustration of an exemplary expression vector of the present invention.

[0101] Figure 2 is a set of representative dot plots of gated CD3+CD8+ T cells in placenta and decidua and the frequency of OTI positive cells.

[0102] Figure 3 is a schematic illustration of an exemplary expression vector of the present invention and the sequence thereof and having a sequence of SEQ ID NO: 24. Both forward and reverse nucleotide sequences are shown. The reverse sequence is underlined.

[0103] Figures 4 and 5 provide a schematic of the experiment described in Example 2 and additional experimental schematics.

[0104] Figure 6 provides a photo of tissue and anatomical drawings of a pregnant mouse from Erlebacher, Nature Reviews Immunology 13: 23-33 (2013).

[0105] Figure 7 is a schematic illustration of an exemplary expression B45 vector expressing Gp96-Ig and ZIKA Pre-Membrane and Envelope protein of the present invention and the sequence thereof and having a sequence of SEQ ID NO: 35. Gp96-Ig construct was cloned in the first expression cassette and ZIKA Pre-membrane Envelope construct was cloned in the second expression cassette.

[0106] Figure 8A-E illustrate single cell cloning of vaccine 293 cells expressing gp96-Ig and ZIKA envelope. Figure 8A is a Western blot showing that transfected cells were successfully transfected with ZIKA envelope (lane 2). Figure 8B shows MBS430270 (MyBiosource) Anti-EnvelopeE (ZikaVirus) Polyclonal antibody primary antibody (1:1000) and HRP-anti-rabbit IgG as a secondary antibody (Jackson Immuno research) (1:5000). Figure 8C shows ELISA for gp96-Ig for clone 5G4 and Figure 8D is a western blot for ZIKA env and Figure 8E shows for clone 5G4.

[0107] Figure 9 is a confocal microscopy image illustrating the expression of DNA vaccination with *in vivo* electroporation (EP) of Gp96-Ig-gfp DNA vaccine.

[0108] Figure 10A-C is a histological staining and a bar graph illustrating that secreted gp96-Ig vaccination during mouse pregnancy is safe. Pregnant Wildtype (WT) mice were injected s.c. with PBS control (Figure 10A) or with 293-gp96-Ig-ZIKAEnv (Figure 10B) at GD 7.5. Figure 10C is a bar graph showing the total number of live born pups at GD 19-21, from control (CTRL) dams that have received only PBS or dams that received 293-gp96-Ig-ZIKA at GD 7.5. (n=8-12)

[0109] Figure 11 is a histological staining showing accumulation of CD8 T cells in the maternal decidua after secreted Gp96ZIKAEnv-Ig vaccination.

[0110] Figure 12A-B is a set of bar graphs illustrating that Gp96-Ig-ZIKVEnv vaccine induces antigen experienced (CD11a+) effector memory CD8 T cells in decidua. Figure 12A shows the percentage of CD8+ cells with CD3+ cells in control and vaccinated mice. Figure 12B shows decidua CD8+ T cells in control and vaccinated mice.

[0111] Figure 13A-B is a confocal microscopy image illustrating the Co-expression of CD11a and CD8 in placenta of Gp96-Ig-ZIKVEnv vaccinated mice. Figure 13A shows the expression in control and Figure 13B shows the expression in Zika vaccinated.

[0112] Figure 14A-B is a set of bar graphs illustrating Gp96-Ig-ZIKVEnv vaccine induced ZIKV-Env-specific CD8+ T lymphocyte responses, as assessed by multiparameter intracellular cytokine staining (ICS) assays. Figure 14A shows the percentage of cytokines within CD8+ cells in control and vaccinated mice and Figure 14B shows the percentage of cytokines within CD8+CD11a+ T cells.

DETAILED DESCRIPTION

[0113] The present invention is based, in part, on the surprising discovery that an expression vector system described herein was able to stimulate immune responses in the placenta thereby providing direct protection against ZIKV infection to the unborn fetus.

[0114] *Expression Vectors and Host Cells*

[0115] The present invention provides an expression vector system comprising (i) a nucleic acid encoding a fusion protein comprising a chaperone protein and an immunoglobulin, or a fragment thereof, (i) a nucleic acid encoding a flavivirus protein, or an antigenic portion thereof, wherein each nucleic acid is operably linked to a promoter. In exemplary embodiments, the flavivirus protein is a Zika virus (ZIKV) protein. Accordingly, the present invention provides an expression vector system comprising (i) a nucleic acid encoding a fusion protein comprising a chaperone protein and an immunoglobulin, or a fragment thereof, (i) a nucleic acid encoding a Zika virus (ZIKV) protein, or an antigenic portion thereof, wherein each nucleic acid is operably linked to a promoter.

[0116] As used herein, the term “expression vector system” refers to one expression vector comprising all components or a set of two or more expression vectors designed to function together. For purposes herein, the term “expression vector” means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the expression vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The expression vector(s) of the disclosure are not naturally-occurring as a whole. However, parts of the vectors can be naturally-occurring.

[0117] The expression vectors of the present invention comprise any type of nucleotides, including, but not limited to DNA and RNA, which may be single- stranded or double-stranded, synthesized or obtained in part from natural sources, and which in exemplary aspects contain natural, non-natural or altered nucleotides. In exemplary aspects, the altered nucleotides or non-naturally occurring internucleotide linkages do not hinder the transcription or replication of the vector. In exemplary aspects, the expression vector system comprises one or more modified or non-natural nucleotides selected from the group consisting of: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5- carboxymethylaminomethyl-2-

thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D- galactosyl queuosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N -substituted adenine, 7-methylguanine, 5-methylammomethyluracil, 5- methoxyaminomethyl-2-thiouracil, beta-D-mannosyl queuosine, 5'- methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queuosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3- (3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine.

[0118] The expression vectors disclosed herein in illustrative aspects comprise naturally-occurring or non-naturally-occurring internucleotide linkages, or both types of linkages. In exemplary aspects, the expression vector system comprises one or more modified internucleotide linkages such as phosphoroamidate linkages and phosphorothioate linkages.

[0014] The expression vector system of the present invention may comprise any one or more suitable expression vectors, and may include one or more expression vectors used to transform or transfect any suitable host. Suitable expression vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. In various embodiments, the expression vector system in exemplary aspects comprises one or more expression vectors such as those from the pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, CA). Bacteriophage vectors, such as λ GT10, λ GT11, λ ZapII (Stratagene), λ EMBL4, and λ NMI 149, also can be used. Examples of plant expression vectors include pBIOL, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-Cl, pMAM and pMAMneo (Clontech). In exemplary aspects, the expression vector system comprises a pBCMGSNeo expression vector and/or a pBCMGRHis expression vector, as described in Yamazaki et al., 1999, *supra*. In exemplary aspects, the expression vector system comprises a viral vector, e.g., a retroviral vector, an adenovirus vector, an adeno-associated virus (AAV) vector, or a lentivirus vector.

[0015] The expression vectors and systems comprising the expression vectors of the present invention can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual, and Ausubel et al., Current Protocols in Molecular Biology (1994). Constructs of expression vectors, which are

circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, *e.g.*, from ColEI, 2 μ plasmid, λ , SV40, bovine papilloma virus, and the like.

[0016] The expression vector system may be designed for either transient expression, for stable expression, or for both. In exemplary aspects, the recombinant expression vector system comprises elements necessary for integration into the host genome. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression. For example, the recombinant expression vector system may comprise one or more suicide genes and/or one or more constitutive or inducible promoters.

[0017] In exemplary aspects, the expression vector system comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (*e.g.*, bacterium, fungus, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA-based.

[0018] The expression vector system in exemplary aspects comprises a native promoter operably linked to the nucleic acid comprising a nucleotide sequence encoding the fusion protein or the flavivirus (*e.g.*, ZIKV) protein, or an antigenic portion thereof, or the nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the fusion protein or the flavivirus (*e.g.*, ZIKV) protein, or an antigenic portion thereof. The selection of promoters, *e.g.*, strong, weak, inducible, tissue-specific and developmental- specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter or a viral promoter, *e.g.*, a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, metallothionein (Mth) promoter, or a promoter found in the long-terminal repeat of the murine stem cell virus.

[0019] An expression vector also can include transcription enhancer elements, such as those found in SV40 virus, Hepatitis B virus, cytomegalovirus, immunoglobulin genes, metallothionein, and β -actin (see, Bittner *et al.*, *Meth Enzymol* 1987, 153:516-544; and Gorman, *Curr Op Biotechnol* 1990, 1:36-47). In addition, an expression vector can contain sequences that permit maintenance and replication of the vector in more than one type of host cell, or integration of the vector into the host chromosome. Such sequences include, without

limitation, to replication origins, autonomously replicating sequences (ARS), centromere DNA, and telomere DNA.

[0119] In exemplary aspects, the nucleic acid encoding the fusion protein is operably linked to the same promoter which is also operably linked to the nucleic acid encoding the flavivirus (*e.g.*, ZIKV) protein or an antigenic portion thereof. In exemplary aspects, the nucleic acid encoding the fusion protein is operably linked to a promoter which is different from the promoter which is operably linked to the nucleic acid encoding the flavivirus (*e.g.*, ZIKV) protein, or an antigenic portion thereof. In exemplary aspects, the nucleic acid encoding the fusion protein is operably linked to a CMV promoter. In exemplary aspects, the nucleic acid encoding the flavivirus (*e.g.*, ZIKV) protein, or an antigenic portion thereof, is operably linked to a Mth promoter

[0120] In exemplary aspects, the expression vector system of the present invention comprises only one recombinant expression vector. In exemplary aspects, the nucleic acid encoding the fusion protein and the nucleic acid encoding the flavivirus (*e.g.*, ZIKV) protein, or antigenic portion thereof, are present on the same expression vector.

[0121] Alternatively, the expression vector system comprises more than one expression vector. In exemplary aspects, the expression vector system comprises one expression vector comprising the nucleic acid encoding the fusion protein and one expression vector per number of different flavivirus (*e.g.*, ZIKV) proteins, or antigenic portion, encoded by the system. In exemplary aspects, the expression vector system comprises a nucleic acid encoding the fusion protein and one or two different flavivirus (*e.g.*, ZIKV) protein, or antigenic portion, and thereby comprises three expression vectors. In exemplary aspects, the recombinant expression vector system comprises two, three, four, five, or more recombinant expression vectors. In exemplary aspects, the expression vector system comprises at least two expression vectors and the nucleic acid encoding the fusion protein is present on an expression vector which is different from the expression vector comprising the nucleic acid encoding the flavivirus (*e.g.*, ZIKV) protein, or antigenic portion thereof.

[0122] The expression vector system of the present invention in exemplary aspects comprises additional components. For example, in exemplary aspects, each vector of the recombinant expression vector system comprises a selectable marker. In exemplary aspects, the selectable marker is a gene product which confers resistance to an antibiotic, including but not limited to ampicillin, kanamycin, neomycin/G418, tetracycline, geneticin, triclosan,

puromycin, zeocin, and hygromycin. In exemplary aspects, the selectable marker is one or more of kanamycin resistance genes, puromycin resistance genes, zeocin resistance genes, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, geneticin resistance genes, triclosan resistance genes, R-fluoroorotic acid resistance genes, 5-fluorouracil resistance genes and ampicillin resistance genes. Combination of any of the selectable markers described herein is contemplated. In exemplary aspects, when the system comprises more than one recombinant expression vector, each vector comprises a selectable marker. In exemplary aspects, each vector has the same selectable marker. Alternatively, each vector within the system comprises a different selectable marker.

[0123] In some embodiments, the expression vector system further comprises a nucleic acid encoding a bovine papilloma virus (BPV) protein. The BPV early region encodes nonstructural proteins E1 to E7. E1 and E2 are nonstructural proteins derived from bovine papilloma virus (BPV). E5, E6 and E7 are viral oncoproteins derived from BPV and have the Gene Accession ID Numbers 1489021, 3783667 and 3783668, respectively. In exemplary aspects, the expression vector system further comprises a nucleotide sequence which encodes a BPV E1 and/or a BPV E2. In exemplary aspects, the expression vector system further comprises a nucleic acid encoding an E1 amino acid sequence of SEQ ID NO: 19 and/or an E2 amino acid sequence of SEQ ID NO: 22. In exemplary aspects, the expression vector system does not comprise a nucleic acid encoding a BPV viral oncoprotein. In exemplary aspects, the expression vector system does not comprise a nucleic acid encoding E5, E6, and/or E7. In exemplary aspects, the expression vector system does not comprise nucleotides 3878 to 4012 of GenBank Accession No. NC_001522.1 encoding E5, nucleotides 91 to 519 of GenBank Accession No. NC_007612.1 encoding E6, and/or nucleotides 522 to 836 of GenBank Accession No. NC_007612.1 encoding E7. In exemplary aspects, the expression vector system does not comprise any one of SEQ ID NOs: 32-34.

[0124] In some embodiments, the expression vector system comprises one or more elements shown in Figure 1. In some embodiments, the expression vector system comprises the vector shown in Figure 1 or Figure 3. In exemplary aspects, the expression vector system of the present invention comprises the sequence of SEQ ID NO: 24 and/or 25.

[0125] In various embodiments, the expression vector system of the present invention encodes proteins that can be expressed in prokaryotic and eukaryotic cells. In various embodiments, expression vectors can be introduced into host cells for producing the fusion

protein and the ZIKV proteins. There are a variety of techniques available for introducing nucleic acids into viable cells. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, polymer-based systems, DEAE-dextran, viral transduction, the calcium phosphate precipitation method, *etc.* For *in vivo* gene transfer, a number of techniques and reagents may also be used, including electroporation, liposomes; natural polymer-based delivery vehicles, such as chitosan and gelatin; viral vectors are also suitable for *in vivo* transduction.

[0126] The present invention further provides a cell (*e.g.*, a host cell) comprising the expression vector system described herein. Cells (*e.g.*, host cells) may be cultured *in vitro* or genetically engineered, for example. Host cells can be obtained from normal or affected subjects, including healthy humans, patients infected with the ZIKA virus, private laboratory deposits, public culture collections such as the American Type Culture Collection, or from commercial suppliers.

[0127] Cells that can be used include, without limitation, epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, or granulocytes, various stem or progenitor cells, such as hematopoietic stem or progenitor cells (*e.g.*, as obtained from bone marrow), umbilical cord blood, peripheral blood, fetal liver, *etc.*, and tumor cells (*e.g.*, human tumor cells). The choice of cell type can be determined by one of skill in the art. In various embodiments, the cells are irradiated.

[0128] *Flavivirus*

[0129] As used herein, the term “flavivirus” refers to any one of the genus of viruses in the family *Flaviviridae*, including, but not limited to the West Nile virus, dengue virus, tick-borne encephalitis virus, yellow fever virus, Zika virus and several other viruses which may cause encephalitis. The term “flavivirus” also refers to any insect-specific flaviviruses, including, for example, cell fusing agent virus (CFAV), Palm Creek virus (PCV), and Parramatta River virus (PaRV) (McLean et al., *Virology* 486: 272-283 (2015)). In exemplary aspects, the flavivirus is Dengue virus, West Nile virus, or Yellow fever virus.

[0130] *Flavivirus Proteins*

[0131] In various embodiments, the expression vector system of the present invention comprises a nucleic acid encoding a flavivirus protein, or an antigenic portion thereof. In some embodiments, the expression vector comprises two or more nucleic acids each

encoding a different flavivirus protein, or an antigenic portion thereof. In exemplary aspects, the flavivirus is Dengue virus, West Nile virus, or Yellow fever virus and the flavivirus protein is selected from the group consisting of: flavivirus polyprotein [Dengue virus 2] which is described in the NCBI database as NCBI Reference Sequence: NP_056776.2; polyprotein precursor [Yellow fever virus] which is described in the NCBI database as NCBI Reference Sequence: NP_041726.1; and flavivirus polyprotein [West Nile virus] which is described in the NCBI database as NCBI Reference Sequence: NP_041724.2.

[0132] In various embodiments, the flavivirus is ZIKV. In some embodiments, the expression vector system of the present invention comprises a nucleic acid encoding a Zika virus (ZIKV) protein, or an antigenic portion thereof. In exemplary aspects, the expression vector comprises two or more nucleic acids each encoding a different ZIKV protein, or an antigenic portion thereof. The structure of the ZIKV virus is known. See, for example, Kostyuchenko *et al.* (2016) *Nature* 533:425-428, the contents of which are hereby incorporated by reference. In various embodiments, the expression vector system of the invention comprises a nucleic acid encoding any of the known ZIKV protein or an antigenic portion, fragments, or variants thereof. In various embodiments, the ZIKV protein is one or more of membrane glycoprotein precursor M, envelope protein E, nonstructural protein NS1, nonstructural protein NS2A, nonstructural protein NS2B, nonstructural protein NS3, nonstructural protein NS4A, and nonstructural protein NS4B, or antigenic portions, fragments, or variants thereof. In some embodiments, the membrane glycoprotein precursor M comprises the amino acid sequence of SEQ ID NO: 10, the envelope protein E comprises the amino acid sequence of SEQ ID NO: 11, the nonstructural protein NS1 comprises the amino acid sequence of SEQ ID NO: 12, the nonstructural protein NS2A comprises the amino acid sequence of SEQ ID NO: 13, the nonstructural protein NS2B comprises the amino acid sequence of SEQ ID NO: 14, the nonstructural protein NS3 comprises the amino acid sequence of SEQ ID NO: 15, the nonstructural protein NS4A comprises the amino acid sequence of SEQ ID NO: 16, and the nonstructural protein NS4B comprises the amino acid sequence of SEQ ID NO: 17. In some embodiments, the expression vector system comprises a nucleic acid encoding the ZIKV protein membrane glycoprotein precursor M and a nucleic acid encoding the ZIKV protein envelope protein E, or antigenic portions, fragments, or variants thereof. In some embodiments, the expression vector system comprises a nucleic acid encoding the amino acid sequence of SEQ ID NO: 10 and a nucleic acid encoding the amino acid sequence of SEQ ID NO: 11.

[0133] Alternatively, in some embodiments, the expression vector system of the present invention may comprise a nucleic acid encoding a ZIKV protein variant that contains one or more substitutions, deletions, or additions as compared to any known wild type amino acid sequence of the ZIKV protein or a ZIKV amino acid sequence disclosed herein.

[0134] In various embodiments, the ZIKV protein may comprises an amino acid sequence that has at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with any known wild type amino acid sequence of the ZIKV protein or a ZIKV amino acid sequence disclosed herein (e.g. about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% sequence identity).

[0135] Thus, in some embodiments, the ZIKV protein portion of the nucleic acid can encode an amino acid sequence that differs from any known wild type amino acid sequence of the ZIKV protein or a ZIKV amino acid sequence disclosed herein at one or more amino acid positions, such that it contains one or more conservative substitutions, non-conservative substitutions, splice variants, isoforms, homologues from other species, and polymorphisms.

[0136] In some embodiments, present invention provides an expression vector system comprising (i) a nucleic acid encoding the amino acid sequence of SEQ ID NO: 2, optionally lacking the terminal KDEL sequence and (ii) a nucleic acid encoding the amino acid sequence of SEQ ID NO: 11, wherein each nucleic acid is operably linked to a promoter. In

some embodiments, present invention provides a method of treating or preventing a flavivirus infection in a subject, comprising administering to the subject this expression.

[0137] In some embodiments, present invention provides a biological cell comprising a first recombinant protein having an amino acid sequence of at least about 90%, or at least about 95% or at least about 97%, or at least about 98%, or at least about 99% sequence identity with SEQ ID NO: 2, optionally lacking the terminal KDEL sequence and a second recombinant protein having an amino acid sequence of at least about 90%, or at least about 95% or at least about 97%, or at least about 98%, or at least about 99% sequence identity with SEQ ID NO: 11. In some embodiments, present invention provides a method of treating or preventing a flavivirus infection in a subject, comprising administering to the subject this expression.

[0138] As defined herein, a “conservative substitution” denotes the replacement of an amino acid residue by another, biologically similar, residue. Typically, biological similarity, as referred to above, reflects substitutions on the wild type sequence with conserved amino acids. For example, conservative amino acid substitutions would be expected to have little or no effect on biological activity, particularly if they represent less than 10% of the total number of residues in the polypeptide or protein. Conservative substitutions may be made, for instance, on the basis of similarity in polarity, charge, size, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the amino acid residues involved. The 20 naturally occurring amino acids can be grouped into the following six standard amino acid groups: (1) hydrophobic: Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe. Accordingly, conservative substitutions may be effected by exchanging an amino acid by another amino acid listed within the same group of the six standard amino acid groups shown above. For example, the exchange of Asp by Glu retains one negative charge in the so modified polypeptide. In addition, glycine and proline may be substituted for one another based on their ability to disrupt α -helices. Additional examples of conserved amino acid substitutions, include, without limitation, the substitution of one hydrophobic residue for another, such as isoleucine, valine, leucine, or methionine, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term “conservative substitution” also includes the use of a substituted amino acid residue in place of an un-substituted parent amino acid residue,

provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

[0139] As used herein, “non-conservative substitutions” are defined as exchanges of an amino acid by another amino acid listed in a different group of the six standard amino acid groups (1) to (6) shown above.

[0140] In various embodiments, the substitutions may also include non-classical amino acids (e.g. selenocysteine, pyrrolysine, *N*-formylmethionine β -alanine, GABA and δ -Aminolevulinic acid, 4-aminobenzoic acid (PABA), D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ -Abu, ϵ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteine, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β methyl amino acids, C α -methyl amino acids, N α -methyl amino acids, and amino acid analogs in general).

[0141] Mutations may also be made to the nucleotide sequences of the present ZIKV protein sequence by reference to the genetic code, including taking into account codon degeneracy.

[0142] *Chaperones/Fusion proteins*

[0100] In various embodiments, the expression vector system of the present invention comprises a nucleic acid encoding a fusion protein comprising a chaperone protein and an immunoglobulin, or a fragment thereof. In exemplary aspects, the chaperone protein is any one of: gp96, Heat shock protein 70 (Hsp70), BiP, or Grp78. The amino acid sequences of such chaperone proteins are known in the art and are provided herein as, SEQ ID NOs: 29-31. In exemplary aspects, the chaperone protein is gp96. The coding region of human gp96 is 2,412 bases in length, and encodes an 803 amino acid protein that includes a 21 amino acid signal peptide at the amino terminus, a potential transmembrane region rich in hydrophobic residues, and an ER retention peptide sequence at the carboxyl terminus (GENBANK® Accession No. X15187; see, Maki *et al.*, *Proc Natl Acad Sci USA* 1990, 87:5658-5562). The DNA and protein sequences of human gp96 are provided below:

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 attgtaa (SEQ ID NO: 1)

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LSLNIDPDAKVEEEPEEEPEETAEDTTEDTEQDEDEEMDVGTDDEEEETAKESTAEEKDEL (SEQ ID NO: 2).

[0020] In exemplary aspects, the gp96 comprises the amino acid sequence of SEQ ID NO: 2. In exemplary aspects, the gp96 comprises the amino acid sequence of SEQ ID NO: 2 but without the terminal KDEL sequence,

[0021] In various embodiments, the gp96 portion of the fusion protein comprises an amino acid sequence that has at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with any known wild type amino acid sequences of gp96 or a gp96 amino acid sequence disclosed herein (e.g. about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% sequence identity).

[0022] Thus, in some embodiments, the gp96 portion of nucleic acid encoding a gp96-Ig fusion polypeptide can encode an amino acid sequence that differs from the wild type gp96 polypeptide at one or more amino acid positions, such that it contains one or more conservative substitutions, non-conservative substitutions, splice variants, isoforms, homologues from other species, and polymorphisms as described previously.

[0023] In various embodiments, the expression vector system of the present invention comprises a nucleic acid encoding a fusion protein comprising a chaperone protein and an immunoglobulin, or a fragment thereof having the SEQ ID NO: 35.

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cagggtgtgacgtctgataccccaccgagacccattggggccaatacgcgcggttcttcttcttcc

ccaccccccccccaagtcgggtgaaggccagggtctgcagccaacgtcgggcgggcaagccctg
ccatagccacgggccccgtgggttagggacggcggatcgcgggccc (SEQ ID NO: 35).

[0024] In various embodiments, the expression vector system comprises a nucleotide sequence that has at least about 60%, or at least about 61%, or at least about 62%, or at least about 63%, or at least about 64%, or at least about 65%, or at least about 66%, or at least about 67%, or at least about 68%, or at least about 69%, or at least about 70%, or at least about 71%, or at least about 72%, or at least about 73%, or at least about 74%, or at least about 75%, or at least about 76%, or at least about 77%, or at least about 78%, or at least about 79%, or at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99% sequence identity with SEQ ID NO: 35 (e.g. about 60%, or about 61%, or about 62%, or about 63%, or about 64%, or about 65%, or about 66%, or about 67%, or about 68%, or about 69%, or about 70%, or about 71%, or about 72%, or about 73%, or about 74%, or about 75%, or about 76%, or about 77%, or about 78%, or about 79%, or about 80%, or about 81%, or about 82%, or about 83%, or about 84%, or about 85%, or about 86%, or about 87%, or about 88%, or about 89%, or about 90%, or about 91%, or about 92%, or about 93%, or about 94%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% sequence identity with SEQ ID NO: 35).

[0025] Mutations may also be made to the nucleotide sequences of the present fusion proteins by reference to the genetic code, including taking into account codon degeneracy.

[0026] In some embodiments, the chaperone protein may be a heat shock protein. In various embodiments, the heat shock protein is one or more of hsp40, hsp60, hsp70, hsp90, and hsp110 family members, inclusive of fragments, variants, mutants, derivatives or combinations thereof (Hickey, *et al.*, 1989, *Mol. Cell. Biol.* 9:2615-2626; Jindal, 1989, *Mol. Cell. Biol.* 9:2279-2283).

[0027] In various aspects, the fusion protein comprises an immunoglobulin or antibody. The antibody may be any type of antibody, i.e., immunoglobulin, known in the art. In illustrative embodiments, the antibody is an antibody of class or isotype IgA, IgD, IgE, IgG, or IgM. In illustrative embodiments, the antibody described herein comprises one or more

alpha, delta, epsilon, gamma, and/or mu heavy chains. In illustrative embodiments, the antibody described herein comprises one or more kappa or light chains. In illustrative aspects, the antibody is an IgG antibody and optionally is one of the four human subclasses: IgG1, IgG2, IgG3 and IgG4. Also, the antibody in some embodiments is a monoclonal antibody. In other embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is structurally similar to or derived from a naturally-occurring antibody, *e.g.*, an antibody isolated and/or purified from a mammal, *e.g.*, mouse, rabbit, goat, horse, chicken, hamster, human, and the like. In this regard, the antibody may be considered as a mammalian antibody, *e.g.*, a mouse antibody, rabbit antibody, goat antibody, horse antibody, chicken antibody, hamster antibody, human antibody, and the like. In illustrative aspects, the antibody comprises sequence of only mammalian antibodies.

[0143] In illustrative aspects, the fusion protein comprises a fragment of an immunoglobulin or antibody. Antibody fragments include, but are not limited to, the F(ab')₂ fragment which may be produced by pepsin digestion of the antibody molecule; the Fab' fragments which may be generated by reducing the disulfide bridges of the F(ab')₂ fragment, and the two Fab' fragments which may be generated by treating the antibody molecule with papain and a reducing agent. In exemplary aspects, the fusion protein comprises an Fc fragment of an antibody.

[0144] DNAs encoding immunoglobulin light or heavy chain constant regions are known or readily available from cDNA libraries. See, for example, Adams *et al.*, *Biochemistry* 1980, 19:2711-2719; Gough *et al.*, *Biochemistry* 1980 19:2702-2710; Dolby *et al.*, *Proc Natl Acad Sci USA* 1980, 77:6027-6031; Rice *et al.*, *Proc Natl Acad Sci USA* 1982, 79:7862-7865; Falkner *et al.*, *Nature* 1982, 298:286-288; and Morrison *et al.*, *Ann Rev Immunol* 1984, 2:239-256.

[0145] In some embodiments, a gp96 peptide can be fused to the hinge, CH2 and CH3 domains of murine IgG1 (Bowen *et al.*, *J Immunol* 1996, 156:442-449). This region of the IgG1 molecule contains three cysteine residues that normally are involved in disulfide bonding with other cysteines in the Ig molecule. Since none of the cysteines are required for the peptide to function as a tag, one or more of these cysteine residues can be substituted by another amino acid residue, such as, for example, serine.

[0146] In illustrative aspects, the fusion protein comprises an Fc fragment of an IgG1 antibody. In illustrative aspects, the Fc fragment comprises the amino acid sequence of SEQ ID NO: 5.

[0147] In exemplary aspects, the fusion protein comprises a gp96 chaperone protein fused to a Fc fragment of an IgG1 antibody. In illustrative aspects, the fusion protein comprises the amino acid sequence of SEQ ID NO: 8.

[0148] A nucleic acid encoding a gp96-Ig fusion sequence can be produced using the methods described in U.S. Patent No. 8,685,384, which is incorporated herein by reference in its entirety. In some embodiments, the gp96 portion of a gp96-Ig fusion protein can contain all or a portion of a wild type gp96 sequence (*e.g.*, the human sequence set forth herein). For example, a secretable gp96-Ig fusion protein can include the first 799 amino acids of the human gp96 sequence provided herein, such that it lacks the C-terminal KDEL sequence. Alternatively, the gp96 portion of the fusion protein can have an amino acid sequence that contains one or more substitutions, deletions, or additions as compared to any known wild type amino acid sequences of gp96 or a gp96 amino acid sequence disclosed herein.

[0149] In various embodiments, the gp96-Ig fusion protein and/or the flavivirus protein or an antigenic portion thereof, further comprises a linker. In various embodiments, the linker may be derived from naturally-occurring multi-domain proteins or are empirical linkers as described, for example, in Chichili *et al.*, (2013), Protein Sci. 22(2):153-167, Chen *et al.*, (2013), Adv Drug Deliv Rev. 65(10):1357-1369, the entire contents of which are hereby incorporated by reference. In some embodiments, the linker may be designed using linker designing databases and computer programs such as those described in Chen *et al.*, (2013), Adv Drug Deliv Rev. 65(10):1357-1369 and Crasto *et. al.*, (2000), Protein Eng. 13(5):309-312, the entire contents of which are hereby incorporated by reference. In some embodiments, the linker is a synthetic linker such as PEG. In other embodiments, the linker is a polypeptide. In some embodiments, the linker is less than about 100 amino acids long. For example, the linker may be less than about 100, about 95, about 90, about 85, about 80, about 75, about 70, about 65, about 60, about 55, about 50, about 45, about 40, about 35, about 30, about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 12, about 11, about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, or about 2 amino acids long. In some embodiments, the linker is flexible. In another embodiment, the linker is rigid. In various embodiments, the linker is substantially comprised

of glycine and serine residues (*e.g.* about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 95%, or about 97% glycines and serines).

[0150] In various embodiments, the linker is a hinge region of an antibody (*e.g.*, of IgG, IgA, IgD, and IgE, inclusive of subclasses (*e.g.* IgG1, IgG2, IgG3, and IgG4, and IgA1 and IgA2)). The hinge region, found in IgG, IgA, IgD, and IgE class antibodies, acts as a flexible spacer, allowing the Fab portion to move freely in space. In contrast to the constant regions, the hinge domains are structurally diverse, varying in both sequence and length among immunoglobulin classes and subclasses. For example, the length and flexibility of the hinge region varies among the IgG subclasses. The hinge region of IgG1 encompasses amino acids 216-231 and, because it is freely flexible, the Fab fragments can rotate about their axes of symmetry and move within a sphere centered at the first of two inter-heavy chain disulfide bridges. IgG2 has a shorter hinge than IgG1, with 12 amino acid residues and four disulfide bridges. The hinge region of IgG2 lacks a glycine residue, is relatively short, and contains a rigid poly-proline double helix, stabilized by extra inter-heavy chain disulfide bridges. These properties restrict the flexibility of the IgG2 molecule. IgG3 differs from the other subclasses by its unique extended hinge region (about four times as long as the IgG1 hinge), containing 62 amino acids (including 21 prolines and 11 cysteines), forming an inflexible poly-proline double helix. In IgG3, the Fab fragments are relatively far away from the Fc fragment, giving the molecule a greater flexibility. The elongated hinge in IgG3 is also responsible for its higher molecular weight compared to the other subclasses. The hinge region of IgG4 is shorter than that of IgG1 and its flexibility is intermediate between that of IgG1 and IgG2. The flexibility of the hinge regions reportedly decreases in the order IgG3>IgG1>IgG4>IgG2.

[0151] Additional illustrative linkers include, but are not limited to, linkers having the sequence LE, GGGGS, (GGGS)_n (n=1-4), (Gly)₈, (Gly)₆, (EAAAK)_n (n=1-3), A(EAAAK)_nA (n = 2-5), AEAAAKEAAKA, A(EAAAK)₄ALEA(EAAAK)₄A, PAPAP, KESGSVSSEQLAQFRSLD, EGKSSSGSGSESKST, GSAGSAAGSGEF, and (XP)_n, with X designating any amino acid, *e.g.*, Ala, Lys, or Glu.

[0152] In various embodiments, the linker may be functional. For example, without limitation, the linker may function to improve the folding and/or stability, improve the expression, improve the pharmacokinetics, and/or improve the bioactivity of the present compositions. In another example, the linker may function to target the compositions to a particular cell type or location.

[0153] *Host Cells*

[0154] Also provided by the present invention is a host cell comprising any one of the expression vector systems described herein. As used herein, the term "host cell" refers to any type of cell that can contain the inventive expression vector system. The host cell can be a eukaryotic cell, *e.g.*, plant, animal, fungi, or algae, or can be a prokaryotic cell, *e.g.*, bacteria or protozoa. The host cell can be a cultured cell or a primary cell, *i.e.*, isolated directly from an organism, *e.g.*, a human. The host cell can be an adherent cell or a suspended cell, *i.e.*, a cell that grows in suspension. In illustrative aspects, the host cell is a mammalian host cell. In illustrative aspects, the host cell is a human host cell. In illustrative aspects, the human host cell is an NIH 3T3 cell or an HEK 293 cell. The presently disclosed host cells are not limited to just these two types of cells, however, and may be any cell type described herein. For example, the cells that can be used include, without limitation, epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, or granulocytes, various stem or progenitor cells, such as hematopoietic stem or progenitor cells (*e.g.*, as obtained from bone marrow), umbilical cord blood, peripheral blood, fetal liver, *etc.*, and tumor cells (*e.g.*, human tumor cells). The choice of cell type can be determined by one of skill in the art. In various embodiments, the cells are irradiated.

[0155] Also provided by the present invention is a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly host cells (*e.g.*, consisting essentially of) comprising the expression vector(s). The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising the recombinant expression vector(s), such that all cells of the population comprise the recombinant expression vector(s). In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising the expression vector(s) as described herein. In illustrative aspects, the cell population of the present invention is one wherein at least 50% of the cells are host cells as described herein. In illustrative aspects, the cell population of the present invention is one wherein at least 60%, at least 70%, at least 80% or at least 90% or more of the cells are host cells as described herein.

[0156] *Compositions*

[0157] The present invention also provides a composition comprising an expression vector system or a host cell or a population of cells, as described herein, and an excipient, carrier, or diluent. In exemplary aspects, the composition is a pharmaceutical composition. In illustrative aspects, the composition may comprise virus particles containing the vector expression system. In illustrative aspects, the composition is a sterile composition and optionally is suitable for administration to a human. In illustrative aspects, the composition is ready for use. In illustrative aspects, the composition comprises a unit dose of host cells. In illustrative aspects, the unit dose is about 10^5 , about 10^6 , about 10^7 , about 10^8 , about 10^9 , about 10^{10} , about 10^{11} , about 10^{12} , about 10^{13} , about 10^{14} , about 10^{15} , or more host cells transfected with the expression vector system.

[0158] The pharmaceutical composition can comprise any pharmaceutically acceptable ingredient, including, for example, acidifying agents, additives, adsorbents, aerosol propellants, air displacement agents, alkalizing agents, anticaking agents, anticoagulants, antimicrobial preservatives, antioxidants, antiseptics, bases, binders, buffering agents, chelating agents, coating agents, coloring agents, desiccants, detergents, diluents, disinfectants, disintegrants, dispersing agents, dissolution enhancing agents, dyes, emollients, emulsifying agents, emulsion stabilizers, fillers, film forming agents, flavor enhancers, flavoring agents, flow enhancers, gelling agents, granulating agents, humectants, lubricants, mucoadhesives, ointment bases, ointments, oleaginous vehicles, organic bases, pastille bases, pigments, plasticizers, polishing agents, preservatives, sequestering agents, skin penetrants, solubilizing agents, solvents, stabilizing agents, suppository bases, surface active agents, surfactants, suspending agents, sweetening agents, therapeutic agents, thickening agents, tonicity agents, toxicity agents, viscosity-increasing agents, water-absorbing agents, water-miscible cosolvents, water softeners, or wetting agents.

[0159] The pharmaceutical compositions may be formulated to achieve a physiologically compatible pH. In some embodiments, the pH of the pharmaceutical composition may be at least 5, at least 5.5, at least 6, at least 6.5, at least 7, at least 7.5, at least 8, at least 8.5, at least 9, at least 9.5, at least 10, or at least 10.5 up to and including pH 11, depending on the formulation and route of administration, for example between 4 and 7, or 4.5 and 5.5. In illustrative embodiments, the pharmaceutical compositions may comprise buffering agents to achieve a physiological compatible pH. The buffering agents may include any compounds capable of buffering at the desired pH such as, for example, phosphate buffers (*e.g.*, PBS),

triethanolamine, Tris, bicine, TAPS, tricine, HEPES, TES, MOPS, PIPES, cacodylate, MES, acetate, citrate, succinate, histidine or other pharmaceutically acceptable buffers.

[0160] The present invention therefore provides compositions including pharmaceutical compositions containing an expression vector system or a cell containing the expression vector system as described herein, in combination with a physiologically and pharmaceutically acceptable carrier. In various embodiments, the physiologically and pharmaceutically acceptable carrier can include any of the well-known components useful for immunization. The carrier can facilitate or enhance an immune response to an antigen administered in a vaccine. The cell formulations can contain buffers to maintain a preferred pH range, salts or other components that present an antigen to an individual in a composition that stimulates an immune response to the antigen. The physiologically acceptable carrier also can contain one or more adjuvants that enhance the immune response to an antigen. Pharmaceutically acceptable carriers include, for example, pharmaceutically acceptable solvents, suspending agents, or any other pharmacologically inert vehicles for delivering compounds to a subject. Pharmaceutically acceptable carriers can be liquid or solid, and can be selected with the planned manner of administration in mind so as to provide for the desired bulk, consistency, and other pertinent transport and chemical properties, when combined with one or more therapeutic compounds and any other components of a given pharmaceutical composition. Typical pharmaceutically acceptable carriers include, without limitation: water, saline solution, binding agents (*e.g.*, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose or dextrose and other sugars, gelatin, or calcium sulfate), lubricants (*e.g.*, starch, polyethylene glycol, or sodium acetate), disintegrates (*e.g.*, starch or sodium starch glycolate), and wetting agents (*e.g.*, sodium lauryl sulfate). Compositions can be formulated for subcutaneous, intramuscular, or intradermal administration, or in any manner acceptable for administration.

[0161] An adjuvant refers to a substance which, when added to an immunogenic agent such as a cell containing the expression vector system of the invention, nonspecifically enhances or potentiates an immune response to the agent in the recipient host upon exposure to the mixture. Adjuvants can include, for example, oil-in-water emulsions, water-in oil emulsions, alum (aluminum salts), liposomes and microparticles, such as, polystyrene, starch, polyphosphazene and polylactide/polyglycosides.

[0162] Adjuvants can also include, for example, squalene mixtures (SAF-I), muramyl peptide, saponin derivatives, mycobacterium cell wall preparations, monophosphoryl lipid A,

mycolic acid derivatives, nonionic block copolymer surfactants, Quil A, cholera toxin B subunit, polyphosphazene and derivatives, and immunostimulating complexes (ISCOMs) such as those described by Takahashi *et al.*, *Nature* 1990, 344:873-875. For veterinary use and for production of antibodies in animals, mitogenic components of Freund's adjuvant (both complete and incomplete) can be used. In humans, Incomplete Freund's Adjuvant (IFA) is a useful adjuvant. Various appropriate adjuvants are well known in the art (see, for example, Warren and Chedid, *CRC Critical Reviews in Immunology* 1988, 8:83; and Allison and Byars, in *Vaccines: New Approaches to Immunological Problems*, 1992, Ellis, ed., Butterworth-Heinemann, Boston). Additional adjuvants include, for example, bacille Calmett-Guerin (BCG), DETOX (containing cell wall skeleton of *Mycobacterium phlei* (CWS) and monophosphoryl lipid A from *Salmonella minnesota* (MPL)), and the like (see, for example, Hoover *et al.*, *J Clin Oncol* 1993, 11:390; and Woodlock *et al.*, *J Immunother* 1999, 22:251-259).

[0163] *Routes of Administration*

[0164] Methods of administering cells to a subject are well-known, and include, but not limited to perfusions, infusions and injections. See, *e.g.*, Burch *et al.*, *Clin Cancer Res* 6(6): 2175-2182 (2000), Dudley *et al.*, *J Clin Oncol* 26(32): 5233-5239 (2008); Khan *et al.*, *Cell Transplant* 19:409-418 (2010); Gridelli *et al.*, *Liver Transpl* 18:226-237 (2012)).

[0165] *Methods of Use*

[0166] Without being bound to a particular theory, the methods of the present invention advantageously rely on the chaperone function of the secreted fusion protein. The fusion protein chaperones the one or more ZIKV proteins or antigen portions thereof, which are efficiently taken up by activated antigen presenting cells (APCs). The APCs act to cross-present the ZIKV proteins or antigen portions thereof via MHC I to CD8⁺ CTLs, whereupon an avid, antigen specific, cytotoxic CD8⁺ T cell response is stimulated. Without being bound to a particular theory, the expression vector systems of the present invention are advantageously capable of initiating both an innate immune response (including, *e.g.*, activation of APCs, pro-inflammatory cytokine release, activation of NK cells), and an adaptive immune response (including, *e.g.*, priming, activation and proliferation of antigen specific CTLs). Such dual-activation leads to successful clearance of the antigen/pathogen.

[0101] Accordingly, in various embodiments, the present invention provides a method of eliciting an immune response against a flavivirus, *e.g.*, Zika virus (ZIKV), in a subject. In

illustrative embodiments, the method comprises administering to the subject the expression vector as disclosed herein, or a population of cells transfected with the expression vector. If the subject is a pregnant female, the immune response is detected in the placenta and/or decidua in various embodiments. For example, in various instances, the method inhibits or reduces the severity of infection in a fetus and/or inhibits or reduces the severity of the biological effects of ZIKV infection of the fetus. In some embodiments, methods of the invention induce immune responses in the placenta and prevent flavivirus (*e.g.*, ZIKV) infection to the embryo or fetus. In some embodiments, methods of the invention induce virus-specific T cell response (*e.g.*, CD8 T cells or cytotoxic T lymphocytes) in the placenta and/or the decidua resulting in clearance of the virus (for example, before it reaches the embryo or fetus).

[0102] In various embodiments, the present methods stimulate an immune response, *e.g.* against a flavivirus, *e.g.*, Zika virus, in various structures within the placenta and/or the decidua, where the flavivirus, *e.g.*, Zika virus, is transmitted and/or infects the placenta, without wishing to be bound by theory, via the blood-syncytiotrophoblast interface and/or the uterus-trophoblast interface.

[0103] The present invention also provides a method of treating or preventing a Zika virus infection in a subject, comprising administering to the subject the expression vector as disclosed herein, or a population of cells transfected with the expression vector. In various embodiments, the subject is an embryo or fetus.

[0167] The present invention also provides a method of treating or preventing one or more birth defects in an embryo or fetus, including fetal brain defects (such as microcephaly), eye defects, hearing loss, and impaired growth. fever, rash, joint pain, or conjunctivitis (red eyes). The present invention also provides a method of treating or preventing one or more symptoms/associated diseases of Zika infection including without limitation Guillain-Barre syndrome, muscle pain, headache, fever, rash, joint pain, and conjunctivitis.

[0168] As used herein, the term “treat,” as well as words related thereto, do not necessarily imply 100% or complete treatment. Rather, there are varying degrees of treatment of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the methods of treating a ZIKV infection of the present invention can provide any amount or any level of treatment. Furthermore, the treatment provided by the method of the present invention may include treatment of one or more conditions or symptoms or signs

of the infection, being treated. Also, the treatment provided by the methods of the present invention may encompass slowing the progression of the infection. For example, the methods can treat the infection by virtue of eliciting an immune response against ZIKV, stimulating or activating CD8+ T cells specific for ZIKV to proliferate, and the like.

[0169] As used herein, the term “prevent” and words stemming therefrom encompasses inhibiting or otherwise blocking infection by ZIKV. As used herein, the term “inhibit” and words stemming therefrom may not be a 100% or complete inhibition or abrogation. Rather, there are varying degrees of inhibition of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the presently disclosed expression vector systems or host cells may inhibit ZIKV infection to any amount or level. In illustrative embodiments, the inhibition provided by the methods of the present invention is at least or about a 10% inhibition (*e.g.*, at least or about a 20% inhibition, at least or about a 30% inhibition, at least or about a 40% inhibition, at least or about a 50% inhibition, at least or about a 60% inhibition, at least or about a 70% inhibition, at least or about a 80% inhibition, at least or about a 90% inhibition, at least or about a 95% inhibition, at least or about a 98% inhibition).

[0104] In various embodiments, methods of the invention prevent, alleviate, and/or treat one or more symptoms associated with ZIKV infection. Illustrative symptoms that may be treated include, but are not limited to fever, rash (*e.g.*, skin rash), muscle and/or joint pain, swollen joints, malaise, headache, conjunctivitis (red eyes), post-infection asthenia, digestive problems including abdominal pain, diarrhea, constipation, mucous membrane ulcerations (aphthae), pruritus, meningoencephalitis, and Guillain-Barré syndrome.

[0105] In various embodiments, methods of the invention may prevent, alleviate, and/or treat one or more symptoms associated with ZIKV infection in pregnant women including those symptoms described above. Additionally, methods of the invention may prevent spontaneous abortions in pregnant women.

[0106] Without wishing to be bound by theory, it is believed that the present invention enhances placental functions against ZIKV infection. In various embodiments, administration of the expression vector system of the invention or the cell including the expression vector system prevents and/or reduces pathological changes in the placenta induced by ZIKV infection.

[0107] Without wishing to be bound by theory, it is believed that methods of the invention prevent the placenta from conveying the ZIKV to the embryo or fetus. For example, methods of the invention may prevent the ZIKV from reaching the fetal brain by transplacental passage, leakage through the trophoblastic plugs, and/or diffusion into the amniotic and yolk sac during embryogenesis. In some embodiments, methods of the invention may prevent the transmission of ZIKV to the placenta through the blood-syncytiotrophoblast interface where the placenta is bathed in maternal blood. In another embodiment, methods of the invention may prevent the transmission of ZIKV to the placenta through the uterus-trophoblast interface where the extra villous trophoblast in humans invade the uterine epithelium and come in contact with maternal immune cells. Alternatively, or additionally, methods of the invention may prevent the placenta from mounting an abnormal response against the ZIKV thus causing defects in the embryo or the fetus. In some embodiments, methods of the invention prevent the ZIKV in semen from reaching the embryo or fetus.

[0108] Accordingly, in various embodiments, methods of the invention prevent, alleviate, and/or treat one or more symptoms associated with ZIKV infection in an embryo or a fetus including an unborn embryo or fetus. In some embodiments, the expression vector system of the invention stimulates immune responses in the placenta and prevent the embryo or fetus from developing congenital abnormalities including microcephaly and other fetal brain defects. Additionally, methods of the invention may prevent the embryo or fetus from developing other health issues including, but not limited to, eye defects, hearing loss, and impaired growth.

[0109] The present expression vector system and cells comprising the same may be administered by any route considered appropriate by a medical practitioner. Illustrative routes of administration include, for example: oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, by electroporation, or topically. Administration can be local or systemic.

[0170] In illustrative aspects, the method comprises intramuscular (IM) administration of the expression vector. In illustrative aspects, the method comprises electroporation or electroporation following the IM administration of expression vector. In various embodiments, electroporation is used to help deliver vectors (genes) into the cell by applying short and intense electric pulses that transiently permeabilize the cell membrane, thus allowing transport of molecules otherwise not transported through a cellular membrane.

Methods for electroporating a nucleic acid construct into cells and electroporation devices for such delivery are known. See, for example, Flanagan *et al.* Cancer Gene Ther (2012) 18:579-586, WO 2014/066655, US 9,020,605, the entire contents are incorporated by reference.

[0171] In exemplary aspects, DNA (50 µg) containing expression vector that contains gp96-Ig and ZIKV antigens in 50 µL of saline is injected in the tibialis anterior muscle of anesthetized wild-type C57BL/6 mice. A two-needle array electrode pair is inserted into muscle immediately after DNA delivery and the injection site is electroporated with field strength of 50 V/cm (constant) and six electric pulses of 50 ms each by using the AgilePulse *in Vivo* System (BTX, Harvard Apparatus).

[0172] In illustrative aspects, the method comprises subcutaneously administering the population of cells. In illustrative aspects, the method comprises subcutaneously administering the population of cells to an arm or leg of the subject.

[0173] In various embodiments, the vector or the cell can be administered to a subject one or more times (*e.g.*, once, twice, two to four times, three to five times, five to eight times, six to ten times, eight to 12 times, or more than 12 times). A vector or a cell as provided herein can be administered one or more times per day, one or more times per week, every other week, one or more times per month, once every two to three months, once every three to six months, or once every six to 12 months. A vector or a cell can be administered over any suitable period of time, such as a period from about 1 day to about 12 months. In some embodiments, for example, the period of administration can be from about 1 day to 90 days; from about 1 day to 60 days; from about 1 day to 30 days; from about 1 day to 20 days; from about 1 day to 10 days; from about 1 day to 7 days. In some embodiments, the period of administration can be from about 1 week to 50 weeks; from about 1 week to 50 weeks; from about 1 week to 40 weeks; from about 1 week to 30 weeks; from about 1 week to 24 weeks; from about 1 week to 20 weeks; from about 1 week to 16 weeks; from about 1 week to 12 weeks; from about 1 week to 8 weeks; from about 1 week to 4 weeks; from about 1 week to 3 weeks; from about 1 week to 2 weeks; from about 2 weeks to 3 weeks; from about 2 weeks to 4 weeks; from about 2 weeks to 6 weeks; from about 2 weeks to 8 weeks; from about 3 weeks to 8 weeks; from about 3 weeks to 12 weeks; or from about 4 weeks to 20 weeks.

[0174] Embodiments that relate to methods of treatment are also envisioned to apply to medical uses and uses in manufacture of medicaments.

[0175] *Combination Therapy*

[0176] In various embodiments, the composition of the present invention is co-administered in conjunction with additional therapeutic agent(s). Co-administration can be simultaneous or sequential.

[0177] In some embodiments, the additional therapeutic agent is an agent that is used to provide relief to symptoms of ZIKV infections. Such agents include anti-inflammatory agents such as a steroidal anti-inflammatory agent or a non-steroidal anti-inflammatory agent (NSAID). Steroids, particularly the adrenal corticosteroids and their synthetic analogues, are well known in the art. Examples of corticosteroids useful in the present invention include, without limitation, hydroxyltriamcinolone, alpha-methyl dexamethasone, beta-methyl betamethasone, beclomethasone dipropionate, betamethasone benzoate, betamethasone dipropionate, betamethasone valerate, clobetasol valerate, desonide, desoxymethasone, dexamethasone, diflorasone diacetate, diflucortolone valerate, fludrenolone, flucorolone acetonide, flumethasone pivalate, fluosinolone acetonide, fluocinonide, flucortine butylester, flucortolone, fluprednidene (fluprednylidene) acetate, flurandrenolone, halcinonide, hydrocortisone acetate, hydrocortisone butyrate, methylprednisolone, triamcinolone acetonide, cortisone, cortodoxone, flucetonide, fludrocortisone, difluorosone diacetate, fluradrenolone acetonide, medrysone, amcinafel, amcinafide, betamethasone and the balance of its esters, chloroprednisone, clocortelone, clescinolone, dichlorisone, difluprednate, flucoronide, flunisolid, fluoromethalone, fluperolone, fluprednisolone, hydrocortisone, meprednisone, paramethasone, prednisolone, prednisone, beclomethasone dipropionate. (NSAIDS) that may be used in the present invention, include but are not limited to, salicylic acid, acetyl salicylic acid, methyl salicylate, glycol salicylate, salicylmides, benzyl-2,5-diacetoxybenzoic acid, ibuprofen, flindac, naproxen, ketoprofen, etofenamate, phenylbutazone, and indomethacin.

[0178] In an embodiment, the additional therapeutic agent is chloroquine, including chloroquine phosphate.

[0179] In an embodiment, the additional therapeutic agent is a composition comprising all-natural herbal ingredients as disclosed in US 2016/0250181, the contents of which are hereby incorporated by reference. In some embodiments, the composition comprises a combination of one or more artemisinin, berberine, capsaicin and *Tinospora Cordifolia*.

[0180] In various embodiments, the additional therapeutic agent is a Zika vaccine (*e.g.* which allows use of two vaccines in a subject for possible improved response), including

GLS-5700 (Inovio Pharmaceuticals) and VRC-ZKADNA085-00-VP (NIAID). For instance, in various embodiments, the present vaccines in combination with known Zika vaccines allows for stimulation of both humoral- and T cell-based immunity.

[0181] *Subjects*

[0182] In illustrative embodiments, the subject is a mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits, mammals from the order Carnivora, including Felines (cats) and Canines (dogs), mammals from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). In some aspects, the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes).

[0183] In various embodiments, the mammal is a human. In some embodiments, the human is an adult aged 18 years or older. In some embodiments, the human is a child aged 17 years or less. In an embodiment, the subject is male, *e.g.*, a male human. In another embodiment, the subject is a female subject. In illustrative embodiments, the subject is a female subject, *e.g.*, a female human, aged from about 16 years to about 50 years. In illustrative embodiments, the female human is capable of giving birth. In illustrative embodiments, the subject is a pregnant female. In illustrative embodiments, the human pregnant female is in the first trimester, second trimester, or third trimester of pregnancy. In illustrative embodiments, the subject is a female and the population of cells is administered prior to pregnancy. In illustrative embodiments, the subject is not pregnant. In various embodiments, the subject is an embryo or a fetus including an unborn embryo or fetus. As referred to herein, an embryo is developed from the time of fertilization until the end of the eighth week of gestation, at which time it is referred to as a fetus.

[0184] *Kits*

[0185] Kits comprising host cells (or a cell population comprising the same) or expression vector systems or a composition comprising any one of the foregoing of the present invention are also provided. In illustrative aspects, the kits comprise a unit dose of cells comprising the expression vector systems of the present invention. In illustrative aspects, the kit comprises a sterile, GMP-grade unit dose of the cells. In illustrative aspects, a unit dose of cells comprises 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , or more than 10^{15} cells comprising the expression vector system of the present invention.

[0186] In illustrative aspects, the unit dose of cells are packaged in an intravenous bag. In illustrative aspects, the unit dose of cells are provided in a cryogenic form. In illustrative aspects, the unit dose of cells are ready to use. In illustrative aspects, the unit dose of cells are provided in a tube, a flask, a dish, or like container.

[0187] In illustrative aspects, the cells are cryopreserved. In illustrative aspects, the cells are not frozen.

[0188] The following examples are given merely to illustrate the present invention and not in any way to limit its scope.

EXAMPLES

Example 1

[0028] This example demonstrates, *inter alia*, a method of constructing an expression vector encoding a gp96-Ig fusion protein.

[0029] An expression vector comprising a nucleic acid encoding a gp96-IgFc fusion protein and one or more ZIKV antigens is constructed. The expression vector is shown in Figure 1. For reference, Yamazaki et al., *The Journal of Immunology* 163: 5178–5182 (1999) describes the construction of a similar expression vector comprising a nucleic acid encoding gp96-Ig fusion protein and the transfection of the expression vector into cells. Briefly, Yamazaki et al., 1999, *supra*, teaches that the KDEL sequence was deleted and replaced with the hinge, CH2 and CH3 domains of murine IgG1 (see, References 16–23 of Yamazaki et al., 1999, *supra*); double-stranded cDNA was prepared from Jurkat DNA (see, Reference 24 of Yamazaki et al., 1999, *supra*) with the GeneAmp RNA PCR Kit (Perkin-Elmer Cetus, Norwalk, CT) and amplified by PCR. The PCR primers were 5'-ATTACTCGAGGGCCGCACGC CATGAGGG-3' and 5'-GCCCCGATCCTTCAGCTGTAGATTTCCTT TGC-3' (Maki et al., *PNAS USA* 87: 5658(1990); Maki et al., *Somat Cell Mol Genet* 19:73 (1993)). The PCR primers included an *Xho*I site (forward primer) and a *Bam*HI site (reverse primer). The hinge, CH2 and CH3 domains of murine IgG1, was amplified by using murine IgG1 cDNA as a template and mutating the three cysteines of the hinge portion to serines (see, References 21, 25 of Yamazaki et al., 1999, *supra*). The PCR primers were 5'-GCGAGGATCCGTGCCCAGGGATTCTGGTT CTAAG-3' and 5'-CTAAGCGGCCGCAAGGACACTGGGATCATTT ACCAGG-3'. The PCR primers included a *Bam*HI site (forward primer) and *Not*I site (reverse primer). Gp96 was inserted into *Xho*I and

*Bam*HI sites of the eukaryotic expression vector, pBCMGSNeo and pBCMGLHis (see References 26–29 of Yamazaki et al., 1999, *supra*) and transfected into SCLC-2, SCLC-7, B16F10, MC57, LLC NIH3T3, EL4, E.G7, and P815. Transfected cells were selected with 1 mg/ml of G418 or 2.5–10 mM of L-Histidinol (Sigma, St. Louis, MO). A second expression vector encoding chicken ovalbumin (OVA) named apc-NEO-OVA was co-transfected into the cells that were transfected with pBCMGSneo expressing gp96-Ig. The production of the gp96-Ig fusion protein by transfected cells and the secretion of the fusion protein into the culture medium was assayed via ELISA. Transfected cells were administered to mice and shown to enhance the CD8 T cell immune response.

Example 2

[0030] This example demonstrates the induction of antigen specific CTL responses in placenta and decidua of pregnant B6 mice by secreted gp96^{OVA}-Ig.

[0031] The placenta acts as a barrier against infections, due to multiple unique structural, cellular, and immune properties. The detrimental effects of congenital viruses on pregnancy and fetal outcomes occur in part because of impaired placental function and profound pathological changes in ZIKV-infected placentas have been observed. Induction of appropriate immune responses in placenta are key to successful prevention of viral infections from developing fetus. Induction of virus-specific CD8 T cells responses in the placenta is hypothesized to lead to clearance of the pathogen/virus.

[0032] To test the ability of vaccine cells to stimulate CD8 T cells in the placenta and decidua, NIH3T3 cells were co- transfected with a gp96-Ig expression vector were made as described in Example 1 and Yamazaki et al., 1999, *supra*, and a second expression vector encoding chicken ovalbumin (OVA) named apc-NEO-OVA. Such cells are referred to as 3T3-gp96-OVA-Ig cells. An illustrative schematic of the experiment is depicted in Figure 4.

[0033] B6 females were mated with B6 mice and gestation day (GD) 0.5 was determined by the presence of vaginal plug. At GD 5.5, 1 million of OT1 cells (T cells comprising T cell receptors specific for the OVA antigen) were injected in the tail vein. Two days' post-injection, 2.5 million 3T3-gp96-OVA-Ig or 3T3-gp96-Ig cells were injected subcutaneously. Five days later, mice were euthanized. Pregnant uteri were collected and from individual fetal/placental units (FPU) placental disk was separated form decidua (maternal endometrium). Placental and decidual single cells suspension was obtained by collagenase D digestion. Cells were labeled with antiCD8 antibody and frequency of OT1 cells was

determent by flow cytometry. Representative dot plots of gated CD3+CD8+ T cells in the placenta and decidua and the frequency of OT1 positive cells are shown in Figure 2.

[0034] After subcutaneous injection of vaccine cells (3T3-OVA-gp96-Ig) into pregnant B6 mice, OVA specific CD8 T cells (OT1) were detected in high frequencies in placenta and decidua (10-15% of all CD8 T cells in placenta and decidua were OVA specific T cells) (Figure 2) without damaging maternal-fetal interface and preserving healthy fetus.

[0035] These data support that complexes of gp96-Ig fusion protein chaperoning the OVA antigens are recognized by antigen presenting cells (APCs) local to the site of SC injection. The APCs prime or activate the innate and adaptive arms of the immune system, and activate CD8-specific T cells having T cell receptors that specifically bind and recognize the OVA antigen chaperoned by the gp96-Ig fusion protein, thereby causing proliferation of these T cells and priming of these T cells. Primed cells circulate in the blood and make their way into the placenta through means of normal circulation.

[0036] This example provides proof-of-principle that the administration of cells expressing gp96-Ig fusion protein and an antigen of interest can lead to stimulation of antigen-specific CD8 T cells located in the placenta and decidua of pregnant subjects.

Example 3

[0037] This example demonstrates a method of administering cells comprising an illustrative expression vector of the present invention.

[0038] An expression vector encoding the gp96-Ig fusion protein and ZIKV antigens was constructed. A map of the expression vector made is shown in Figure 1. HEK293 cells were then transfected with the gp96-Ig-ZIKV antigen expression vector.

[0039] Transfected HEK293 cells are first irradiated (12,000 rads) and then suspended in freezing medium containing 10%DMSO, 25% human serum albumin, sodium bicarbonate 8.4% and sodium chloride 0.9% and subcutaneously administered to the upper outer arm or thigh of a human female. The number of cells that are injected depends on the amount of secreted gp96-Ig: equivalent number of cells that produce 10-20 micrograms of gp96-Ig in in vitro established assay. Based on previous experiments (*e.g.*, J Immunol. 2013 Mar 15;190(6):2495-9. doi: 10.4049/jimmunol.1202655. Epub 2013 Feb 11. PMID: 23401588; Vaccine. 2011 Mar 21;29(14):2619-25. doi: 10.1016/j.vaccine.2011.01.044. Epub 2011 Jan 28. PMID:21277409; Mucosal Immunol. 2010 Mar;3(2):182-92. doi: 10.1038/mi.2009.127. Epub 2009 Nov 18. PMID: 19924120; J Immunol. 2007 Aug 15;179(4):2310-7.

PMID:17675492), the complexes of gp96-Ig chaperoning the ZIKV antigens are recognized by APCs local to the injection site. The APCs prime or activate the innate and adaptive arms of the immune system, and activate CD8-specific T cells having T cell receptors that specifically bind and recognize the ZIKV antigens chaperoned by the gp96-Ig fusion protein, thereby causing proliferation of these T cells and priming of these T cells. Primed T cells circulate in the blood and make their way into the placenta through means of normal circulation. In this experiment, the number of cells injected depends on the amount of secreted gp96-Ig, *i.e.*, equivalent number of cells are used that produces 10-20 micrograms of gp96-Ig in in vitro established assays.

Example 4

[0040] This example demonstrates a method of administering illustrative expression vectors of the present invention.

[0041] The expression vector of Figure 1 is generated and formulated into a sterile composition. The composition is injected into the muscle of the upper outer arm or thigh of a human female subject. Subsequently, electroporation (EP) pulses are delivered, a slow resealing of the membrane occurs on the second to minutes' time scale. The end result of the process is that upwards of 100–1000-fold enhancement of vector delivery and increased expression of the encoded ZIKV antigens and gp96-Ig fusion protein, which in turn, leads to elevated immune responses relative to delivery of DNA alone without electroporation. *See, e.g.*, Luckay et al., J Virol 81:5257-5269 (2007); Liu et al., J Virol 82: 4844-4852 (2008); Hirao et al., Vaccine 26:3112-3120 (2008); Rosati et al., Vaccine 26: 5223-5229 (2008); Simon et al., Vaccine 26:5202-5209 (2008); Hirao et al., Mol Ther 18:1568-1576 (2010); Dobano et al., Vaccine 25: 6635-6645 (2007); LeBlanc et al., Vaccine 26:185-192 (2008); Ahlen et al., J Immunol 179:4741-4753 (2007); Luxembourg et al., Vaccine 26: 4025-4033 (2008); van Drunen Littel-van den Hurk et al., Vaccine 26: 5503-5509 (2008); Kim et al., Exp Mol Med 40: 669-676 (2008); Nystrom et al., J Infect Dis 201: 1867-1879 (2010); Trollet et al., Infect Immun 77:2221-2229 (2009); Best et al., Vaccine 27: 5450-5459 (2009); Seo et al., Vaccine 27: 5906-5912 (2009); Livingston et al., Vaccine 28: 1056-1061 (2010); Laddy et al., J Virol 83: 4624-4630 (2009); Laddy et al., PLoS One 3:e2517 (2008); and Hirao et al., Vaccine 26: 440-448 (2008).

[0042] Experiments are also performed in mice. Specifically, 50 micrograms of DNA containing expression vector that contains gp96-Ig and ZIKV antigens in 50 micro liters of

saline was injected in the tibialis anterior muscle of anesthetized wild-type C57BL/6 mice. A two-needle array electrode pair was inserted into muscle immediately after DNA delivery and the injection site was electroporated with field strength of 50 V/cm (constant) and six electric pulses of 50 ms each by using the AgilePulse *In Vivo* System (BTX, Harvard Apparatus).

Example 5

[0043] This example demonstrates the generation of gp96-Ig and ZIKA envelope vaccine and induction of ZIKV-Env-specific CD8⁺ T lymphocyte responses.

[0044] HEK-293-gp96-Ig was transfected with the B45 vector expressing gp9-Ig and ZIKV-envelope. The cells were transfected using Effectin-based transfection protocol and subsequently selected in the presence of selection marker (geneticin, G418). a stable transfection of HEK-293 cells that co express gp96-Ig and ZIKV-envelope as shown in the figure below was established (Figure 8A). Production of gp96-Ig was confirmed by ELISA using human IgG1 as a standard. Western blotting was also used to confirm that cells express ZIKA envelope (Figure 8B, lane 2). MBS430270 (MyBiosource) Anti-Envelope E (ZikaVirus) Polyclonal antibody as a primary antibody (1:1000) and HRP-anti-rabbit IgG as a secondary antibody (Jackson ImmunoResearch) (1:5000) were used to confirm (Figure 8B). The expression of gp96-Ig production was improved in an already established cell line, 293-gp96-Ig-ZIKVenv (Figure 8A, C) by performing single cell cloning. Data showed several highly gp96-Ig- expressing cell clones (Figure 8C). Clone 5G4 was further expanded, high production of gp96-Ig confirmed by ELISA (Figure 8D) and ZIKA envelope expression confirmed by Western blotting (Figure 8E). Vaccine cells were irradiated and stored in freezing media at concentration 10⁶/500ul at -135 °C and used for in vivo experiments.

[0045] Data shows that continuous cell secretion of gp96-Ig in vivo is more effective for CD8 CTL induction than injection of purified gp96-Ig. This increased efficiency is attributed to continuous secretion and stimulation of the immune system, similar to attenuated viral vaccines. However, DNA vaccines are more attractive due to their safety and ease of engineering and manufacturing compared to cell-based vaccine approach.

[0046] In order to confirm efficacy of DNA vaccine delivery, transfection efficiency of B45 plasmid entry into target (muscle) cells after *in vivo* electroporation (EP) by fluorescent microscopy was measured. B45 plasmid containing secreted gp96-Ig (in the 1st expression cassette) and EGFP (in the 2nd expression cassette) was injected into muscle (tibialis anterior) (50 micrograms) followed by a brief electric field pulse that induces temporary and

reversible pores in the muscle cell membrane. B45 plasmid (prepared using Endotoxin-free DNA purification kit, Qiagen, CA) at previously determined dose of 50 micrograms was used. *In vivo* electroporation was applied after injection of the plasmid(s) into muscles using AgilePulse IM *in Vivo* gene delivery system (BTX Harvard Apparatus). Left muscle was injected with PBS. Expression of EGFP protein was analyzed in longitudinal sections of the tibialis anterior muscle 48 h after injection (Figure 9). Frozen tissue samples were cut in 10 micron sections, stained with anti-gfp-AF488 antibody and examined under the fluorescent microscope. Nuclei were stained with DAPI (blue). Results indicated very high level of expression of gp96-Ig-gfp in the injected site. This results confirms that *in vivo* transfection of gp96-Ig is successful and that immunization protocols can begin with gp96-Ig-ZIKV DNA and electroporation.

[0047] Without wishing to be bound by theory, a critical issue for protective ZIKV vaccines is induction of protective immune responses at maternal/fetal interface. In order to develop ZIKV protective vaccine, immunogenicity and efficacy was tested in a pregnant mouse model to measure immunogenicity of secreted gp96-Ig vaccine (Figure 10A-C and Figure 11). First, to confirm preliminary data that vaccination with gp96-Ig during pregnancy is safe, female mice were first evaluated for the stage of the estrous cycle by visual observation for 2 weeks. Females in the proestrus cycle were put with one male for 24 h. Appearance of a vaginal plug was marked as day 0.5 of pregnancy, gestation day (GD) 0.5 at which time male mice were removed. Pregnant mice at GD 7.5 were vaccinated with established vaccine cells (293-gp96-Ig-ZIKVenv) or with mock control (PBS). Mice were followed up until the end of pregnancy (GD 19.5-21.5) or humanely euthanized 5 days after vaccination (GD 12.5) (Figure 10). Results indicated that vaccinated mice have the same number of live-born pups as control mice (n=12). In addition, histological findings confirm that there was no difference among fetal-placental units from control vs vaccinated dams with an overall conclusion that gp96-Ig vaccination during pregnancy does not induce pathological changes in placenta or in fetus. (Figure 10A, B and C).

[0048] To confirmation of preliminary data showing that gp96-Ig vaccination induces CD8 T cell responses in placenta. Pregnant WT mice were injected s.c with 293-gp96-Ig-ZIKAEnv at GD 7.5. Individual Fetal-Placental Unit at GD 12.5 was fixed in 10% neutral buffered formalin solution and embedded in paraffin. Deparaffinized sections were fixed in acetone and staining was performed using primary antibody against CD8 (clone YTS169) followed by incubation with secondary goat-anti-rabbit antibody and peroxidase-linked

avidin (Dako). 3 Amino-9 ethylcarbazole (Sigma-Aldrich) was used as the chromogen resulting in the brown staining. Serial section incubated with an Ab of the same isotype were used for control staining. White arrows correspond to the same section area of black arrows (Figure 11). Results showed that dams vaccinated s.c. with 293-gp96-Ig-ZIKVenv (0.2×10^6) at GD 7.5, accumulate CD8 specific cells in the maternal decidua (Figure 11). This is the first finding that confirms the presence of gp96-Ig-ZIKVenv-induced CD8 T cells around arteries and larger blood vessels in the maternal decidua.

[0049] CD8 T cells in decidua was evaluated by flow cytometry (Figure 12). Pregnant WT mice were injected s.c with 293-gp96-Ig-ZIKAEnv or PBS (CTRL) at GD 7.5. Individual Fetal-Placental Unit at GD 12.5 were collected and decidua was dissected from the placental tissue. Decidual single cell suspension was prepared by physical dissociation, cells were passed through the mesh and stained for following cell surface markers: live/dead marker, CD45, CD3, CD11a, CD69, CD25, CD62L. After surface staining cells were fixed/permeabilized and stained for Ki67. Cells were analyzed on Fortessa (BD) flow cytometer. Live, CD45⁺ CD3⁺ lymphocytes were analyzed for expression of CD8 and frequency of CD8⁺ T cells is shown ($n=6 \pm$ SEM, $p=0.05$). Gated CD8 T cells were analyzed for the expression of following markers: CD11a, Ki67, CD69, CD25 and CD62L. Results indicated that frequency of decidual CD8 T cells was significantly increased in vaccinated vs control (PBS treated) pregnant mice. Phenotype of decidual CD8 T cells revealed that in vaccinated dams CD8 T cells were highly proliferating cells (high Ki67 expression), activated (high CD69 and CD25 expression) effector memory CD8 T cells (high CD11a and lower CD62L expression) compared to non- vaccinated controls (Figure 12). Increased expression of integrin molecule CD11a on CD8 T cells after various types of infections and/or antigen stimulations can distinguish naïve from antigen-experienced effector and memory CD8 T cells in both lymphoid and non-lymphoid tissues. Thus, CD11a marker can be used to determine the magnitude and kinetics of most, if not all, antigen-specific polyclonal CD8 T cell responses.

[0050] CD11a marker was confirmed by immunofluorescence (IF) staining of frozen placental tissue, predominant distribution of CD11a marker (red) on CD8 (green) positive cells (shown as orange overlay) (Figure 13) in placenta from vaccinated pregnant animals. Briefly, pregnant WT mice were injected s.c with 293-gp96-Ig-ZIKAEnv or PBS (CTRL) at GD 7.5. Individual Fetal-Placental Unit at GD 12.5 were collected and total placental tissue dissected and frozen in OCT medium. 7um tissue sections were fixed with paraformaldehyde,

permeabilized with 0.3% Triton X-100 in PBS and blocked with 5% serum for 1 hour at 24°C. Samples were incubated with primary antibodies anti-CD8-FITC and anti-CD11a-PECy5 for 3 hours at RT. Tissues were stained with DAPI (DNA stain) for 1 min. Representative samples for vaccinated and control PBS placenta are shown.

[0051] Decidua CD8 T cells ZIKV specificity was confirmed by stimulation of isolated decidual cells with overlapping ZIKV envelope peptide library and intracellular cytokine staining assay (ICS) (Figure 14). Pregnant WT mice were injected s.c with 293-gp96-Ig-ZIKAEnv or PBS (CTRL) at GD 7.5. Individual Fetal-Placental Unit at GD 12.5 were collected and decidua was dissected from the placental tissue. ZIKV-specific CD8⁺ T lymphocyte responses were assessed using decidual cells and analyzed by flow cytometry. Cells were stimulated for 6 h at 37°C with 2 µg/ml of overlapping 15-amino-acid peptides covering the prM or Env proteins (JPT, Berlin, Germany). Following 4 h incubation, brefeldin-A and monensin (BioLegend, CA, USA) were added, and samples were incubated for total of 6 h at 37°C. Cells were then washed, stained, permeabilized with Cytofix/Cytoperm (BD Biosciences, CA, USA). Data was acquired using an Fortessa flow cytometer (BD Biosciences, CA, USA) and analyzed using FlowJo v.10.2 (Treestar, OR, USA). Monoclonal antibodies included: CD45, CD3, CD8α, CD11a, IL-2, IFN-γ and TNFα. Antibodies were purchased from BD Biosciences, eBioscience, or BioLegend, CA, USA. Vital dye exclusion (LIVE/DEAD) was purchased from Life Technologies, CA, USA. (n=4). The Gp96-Ig-ZIKAenv vaccine induced ZIKAEnv-specific CD8⁺ lymphocyte that produce IFN-γ, TNFα and IL-2 after ZIKA envelope peptide stimulation. In addition, 50% of all CD11a⁺ CD8⁺ T cells represent ZIKA-specific CD8 T cells (Figure 14).

[0052] Overall the results indicate, inter alia, success in generating a B45 plasmid that expresses gp96-Ig and ZIKV envelope protein and also confirms that vaccination with cell based secreted gp96-Ig-ZIKA is safe and induces CD8 T cell responses in maternal decidua. Additionally, *in vitro* stimulation with Zika peptide activates CD8⁺ specific antigen, indicating an antigen specific effect. This T-cell specific response is beneficial as it improves over existing methods directed to antibody protection only. Vaccination with gp96 provides a unique T-cell mediated response that is protective in the placenta.

[0053] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0054] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (*i.e.*, meaning “including, but not limited to,”) unless otherwise noted.

[0055] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range and each endpoint, unless otherwise indicated herein, and each separate value and endpoint is incorporated into the specification as if it were individually recited herein.

[0056] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or illustrative language (*e.g.*, “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0057] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0058] As used herein, all headings are simply for organization and are not intended to limit the disclosure in any manner. The content of any individual section may be equally applicable to all sections.

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CLAIMS:

What is claimed is

1. An expression vector system comprising (i) a nucleic acid encoding a fusion protein comprising a chaperone protein and an immunoglobulin, or a fragment thereof, and (ii) a nucleic acid encoding a flavivirus protein, or an antigenic portion thereof, wherein each nucleic acid is operably linked to a promoter.

2. The expression vector system of claim 1, wherein the nucleic acid encoding the fusion protein is operably linked to a promoter which is different from the promoter which is operably linked to the nucleic acid encoding the flavivirus protein, or an antigenic portion thereof.

3. The expression vector system of claim 2, wherein the nucleic acid encoding the fusion protein is operably linked to a CMV promoter.

4. The expression vector system of claim 2 or 3, wherein the nucleic acid encoding the flavivirus protein, or an antigenic portion thereof, is operably linked to an Mth promoter.

5. The expression vector system of any one of claims 1 to 4, wherein the nucleic acid encoding the fusion protein and the nucleic acid encoding the flavivirus protein, or antigenic portion thereof, are present on the same expression vector.

6. The expression vector system of any one of claims 1 to 4, wherein the nucleic acid encoding the fusion protein is present on an expression vector which is different from the expression vector comprising the nucleic acid encoding the flavivirus protein, or antigenic portion thereof.

7. The expression vector system of any one of claims 1 to 6, comprising two or more nucleic acids each encoding a different flavivirus protein, or an antigenic portion thereof.

8. The expression vector system of any one of the previous claims, wherein the chaperone protein is selected from the group consisting of: gp96, Hsp70, BiP, and Grp78.

9. The expression vector system of any one of the previous claims wherein the chaperone protein comprises an amino acid sequence of any one of SEQ ID NOs: 2, 29, 30, and 31.

10. The expression vector system of claim 8, wherein the chaperone protein is gp96 comprising the amino acid sequence of SEQ ID NO: 2.

11. The expression vector system of any one of the previous claims, wherein the fusion protein comprises an Fc fragment of an immunoglobulin.

12. The expression vector system of claim 11, wherein the immunoglobulin is an IgG1 immunoglobulin.

13. The expression vector system of claim 11 or 12, wherein the Fc fragment comprises the amino acid sequence of SEQ ID NO: 5.

14. The expression vector system of any one of the previous claims, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 8.

15. The expression vector system of any one of the previous claims, wherein the flavivirus protein is a Zika virus protein, Dengue virus protein, Yellow Fever virus protein, or West Nile virus protein.

16. The expression vector system of claim 15, wherein the flavivirus protein is selected from the group consisting of: flavivirus polyprotein from Dengue Virus 2, polyprotein precursor from Yellow Fever Virus, or flavivirus polyprotein from West Nile Virus.

17. The expression vector system of claim 16, wherein the flavivirus protein comprises an amino acid sequence of any one of SEQ ID NOs: 26-28.

18. The expression vector system of any one of the previous claims, wherein the flavivirus protein is a ZIKV protein selected from the group consisting of: membrane

glycoprotein precursor M, envelope protein E, nonstructural protein NS1, nonstructural protein NS2A, nonstructural protein NS2B, nonstructural protein NS3, nonstructural protein NS4A, and nonstructural protein NS4B.

19. The expression vector system of claim 18, wherein the membrane glycoprotein precursor M comprises the amino acid sequence of one or more of SEQ ID NO: 10, the envelope protein E comprises the amino acid sequence of SEQ ID NO: 11, the nonstructural protein NS1 comprises the amino acid sequence of SEQ ID NO: 12, the nonstructural protein NS2A comprises the amino acid sequence of SEQ ID NO: 13, the nonstructural protein NS2B comprises the amino acid sequence of SEQ ID NO: 14, the nonstructural protein NS3 comprises the amino acid sequence of SEQ ID NO: 15, the nonstructural protein NS4A comprises the amino acid sequence of SEQ ID NO: 16, and the nonstructural protein NS4B comprises the amino acid sequence of SEQ ID NO: 17.

20. The expression vector system of any one of the previous claims, comprising a nucleic acid encoding the ZIKV protein membrane glycoprotein precursor M and/or a nucleic acid encoding the ZIKV protein envelope protein E.

21. The expression vector system of any one of the previous claims, comprising a nucleic acid encoding the amino acid sequence of SEQ ID NO: 10 and/or a nucleic acid encoding the amino acid sequence of SEQ ID NO: 11.

22. The expression vector system of any one of the previous claims, further comprising a nucleic acid encoding a bovine papillomavirus (BPV) E1 protein and/or a BPV E2 protein.

23. The expression vector system of any one of the previous claims, further comprising a nucleic acid encoding a BPV E1 protein of SEQ ID NO: 19 and/or a BPV E2 protein of SEQ ID NO: 22.

24. The expression vector system of any one of the previous claims, which does not comprise a nucleic acid encoding an E5 sequence, E6 sequence, E7 sequence.

25. The expression vector system of any one of the previous claims, comprising one or more elements shown in Figure 1 or Figure 3.

26. The expression vector system of any one of the previous claims, comprising the sequence of any one of SEQ ID NO: 24, 25, and 35.

27. A host cell comprising the expression vector system of any one of the previous claims.

28. The host cell of claim 27, which is a mammalian host cell.

29. The host cell of claim 28, which is a human host cell.

30. The host cell of claim 29, which is an NIH 3T3 cell or an HEK 293 cell.

31. A population of cells wherein at least 50% of the cells are host cells according to any one of claims 28 to 30.

32. A composition comprising an expression vector system of any one of claims 1 to 26 or a host cell of any one of claims 27 to 30, or a population of cells of claim 31, and an excipient, carrier, or diluent.

33. The composition of claim 32, which is a sterile composition.

34. The composition of any one of claims 32 or 33, which is suitable for administration to a human.

35. The composition of any one of claims 32 to 34, which is ready for use.

36. The composition of any one of claims 32 to 35, comprising at least or about 10^6 cells transfected with the expression vector system.

37. A kit comprising an expression vector system of any one of claims 1 to 26 or a host cell of any one of claims 27 to 30 or a population of cells of claim 31 or a composition of any one of claims 32 to 36.

38. A method of eliciting an immune response against flavivirus in a subject, comprising administering to the subject the expression vector of any one of claims 1 to 26, or a population of cells transfected with the expression vector.

39. A method of treating or preventing a flavivirus infection in a subject, comprising administering to the subject the expression vector of any one of claims 1 to 26, or a population of cells transfected with the expression vector.

40. The method of claim 38 or 39, wherein the flavivirus is selected from the group consisting of: Dengue virus, Yellow Fever virus, West Nile virus, and Zika virus (ZIKV).

41. The method of claim 40, wherein the flavivirus is ZIKV.

42. The method of claim 38 or 39, wherein the flavivirus infection is selected from the group consisting of: Dengue virus infection, Yellow Fever virus infection, West Nile virus infection, and Zika virus (ZIKV) infection.

43. The method of claim 40, wherein the flavivirus infection is a ZIKV infection.

44. The method of any one of claims 38 to 42, wherein the subject is female.

45. The method of claim 44, wherein the subject is a pregnant female.

46. The method of claim 45, wherein the pregnant female is human.

47. The method of claim 46, wherein the human pregnant female is in the first trimester of pregnancy.

48. The method of any one of claims 38 to 42, wherein the subject is male.

49. The method of any one of claims 38 to 42, wherein the subject is not pregnant.

50. The method of claim 49, wherein the subject is a female and the population of cells is administered prior to pregnancy.

51. The method of any one of claims 38 to 50, comprising subcutaneously administering the population of cells to the subject.

52. The method of claim 51, comprising subcutaneously administering the population of cells to an arm or leg of the subject.

53. The method of any one of claims 38 to 50, comprising intramuscularly administering the expression vector.

54. The method of any one of claims 38 to 53, comprising electroporating an expression vector containing ZIKV antigens and gp96-Ig.

55. A method of treating or preventing a flavivirus infection in a subject, comprising administering to the subject an expression vector comprising a sequence having at least 90% identity with SEQ ID NO: 35 or a population of cells transfected an expression vector comprising a sequence having at least 90% identity with SEQ ID NO: 35.

56. A method of treating or preventing a flavivirus infection in a subject, comprising administering to the subject an expression vector comprising a sequence having at least 95% identity with SEQ ID NO: 35 or a population of cells transfected an expression vector comprising a sequence having at least 95% identity with SEQ ID NO: 35.

57. An expression vector system comprising (i) a nucleic acid encoding the amino acid sequence of SEQ ID NO: 2 and (ii) a nucleic acid encoding the amino acid sequence of SEQ ID NO: 11, wherein each nucleic acid is operably linked to a promoter.

58. The expression vector system of claim 57, wherein SEQ ID NO: 2 lacks the terminal KDEL sequence.

59. A method of treating or preventing a flavivirus infection in a subject, comprising administering to the subject the expression vector of claim 57 or claim 58.

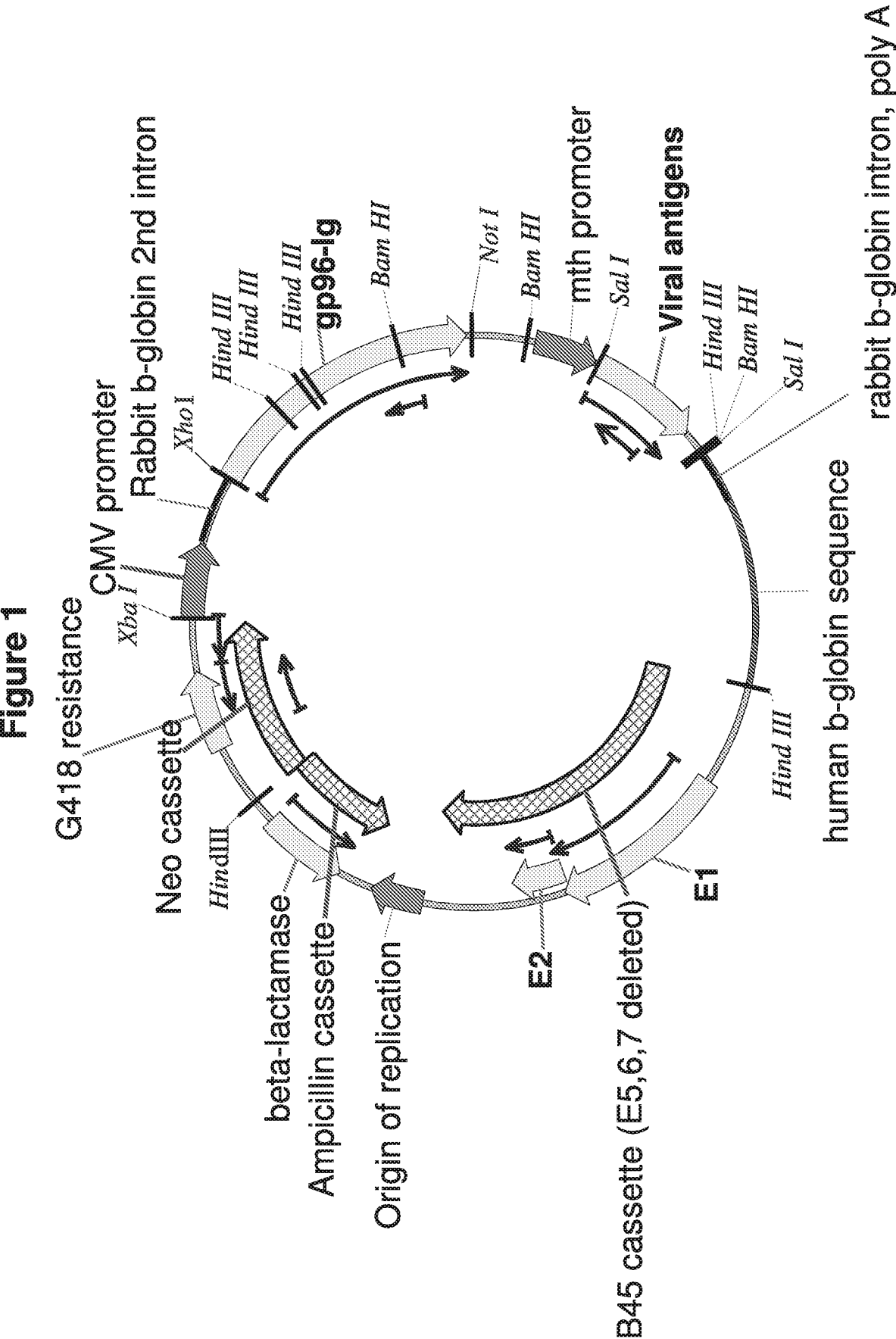
60. A biological cell comprising a first recombinant protein having an amino acid sequence of at least 95% sequence identity with SEQ ID NO: 2 and a second recombinant protein having an amino acid sequence of at least 95% sequence identity with SEQ ID NO: 11.

61. The biological cell of claim 60, wherein the first recombinant protein has at least 97% sequence identity with SEQ ID NO: 2 and the second recombinant protein having an amino acid sequence of at least 97% sequence identity with SEQ ID NO: 11.

62. The biological cell of claim 60, wherein the first recombinant protein has at least 98% sequence identity with SEQ ID NO: 2 and the second recombinant protein having an amino acid sequence of at least 98% sequence identity with SEQ ID NO: 11.

63. The biological cell of any one of claims 60-62, wherein SEQ ID NO: 2 lacks the terminal KDEL sequence.

64. A method of treating or preventing a flavivirus infection in a subject, comprising administering to the subject the biological cell of any one of claims 60-63.



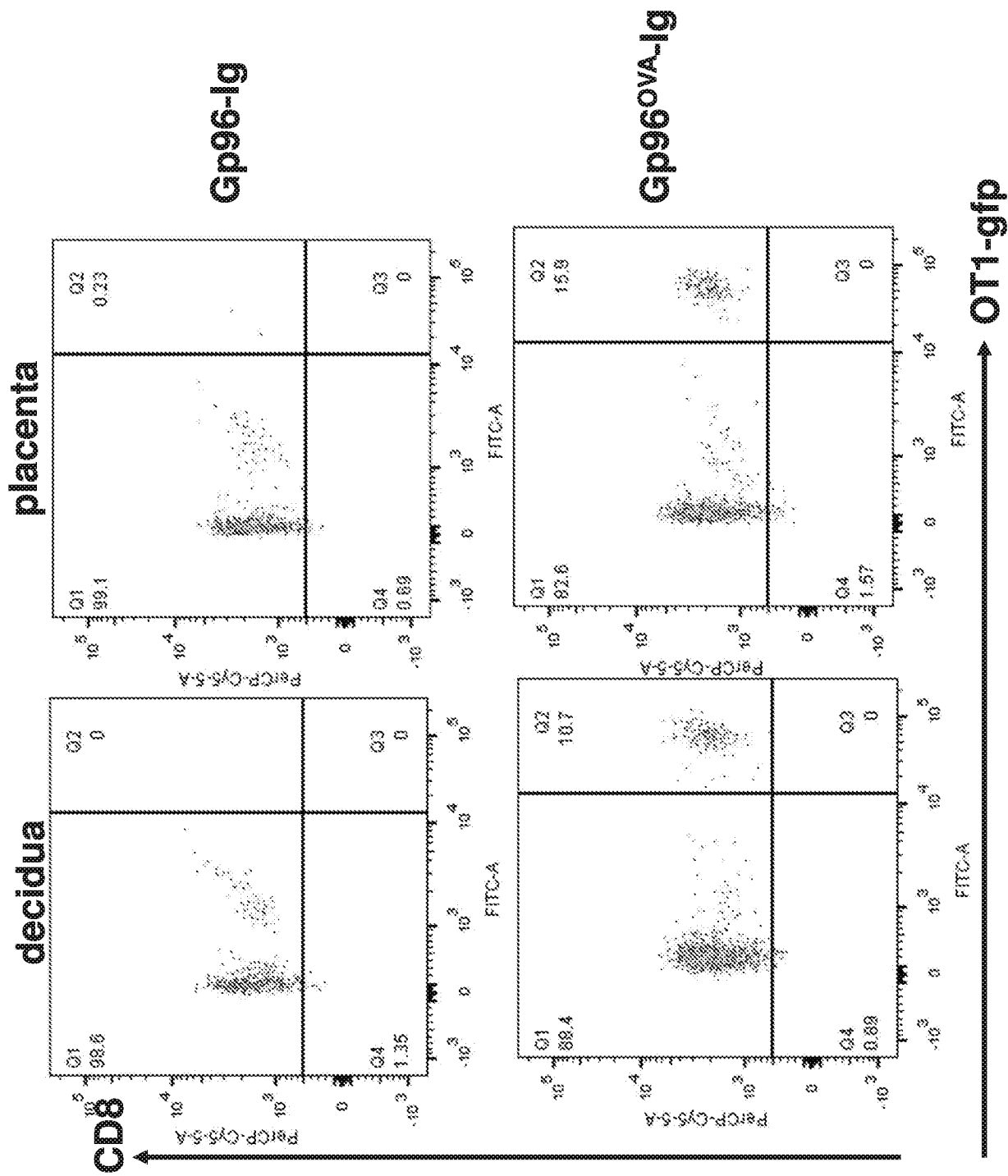
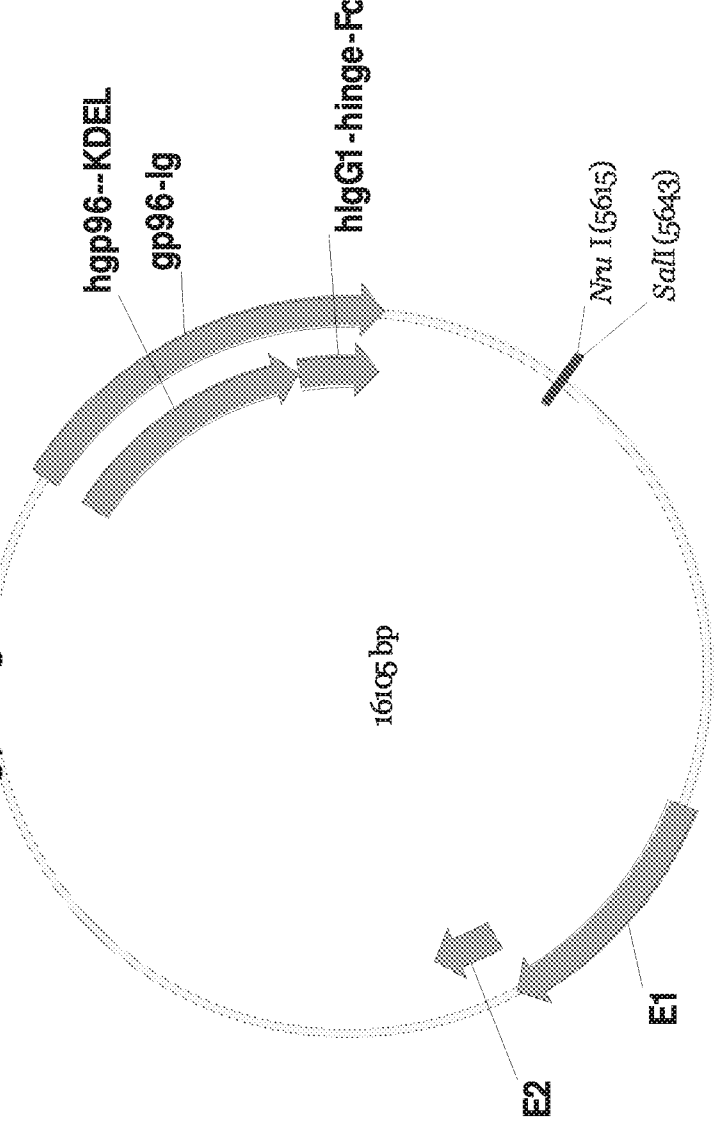


Figure 2

Figure 3

B45- 1st cassette hgp96igG1-Fc 2nd cassette NRU SFI SAL



1	TCTAGAGAGC TTGGCCCAATT GCATACGTTG TATCCATATC ATAATAATGTA CATTATATT GGCTCATGTC CAACATTACC GCCATGTTGA CATTGATTAT AGATCTCTCG AACCGGGTAA CGTATGCAAC ATAGGTATAG TATTATACAT GTAAATATAA CCGAGTACAG GTTGTAATGG CGGTACAACCT GTAACATAA TGACTAGTTA TTAATAGTAA TCAATTACGG ATAACTTACG GTAAATGGCC CGCCTGGCTG ACTGATCAAT AATTATCAAT AGTTAATGCC CCAGTAATCA AGTATCGGT ATATACCTCA AGCGCAATG TATTGAATGC CATTTACCGG GCGGACCGAC ACCGCCCAAC GACCCCGGCC CATTGACGTC AATAATGACG TAAGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC GTCAAATGGGT GGAGTATTAA TGCGGGGTTG CTGGGGGCGG GTAACTGCAG TTATTACTGC ATACAAGGT ATCATTGCGG TTATCCCTGA AAGGTAACCTG CAGTTACCCA CCTCATAAAT CGGTAAACTG CCCACTTGGC AGTACATCAA ACGGTAAATG GCCCGCCTGG CATTATGCC GCCATTGAC GGGTGAACCG TCATGTAGTT CACATAGTAT ACGGTTTCATG CGGGGGATAA CTGCAGTTAC TGCCATTTC CGGCGGGACC GTAAATACGGG AGTACATGAC CTTATGGGAC TTTCCCTACTT GGCAGTACAT CTACGTATTA GTCATCGCTA TTACCATGGT GATGCGGTTT TGGCAGTACA TCAATGGGCG TCATGTACTG GAATACCCCTG AAAGGATGAA CCGTCAATGA GATGCATAAT CAGTAGCGAT AATGGTACCA CTACGCCAAA ACCGTCATGT AGTTACCCCG TGATAGCGG TTTGACTCAC GGGGATTTCC AAGTCTCCAC CCCATGACG TCAATGGGAG TTTGTTTGG CACCAAAATC AACGGGACTT TCCAAAATGT ACCTATCGCC AAACCTGAGTG CCCCTAAAGG TTCAGAGGTG GGGTAACTGC AGTTACCCCTC AAACAAAACC GTGGTTTTAG TTGCCCTGAA AGGTTTACA CGTAACAACT CCGCCCCCAT T GACGCAAAATG CTCGTTTAGT GAACCGTCAG ATCGCCTGGA GCATTGTTGA GCGGGGGTAA CTGCGTTTAC CCGCCATCCG CACATGCCAC CCTCCAGATA TATTGCTCTC GAGCAAAATCA CTTGGCAGTC TAGCGGACCT GACGCCATCC ACGCTGTTTT GACCTCCATA ATCCTGAGAA CTTCAGGGTG AGTTTGGGGA
101	
201	
301	
401	
501	
601	
701	

Figure 3 (continued)

	CTGCGGTAGG TCGACAAAA CTGGAGGTAT CTTCTGTGGC CCTGGCTAGG TCGGAGGCCA GCTAGCTGGC TAGGACTCTT GAAGTCCAC TCAAAACCCCT
801	CCCTTGATTG TTCCTTCTTT TTCGCTATTG TAAAAATTCAT GTTATATGGA GGGGGCAAAG TTTTCAGGGT GTTGTTTAGA ATGGAAGAT GTCCCTTGTA GGGAACCTAAC AAGAAAGAAA AAGCGATAAC ATTTTAAAGTA CAATATACCT CCCCCGTTTC AAAAGTCCCA CAACAAATCT TACCCTTCTA CAGGGAACAT
901	TCACCATGGA CCTCATGAT AATTTTGTTT CTTTCACTTT CTACTCTGTT GACAACCATT GTCTCCTCTT ATTTTCTTTT CATTTCCTGT AACTTTTTCG AGTGGTACCT GGGAGTACTA TTAACAACAAA GAAAGTGAAA GATGAGACAA CTGTTGTAA CAGAGGAGAA TAAAGAAAA GTAAAAGACA TTGAAAAAGC
1001	TTAAACTTTA GCTTGCATTT GTAACGAATT TTAAATTC ATTTTGTTTA TTTGTCAGAT TGTAAGTACT TTCCTCTAATC ACTTTTTTTT CAAGGCAATC AATTTGAAAT CGAACGTAA CATTGCTTAA AAATTTAAGT GAAACACAAAT AAACAGTCTA ACATTCAATGA AAGAGATTAG TGAATAAAAA GTTCCGTTAG AGGGTATATT ATATTGTACT TCAGCACAGT TTTAGAGAAC AATTGTTATA ATTAAATGAT AAGGTAGAAT ATTTCTGCAAT ATAAATTCTG GCTGGCGTGG TCCCATATAA TATAACATGA AGTCGTGTCA AAATCTCTTG TTAACAATAT TAATTTACTA TTCCATCTTA TAAAGACGTA TATTTAAGAC CGACCGCACC
1201	AAATAATTCTT ATTGGTAGAA ACAACTACAC CCTGGTCATC ATCCTGCCTT TCTCTTTATG GTTACAAATGA TATACACTGT TTGAGATGAG GATAAAATAC TTTATAAGAA TAACCAATCTT TGTGATGTG GGACCAGTAG TAGGACGGAA AGAGAAATAC CAAATGTTACT ATATGTGACA AACTCTACTC CTATTTTATG TCTGAGTCCA AACCGGGCCC CTCTGCTAAC CATGTTTCATG CCTTCTTCTC TTTCTTACAG CTCCTGGGCA ACGTGCTGGT TGTGTGCTG TCTCATCAT AGACTCAGGT TTGGCCCCGG GAGACGATTG GTACAAGTAC GGAAGAAGAG AAAGGATGTC GAGGACCCCGT TGCACGACCA ACAACACGAC AGAGTAGTAA
1401	TTGGCAAAGA ATTCGAAGCC TCGAGATGAT GAAACTTATC ATCAATTAT TGTATAAAAA TAAAGAGATT TTCCCTGAGAG AACTGATTTC AAATGCTTCT AACCCTTTCT TAAGCTTCGG AGCTCTACTA CTTTGAATAG TAGTTAAGTA ACAATATTTT ATTCTCTAA AAGGACTCTC TTGACTAAAG TTTACGAAGA

Figure 3 (continued)

1501	GATGCTTTAG ATAAGATAAG GCTAATATCA CTGACTGATG AAAATGCTCT TTTCTGGAAAT GAGGAACATAA CAGTCAAAAAT TAAGTGTGAT AAGGAGAAGA CTACGAAATC TATTCTATTC CGATTATAGT GACTGACTAC TTTTACGAGA AAGACCTTTA CTCCTTGATT GTCAGTTTTA ATTACACACTA TTCCTCTTCT
1601	ACCTGCTGCA TGTACACAGAC ACCGGTGTAG GAATGACCAG AGAAGAGTTG GTTAAAAACC TTGTTACCAT AGCCAAATCT GGGACAAGCG AGTTTTTAAA TGGACGACGT ACAGTGTCTG TGGCCACATC CTTACTGGTC TCTTCTCAAC CAATTTTGG AACCATGGTA TCGGTTTAGA CCTGTTCGC TCAAAAATTT
1701	CAAAATGACT GAAGCACAGG AAGATGGCCA GTCAACTTCT GAATTGATTG GCCAGTTTGG TGTGCGTTTC TATTCCGCCCT TCCTTGTAGC AGATAAGGTT GTTTTACTGA CTTCTGTCTC TTCTACCGGT CAGTTGAAGA CTTAACTAAC CGGTCAAACC ACAGCCCAAAG ATAAGGCGGA AGGAACATCG TCTATTCCAA
1801	ATTGTCACTT CAAAACACAA CAACGATACC CAGCACATCT GGGAGTCTGA CTCCAATGAA TTTTCTGTAA TTGCTGACCC AAGAGGAAAC ACTCTAGGAC TAACAGTGAA GTTTTGTGTT GTTGCTATGG GTCGTGTAGA CCTCAGACT GAGGTTACTT AAAAGACATT AACGACTGGG TTCTCCTTTG TGAGATCCTG
1901	GGGGAACGAC AATTACCCCTT GTCTTAAAAG AAGAAGCATC TGATTACCTT GAATTGGATA CAATTAAAAA TCTCGTCAAA AAATATTCAC AGTTCAATAA CCCCTTGCTG TTAATGGGA CAGAAATTTT TCTTTCGTAG ACTAATGGAA CTTAACCTAT GTTAATTTTT AGAGCAGTTT TTTATAAGTG TCAAGTATTT
2001	CTTTCCCTATT TAIGTATGGA GCAGCAAGAC TGAAACTGTT GAGGAGCCCCA TGGAGGAAGA AGAAGCAGCC AAAGAAGAGA AAGAAGAATC TGAATGATGA GAAAGGATAA ATACATACCT CGTCGTTCTG ACTTTGACAA CTCCTCGGGT ACCTCCTTCT TCTTCGTCGG TTTCTTCTCT TTCTTCTTAG ACTACTACTT
2101	GCTGCAGTAG AGGAAGAAGA AGAAGAAAAAG AAACCAAAGA CTAAAAAAGT TGAAAAAACT GTCTGGGACT GGGAACCTTAT GAATGATATC AAACCAATAT CGACGTCAIC TCCTTCTTCT TCTTCTTTTC TTGGTTTCT GATTTTTC AACTTTTGA CAGACCCCTGA CCCTTGAATA CTTACTATAG TTTGGTTATA
2201	GGCAGAGACC ATCAAAAGAA GTAGAAGAAG ATGAATACAA AGCTTTCTAC AAATCATTTT CAAAGGAAAG TGATGACCCC ATGGCTTATA TTCACTTTAC

Figure 3 (continued)

2301	CCGTCCTCTGG TAGTTTCTT CACTCTCTC TACTTATGTT TCGAAAGATG TTAGTAAAA GTTTCCTTTC
	ACTACTGGGG TACCGAATAT AAGTGAAAATG
2301	TGCTGAAGGG GAAGTTACCT TCAAATCAAT TTTATTGTA CCCACATCTG CTCCACGTGG TCTGTTTGAC
	GAATATGGAT CTAAAAAGAG CGATTACATT
	ACGACTTCCC CTTCAATGGA AGTTTAGTTA AATAAACAT GGGGTAGAC GAGGTGCACC AGACAAAACTG
	CTTATACCTA GATTTTCTC GCTAATGTAA
2401	AAGCTCTATG TCGGCCGTGT ATTCAICACA GACGACTTCC ATGATATGAT GCCTAAATAC CTCAATTTTG
	TCAAAGGGTGT GGTGGACTCA GATGATCTCC
	TTCGAGATAC ACGCGGCACA TAAGTAGTGT CTGCTGAAGG TACTATACTA CGGATTTATG GAGTTAAAAAC
	AGTTCCCA CA CCACCTGAGT CTACTAGAGG
2501	CCTTGAATGT TTCCCGCGAG ACTCTTCAGC AACATAAACT GCTTAAGGTG ATTAGGAAGA AGCTTGTTCG
	TAAACGCTG GACATGATCA AGAAGATTGC
	GGAACCTTACA AAGGCGCTC TGAGAACTCG TTGTATTGA CGAATTCCAC TAATCCTTCT TCGAACCAAGC
	ATTTTGGCAG CTGTACTAGT TCTTCTAACG
2601	TGATGATAAA TACAAIGATA CTTTTTGAA AGAATTGGT ACCAACATCA AGCTTGGTGT GATTGAAGAC
	CACCTCGAATC GAACACGTCT TGCTAAACTT
	ACTACTATTT ATGTTACTAT GAAAAACCTT TCTTAAACCA TGGTTGTAGT TCGAACCCACA CTAACCTTCG
	GTGAGCTTAG CTTGTGCAGA ACGATTTGAA
2701	CTTAGGTTCC AGTCTTCTCA TCATCCAACT GACATTACTA GCCTAGACCA GTATGTGGAA AGAATGAAGG
	AAAAACAAGA CAAAATCTAC TTCATGGCTG
	GAATCCCAAGG TCAGAAGAGT AGTAGGTTGA CTGTAATGAT CGGATCTGGT CATAACCTT TCTTACTTCC
	TTTTTGTCT GTTTTAGATG AAGTACCGAC
2801	GGTCCAGCAG AAAAGAGGCT GAATCTTCTC CATTTGTGA GCGACTTCTG AAAAAGGGCT ATGAAGTTAT
	TTACCTCACA GAACCTGTGG ATGAATACTG
2901	CCAGGTCGTC TTTTCCTCGA CTTAGAAGAG GTAAACAACT CGCTGAAGAC TTTTCCCGA TACTTCAATA
	AATGGAGTGT CTTGGACACC TACTTATGAC
	TATTCAGGCC CTTCCCGAAT TTGATGGGAA GAGGTTCCAG AATGTTGCCA AGGAAGGAGT GAAGTTCGAT
	GAAAGTGAGA AACTAAGGA GAGTCGTGAA
2901	ATAAGTCCGG GAAGGCTTA AACTACCCTT CTCCAAGGTC TTACAACGGT TCCTTCTCTCA CTTCAAGCTA
	CTTTCACCTCT TTGATTCTC CTCAGCACTT

Figure 3 (continued)

3001	GCAGTTGAGA AAGAAATTGA GCCTCTGCTG AATTGGATGA AAGATAAAGC CCTTAAGGAC AAGATTGAAA AGGCTGTGGT GTCTCAGCGC CTGACAGAAAT CGTCAACTCT TCTTAAACT CGGAGACGAC TTAACCTACT TTCTATTTCG GGAATTCCCTG TTCTAACTTT TCCGACACCA CAGAGTCGGG GACTGTCTTA
3101	CTCCGIGTGC TTTGGTGGCC AGCCAGTACG GATGGTCTGG CAACATGGAG AGAATCAATGA AAGCACAAAGC GTACCAAACG GGCAAGGACA TCTCTACAAA GAGGCACACG AAACCACCGG TCGGTCAATG CTACCAGACC GTTGTAACCTC TCTTAGTACT TTCGTGTTTCG CATGGTTTGC CCGTTCCTGT AGAGATGTTT
3201	TTACTATGCG AGTCAGAAGA AAACATTTGA AATTAATCCC AGACACCCGC TGATCAGAGA CATGCTTCGA CGAATTAAGG AAGATGAAGA TGATAAAACA AATGATACGC TCAGTCTTCT TTTGTAAACT TTAATTAGGG TCTGTGGCGG ACTAGTCTCT GTACGAAAGCT GCTTAATTCC TTCTACTTCT ACTATTTTGT
3301	GTTTTGGATC TTGCTGTGGT TTTGTTTGA ACAGCAACGC TTCGGTCAGG GTATCTTTA CCAGACACTA AAGCATATGG AGATAGAATA GAAAGAATGC CAAAACCTAG AACGACACCA AAACAAACTT TGTCGTTCG AAGCCAGTCC CATAGAAAAT GGTCTGTGAT TTCGTATACC TCTATCTTAT CTTTCTTACG
3401	TTCGCCCTCAG TTGGAACATT GACCCCTGATG CAAAGGTGGA AGAAGAGCCC GAAGAAGAAC CTGAAGAGAC AGCAGAAGAC ACAACAGAAG ACACAGAGCA AAGCGGAGTC AACTTGTAA CTGGGACTAC GTTCCACCT TCTTCTCGGG CTTCTTCTTG GACTTCTCTG TCGTCTTCTG TGTGTCTTC TGTGTCTCGT
3501	AGACGAAGAT GAAGAAATGG ATGTGGGAAC AGATGAAGAA GAAGAAACAG CAAAGGAATC TACAGCTGAA GGATCCTGIG ACAAAACTCA CACATGCCCA TCTGCTTCTA CTTCTTTACC TACACCCTTG TCTACTTCTT CTTCTTTGTC GTTCCCTTAG ATGTCGACTT CCTAGGACAC TGTTTTGAGT GTGTACGGGT
3601	CCGTGCCCCAG CACCTGAAC CTGTGGGGGA CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGGACCCCT GAGGTCACAT GGCACGGGIC GTGGACTTGA GGACCCCCCT GGCAGTCAGA AGGAGAAGGG GGGTTTGGG TTCTGTGGG AGTACTAGAG GGCCTGGGA CTCCAGTGTA
3701	GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC AAGACAAAGC CGCGGGAGGA

Figure 3 (continued)

3801	CGCACCA	CGTGC	ACTCG	GTGCT	TCTGG	GACTC	CAGTT	CAAGTT	GACC	ATGCAC	CTGC	CGCAC	CTCCA
	CGTATT	ACGG	TTCTG	TTTCG	GCGCC	CTCCT							
	GCAGTACA	AGCACG	TACC	GTGTG	TCAG	CGTCC	TCACC	GTCC	TGCACC	AGGACT	GGCT	GAATG	GGCAAG
	GAGTACAA	GT	GCTC										
3901	CGTCA	GTG	TCATGG	CACAC	CAGTC	GCAGG	AGTGG	CAGGAC	GTGG	TCTGAC	CGA	CTTACC	GTTCC
	CTCATG	TTCA	CGTTC	CAGAG	GTTG	TTTCGG							
	CTCCCAG	CCC	CCATC	GAGAA	AACCA	TCCTC	AAAGC	CAAAG	GGCAG	CCCCG	AGAACC	ACAG	GTGTAC
	TGCCCCC	ATC	CCGGG	ATGAG	CTGAC	CAAGA							
4001	GAGGT	CGGG	GGTAG	CTCTT	TTGGT	AGAGG	TTTCG	GTTTC	CCGTC	GGGGC	TCTTGG	TGTC	CACATG
	ACGGGG	G	GTAG	CTCTC	GACTG	GTCT							
	ACCAGG	TCAG	CTGAC	CTCG	CTGGT	CAAG	GCTTCT	ATCC	CAGCG	ACATC	GCCGTG	GAGT	GGGAGAG
	TGGGAG	CCG	GAGAA	CAACT	ACAAG	ACCAC							
4101	TGGTCC	AGTC	GGACT	GGACG	GACCA	GTTC	CGAAG	ATAGG	GTGCT	GTAG	CGGCAC	CTCA	CCCTCT
	ACCCGT	CGGC	CTCTT	GTGTA	TGTTCT	GTGTG							
	GCCTCC	CGTG	CTGAC	CTCG	ACGGT	CTCCT	CTTCT	CTAC	AGCAAG	CTCA	CCGTG	GACAA	GAGCAGG
	CAGCAG	GGGA	ACGTCT	TCTC	ATGCT	CCGTG							
4201	CGGAGG	GCAC	GACCT	GAGGC	TGCCG	AGGAA	GAAGG	AGATG	TCGTT	CGAGT	GGCAC	CTGT	CTCCAC
	GTCGT	CCCC	TGCAG	AAGAG	TACGAG	GCAC							
	ATGCAT	GAGG	CTCTG	CACAA	CCACT	TACAG	CAGAAG	AGCC	TCTCC	CTGTC	TCCGGG	TAAA	TGACTCG
	CAGACT	AGTC	AAATT	AAGCC	GAATT	CTGCA							
4301	TACGTAC	TCC	GAGAC	GTGTT	GGTGA	TGTG	GTCTT	CTCGG	AGAGG	GCACAG	AGGCCCAT	TTT	ACTGAGC
	GTC	TGATCAG	TTTAA	TTCCG	CTTAA	GACGT							
	GATATC	CATC	ACACT	GGCGG	CCGCT	GGAAT	TCACT	CTCTCA	GGTGC	AGGCT	GCCTAT	CAGA	AGGTGGT
	TGGTGT	GGCC	AATGC	CCCTGG	CTCAC	AAATA							
4401	CTATAG	G	TAGT	ACC	CGC	GACCTTA	AGTGAG	GAGT	CCACG	TCCGA	CGGATAG	TCT	TCCACCAC
	ACCACAC	CCGG	TTACG	GGGACC	GAGTGT	TTAT							
	CCACTG	AGAT	CTTTT	TCCCT	CTGCCA	AAAAA	TTATG	GGGAC	ATCATG	AAGC	CCCTTG	AGCA	TCTGACT
	GGCTAA	TAAA	GGAA	TTTTAT	TTTCAT	TGCA							
	GGTGAC	TCTA	GAAA	AAGGA	GACGG	TTTTT	AATACC	CCCTG	TAGTACT	TTCG	GGGAACT	CTGT	AGACTGA
	CCGATT	TATT	CCITT	AAATA	AAAGT	AACGT							

Figure 3 (continued)

4501	ATAGTGTGTT GGAATTTTTT GTGTCTCTCA CTCGGAAGGA CATATGGGAG GGCAAAATCAT TTAAAACATC AGAAATGAGTA TTGGTTTAG AGTTTGGCAA TATCACACAA CCTTAAAAAA CACAGAGAGT GAGCCTTCCT GTATACCCTC CCGTTTAGTA AATTTGTAG TCTTACTCAT AAACCAAATC TCAAACCGTT	ATGAAACATC
4601	CATATGCCCA TATGCTGGCT GCCATGAACA AAGGTGGCT ATAAAGAGGT CATCAGTATA TGAAACAGCC CCCTGCTGTC CATTCCTTAT TCCATAGAAA GTATACGGGT ATACGACCGA CGTACTTGT TTCCAACCGA TATTTCTCCA GTAGTCATAT ACTTGTGCGG GGGACGACAG GTAAGGAATA AGTATCTTT	TGAAACAGCC
4701	AGCCTTGACT TGAGGTTAGA TTTTTTTTAT ATTTTGTTTT GTGTTATTTT TTTCTTTAAC ATCCCTAAAA TTTTCTTAC ATGTTTACT AGCCAGATT TCGGAACTGA ACTCCAATCT AAAAAAATA TAAAACAAAA CACAATAAAA AAAGAAATTG TAGGGATTTT AAAAGGAAATG TACAAAATGA TCGGTCTAAA	ATCCCTAAAA
4801	TTCTCTCCTCT CCTGACTACT CCCAGTCATA GCTGTCCCTC TTCTCTTATG GAGATCCCTC GACGGATCCC TAGAGTCGAG GCGATGCGG CACGACCCAT AAGGAGGAGA GGAATGATGA GGTCTAGTAT CGACAGGGAG AAGAGAATAC CTCTAGGGAG CTGCCTAGGG ATCTCAGCTC CGTACGCCG CGTCGTGGTA	CTGCCTAGGG
4901	GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTAGGTAC CTGTGTTTTT AAAACCAGCC TGGAGTAGAG CAGATGGGTT AAGTGAGTG ACCCTCAGC CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAACCAAAAA TTTTGGTCCG ACCTCATCTC GTCTACCCAA TTCCACTCAC TGGGAGTGC	ACCTCATCTC
5001	CCTGGACATT CTTAGATGAG CCCCTCAGG AGTAGAGAAAT AATGTTGAGA TGAGTTCTGT TGGCTAAAAAT AATCAAGGCT AGTCTTATA AACTGTCTC GGACCTGTAA GAATCTACTC GGGGAGTCC TCATCTCTTA TTACAACCTCT ACTCAAGACA ACCGATTTTA TTAGTTCCGA TCAGAAATAT TTTGACAGAG	ACCGATTTTA
5101	CTCTTCTCCT AGCTTCGATC CAGAGAGAGA CCTGGGCGGA GCTGGTCGCT GCTCAGGAAC TCCAGGAAAG GAGAACTGA GGTACCACG CTGCGAATGG GAGAAAGAGA TCGAAGCTAG GTCTCTCTT GGACCCGCCT CGACCAGCGA CGAGTCTTIG AGGTCCTTC CTCTTCGACT CCAATGGTGC GACGCTTACC	AGGTCCTTC
5201	GTTTACGGAG ATAGCTGGCT TTCCGGGGTG AGTTCTCGTA AACTCCAGAG CAGCGATAGG CCGTAATATC GGGAAAGCA CTATAGGGAC ATGATGTTCC	CCGTAATATC

Figure 3 (continued)

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CAAAATGCCCTC TATCGACCGA AAGGCCCCAC TCAAGAGCAT TTGAGGTCTC GTCGCTATCC GGCATTATAG
CCCCTTTCGT GATATCCCTG TACTACAAGG
5301 ACACGTCACA TGGGTCGTCC TATCCGAGCC AGTCGTGCCA AAGGGGCGGT CCCGCTGTGC ACACTGGCGC
TCCAGGGAGC TCTGCACTCC GCCCGAAAAG
TGTGCAGTGT ACCCAGCAGG ATAGGCTCGG TCAGCACGGT TTCGCCGCCA GGGCGACACG TGTGACCGCG
AGTCCCTCG AGACGTGAGG CGGCTTTTC
5401 TGGCTCGGC TCTGCCAGGA CGCGGGCGC GTGACTATGC GTGGGCTGGA GCAACCGCCT GCTGGGTGCA
AACCCTTTGC GCCCGGACTC GTCCAACGAC
ACGCGAGCCG AGACGGTCTT CGCCCCCGCG CACTGATACG CACCCGACCT CGTTGGCGGA CGACCCACGT
TTGGGAAACG CGGGCCTGAG CAGGTTGCTG
5501 TATAAAGAGG GCAGGCTGTC CTCTAAGCGT CACCACGACT TCAACGTCCT GAGTACCTTC TCCTCACTTA
CTCCGTAGCT CCAGCTTCAC CACCAAGCTC
ATATTTCTCC CGTCCGACAG GAGATTGCGA GTGGTGCTGA AGTTGCAGGA CTCAITGAAG AGGAGTGAAT
GAGGCATCGA GGTGGAAGTG GTGGTTCGAG
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5601 CTCGACGTCG ATCGCGAAGC TTGGCCCCCT TTGGCCTTAG CGTCGACCGA TCCIGAGAAC
TTCAGGGTGA GTTTGGGGAC CCTTGATTGT TCTTCTTTT
GAGCTGCAGC TAGCGCTTCG AAACCGGGGA AACCGGAATC GCAGCTGGCT AGGACTCTTG AAGTCCCACT
CAAACCCCTG GGAACATAACA AGAAAGAAAA
5701 TCGCTATTGT AAAATTTCATG TTATATGGAG GGGGCAAAGT TTTCAGGGTG TTGTTTAGAA
TGGGAAGAIG TCCCTTGTAT CACCAITGGAC CCTCATGATA
AGCGATAACA TTTTAAGTAC AATATACCTC CCCCCTTCA AAGTCCAC AACAAATCTT ACCCTTCTAC
AGGGAACATA GTGGTACCTG GGAGTACTAT
5801 ATTTTGTTC TTTCACITTC TACTCTGTTG ACAACCAITG TCTCCTCTTA TTTTCTTTC
ATTTTCTGTA ACTTTTTCGT TAAACTTTAG CTITGCATTG
TAAACAAAG AAAGTGAAG ATGAGACAAC TGTGGTAAC AGAGGAGAAT AAAAGAAAAG TAAAGACAT
TGAAAAAGCA ATTTGAAATC GAACGTAAAC
5901 TAACGGAATT TTAAATTAC TTTTGTTTAT TTGTCAGATT GTAAGTACTT TCTCTAATCA
CTTTTCTTC AAGGCAATCA GGGTATATTA TATTGTACTT
Sali
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Figure 3 (continued)

ATTGCTTAA AATTTAAGTG AAAACAAATA AACAGTCTAA CATTCAATGAA AGAGATTAGT GAAAAAAAAG
 TTCCGTTAGT CCCATATAAT ATAACATGAA
 6001 CAGCACAGTT TTAGAGAACA ATTGTTATAA TTAATGATA AGGTAGAATA TTTCTGCATA
 TAAATTCTGG CTGGCGTGG AATATTCTTA TTGGTAGAAA
 GTCGIGTCAA AATCTCTTGT TAACAATATT AATTACTAT TCACTTTAT AAAGACGTAT ATTAAGACC
 GACCGCACCT TTATAAGAAAT AACCATCTTT
 6101 CAACTACACC CTGGTCATCA TCCTGCCCTTT CTCTTTATGG TTACAATGAT ATACACTGTT
 TGAGATGAGG ATAAAATACT CTGAGTCCAA ACCGGGCCCC
 GTTGAITGG GACCAGTAGT AGGACGGAAA GAGAAATACC AATGTTACTA TATGTGACAA ACTCTACTCC
 TATTTTATGA GACTCAGGT TGGCCCGGG
 6201 TCTGCTAACC ATGTTATGCT CTCTTCTCT TTCCTACAGC TCCTGGGCAA CGTGTGGTT
 GTTGIGCTGT CTCATCATTT TGGCAAAGAA TTCCTCGACC
 AGACGATTGG TACAAGTACG GAAGAAGAGA AAGGATGTCG AGGACCCGTT GCACGACCAA CAACACGACA
 GAGTAGTAAA ACCGTTTCTT AAGGAGCTGG
 6301 AGTGCAGGCT GCCTATCAGA AAGTGTGGC TGGTGTGGT AATGCCCTGG CCCACAAGTA
 TCACTAAGCT CGCTTCTTG CTGTCCAATT TCTATTAAAG
 TCACGTCCGA CGGATAGTCT TTCACCACCG ACCACACCGA TTACGGGACC GGGTGTTCAT AGTGATTCCA
 GCGAAAGAAC GACAGGTTAA AGATAATTTC
 6401 GTTCCTTTGT TCCCTAAGTC CAACTACTAA ACTGGGGGAT ATTATGAAGG GCCTTGAGCA
 TCTGGATTCT GCCTAATAAA AAACATTTAT TTCAATTGCA
 CAAGGAAACA AGGGATTTCAG GTTGATGATT TGACCCCTTA TAATACTTCC CGGAACCTCGT AGACCTAAGA
 CGGATTATTT TTTGTAAATA AAAGTAACGT
 6501 ATGATGTATT TAAATTATTT CTGAATATTT TACTAAAAAG GGAATGTGGG AGGTCAGTGC
 ATTTAAAAA TAAAGAAATG AAGAGCTAGT TCAACCTTG
 TACTACATAA ATTTAATAAA GACTTATAAA ATGATTTTC CCTTACACCC TCCAGTCACG TAAATTTTGT
 ATTTCTTTAC TTCCTGATCA AGTTTGGAAAC
 6601 GGAATAACA CTATATCTTA AACTCATGA AAGAAGTGA GGTGCAAAAC AGCTAATGCA
 CATTGGCAAC AGCCCCTGAT GCCTATGCTT TATTCATCCC
 CCTTTTATGT GATATAGAAAT TTGAGGTACT TTCTTCCACT CCGACGTTTG TCGATTACGT GTAACCGTTG
 TCGGGGACTA CGGATACGGA ATAAGTAGGG

Figure 3 (continued)

6701 TCAGAAAAAGG ATTCAAGTAG AGGCTTGATT TGGAGGTAA AGTTTTGCTA TGCTGTATTT
 TACATTACTT ATTGTTTTAG CTGTCTCTCAT GAAATGCTTT
 AGTCTTTTCC TAAGTTCATC TCCGAACATA ACCTCCAATT TCAAAACGAT ACGACATAAA ATGTAATGAA
 TAACAAAATC GACAGGAGTA CTTACAGAAA
 6801 TCACTACCCA TTTGCTTATC CTGCATCTCT CAGCCTTGAC TCCACTCAGT TCTCTTGCTT
 AGAGATACCA CCTTTCCCTT GAAGTGTTCC TTCCATGTTT
 AGTGAIGGGT AAACGAATAG GACGTAGAGA GTCGGAACGT AGGTGAGTCA AGAGAACGAA TCTCTATGGT
 GGAAGGGGA CTTACAAAGG AAGTACAAA
 6901 TACGGCGAGA TGGTTTCTCC TCGCCTGGCC ACTCAGCCTT AGTTGTCTCT GTTGTCTTAT
 AGAGGTCTAC TTGAAGAAGG AAAAACAGGG GGCAATGGTT
 ATGCCGCTCT ACCAAAGAGG AGCGGACCGG TGAGTCGGAA TCAACAGAGA CAACAGAAATA TCTCCAGATG
 AACTTCTTCC TTTTGTCCC CCGTACCAA
 7001 GACTGTCTG TGAGCCCTTC TTCCCTGCCT CCCCCACTCA CAGTGACCCG GAATCTGCAG
 TGCTAGTCTC CCGGAACAT CACTCTTTCA CAGTCTGCTT
 CTGACAGGAC ACTCGGGAAG AAGGACGGA GGGGTGAGT GTCAGTGGC CTTAGACGTC ACGATCAGAG
 GGCTTGATA GTGAGAAAGT GTCAGACGAA
 7101 TGGAAAGGACT GGGCTTAGTA TGAAGAAGTA GGACTGAGAA GAAATTGAAA GGGGGCTTTT
 TGTAGCTTGA TATTCACCTAC TGCTTTATTA CCTATCATA
 ACCTTCTGA CCCGAATCAT ACTTTTCAAT CCTGACTCTT CTTAAACTTT CCCCCGAAA ACATCGAACT
 ATAAAGTATG ACAGAATAAT GGGATAGTAT
 7201 GGCCCAACCC AAATGGAAGT CCCATTCTTC CTCAGGATGT TTAAGATTAG CATTACAGAA
 GAGATCAGAG GTCTGTGGC TCCCTTATCA TGTCCCTTAT
 CCGGTGGGG TTTACCTTCA GGTAAAGAAG GAGTCTTACA AATTCTAATC GTAAGTCTT CTCTAGTCTC
 CAGACGACCG AGGGAATAGT ACAGGGAATA
 7301 GGTCCTTCTG GCTCTGCAGT TATTAGCATA GTGTACCAT CAACCACCTT AACTTCATTT
 TTCTTATTCA ATACCTAGGT AGGTAGATGC TAGATTCTGG
 CCACGAAGAC CGAGACGTCA ATAATCGTAT CACAATGGTA GTTGGTGGAA TTGAAGTAAA AAGAATAAGT
 TATGGATCCA TCCATCTACG ATCTAAGACC
 7401 AAATAAAATA TGAGTCTCAA GTGGTCTCTG TCCTCTCTCC CAGTCAAATT CTGAATCTAG
 TTGGCAAGAT TCTGAAATCA AGGCATATAA TCAGTAATAA

Figure 3 (continued)

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TTTTATTTTAT ACTCAGAGTT CACCAGGAAC AGGAGAGAGG GTCAGTTTAA GACTTAGATC AACCGTTCTA
AGACTTTTAGT TCCGTATATT AGTCATTATT
7501 GTGATGATAG AAGGGTATAT AGAAGAAATTT TATTATATGA GAGGGTGAAA TCCCAGCAAT
TTGGGAGGGCT GAGGCAGGAG AATCGCTTGA TCCTGGGAGG
CACTACTATC TTCCCATATA TCTTCTTAAA ATAATATACT CTCACACTTT AGGGTCGTAA AACCTCCGA
CTCCGTCCCTC TTAGCGAACT AGGACCCCTCC
7601 CAGAGGTTGC AGTGAGCCAA GATTGGCCA CTGCATTCCA GCCAGGTGA CAGCATGAGA
CTCCGTCA CA AAAAAAAAAAG AAAAAAAAAAG GGGGGGGGG
GTCCTCAAACG TCACTCGGTT CTAACACGGT GACGTAAGT CCGGTCCACT GTCGTACTCT GAGGCAGTGT
TTTTTTTTC TTTTTTTTC CCCCCCCCCC
7701 CGGTGGAGCC AAGATGACCG AATAGGAACA GCTCCAGTAC TATAGCTCCC ATCGTGAGTG
ACGCAGAAAG CGGGTGATTT CTGCATTTCC AAC TGAGGTA
GCCACCTCGG TTCTACTGCG TTATCCTTGT CGAGGTCATG ATATCGAGGG TAGCACTCAC TCGGTCTTCT
GCCCACTAA GACGTAAAGG TTGACTCCAT
7801 CCAGGTTTCA CTCACAGGA AGTGCCAGG AGTGGGIGCA GGACAGTAGG TGCAGTGCAC
TGTCATGAG CCGAAGCAGG GACGAGGCAT CACCTCACCC
GGTCCAAGTA GAGTGTCCT TCACGGTCCG TCACCCACGT CCTGTCAATC ACGTCACGTG ACAGTACTC
GGCTTCGTCC CTGCTCCGTA GGGAGTGGG
7901 GGAAGCACA AGGGTTCAG GAATTCCTT TCCTAGTCAA AGAAAAGGT GACAGATGGC
ACCTGGA AAA TCGGTCACT CCGCCCTAA TACTGCGTC
CCCTTCGTGT TCCCCAGTCC CTTAAGGGAA AGGATCAGT TCTTTTCCCA CTGTCTACCG TGGACCTTTT
AGCCCA GTGA GGGCGGATT ATGACGCGAG
8001 TTCCAACAAG CTGTCTTTG GAAAATAGAT CAATTTCCCT TGGGAAGAAG ATTTTAGCA
CAGCAAGGG CAGGATGTT CACTGTGAGA AACGAAGAA
AAGGTGTTC GAACAGAAAC CTTTATCTA GTTAAAGGA ACCCTTCTT TAAAAATCGT GTCGTTCCCC
GTCCTACAAG TTGACACTCT TTTGCTTCTT
8101 TTAGCCAAA AACCTCCAGT AAGCCTGCAA AAAAAAAAAA AAAATAAAG CTAAGTTTCT
ATAAATGTT TGTAATGTA AACAGAAG TAAGTCACT
AATCGGTTTT TTGAAGGTCA TTCGGACGTT TTTTTTTTT TTTTATTTT GATTCAAAGA TATTACAAG
ACATTTACAT TTGTCCTCC ATTCAGTTGA

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Figure 3 (continued)

8201 GCACCTAATA AAAATCACTT AATAGCAATG TGCTGTGTCA GTTGTTTATT GGAACCACAC
 CCGGTACACA TCCTGTCCAG CATTGTCAGT GCGTGCATTG
 CGTGGATTAT TTTAGTGAA TTATCGTTAC ACGACACAGT CAACAAATAA CCTTGGTGTG GGCCATGTGT
 AGGACAGGTC GTAAACGTCA CGCACGTAAC
 8301 AATTATTGTG CTGGCTAGAC TTCAITGGCG CTGGCACCGA ATCCIGCCIT CTCAGCGAAA
 ATGAATAATT GCTTTGTTGG CAAGAAACTA AGCATCAATG
 TTAATAACAC GACCGATCTG AAGTACCGG GACCGTGGCT TAGGACGGAA GAGTCGCTTT TACTTATTAA
 CGAAACAACC GTTCTTTGAT TCGTAGTTAC
 8401 GGACGCGTGC AAAGCACCGG CGCGGTAGA TCGGGGTAA GTACTGAATT TTAATTTCGAC
 CTATCCCGGT AAAGCGAAAG CGACACGCTT TTTTTCACA
 CCTGCGCAG TTTCTGTGGC GCGCCATCT ACGCCCCATT CATGACTTAA AATTAAGCTG GATAGGGCCA
 TTTGCTTTC GCTGTGCGAA AAAAAAGTGT
 8501 CATAGCGGA CCGAACACGT TATAAGTATC GATTAGTCT ATTTTGTCT CTCGTGCGGA
 ACCAGAACTG GTAAAAGTTT CCATTGCGTC TGGCTTGTG
 GTATCGCCCT GGCTTGIGCA ATATTTCATG CTAATCCAGA TAAAAACAGA GAGACAGCCT TGGTCTTGAC
 CATTTTCAA GGTAAACGAG ACCCGAACAG
 8601 TATCATTGCG TCTCTATGGT TTTTGAGGA TTAGACGGG CCACCAGTAA TGGTGCAATG
 CGGATGTCTG TACCGCCATC GGTGCACCGA TATAGGTTG
 ATAGTAACGC AGAGATACCA AAAACCTCCT AATCIGCCCC GGTGGTCATT ACCACGTATC GCCTACAGAC
 ATGGCGGTAG CCACGTGGCT ATATCCAAAC
 8701 GGGTCCCCA AGGGACTGCT GGGATGACAG CTTCATATTA TATTGAATGG GCGCATAATC
 AGCTTAATIG GTGAGGACAA GCTACAAGTT GTAACCTGAT
 CCGGAGGGGT TCCCTGACGA CCTACTGTC GAAGTATAAT ATAACCTACC CGCGTATTAG TCGAATTAAAC
 CACTCCTGTT CGATGTTCAA CATTGGACTA
 8801 CTCACAAAAG TACGTTGCCG GTCGGGGTCA AACCGTCTTC GGTGCTCGAA ACCGCCTTAA
 ACTACAGACA GGTCCCAGCC AAGTAGGCGG ATCAAAACCT
 GAGGIGTTTC ATGCAACGGC CAGCCCCAGT TTGGCAGAAG CCACGAGCTT TGGCGGAATT TGAIGTCTGT
 CCAGGGTCGG TTCATCCGCC TAGTTTGGG
 8901 CAAAAAGCGG GGAGCCCAATC AAAATGCAGC ATTATATTTT AAGCTCACCG AAACCGGTAA
 GTAAAGACTA TGTATTTTTT CCCAGTGAAT AATTGTGTT

Figure 3 (continued)

GTTTTCCGC CCTCGGTTAG TTTTACGTCG TAATATAAAA TTCGAGTGGC TTTGGCCATT CATTTCTGAT
 ACATAAAAAA GGGTCACCTTA TTAACAACAA
 9001 AACTATAAAA AGCGTCATGG CAAACGATAA AGTAGCAAT TGGGATTCGG GCTTGGGATG
 CTCATATCTG CTGACTGAGG CAGAAATGTGA AAGTGACAAA
 TTGATAATTT TCGCAGTACC GTTIGCTATT TCCATCGTTA ACCCTAAGCC CGAACCCCTAC GAGTATAGAC
 GACTGACTCC GTCCTTACACT TTCACTGTTT
 9101 GAGAAAGAGG AACCCGGGGC AGGIGTAGAA CTGTCTGTGG AATCTGATCG GTATGATAGC
 CAGGATGAGG ATTTTGTGA CAATGCATCA GTCITTCAGG
 CTCCTTACTCC TTGGGCCCCG TCCACATCTT GACAGACACC TTAGACTAGC CATACTATCG GTCCTACTCC
 TAAAACAACT GTTACGTAGT CAGAAAGTCC
 9201 GAAATCACCT GGAGGTCTTC CAGGCATTAG AGAAAAAGGC GGGTGAGGAG CAGATTTTAA
 ATTTGAAAAG AAAAGTATTG GGGAGTTCCG AAAACAGCAG
 CTTTAGTGGA CCTCCAGAAG GTCCGTAATC TCTTTTCCG CCCACTCCTC GTCATAAATT TAAACTTTTC
 TTTTCATAAC CCTCAAGCG TTTTGTCTGC
 9301 CGGTTCCGAA GCATCTGAAA CTCCAGTTAA AAGACGGAAA TCAGGAGCAA AGCGAAGATT
 ATTTGCTGAA AATGAAGCTA ACCGTGTTCT TACGCCCTC
 GCCAAGGCTT CGTAGACTTT GAGGTCAATT TTCIGCCTTT AGTCCTCGTT TCGCTTCTAA TAAACGACTT
 TTAATTTCGAT TGGCACAAGA ATGCGGGGAG
 9401 CAGGTACAGG GGGAGGGGA GGGAGGCAA GAACTTAATG AGGAGCAGGC AATTAGTCAT
 CTACAICTGC AGCTTGTTAA ATCTAAAAAT GCTACAGTTT
 GTCCAIGTCC CCTCCCCCT CCCCTCCGTT CTTGAATTAC TCCTCGTCCG TTAATCAGTA GATGTAGACG
 TCGAACCAATT TAGATTTTGA CGAIGTCAAA
 9501 TTAAGCTGGG GCTCTTTAA TCTTTGTTCC TTTGTAGCTT CCAATGATATT ACGAGGTGTG
 TTAAGAATGA TAAGACCACT AATCAGCAAT GGGTGTGGC
 AATTGACCC CGAGAAATTT AGAAACAAGG AAACATCGAA GGTACTATAA TGCCTCAACA AATTCTTACT
 ATTCTGGTGA TTAGTCGTTA CCCACGACCG
 9601 TGTGTTTGGC CTITGCAGAGG TGTITTTTGA GCGAGTTTC GAACCTCTAA AGAAGCAGTG
 TAGTTTTCTG CAGATGCAAA AAAGATCTCA TGAAGGAGGA
 ACACAAACCG GAACGTCTCC ACAAATAACT CCGCTCAAAG CTTGAGGATT TCTTCGTCTAC ATCAAAAGAC
 GTCTACGTTT TTCTAGAGT ACTTCCTCCT

Figure 3 (continued)

9701 ACTTGTGCAG TTTACTTAAT CTGCTTTAAC ACAGCTAAAA GCAGAGAAAC AGTCCGGAAT
 CTGATGGCAA ACATGCTAAA TGTAAGAGAA GAGTGTTTGA
 TGAACACGTC AAATGAATTA GACGAAATTG TGTCGATTTT CGTCTCTTTG TCAGGCCCTTA GACTACCGTT
 TGTACGATTT ACATTCTCTT CTCACAAACT
 9801 TGCTGCAGCC ACCTAAAAAT CGAGGACTCA GCGCAGCTCT ATTCTGGTTT AAAAGTAGTT
 TGTCACCCGC TACACTTAAA CATGGTGCTT TACCTGAGTG
 ACGACGTCGG TGGATTTTAA GCTCCTGAGT CCGGTCGAGA TAAGACCAAA TTTTCATCAA ACAGTGGGCG
 ATGTGAATTT GTACCACGAA ATGGACTCAC
 9901 GATACGGCG CAACTACTC TGAACGAGAG CTTGCAGACC GAGAAATTCG ACTTCGGAAC
 TATGGTGCAA TGGGCCATG ATCACAATA TGCIGAGGAG
 CTATGCCCCG GTTTGAIGAG ACTTGCTCTC GAACGTCIGG CTCTTAAGC TGAAGCCTTG ATACCACGTT
 ACCCGGATAC TAGTGTTTAT ACGACTCCTC
 10001 TCTAAAAATAG CCTATGAATA TGCTTTGGCT GCAGGATCTG ATAGCAATGC ACGGGCTTTT
 TTAGCAACTA ACAGCCAAGC TAAGCATGTG AAGGACTGTG
 AGATTTTATC GGATACCTTAT ACGAAACCGA CGTCCTAGAC TATCGTTACG TGCCCGAAAA AATCGTTGAT
 TGTCGGTTTC ATTGCTACAC TTCCTGACAC
 10101 CAACTATGGT AAGACACTAT CTAAGAGCTG AAACACAAGC ATTAAGCATG CCTGCATAA
 TTAAGCTAG GTGCAAGCTG GCAACTGGGG AAGGAAGCTG
 GTTGATACCA TTCGTGATA GATTCTCGAC TTGTGTTCG TAATTCGTAC GGACGTATAT AATTTCGATC
 CACGTTCGAC CGTTGACCCC TTCCTTCGAC
 10201 GAAGTCTATC CTAACCTTTT TTAACATCA GAATATTGAA TTAATTACCT TTATTAAATGC
 TTTAAAGCTC TGGCTAAAAG GAATTCCAA AAAAACTGT
 CTTCAGATAG GATTGAAAAA AATTGATAGT CTTATAACTT AATTAATGGA AATAATTACG AAATTCGAG
 ACCGATTTTC CTTAAGGTTT TTTTGTGACA
 10301 TTAGCATTTA TTGGCCCTCC AAACACAGGC AAGTCTATGC TCTGCAACTC ATTAATTCAT
 TTTTGGGTG GTAGTGTTTT ATCTTTTGGC AACCATAAAA
 AATCGTAAAT AACCGGGAGG TTGTGTCCG TTCAGATACG AGACGTGAG TAATTAAGTA AAAAACCAC
 CATCACAAA TAGAAAACGG TTGGTATTTT
 10401 GTCACCTTTG GCTTGCTTCC CTAGCAGATA CTAGAGCTGC TTTAGTAGAT GATGCTACTC
 ATGCTTGCTG GAGGTACTTT GACACATACC TCAGAAATGC

Figure 3 (continued)

CAGTGAAAC CGAACGAAGG GATCGTCTAT GATCTCGACG AAATCATCTA CTACGATGAG TACGAACGAC
 CTCCATGAAA CTGTGATGG AGTCTTTACG
 10501 ATTGGATGC TACCCTGTCA GTATTGATAG AAAACACAAA GCAGCGGTTT AAATTAAAGC
 TCCACCCCTC CTGGTAACCA GTAATATTGA TGTGCAGGCA
 TAACCTACCG ATGGGACAGT CATAACTATC TTTTGTGTTT CGTCGCCAAG TTTAATTTCG AGGIGGGGAG
 GACCATTTGGT CATTATAACT ACACGTCCGT
 10601 GAGGACAGAT ATTTGTACTT GCAATGTCGG GTGCAAAACCT TTCGCCTTGA GCAGCCAATGC
 ACAGATGAAT CGGGTGAGCA ACCTTTTAAT ATTACTGATG
 CTCCTGTCTA TAAACATGAA CGTATCAGCC CACGTTTGA AAGCGAAACT CGTCGGTAGG TGTCTACTTA
 GCCCACTCGT TGGAAAATTA TAATGACTAC
 10701 CAGATTGGAA ATCTTTTTT GTAAGTTAT GGGGGCGTTT AGACCTGATT GACGAGGAGG
 AGGATAGTGA AGAGGATGGA GACAGCATGC GAACGTTTAC
 GTCTAACCTT TAGAAAAAAA CATTCCAATA CCCCCGCAA TCTGGACTAA CTGCTCCTCC TCCTATCACT
 TCTCCTACCT CTGTCGTACG CTTGCAAAATG
 10801 ATGCAGCGCA AGAAACACAA ATGCAGTTGA TTGAGAAAAG TAGTGATAAG TTGCAAGATC
 ATATACTGTA CTGGACTGCT GTTAGAACTG AGAACACACT
 TACGTCGCGT TCTTTTGTTT TACGTCAACT AACCTTTTC ATCACTATT C AACGTTCTAG TATATGACAT
 GACCTGACGA CAATCTTGAC TCTTGTTGA
 10901 GCTTTATGCT GCAAGGAAA AAGGGGTGAC TGTCTTAGGA CACTGCAGAG TACCACACTC
 TGTAGTTTGT CAAGAGAGAG CCAAGCAGGC CATTGAAATG
 CGAAATACGA CGTTCCTTTT TTCCCACCTG ACAGGATCCT GTGACGTCTC ATGGTGTGAG ACATCAAACA
 GTTCTCTCTC GGTCGTCCG GTAACTTTAC
 11001 CAGTTGTCTT TGCAGGAGTT AAGCAAAACT GAGTTTGGG ATGAACCATG GTCITTGCTT
 GACACAAGCT GGGACCGATA TATGTCAGAA CCTAAACGGT
 GTCAACAGAA ACGTCCCTCAA TTCGTTTGA CTCAAACCCC TACTTGGTAC CAGAAACGAA CTGTGTTTGA
 CCCTGGCTAT ATACAGTCTT GGATTTGCCA
 11101 GCTTTAAGAA AGCGGCCAGG GTGGTAGAGG TGGAGTTTGA TGGAAATGCA AGCAATACAA
 ACTGGTACAC TGTCTACAGC AATTGTGACA TCGGCACAGA
 CGAAATTCTT TCCGCGGTCC CACCATCTCC ACCTCAAACT ACCTTTACGT TCGTTATGTT TGACCATGTG
 ACAGATGTGCG TTAACAATGT ACGCGTGTCT

Figure 3 (continued)

11201 GGACGGCTGG CAGCTTGCGA AGCTGGGCT GACGGAAC TG GCTCTACTA CTGCACCATG
GCCGTGCTG GACGCATTTA CTATTCTCGC TTGGTGACG
CTGCCGACC GTCGAACGCT TCCGACCCGA CTGCCTTGAC CCGAGATGAT GACGTGGTAC CGGCCACGAC
CTCGGTAAAT GATAAGAGCG AAACCACTGC

11301 AGGCAGCCAG ATTTAGTACA ACAGGCGATT ACTCTGTAAG AGATCAGGAC AGAGTGTATG
CTGGTGTCTC ATCCACCTCT TCTGATTTA GAGATCGCCC
TCCGTGGTC TAAATCATGT TGTCCCGTAA TGAGACATTC TCTAGTCCTG TCTCACATAC GACCACAGAG
TAGGTGGAGA AGACTAAAAT CTCTAGCGGG

11401 AGACGGAGTC TGGTGGCAT CCGAAGGACC TGAAGGAGAC CTTGCAGGAA AAGAAGCCGA
GCCAGCCAG CCGTCTCTT CTTTGTCTGG CTCCCCGCGC
TCTGCCCTCAG ACCCAGCGTA GGCTTCTCTG ACTTCTCTG GGACGTCTCTT TTCTTCGGCT CGGTCCGGTC
GGACAGAGAA GAAACGAGCC GAGGGGGCGG

11501 TCGGTGTTCCA TCAGAGCAGG CCTCGGTTGG GTACGGGACG GTCCTCGCTC GCACCCCTAC
AATTTCTCTG CAGGCTCGGG GGGCTCTATT CTCCGCTCTT
ACGCCAGGGT AGTCTCGTCC GGAGCCAAACC CATGCCCTGC CAGGAGCGAG CGTGGGGATG TTAAAAGGAC
GTCCGAGCCC CCGAGATAA GAGGCGAGAA

11601 CCTCCACCCC GTGCAGGCA CGGTACCGGT GGACTTGGCA TCAAGGCAGG AAGAAGAGGA
GCAGTCGCCC GACTCCACAG AGGAAGAAC AGTGACTCTC
GGAGTGGGG CAGTCCCGT GCCATGGCCA CCTGAACCGT AGTTCCGTCC TTCTTCTCT CGTCAGCGGG
CTGAGGTGTC TCTTCTTGG TCACTGAGAG

11701 CCAAGGCGCA CCACCAATGA TGGATTCCAC CTGTTAAAGG CAGGAGGGTC ATGCTTTGCT
CTAATTTTACG GAACTGCTAA CCAGGTAAAG TGCTATCGCT
GGTTCCGCGT GGTGGTTACT ACCTAAGGTG GACAATTTC GTCTCTCCAG TACGAAACGA GATTAAAGTC
CTTGACGATT GGTCCATTTC ACGATAGCGA

11801 TTCGGGTGAA AAAGAACCAT AGACATCGCT ACGAGAAGTG CACCACCACC TGGTTACAG
TTGCTGACAA CGGTGCTGAA AGACAAGGAC AAGCACAAAT
AAGCCCACTT TTTCTTGGTA TCTGTAGCGA TGCTCTTGAC GTGGTGGTGG ACCAAGTGTC AAGCACTGTT
GCCACGACTT TCTGTTCCTG TTCGTGTTA

11901 ACTGATCACC TTGGATCGC CAAGTCAAAG GCAAGACTTT CTGAACAATG TACCCTACC
TCCTGGAATG AACATTTCG GCTTTACAGC CAGCTTGGAC

Figure 3 (continued)

TGACTAGTGG AACCTAGCG GTTCAGTTTC CGTTCGAAA GACTTTGTAC ATGGTGA TGG AGGACCTTAC
 TTGTAAGGC CGAAATGTCG GTCGAACCTG
 12001 TTCTGATCAC TGCCATTGCC TTTTCTTCAT CTGACTGGTG TACTATGCCA AATCTATGCG
 ACCGCATTAT AAGCCGAAT TCTGCAGATA TCCATCACAC
 AAGACTAGTG ACGGTAACGG AAAAGAAGTA GACTGACCAC ATGATACGGT TTAGATACGC TGGCGTAATA
 TTTTCGGCTTA AGACGTCAT AGGTAGTGTG
 12101 TGGCGGCCAT ATGCCGCTA TCGGGTGTGA AATACCGCAC AGATCGGTAA GGAGAAAATA
 CCGCATCAGG CGCTCTTCCG CTTCCCTCGT CACTGACTCG
 ACCGCCGTA TACCGCGAT ACGCCACACT TTATGGCGTG TCTACGCATT CCTCTTTTAT GCGGTAGTCC
 GCGAGAAGGC GAAGGAGCGA GTGACTGAGC
 12201 CTGCGCTCG TCGTTCGGT GCGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAATACGG
 TTATCCACAG AATCAGGGGA TAACGCAGGA AAGAACAIGT
 GACGCGAGCC AGCAAGCCGA CGCGCTCGC CATAGTCGAG TGAGTTCCG CCATTATGCC AATAGGTGTC
 TTAGTCCCT ATTGCGTCT TCTTGTACA
 12301 GAGCAAAAAG CCAGCAAAAG GCCAGGAACC GTAAAAGGC CGCGTTGCTG GCGTTTTTCC
 ATAGGCTCCG CCCCCCTGAC GAGCATCACA AAAATCGACG
 CTCGTTTTCC GGTGTTTTTC CGGTCTTGG CATTTTCCG GCGCAACGAC CGCAAAAAGG TATCCGAGGC
 GGGGGGACTG CTCGTAGTGT TTTTAGCTGC
 12401 CTCAAGTCAG AGGTGGCGAA ACCGACAGG ACTATAAGA TACCAGGCGT TTCCCCCTGG
 AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT
 GAGTTCAGTC TCCACCGCTT TGGGTGTCC TGATATTCT ATGGTCCGCA AAGGGGGACC TTCGAGGGAG
 CACGCGAGAG GACAAGGCTG GGACGGCGAA
 12501 ACCGGATACC TGTCGGCTT TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA TAGCTCACGC
 TGTAGGTATC TCAGTTCGGT GTAGGTCTGT CGTCCCAAGC
 TGGCCTATGG ACAGGCGGAA AGAGGGAAGC CCTTCGCACC GCGAAAGAGT ATCGAGTGG ACATCCATAG
 AGTCAAGCCA CATCCAGCAA GCGAGTTTCG
 12601 TGGGCTGTGT GCACGAAACC CCCGTTTCAGC CCGACCGCTG CGCCTTATCC GGTAACATATC
 GTCTTGAGTC CAACCCGGTA AGACACGACT TATCGCCACT
 ACCCGACACA CGTGCTTGG GGCAGAGTCG GGCTGGCGAC GCGGAATAGG CCATTGATAG CAGAACTCAG
 GTTGGGCCAT TCTGTGCTGA ATAGCGGTGA

Figure 3 (continued)

12701 GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGCGGGTG CTACAGAGTT
 CTTGAAGTGG TGGCTAACT ACGGCTACAC TAGAAGGACA
 CCGTCGTCGG TGACCAATTGT CCTAATCGTC TCGTCCATA CATCCGCCAC GATGTCTCAA GAACTTCACC
 ACCGGATTGA TGCCGATGTG ATCTTCCTGT
 12801 GTATTIGGTA TCTGCGCTCT GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT
 TGATCCGGCA AACAAACCAC CGCTGGTAGC GGTGGTTTT
 CATAAACCAT AGACGCGAGA CGACTTCGGT CAATGGAAGC CTTTTTCTCA ACCATCGAGA ACTAGGCCGT
 TTGTTTGGTG GCGACCATCG CCACCAAAA
 12901 TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC TCAAGAAGAT CCTTTGATCT
 TTTCTACGGG GCTGACGCT CAGTGAACG AAACTCAG
 AACAAACGTT CGTCGTCTAA TCGCGGTCTT TTTTCCCTAG AGTTCTTCTA GGAAACTAGA AAAGATGCCCC
 CAGACTGCGA GTCACCTTGC TTTGAGTGC
 13001 TTAAGGGATT TTGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC TTTTAAATTA
 AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA
 AATTCCCTAA AACCAGTACT CTAATAGTTT TTCCTAGAAG TGGATCTAGG AAAATTAAAT TTTTACTTCA
 AAATTTAGTT AGATTTTATA TATACTCAT
 13101 ACTTGGTCTG ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA
 TTTTCGTTTCAAT CCATAGTTGC CTGACTCCCC GTCGTGTAGA
 TGAACCAGAC TGTCAAATGGT TACGAATTAG TCATCCGTG GATAGAGTCTG CTAGACAGAT AAAGCAAAGTA
 GGTAICAAACG GACTGAGGGG CAGCACATCT
 13201 TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC TGCAATGATA CCGCGAGACC
 CACGCTCACC GGCTCCAGAT TTAICAGCAA TAAACCAGCC
 ATTGAATGCTA TGCCCTCCCG AATGGTAGAC CGGGGTCACG ACGTTACTAT GCGGCTCTGG GTGCGAGTGG
 CCGAGGTCTA AATAGTCGTT ATTTGGTCGG
 13301 AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCCTCCA TCCAGTCTAT
 TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT
 TCGGCCCTTCC CGGCTCGCGT CTTCACCAGG ACGTTGAAAT AGCGGAGGT AGGTCAGATA ATTAAACAACG
 GCCCTTCGAT CTCATTTCATC AAGCGGTCAA
 13401 AATAGTTTGC GCAACGTTGT TGCCATTGCT GCAGGCATCG TGGTGTACAG CTCGTCTGTTT
 GGTATGGCTT CATTACGCTC CGGTTCCCAA CGATCAAGGC

Figure 3 (continued)

TTATCAAACG CGTTGCAACA ACGTAACGA CGTCCGTAGC ACCACAGTGC GAGCAGCAA CCATACCGAA
 GTAAGTCGAG GCCAAGGGTT GCTAGTTCCG
 13501 GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGCGGTTAG CTCCTTCGGT CCTCCGATCG
 TTGTCAGAAAG TAAGTTGGCC GCAGTGTTAT CACTCATGGT
 CTCAATGATC TAGGGGTAC AACACGTTTT TTCGCCAATC GAGGAAGCCA GGAGGCTAGC AACAGTCTTC
 ATTCAACCCG CGTCACAATA GTGAGTACCA
 13601 TATGGCAGCA CTGCATAATT CTCCTACTGT CATGCCATCC GTAAGATGCT TTTCTGTGAC
 TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGATG
 ATACCGTCTG GACGTATTAA GAGAAATGACA GTACGGTAGG CATTCTACGA AAAGACACTG ACCACTCATG
 AGTTGGTTCA GTAAGACTCT TATCACATAC
 13701 CGCGGACCGA GTTGCTCTTG CCCGGCGTCA ACACGGGATA ATACCGCGCC ACATAGCAGA
 ACTTTAAAAG TGCTCATCAT TGAATAACGT TCTTCGGGGC
 GCCGCTGGCT CAACGAGAAC GGGCCGCAGT TGTGCCCTAT TATGGCGCGG TGTATCGTCT TGAAATTTTC
 ACGAGTAGTA ACCTTTTGCA AGAAGCCCCG
 13801 GAAAACCTCT AAGGATCTTA CCGCTGTTGA GATCCAGTTC GATGTAACCC ACTCGTGCAC
 CCAACTGATC TTCAGCATCT TTTACTTTCA CCAGCGTTTC
 CTTTIGAGAG TTCCTAGAAAT GCGACAACCT CTAGGTCAAG CTACATIGGG TGAGCACGTG GGTGACTAG
 AAGTCGTAGA AAATGAAAGT GGTCGCAAAAG
 13901 TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAAG GGAATAAGG CGACACGGAA
 ATGTTGAATA CTCATACTCT TCCTTTTTCA ATATTATTGA
 ACCCACTCGT TTTTGTCCTT CCGTTTTACG GCGTTTTTC CCTTATTCCC GCTGTGCCCT TACAACTTAT
 GAGTATGAGA AGGAAAAAGT TATAATAACT
 14001 AGCATTATC AGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT
 AAACAAATAG GGGTCCGG CACATTTCCT CGAAAAGTGC
 TCGTAAATAG TCCCAATAAC AGAGTACTCG CCTAIGTATA AACITACATA AATCTTTTA TTTGTTTATC
 CCCAAGGCGC GTGTAAAGGG GCTTTTCACG
 14101 CACCTGACGT CTAAGAAAAC ATTATTATCA TGACATTAACT CTATAAAAAT AGGCGTATCA
 CGAGGCCCTT TCGTCTTCAA GAATTCTCAT GTTTGACAGC
 GTGGACTGCA GATTCTTTGG TAATAATAGT ACTGTAATTG GATATTTTA TCCGCATAGT GCTCCGGGAA
 AGCAGAAGTT CTAAAGAGTA CAACTGTGCTG

Figure 3 (continued)

14201 TTATCATCGA TAAGCTTAC GCTGCCGCAA GCACTCAGGG CGCAAGGGCT GCTAAAGGAA
 GCGGAACACG TAGAAAGCCA GTCCGCAGAA ACGGTGCTGA
 AATAGTAGCT ATTCGAAGTG CGACGGCGTT CGTGAGTCCC GCGTTCGCCA CGATTTCCTT CGCCTTGTGC
 ATCTTTCCGT CAGGCGTCTT TGCCACGACT
 14301 CCCCGGATGA ATGTACAGTA CTGGGCTATC TGGACAAGGG AAAACGCAAG CGCAAAGAGA
 AAGCAGGTAG CTTGCAGTGG GCTTACATGG CGATAGCTAG
 GGGCCCTACT TACAGTCGAT GACCCGATAG ACCTGTTCCT TTTTGGGTTT GCGTTTCTCT TTCGTCCATC
 GAACGTCACC CGAATGTACC GCTATCGATC
 14401 ACTGGGCGGT TTTATGGACA GCAAGCGAAC CGGAATTGCC AGCTGGGGCG CCTCTCTGGTA
 AGGTTGGGAA GCCCTGCAA GTAAACTGGA TGGCTTCTT
 TGACCCGCCA AATACCTGT CGTTCGCTTG GCTTAAACGG TCGACCCCGC GGGAGACCAT TCCAACCCCT
 CGGGACGTTT CATTGACCT ACCGAAAGAA
 14501 GCCGCCAAG ATCTGATGC GCAGGGGATC AAGATCCTGC TTATCCCCG TGGCCCCGTTG
 CTCGCGTTTG CTGGCGGTGT CCCCAGGAAGA AATATATTG
 CGGCGGTTCC TAGACTACCG CGTCCCCTAG TTCTAGGACG AAGTAGGGC ACCGGGCAAC GAGCGCAAC
 GACCGCCACA GGGCCCTTCT TTATATAAC
 14601 CATGTCTTTA GTTCTATGAT GACACAAACC CCGCCCAGCG TCTTGTCAAT GGCGAATTCG
 AACACGCAGA TGCAGTCGGG GCGGCGCGGT CCCAGGTCCA
 GTACAGAAAT CAAGATACTA CTGTGTTTGG GCGGGGTGCG AGAACAGTAA CCGCTTAAGC TTGTGCGTCT
 ACGTCAGCCC CGCCGCGCCA GGTCCAGGT
 14701 CTTCGCATAT TAAGGTGACG CGTGTGGCCT CGAACACCGA GCGACCCCTGC AGCGACCCGC
 TTAACAGCGT CAACAGCGTG CCGCAGATCT GATCAAGAGA
 GAAGCGTATA ATTCCACTGC GCACACCGGA GCTTGTGGCT CGCTGGGACG TCGCTGGGCG AATTGTGCA
 GTTGTGCGAC GCGTCTAGA CTAGTTCTCT
 14801 CAGGATGAG ATCGTTTCG ATGATTGAAC AAGATGGATT GCACGCAGGT TCTCCGGCCG
 CTTGGGTGGA GAGGCTATTC GGCTATGACT GGGCACAACA
 GTCCCTACTCC TAGCAAAGCG TACTAACTTG TTCTACCTAA CGTGGTCCA AGAGCCCGC GAACCCACCT
 CTCCGATAAG CCGATACTGA CCCGTGTTGT
 14901 GACAAATCGC TGCTCTGATG CCGCCGTGTT CCGGCTGTCA GCGCAGGGC GCCCGGTTCT
 TTTTGTCAAG ACCGACCTGT CCGGTGCCCT GAAIGAAC TG

Figure 3 (continued)

CTGTTAGCCG ACGAGACTAC GCGGCACAA GCGCGACAGT CCGGTCCCCG CGGGCCAAGA AAAACAGTTC
 TGGCTGGACA GGCACGGGA CTTACTTGAC
 15001 CAGGACGAG CAGCGCGGT ATCGTGGCTG GCCACGACGG GCGTTCCTTG CGCAGCTGTG
 CTCGACGTTG TCACTGAAG GGAAGGGAC TGGCTGCTAT
 GTCCGTCTCC GTCGCGCCGA TAGCACCGAC CCGTGTCTGC CGCAAGGAAC GCGTCGACAC GAGCTGCAAC
 AGTGACTTCG CCCTTCCCTG ACCGACGATA
 15101 TGGGCGAAGT GCGGGGCAG GATCTCTGT CATCTCACCT TGCTCCTGCC GAGAAAGTAT
 CCATCATGGC TGATGCAATG CCGCGGCTGC ATACGCTTGA
 ACCCGCTTCA CGGCCCGTC CTAGAGGACA GTAGAGTGA ACGAGGACGG CTCTTTCATA GGTAGTACCG
 ACTACGTTAC GCGCGCGACG TATGCGAACT
 15201 TCCGGCTACC TGCCCATTCG ACCACCAAGC GAAACATGC ATCGAGCGAG CACGTACTCG
 GATGGAAGCC GGCTTGTG ATCAGGATGA TCTGGACGAA
 AGCCCGATGG ACGGGTAAGC TGGTGGTTCG CTTTGTAGCG TAGCTCGCTC GTGCATGAGC CTACCTTCGG
 CCAGAACAGC TAGTCCTACT AGACCTGTCT
 15301 GAGCATCAGG GGCTCGGCC AGCCGAACTG TTCGCCAGGC TCAAGGCGCG CATGCCCGAC
 GCGGAGGATC TCGTCGTGAC CCAATGGCGAT GCCTGCTTGC
 CTCGTAGTCC CCGAGCGCGG TCGGCTTGAC AAGCGTCCG AGTCCGCGC GTACGGGCTG CCGTCTCTAG
 AGCAGCACTG GGTACCGCTA CGGACGAACG
 15401 CGAATATCAT GTGGAAAAAT GGCCGCTTTT CTGGATTCAI CGACTGTGGC CGGCTGGGTG
 TGGCGGACCG CTATCAGGAC ATAGCGTTGG CTACCCGTGA
 GCTTATAGTA CCACCTTTTA CCGGCGAAAA GACCTAAGTA GCTGACACCG GCCGACCCAC ACCGCCCTGGC
 GATAGTCCCTG TATCGCAACC GATGGGCAT
 15501 TATTGCTGAA GAGCTTGGC GCGAATGGG TGACCGCTTC CTCGTGCTTT ACGGTATCGC
 CGTCCCGAT TCGACGCGA TCGCCTTCTA TCGCCTTCTT
 ATAACGACTT CTCGAACCGC CGCTTACCG ACTGGCGAAG GAGCACGAAA TGCCATAGCG GCGAGGGCTA
 AGCGTCGCGT AGCGGAAGAT AGCGGAAGAA
 15601 GACGAGTTCT TCTGAGCGGG ACTCTGGGT TCGAAATGAC CGACCAAGCG ACGCCCAACC
 TGCCATCAG AGATTTCGAT TCCACCGCCG CCTTCTATGA
 CTGCTCAAGA AGACTCGCC TGAGACCCCA AGCTTTACTG GCTGGTTCGC TCGGGGTGG ACGGTAGTGC
 TCTAAAGCTA AGGTGGCGGC GGAAGATACT

Figure 3 (continued)

15701 AAGTTGGC TTCGGAATCG TTTTCCGGGA CGCCGGCTGG ATGATCCTCC AGCGGGGGGA
 TCTCATGCTG GAGTTCCTCG CCCACCCCGG GAGATGGGGG
 TTCCAACCCG AAGCCTTAGC AAAAGGCCCT GCGCCGACC TACTAGAGG TCGGCCCTT AGAGTACGAC
 CTCAGAAGC GGTGGGGC CTCTACCCCC
 15801 AGGCTAACTG AAACACGGAA GGAGACAATA CCGGAAGGAA CCCGCGCTAT GAACGGCAAT
 AAAAAGACAG AATAAAACGC ACGGTGTTGG GTCGTTTGT
 TCCGATTGAC TTGTGCCCTT CCTCTGTTAT GGCCTTCCTT GGGCGCGATA CTGCGCGTTA TTTTCTGTC
 TTATTTTGG TGCCACAACC CAGCAACAA
 15901 CATAAACGG GGTTCGGTC CCAGGCTGG CACTCTGTG ATACCCACC GAGACCCCAT
 TGGGCGCAAT ACGCCCGCGT TTCTTCCTT TCCCCACCCC
 GTATTGCGC CCAAGCCAG GGTCCCGACC GTGAGACAGC TATGGGGTGG CTCTGGGGTA ACCCGGTTA
 TCGGGGCGCA AGAAGGAA AGGGTGGG
 16001 ACCCCCCAAG TTCGGTGAA GGCCAGGGC TCGCAGCCAA CGTCGGGGCG GCAAGCCCTG
 CCATAGCCAC GGGCCCCGTG GGTAGGGAC GGCGGATCGC
 TGGGGGTTT AAGCCCACTT CCGGTCCCG AGCGTCGGT GCAGCCCCGC CGTTCGGGAC GGTATCGGTG
 CCGGGGGCAC CCAATCCCTG CCGCCTAGCG
 16101 GGCC
 CCGG

Figure 4

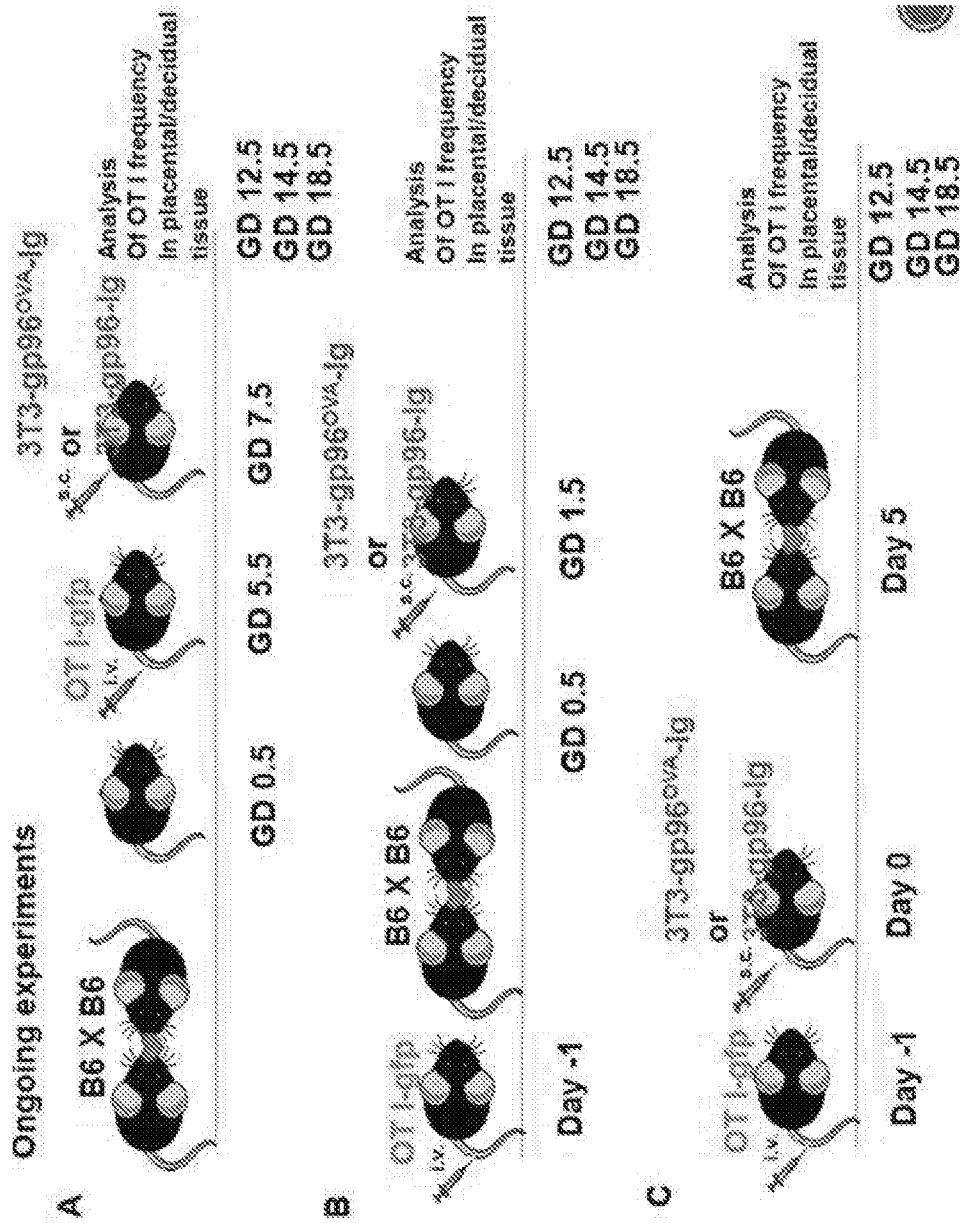


Figure 5

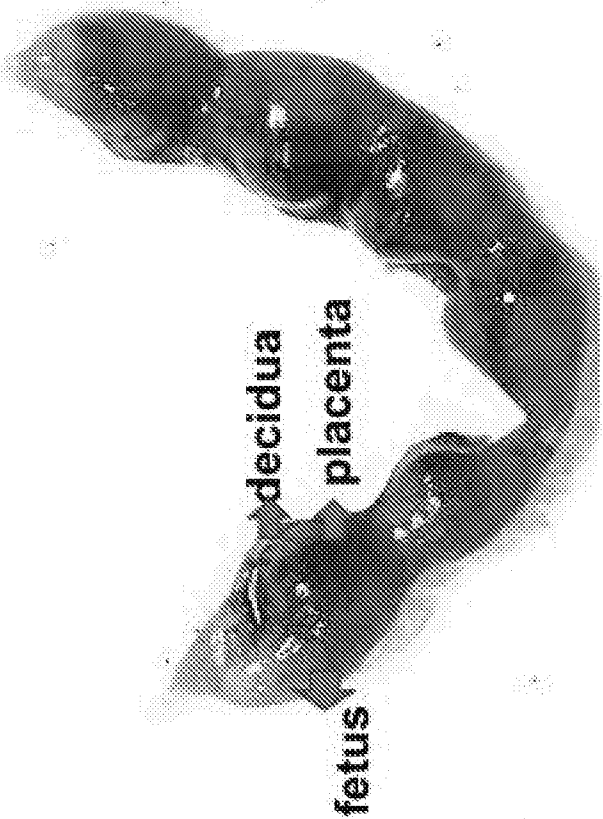
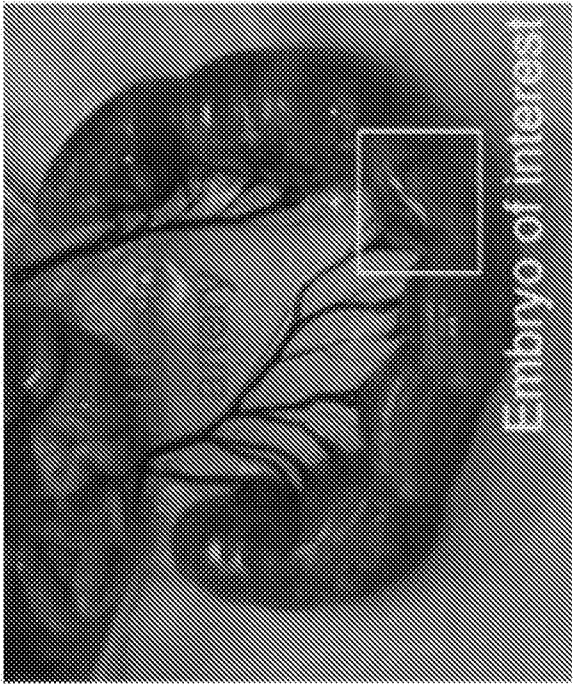
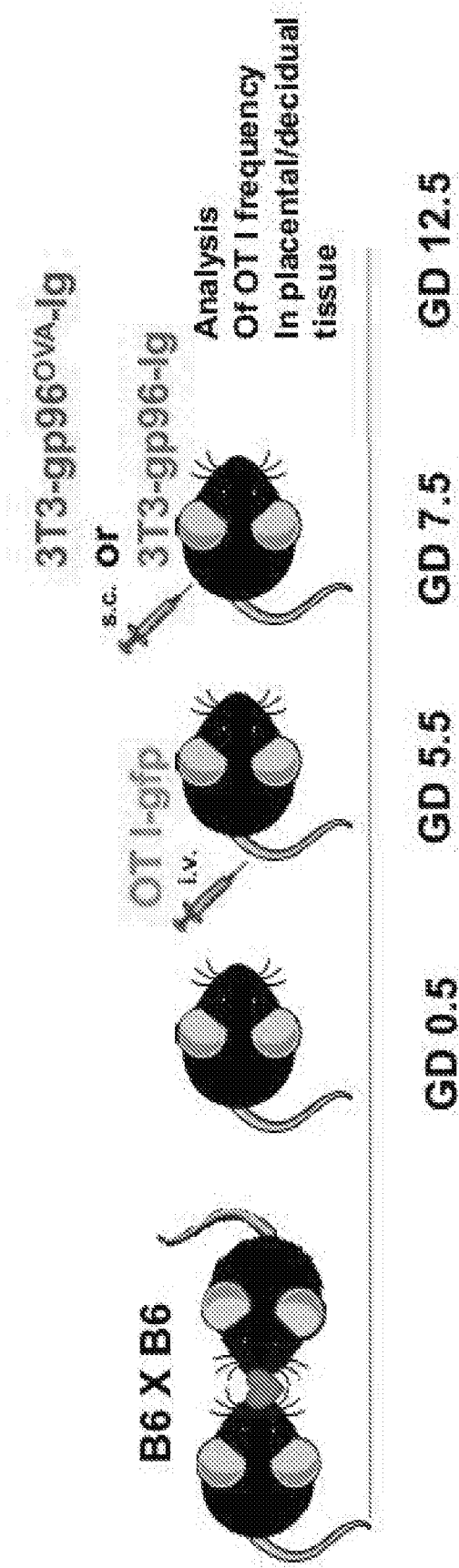


FIGURE 6

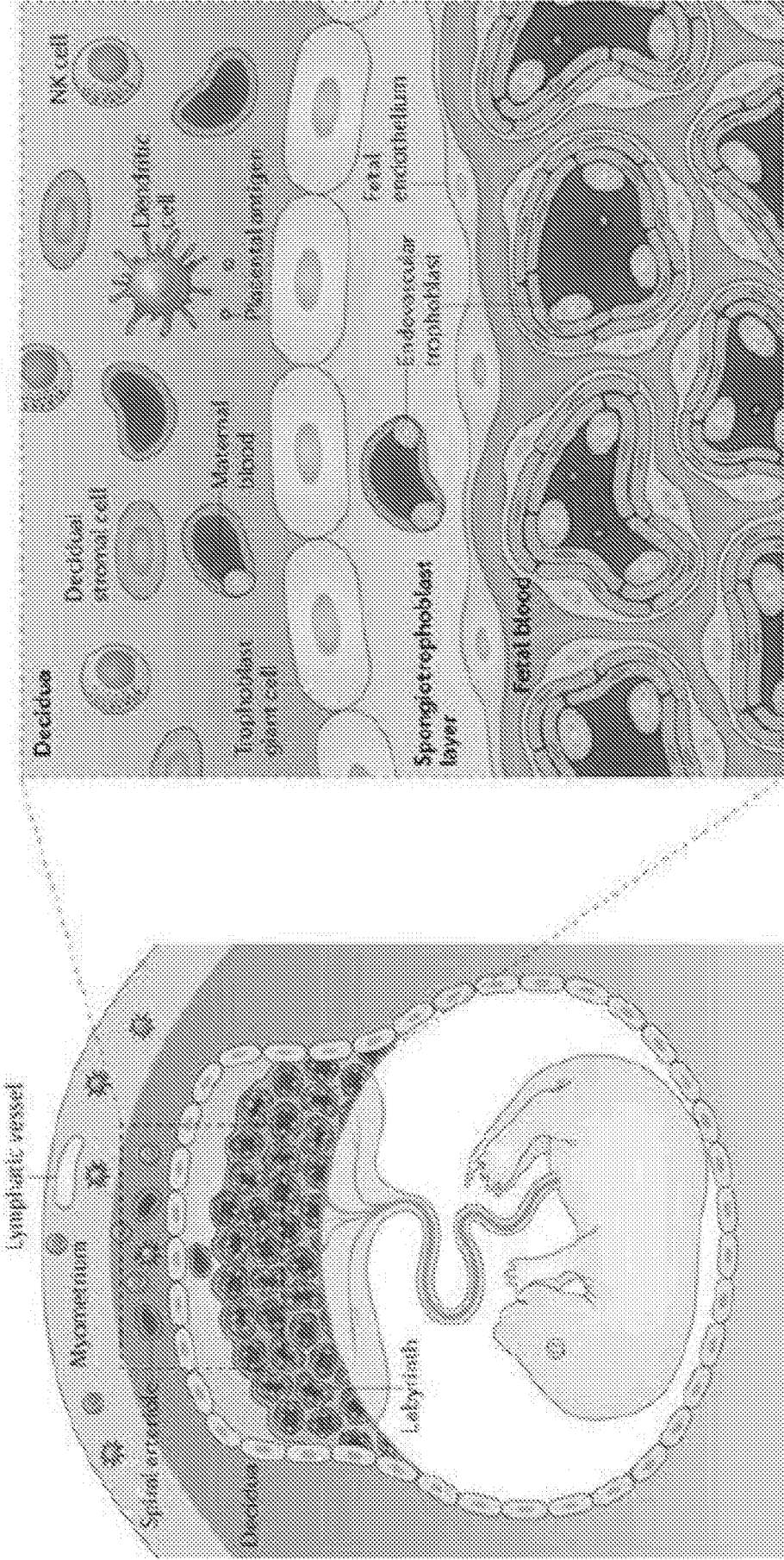
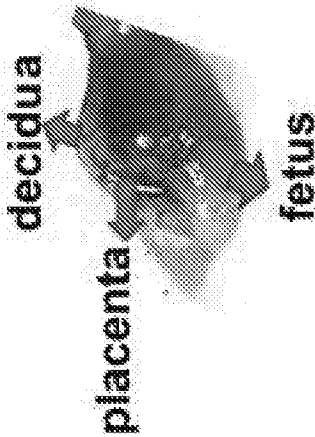


FIGURE 7

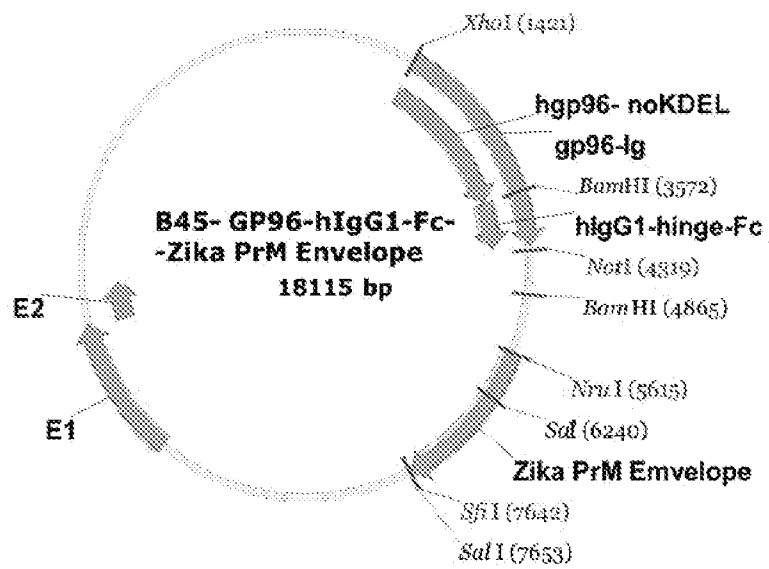


FIGURE 8

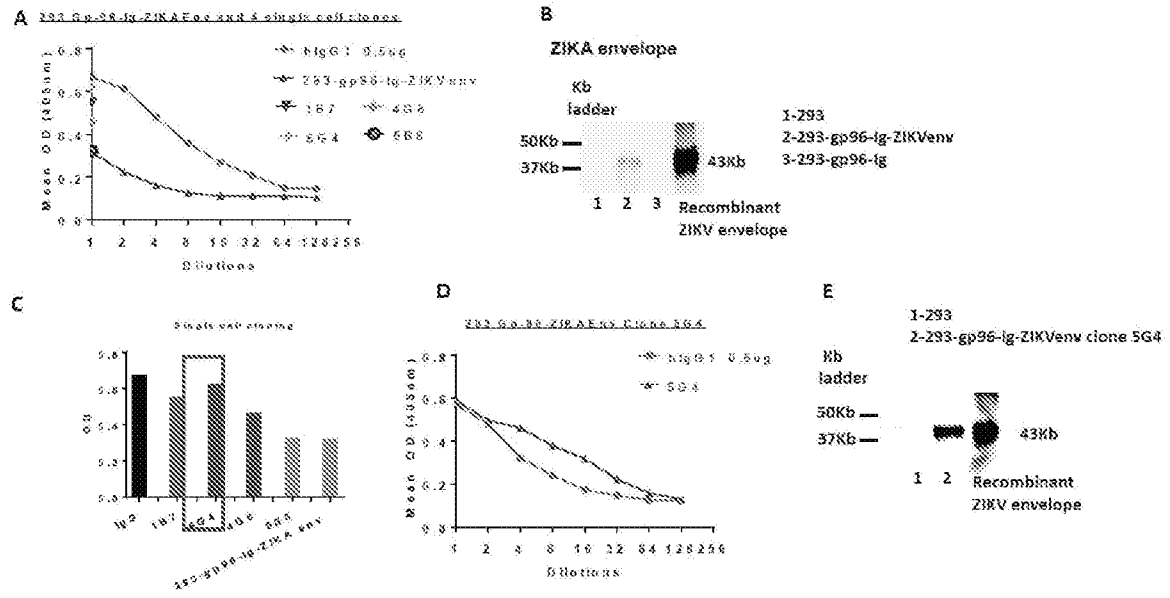


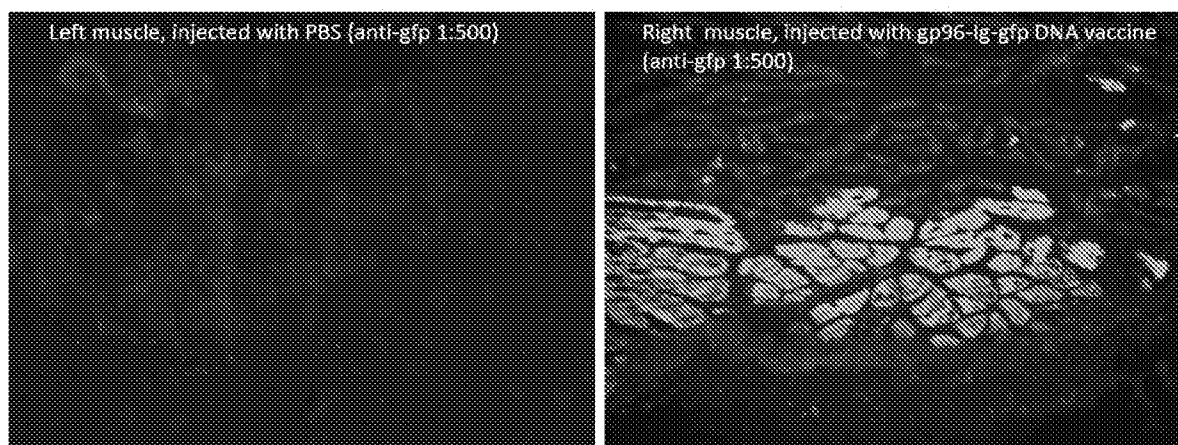
FIGURE 9

FIGURE 10

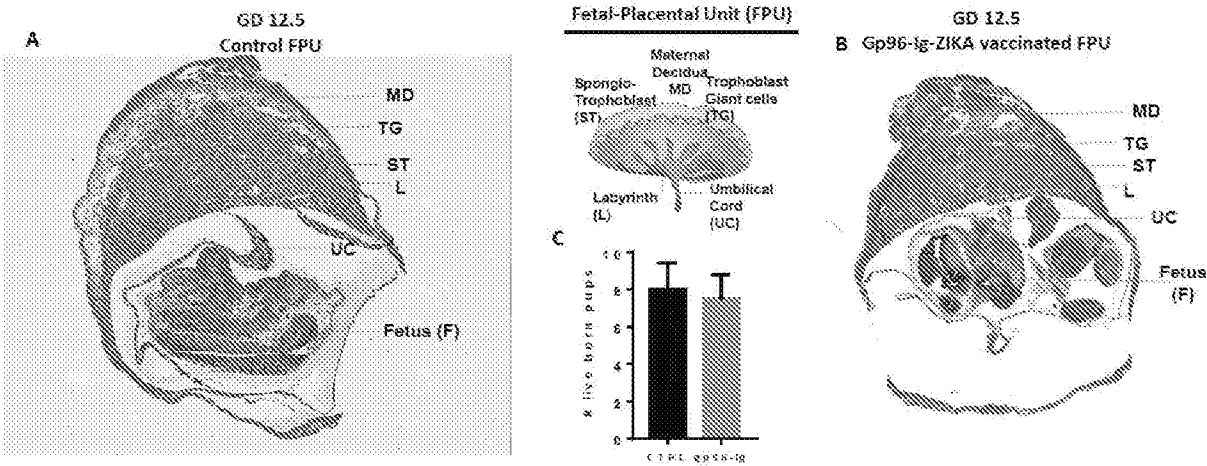


FIGURE 11

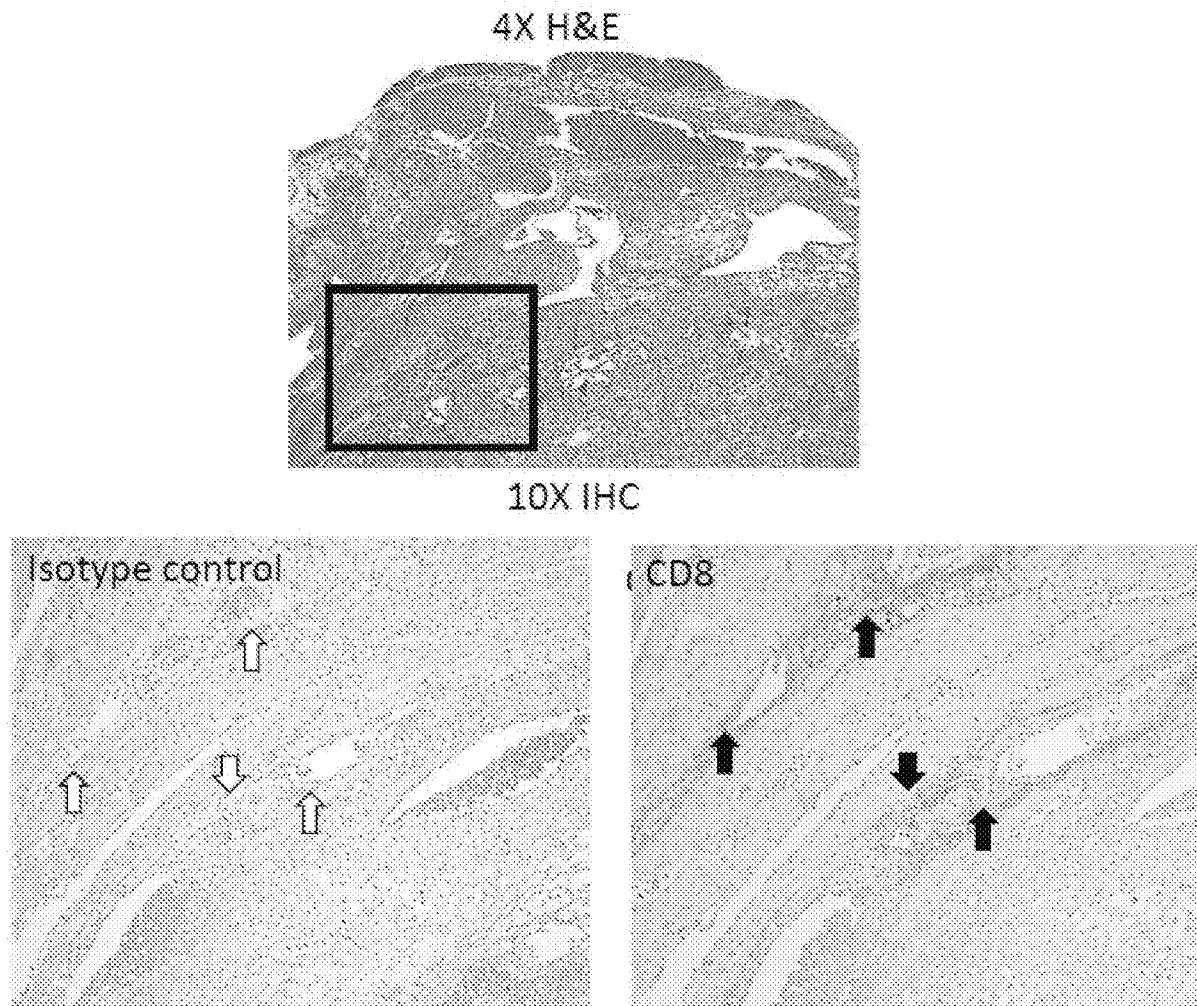


FIGURE 12

Gp96-Ig-ZIKVEnv vaccine induces antigen experienced (CD11a+) effector memory CD8 T cells in decidua

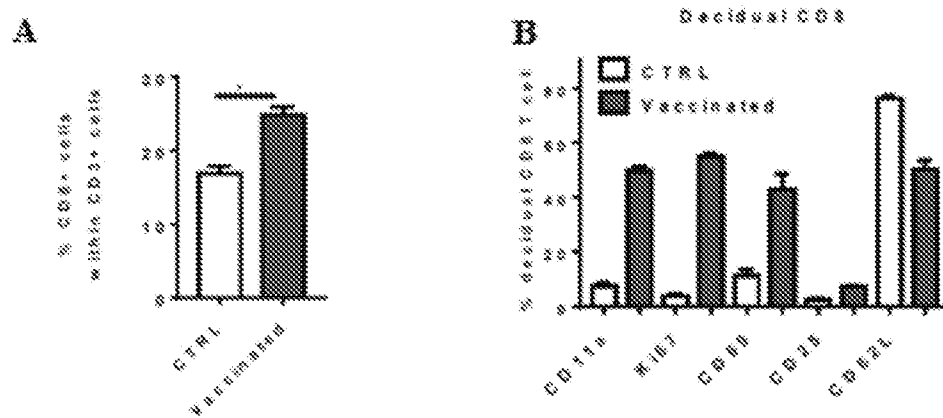
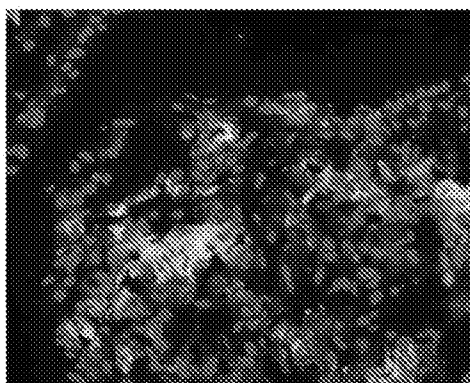


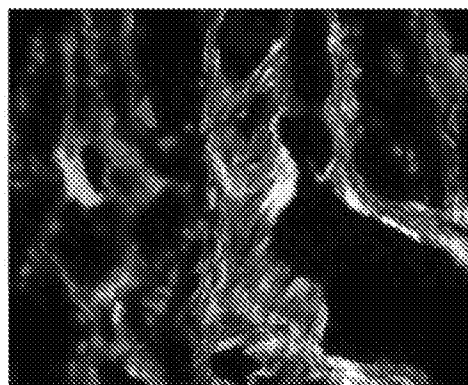
FIGURE 13

A

Control, PBS

**B**

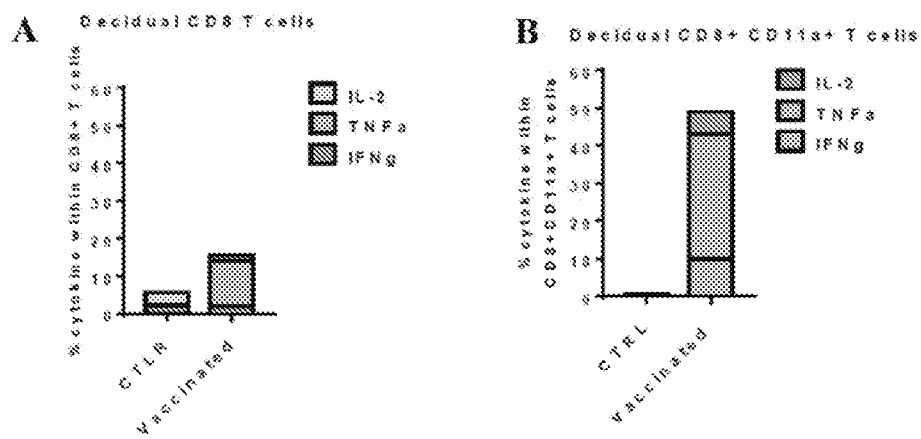
ZIKA vaccinated



CD8-FITC (green)
CD11a-PECy5 (red)
DAPI (blue)

FIGURE 14

Gp96-Ig-ZIKVEnv vaccine induced ZIKV-Env-specific CD8⁺ T lymphocyte responses, as assessed by multiparameter intracellular cytokine staining (ICS) assays



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/55912

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/US17/55912

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.: 5-54, 64
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:

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.:

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/US17/55912

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/12, C07K 14/18; C12N 7/04 (2017.01)

CPC - A61K 39/00, 39/12; C07K 14/005, 14/1825; C12N 7/00, 7/04

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.
Y	US 2014/0127261 A1 (SOUTHERN RESEARCH INSTITUTE) May 8, 2014; paragraphs [0013], [0033], [0035], [0039]; claims 23, 26	1-3, 4/2-3
Y	WO 2015/019253 A2 (INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY – ICGEB) February 12, 2015; abstract; claims 1, 11	1-3, 4/2-3
Y	US 2014/0030761 A1 (PANOUSIS, et al.) January 30, 2014; claim 19	2-3, 4/2-3
A	US 2009/0047338 A1 (SWAMY et al.) February 19, 2009; abstract; claims 1, 5	55-56
A	US 2002/0160496 A1 (GEWIRTH et al.) October 31, 2002; paragraph [0043]	55-56
A	US 2011/0312032 A1 (CHOI, et al.) December 22, 2011; paragraph [0150]	57-58, 59/57-58, 60-62, 63/61-62
A	US 2004/0009469 A1 (APT et al.) Jan 15, 2004; paragraph [0026]	57-58, 59/57-58, 60-62, 63/61-62

☐ 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

04 December 2017 (04.12.2017)

Date of mailing of the international search report

05 JAN 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
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Authorized officer

Shane Thomas

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