fails to teach or suggest that a mutation in NS1 may improve stability of the virus; fails to teach or suggest that a virus harboring the mutation may be used in the development of an effective vaccine against Zika virus; and fails to teach or suggest that such a vaccine may be effective in inducing a robust immune response and providing significant protection from Zika virus infection when used at both low and high doses. Thus, without wishing to be bound by theory, the inventors of the present disclosure have determined that the adaptation mutation in protein NS1 appeared to enhance genetic stability within the Zika virus, resulting in increased/enhanced replication efficiency. Further, the Zika strain harboring an adaptation mutation in protein NS1 was able to be passaged multiple times without developing further mutations. Such a stable Zika virus strain is advantageous as a master virus seed (MVS), or subsequent seeds derived from the MVS, for vaccine production and manufacturing, as the risk of the master virus seed developing undesirable mutations is reduced. Moreover, without wishing to be bound by theory, the adaptation mutation in protein NS1 of the Zika strain of the present disclosure may also reduce or otherwise inhibit the occurrence of undesirable mutations, such as a mutation within the envelope protein E (Env) of the Zika virus strain.

[0008a] In one aspect, the present disclosure relates to a vaccine comprising a Zika virus having a Trp to Gly mutation at position 98 (Trp98Gly) of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1, wherein the Zika virus is inactivated. In another aspect, the present disclosure relates to a use of the vaccine as described herein in the manufacture of a medicament for treating or preventing Zika virus infection in a subject in need thereof, wherein the use comprises that the medicament is to be administered to the subject. In another aspect, the present disclosure relates to a use of the vaccine as described herein in the manufacture of a medicament for inducing an immune response in a subject in need thereof, wherein the use comprises that the medicament is to be administered to the subject.

[0009] Accordingly, certain examples of the present disclosure relate to a vaccine or immunogenic composition containing one or more antigen from a Zika virus, where the Zika virus contains at least one non-human cell adaptation mutation. In some examples, the at least one non-human cell adaptation mutation is in Zika virus Non-structural protein 1 (NS1). In some examples, the at least one adaptation mutation occurs at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some examples, the at least one adaptation mutation is a Trp98Gly mutation.

[0010] In some examples that may be combined with any of the preceding examples, the at least one adaptation mutation enhances genetic stability as compared to a Zika virus lacking the at least one adaptation mutation. In some examples that may be combined with any of the preceding examples, the at least one adaptation mutation enhances viral replication as compared to a Zika virus lacking the at least one adaptation mutation. In some examples that may be combined with any of the preceding examples, the Zika virus does not comprise a mutation in Envelope protein E (Env).

[0011] In some examples that may be combined with any of the preceding examples, the non-human cell is a mammalian cell. In some examples that may be combined with any of the preceding examples, the non-human cell is a monkey cell. In some examples, the monkey cell is from a Vero cell line. In some examples, the Vero cell line is a WHO Vero 10-87 cell line.

[0012] In some examples that may be combined with any of the preceding examples, the Zika virus is an African lineage virus or an Asian lineage virus. In some examples, the Zika virus is an Asian lineage virus. In some examples, the Zika virus is from strain PRVABC59.

[0013] In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition is a purified antigen vaccine or immunogenic composition, a subunit vaccine or immunogenic composition, an inactivated whole virus vaccine or immunogenic composition, or an attenuated virus vaccine or immunogenic composition. In some examples, the vaccine or immunogenic composition is an inactivated whole virus vaccine or immunogenic composition. In some examples, the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus. In some examples, the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus comprising a mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some examples, the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some examples, the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59. In some examples, the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59 comprising the genomic sequence according to SEQ ID NO:2. In some examples, the vaccine or immunogenic composition comprises a purified inactivated whole Zika which differs from strain PRVABC59 in a Trp98Gly mutation at position 98 of SEQ ID NO: 1.

[0014] In some examples that may be combined with any of the preceding examples, the virus was chemically inactivated. In some examples, the virus was chemically inactivated with one or more of a detergent, formalin, hydrogen peroxide, beta-propiolactone (BPL), binary ethylamine (BEI), acetyl ethyleneimine, methylene blue, and psoralen. In some examples, the virus was chemically inactivated with formalin.

[0015] In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition further contains an adjuvant. In some examples, the adjuvant is selected from aluminum salts, toll-like receptor (TLR) agonists, monophosphoryl lipid A (MLA), synthetic lipid A, lipid A mimetics or analogs, MLA derivatives, cytokines, saponins, muramyl dipeptide (MDP) derivatives, CpG oligos, lipopolysaccharide (LPS) of gram-negative bacteria, polyphosphazenes, emulsions, virosomes, cochleates, poly(lactide-co-glycolides) (PLG) microparticles, poloxamer particles, microparticles, liposomes, Complete Freund's Adjuvant (CFA), and/or Incomplete Freund's Adjuvant (IFA). In some examples, the adjuvant is an aluminum salt. In some examples, the adjuvant is selected from the group consisting of alum, aluminum phosphate, aluminum hydroxide, potassium aluminum sulfate, and Alhydrogel 85. In some examples, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the one or more antigens are adsorbed to the adjuvant.

[0016] In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition is a low dose vaccine or immunogenic composition (*e.g.,* containing from about 1μg to about 5 μg antigen). In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition is a high dose vaccine or immunogenic composition (*e.g.,* containing about 10μg antigen). In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition contains from about 0.1 μg to about 25 μg of the one or more antigens. In certain such examples the antigen is a purified inactivated whole virus, such as a Zika virus with a mutation which is a tryptophan to glycine substitution at position 98 of SEQ ID NO:1 or at a position corresponding to position 98 of SEQ ID NO: 1 as described herein. In some examples, the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59. In some examples, the vaccine or immunogenic composition comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59 comprising the genomic sequence according to SEQ ID NO:2. In certain such examples the Zika virus is a plaque purified clonal Zika virus isolate. In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition contains from about 0.1 μg Zika virus or Env to about 100 μg Zika virus or Env. In some examples, the vaccine or immunogenic composition is unadjuvanted. In some examples that may be combined with any of the preceding examples, the Zika virus is a clonal isolate. In some examples, the clonal isolate is substantially free of one or more adventitious agents (*e.g.,* free of one or more adventitious agents that may be co-purified with the parental strain).

[0017] Other examples of the present disclosure relate to a vaccine comprising a Zika virus having a mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some examples, the vaccine comprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59. In some examples, the vaccine omprises a purified inactivated whole Zika virus comprising a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO:1, wherein the Zika virus is derived from strain PRVABC59 comprising the genomic sequence according to SEQ ID NO:2. In certain such examples the Zika virus is a plaque purified clonal Zika virus isolate.

[0018] Other examples of the present disclosure relate to a vaccine or immunogenic composition containing: a) an aluminum salt adjuvant; and b) a purified inactivated whole Zika virus, where the Zika virus contains a non-human cell adaptation mutation, and where the non-human cell adaptation mutation is a Trp98Gly mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1.

[0019] Other examples of the present disclosure relate to a method of treating or preventing Zika virus infection in a subject in need thereof, including administering to the subject a therapeutically effective amount of any of the vaccines or immunogenic compositions described herein.

[0020] Other examples of the present disclosure relate to a method for inducing an immune response in a subject in need thereof, including administering to the subject an immunogenic amount of any of the vaccines or immunogenic compositions described herein. In some examples that may be combined with any of the preceding examples, the subject is a human. In some examples, the subject is pregnant or intends to become pregnant.

[0021] In some examples that may be combined with any of the preceding examples, administration of the vaccine or immunogenic composition induces a protective immune response in the subject. In some examples, the protective immune response induced in the subject is greater than a protective immune response induced in a corresponding subject administered a vaccine or immunogenic composition containing one or more antigens from a Zika virus lacking the at least one non-human cell adaptation mutation. In some examples that may be combined with any of the preceding examples, administration of the vaccine or immunogenic composition induces the generation of neutralizing antibodies to Zika virus in the subject. In some examples, the concentration of neutralizing antibodies generated in the subject is higher than a concentration of neutralizing antibodies generated in a corresponding subject administered a vaccine or immunogenic composition comprising one or more antigens from a Zika virus lacking the at least one non-human cell adaptation mutation.

[0022] In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition is administered by a route selected from subcutaneous administration, transcutaneous administration, intradermal administration, subdermal administration, intramuscular administration, peroral administration, intranasal administration, buccal administration, intraperitoneal administration, intravaginal administration, anal administration and/or intracranial administration. In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition is administered one or more times. In some examples, the vaccine or immunogenic composition is administered as a first (prime) and a second (boost) administration. In some examples, the second (boost) administration is administered at least 28 days after the first (prime) administration.

[0023] Other examples of the present disclosure relate to a method for inactivating a Zika virus preparation, including (a) isolating the Zika virus preparation from one or more non-human cells, where the cells are used to produce the virus preparation, and where the Zika virus contains at least one non-human cell adaptation mutation; and (b) treating the virus preparation with an effective amount of formalin. In some examples, the method further includes (c) neutralizing the formalin-treated virus preparation with sodium metabisulfite. In some examples, the virus preparation is neutralized at least five, at least seven, at least nine, at least 11, or at least 14 days after formalin treatment. In some examples, the method further includes (d) purifying the neutralized virus preparation. In some examples, the neutralized virus preparation is purified by a process selected from cross flow filtration (CFF), multimodal chromatography, size exclusion chromatography, cation exchange chromatography, and/or anion exchange chromatography.

[0024] Other examples of the present disclosure relate to a method for inactivating a Zika virus preparation, including (a) isolating the Zika virus preparation from one or more non-human cells, wherein the

cells are used to produce the virus preparation, and wherein the Zika virus comprises a mutation at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1; and (b) treating the virus preparation with an effective amount of formalin. In some examples, the method further includes (c) neutralizing the formalin-treated virus preparation with sodium metabisulfite. In some examples, the virus preparation is neutralized at least five, at least seven, at least nine, at least 11, or at least 14 days after formalin treatment. In some examples, the method further includes (d) purifying the neutralized virus preparation. In some examples, the neutralized virus preparation is purified by process selected from cross flow filtration (CFF), multimodal chromatography, size exclusion chromatography, cation exchange chromatography, and/or anion exchange chromatography.

[0025] In some examples that may be combined with any of the preceding examples, the virus preparation is mixed with an adjuvant. In some examples, the adjuvant is selected from aluminum salts, toll-like receptor (TLR) agonists, monophosphoryl lipid A (MLA), synthetic lipid A, lipid A mimetics or analogs, MLA derivatives, cytokines, saponins, muramyl dipeptide (MDP) derivatives, CpG oligos, lipopolysaccharide (LPS) of gram-negative bacteria, polyphosphazenes, emulsions, virosomes, cochleates, poly(lactide-co-glycolides) (PLG) microparticles, poloxamer particles, microparticles, liposomes, Complete Freund's Adjuvant (CFA), and/or Incomplete Freund's Adjuvant (IFA). In some examples, the adjuvant is an aluminum salt. In some examples, the adjuvant is selected from alum, aluminum phosphate, aluminum hydroxide, potassium aluminum sulfate, and/or Alhydrogel 85. In some examples, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of one or more antigens in the virus preparation are adsorbed to the adjuvant.

[0026] In some examples that may be combined with any of the preceding examples, the at least one non-human cell adaptation mutation is in Zika virus NS1. In some examples, the at least one adaptation mutation occurs at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some examples, the at least one adaptation mutation is a Trp98Gly mutation. In some examples that may be combined with any of the preceding examples, the at least one adaptation mutation enhances genetic stability as compared to a Zika virus lacking the at least one adaptation mutation. In some examples that may be combined with any of the preceding examples, the at least one adaptation mutation enhances viral replication as compared to a Zika virus lacking the at least one adaptation mutation. In some examples that may be combined with any of the preceding examples, the Zika virus does not comprise a mutation in Envelope protein E (Env).

[0027] Other examples of the present disclosure relate to a method of purifying Zika virus, including the steps: (a) inoculating a plurality of cells with an inoculum containing a population of Zika viruses, and (b) obtaining a Zika virus clonal isolate by plaque purification from one or more of the inoculated cells. In some examples, the cells are non-human cells. In some examples, the cells are insect cells. In some examples, the cells are mosquito cells. In some examples, the cells are mammalian cells. In some examples, the mammalian cells are monkey cells. In come examples, the monkey cells are from a Vero cell line. In some examples, the Vero cell line is a WHO Vero 10-87 cell line.

[0028] In some examples that may be combined with any of the preceding examples, the population of Zika viruses is heterogeneous. In some examples that may be combined with any of the preceding examples, the population of Zika viruses comprises a Zika virus clinical isolate. In some examples, the Zika virus clinical isolate is from strain PRVABC59. In some examples that may be combined with any of the preceding examples, the population of Zika viruses comprises a Zika virus that has been previously passaged one or more times in cell culture. In some examples that may be combined with any of the preceding examples, the inoculum comprises human serum. In some examples that may be combined with any of the preceding examples, the inoculum comprises one or more adventitious agents. In some examples, the Zika virus clonal isolate is substantially free of the one or more adventitious agents.

[0029] In some examples that may be combined with any of the preceding examples, the methods further include one or more additional plaque purifications of the Zika virus clonal isolate. In some examples, the Zika virus clonal isolate is further plaque purified two or more times. In some examples that may be combined with any of the preceding examples, the methods further include passaging the Zika virus clonal isolate one or more times in cell culture. In some examples, the Zika virus clonal isolate is passaged two or more times.

[0030] In some examples that may be combined with any of the preceding examples, the methods further include formulating a vaccine or immunogenic composition comprising one or more antigens from the Zika virus clonal isolate. In some examples, the vaccine or immunogenic composition is a purified antigen vaccine or immunogenic composition, a subunit vaccine or immunogenic composition, an inactivated whole virus vaccine or immunogenic composition, or an attenuated virus vaccine or immunogenic composition. In some examples, the vaccine or immunogenic composition is a purified inactivated whole virus vaccine or immunogenic composition. In some examples, the Zika virus clonal isolate was chemically inactivated. In some examples, the Zika virus clonal isolate was chemically inactivated with one or more of a detergent, formalin, hydrogen peroxide, beta-propiolactone (BPL), binary ethylamine (BEI), acetyl ethyleneimine, methylene blue, and psoralen. In some examples, the Zika virus clonal isolate was chemically inactivated with formalin.

[0031] In some examples that may be combined with any of the preceding examples, the methods further include admixing the vaccine or immunogenic composition with an adjuvant. In some examples, the adjuvant is selected from aluminum salts, toll-like receptor (TLR) agonists, monophosphoryl lipid A (MLA), synthetic lipid A, lipid A mimetics or analogs, MLA derivatives, cytokines, saponins, muramyl dipeptide (MDP) derivatives, CpG oligos, lipopolysaccharide (LPS) of gram-negative bacteria, polyphosphazenes, emulsions, virosomes, cochleates, poly(lactide-co-glycolides) (PLG) microparticles, poloxamer particles, microparticles, liposomes, Complete Freund's Adjuvant (CFA), and Incomplete Freund's Adjuvant (IFA). In some examples, the adjuvant is an aluminum salt. In some examples, the adjuvant is selected from alum, aluminum phosphate, aluminum hydroxide, potassium aluminum sulfate, and Alhydrogel 85. In some examples, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the one or more antigens are adsorbed to the adjuvant. In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition comprises from

about 0.1 μg Env to about 100 μg Env. In certain such examples the Zika virus is a plaque purified clonal Zika virus isolate. In some examples, the vaccine or immunogenic composition is unadjuvanted.

[0032] In some examples that may be combined with any of the preceding examples, the Zika virus clonal isolate is a homogenous genetic population. In some examples, the Zika virus clonal isolate does not contain a mutation in Envelope protein E (Env). In some examples, the Zika virus clonal isolate contains at least one mutation. In some examples, the at least one mutation is in Zika virus Non-structural protein 1 (NS1). In some examples, the at least one mutation occurs at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some examples, the at least one mutation is a Trp98Gly mutation. In some examples the at least one mutation is not in Envelope protein E (Env). In some examples, the at least one mutation enhances genetic stability as compared to a Zika virus lacking the at least one mutation. In some examples, the at least one mutation enhances viral replication as compared to a Zika virus lacking the at least one mutation.

[0033] Other examples of the present disclosure relate to a method of purifying Zika virus for the preparation of a vaccine or immunogenic composition, including one or more (*e.g.,* one or more, two or more, three or more, four or more, five or more, or all six) steps selected from: (a) passing a sample containing a Zika virus through a depth filter to produce an eluate, where the eluate contains the Zika virus; (b) buffer exchanging and/or diluting a sample containing a Zika virus by cross flow filtration (CFF) to produce a retentate, where the retentate contains the Zika virus; (c) binding a sample containing a Zika virus to an ion exchange membrane to produce a bound fraction, where the bound fraction contains the Zika virus, and eluting the bound fraction from the ion exchange membrane; (d) treating a sample containing a Zika virus with an effective amount of a chemical inactivator; (e) neutralizing a sample containing a chemically inactivated Zika virus with sodium metabisulfite; and/or (f) purifying a naturalized sample containing a chemically inactivated Zika virus by cross flow filtration (CFF). In some examples, the method includes all of the steps of: (a) passing a sample containing a Zika virus through a depth filter to produce an eluate, where the eluate contains the Zika virus; (b) buffer exchanging and/or diluting a sample containing a Zika virus by cross flow filtration (CFF) to produce a retentate, where the retentate contains the Zika virus; (c) binding a sample containing a Zika virus to an ion exchange membrane to produce a bound fraction, where the bound fraction contains the Zika virus, and eluting the bound fraction from the ion exchange membrane; (d) treating a sample containing a Zika virus with an effective amount of a chemical inactivator; (e) neutralizing a sample containing a chemically inactivated Zika virus with sodium metabisulfite; and/or (f) purifying a naturalized sample containing a chemically inactivated Zika virus by cross flow filtration (CFF), in any order. In some examples, the method includes the steps of: (a) passing a sample containing a Zika virus through a first depth filter to produce a first eluate, where the first eluate containing the Zika virus; (b) buffer exchanging and/or diluting the first eluate by cross flow filtration (CFF) to produce a first retentate, where the first retentate contains the Zika virus; (c) binding the first retentate to an ion exchange membrane to produce a first bound fraction, where the first bound fraction contains the Zika virus, and eluting the first bound fraction from the ion exchange membrane to produce a second eluate, where the second eluate contains the Zika virus; (d) passing the second eluate through a second depth filter to produce a second retentate, where the second retentate contains the Zika virus; (e) treating the second retentate with an effective amount of a chemical

inactivator; (f) neutralizing the treated second retentate with sodium metabisulfite; and (g) purifying the neutralized second retentate by cross flow filtration (CFF). In some examples that may be combined with any of the preceding examples, the ion exchange membrane is an anion exchange membrane. In some examples that may be combined with any of the preceding examples, the anion exchange membrane comprises quaternary ammonium ligands.

[0034] In some examples that may be combined with any of the preceding examples, the bound fraction of step (c) is eluted in multiple steps, where each step includes an increasing salt concentration. In some examples that may be combined with any of the preceding examples, the salt is sodium chloride. In some examples that may be combined with any of the preceding examples, the salt concentration increases from 250 mM to 750 mM. In some examples that may be combined with any of the preceding examples, the chemical inactivator is one or more of a detergent, formalin, hydrogen peroxide, beta-propiolactone (BPL), binary ethylamine (BEI), acetyl ethyleneimine, methylene blue, and psoralen. In some examples that may be combined with any of the preceding examples, the chemical inactivator is formalin. In some examples that may be combined with any of the preceding examples, the neutralization occurs for at least five, at least seven, at least nine, at least 11, or at least 14 days. In some examples that may be combined with any of the preceding examples, the Zika virus is a Zika virus clonal isolate produced by any of the methods described herein.

[0035] Other examples of the present disclosure relate to a vaccine or immunogenic composition containing one or more antigens from a plaque purified clonal Zika virus isolate. In some examples, the plaque purified clonal Zika virus isolate was plaque purified from cells contacted with an inoculum comprising a population of Zika viruses. In some examples, the cells are non-human cells. In some examples, the cells are insect cells. In some examples, the insect cells are mosquito cells. In some examples, the cells are mammalian cells. In some examples, the mammalian cells are monkey cells. In some examples, the monkey cells are from a Vero cell line. In some examples, the Vero cell line is a WHO Vero 10-87 cell line.

[0036] In some examples that may be combined with any of the preceding examples, the population of Zika viruses was heterogeneous. In some examples that may be combined with any of the preceding examples, the population of Zika viruses comprised a Zika virus clinical isolate. In some examples, the Zika virus clinical isolate is from strain PRVABC59. In some examples that may be combined with any of the preceding examples, the population of Zika viruses comprised a Zika virus that had been previously passaged one or more times in cell culture. In some examples that may be combined with any of the preceding examples, the inoculum comprised human serum. In some examples that may be combined with any of the preceding examples, the inoculum comprised one or more adventitious agents. In some examples, the plaque purified clonal Zika virus isolate is substantially free of the one or more adventitious agents.

[0037] In some examples that may be combined with any of the preceding examples, the plaque purified clonal Zika virus isolate is modified as compared to a wild-type Zika virus. In some examples that may be combined with any of the preceding examples, the plaque purified clonal Zika virus isolate is a homogenous genetic population. In some examples that may be combined with any of the preceding

examples, the plaque purified clonal Zika virus isolate does not include a mutation in Envelope protein E (Env). In some examples that may be combined with any of the preceding examples, the plaque purified clonal Zika virus isolate comprises at least one mutation. In some examples, the at least one mutation is in Zika virus Non-structural protein 1 (NS1). In some examples, the at least one mutation occurs at position 98 of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1. In some examples, the at least one mutation is a Trp98Gly mutation. In some examples, the at least one mutation is not in Zika virus Envelope protein E (Env). In some examples, the at least one mutation enhances genetic stability as compared to a Zika virus lacking the at least one mutation. In some examples, the at least one mutation enhances viral replication as compared to a Zika virus lacking the at least one mutation. In some examples that may be combined with any of the preceding examples, the plaque purified clonal Zika virus isolate is an African lineage virus or an Asian lineage virus. In some examples, the plaque purified clonal Zika virus isolate is an Asian lineage virus.

[0038] In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition is a purified antigen vaccine or immunogenic composition, a subunit vaccine or immunogenic composition, an inactivated whole virus vaccine or immunogenic composition, or an attenuated virus vaccine or immunogenic composition. In some examples, the vaccine or immunogenic composition is an inactivated whole virus vaccine or immunogenic composition. In some examples that may be combined with any of the preceding examples, the plaque purified clonal Zika virus isolate was chemically inactivated. In some examples, the plaque purified Zika virus was chemically inactivated with one or more of a detergent, formalin, hydrogen peroxide, beta-propiolactone (BPL), binary ethylamine (BEI), acetyl ethyleneimine, methylene blue, and psoralen. In some examples, the plaque purified clonal Zika virus isolate was chemically inactivated with formalin.

[0039] In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition further comprises an adjuvant. In some examples, the adjuvant is selected from aluminum salts, toll-like receptor (TLR) agonists, monophosphoryl lipid A (MLA), synthetic lipid A, lipid A mimetics or analogs, MLA derivatives, cytokines, saponins, muramyl dipeptide (MDP) derivatives, CpG oligos, lipopolysaccharide (LPS) of gram-negative bacteria, polyphosphazenes, emulsions, virosomes, cochleates, poly(lactide-co-glycolides) (PLG) microparticles, poloxamer particles, microparticles, liposomes, Complete Freund's Adjuvant (CFA), and Incomplete Freund's Adjuvant (IFA). In some examples, the adjuvant is an aluminum salt. In some examples, the adjuvant is selected from alum, aluminum phosphate, aluminum hydroxide, potassium aluminum sulfate, and Alhydrogel 85. In some examples, at least 75%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% of the one or more antigens are adsorbed to the adjuvant. In some examples that may be combined with any of the preceding examples, the vaccine or immunogenic composition contains from about 0.1 μg Env to about 100 μg Env. In certain such examples the Zika virus is a plaque purified clonal Zika virus isolate. In some examples, the vaccine or immunogenic composition is unadjuvanted.

[0040] It is to be understood that one, some, or all of the properties of the various examples described above and herein may be combined to form other examples of the present disclosure. These and other

examples of the present disclosure will become apparent to one of skill in the art. These and other examples of the present disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] **FIG. 1** shows bright field microscopy images of Vero cell monolayers mock infected (top) or infected with ZIKAV strain PRVABC59 (bottom).

[0042] **FIG. 2** shows growth kinetics of ZIKAV PRVABC59 P1 on Vero cell monolayers, as determined by $TCID_{50}$.

[0043] **FIG. 3** shows potency assay testing ($TCID_{50}$) of Zika virus PRVABC59 P5 clones a-f.

[0044] **FIG. 4** shows bright-field microscopy images depicting the cytopathic effect (CPE) of growth of Zika virus PRVABC59 P6 clones a-f on Vero cell monolayers.

[0045] **FIG. 5** shows potency assay testing ($TCID_{50}$) of Zika virus PRVABC59 P6 clones a-f

[0046] **FIG. 6** shows an amino acid sequence alignment comparing the envelope glycoprotein sequence of Zika virus near residue 330 from Zika virus strains PRVABC59 P6e (SEQ ID NO: 8) and PRVABC59 (SEQ ID NO: 9) with several other flaviviruses (WNV (SEQ ID NO: 10); JEV (SEQ ID NO: 11); SLEV (SEQ ID NO: 12); YFV (SEQ ID NO: 13); DENV 1 16007 (SEQ ID NO: 14); DENV 2 16681 (SEQ ID NO: 15); DENV 3 16562 (SEQ IDNO: 16); and DENV 4 1036 (SEQ ID NO: 17)).

[0047] **FIG. 7** shows an amino acid sequence alignment comparing the NS1 protein sequence of Zika virus near residue 98 from Zika virus strains PRVABC59 P6e (SEQ ID NO: 18) and PRVABC59 (SEQ ID NO: 19) with several other flaviviruses (WNV (SEQ ID NO: 20); JEV (SEQ ID NO: 21); SLEV (SEQ ID NO: 22); YFV (SEQ ID NO: 23); DENV 1 16007 (SEQ ID NO: 24); DENV 2 16681 (SEQ ID NO: 25); DENV 3 16562 (SEQ IDNO: 26); and DENV 4 1036 (SEQ ID NO: 27)).

[0048] **FIG. 8** shows the plaque phenotype of ZIKAV PRVABC59 P6 virus clones a-f compared to ZIKAV PRVABC59 P1 virus.

[0049] **FIG. 9** shows the mean plaque size of ZIKAV PRVABC59 P6 virus clones compared to ZIKAV PRVABC59 P1 virus.

[0050] **FIG. 10** shows the growth kinetics of ZIKAV PRVABC59 P6 clones a-f in Vero cells under serum-free growth conditions.

[0051] **FIG. 11** shows a schematic of the steps taken to prepare PRVABC59 P6b and P6e formulated drug product for the immunization experiments.

CLAIMS

What is claimed is:

1. A vaccine comprising a Zika virus having a Trp to Gly mutation at position 98 (Trp98Gly) of SEQ ID NO: 1, or at a position corresponding to position 98 of SEQ ID NO: 1, wherein the Zika virus is inactivated.

2. The vaccine of claim 1, the Zika virus comprising an envelope protein having an amino acid sequence that has 100% sequence identity with an amino acid sequence encoded by the corresponding sequence in SEQ ID NO: 2.

3. The vaccine of claim 1, wherein the sequence encoding the envelope protein is the same as the corresponding sequence in SEQ ID NO: 2.

4. The vaccine of any one of claims 1 to 3, wherein the Zika virus is derived from strain PRVABC59.

5. The vaccine of claim 4, wherein strain PRVABC59 comprises the genomic sequence according to SEQ ID NO: 2.

6. The vaccine of any one of claims 1 to 5, further comprising an adjuvant.

7. The vaccine of claim 6, wherein the adjuvant is selected from the group consisting of aluminum salts, toll-like receptor (TLR) agonists, monophosphoryl lipid A (MLA), synthetic lipid A, lipid A mimetics or analogs, MLA derivatives, cytokines, saponins, muramyl dipeptide (MDP) derivatives, CpG oligos, lipopolysaccharide (LPS) of gram-negative bacteria, polyphosphazenes, emulsions, virosomes, cochleates, poly(lactide-co-glycolides) (PLG) microparticles, poloxamer particles, microparticles, liposomes, Complete Freund’s Adjuvant (CFA), and Incomplete Freund’s Adjuvant (IFA).

8. The vaccine of claim 6 or 7, wherein the adjuvant is an aluminum salt, optionally wherein the adjuvant is selected from the group consisting of alum, aluminum phosphate, aluminum hydroxide, potassium aluminum sulfate, and Alhydrogel 85.

9. Use of the vaccine of any one of claims 1-8 in the manufacture of a medicament for treating or preventing Zika virus infection in a subject in need thereof, wherein the use comprises that the medicament is to be administered to the subject.

10. Use of the vaccine of any one of claims 1-8 in the manufacture of a medicament for inducing an immune response in a subject in need thereof, wherein the use comprises that the medicament is to be administered to the subject.

11. The use of claim 10, wherein the immune response is a protective immune response.

12. The use of any one of claims 9-11, wherein the subject is a human.

13. The use of any one of claims 9-12, wherein administration of the medicament induces the generation of neutralizing antibodies to Zika virus in the subject.

14. The use of any one of claims 9-13, wherein the use comprises that the medicament is to be administered by a route selected from the group consisting of subcutaneous administration, transcutaneous administration, intradermal administration, subdermal administration, intramuscular administration, peroral administration, intranasal administration, buccal administration, intraperitoneal administration, intravaginal administration, anal administration and intracranial administration.

15. The use of any one of claims 9-14, wherein the use comprises that the medicament is to be administered as a first (prime) and a second (boost) administration, optionally wherein the first (prime) and second (boost) administration are administered at least 1 week apart, optionally wherein the second (boost) administration is administered from 25-30 days, optionally 28 days, after the first (prime) administration.