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(54) **ZIKA VIRAL ANTIGEN CONSTRUCTS**

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(73) Assignees: **GLAXOSMITHKLINE BIOLOGICALS SA**, Rixensart (BE); **THE U.S.A., AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES**, Bethesda, MD (US)

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(60) Provisional application No. 62/568,559, filed on Oct. 5, 2017, provisional application No. 62/485,090, filed on Apr. 13, 2017, provisional application No. 62/423,398, filed on Nov. 17, 2016.

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C12N 7/00	(2006.01)

(52) **U.S. Cl.**
CPC ***C07K 14/005*** (2013.01); ***A61K 39/12*** (2013.01); ***C12N 7/00*** (2013.01); *A61K 2039/53* (2013.01)

(57) **ABSTRACT**

Compounds useful as components of immunogenic compositions for the induction of an immunogenic response in a subject against viral infection, methods for their use in treatment, and processes for their manufacture are provided herein. The compounds comprise a nucleic acid construct comprising a sequence which encodes a Zika virus antigen.

Specification includes a Sequence Listing.

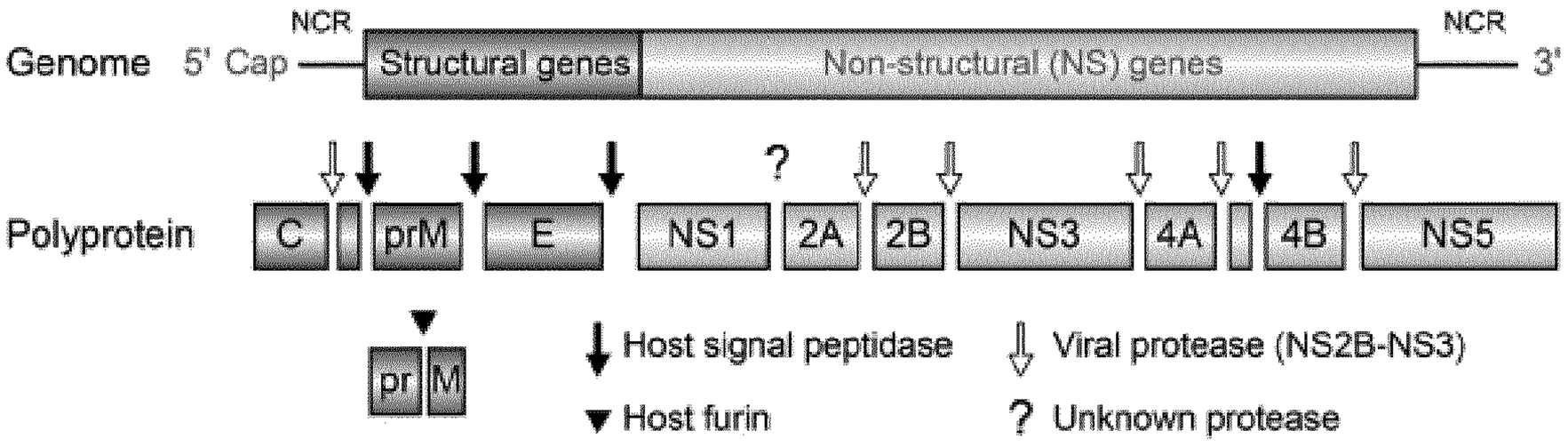


FIG.1

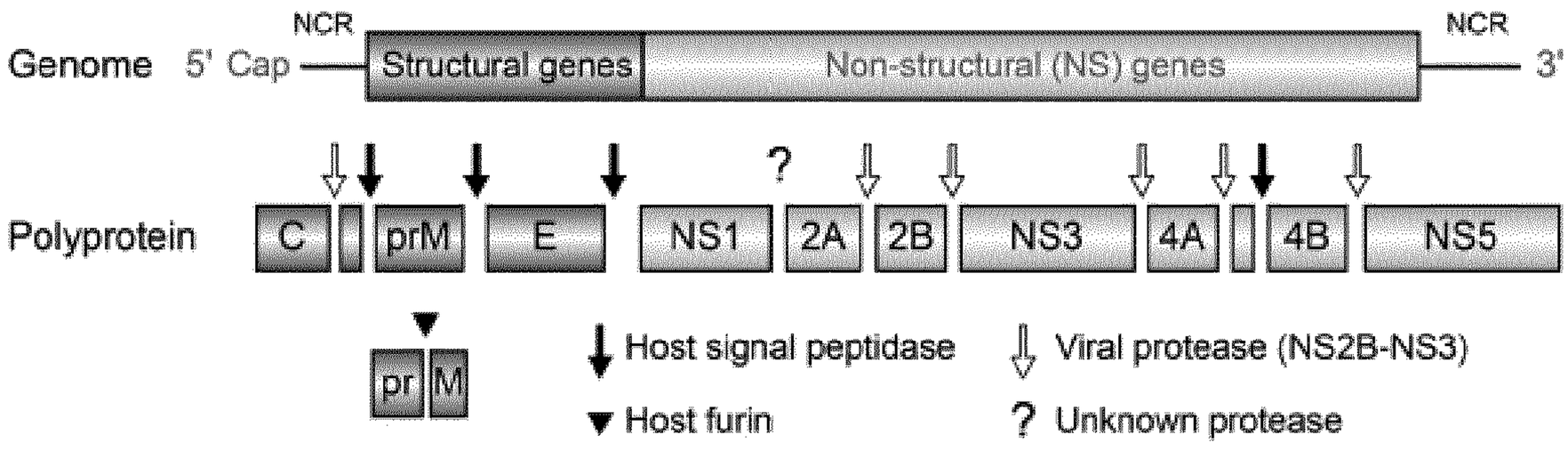

Genome
5' Cap
NCR
Structural genes
Non-structural (NS) genes
NCR
3'
Polyprotein
C
prM
E
NS1
2A
2B
NS3
4A
4B
NS5
?
pr
M
Host signal peptidase
Host furin
Viral protease (NS2B-NS3)
? Unknown protease

FIG.2

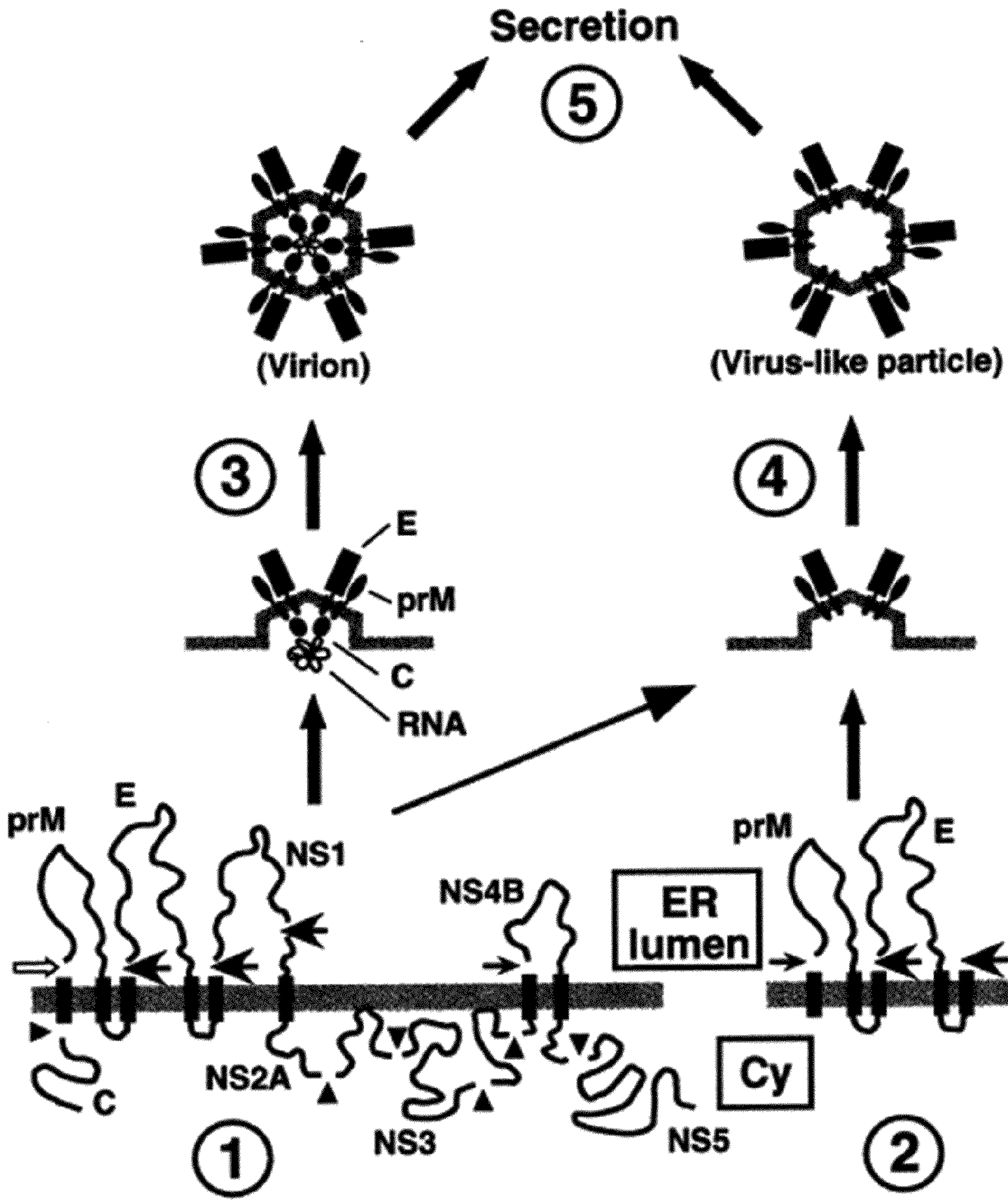
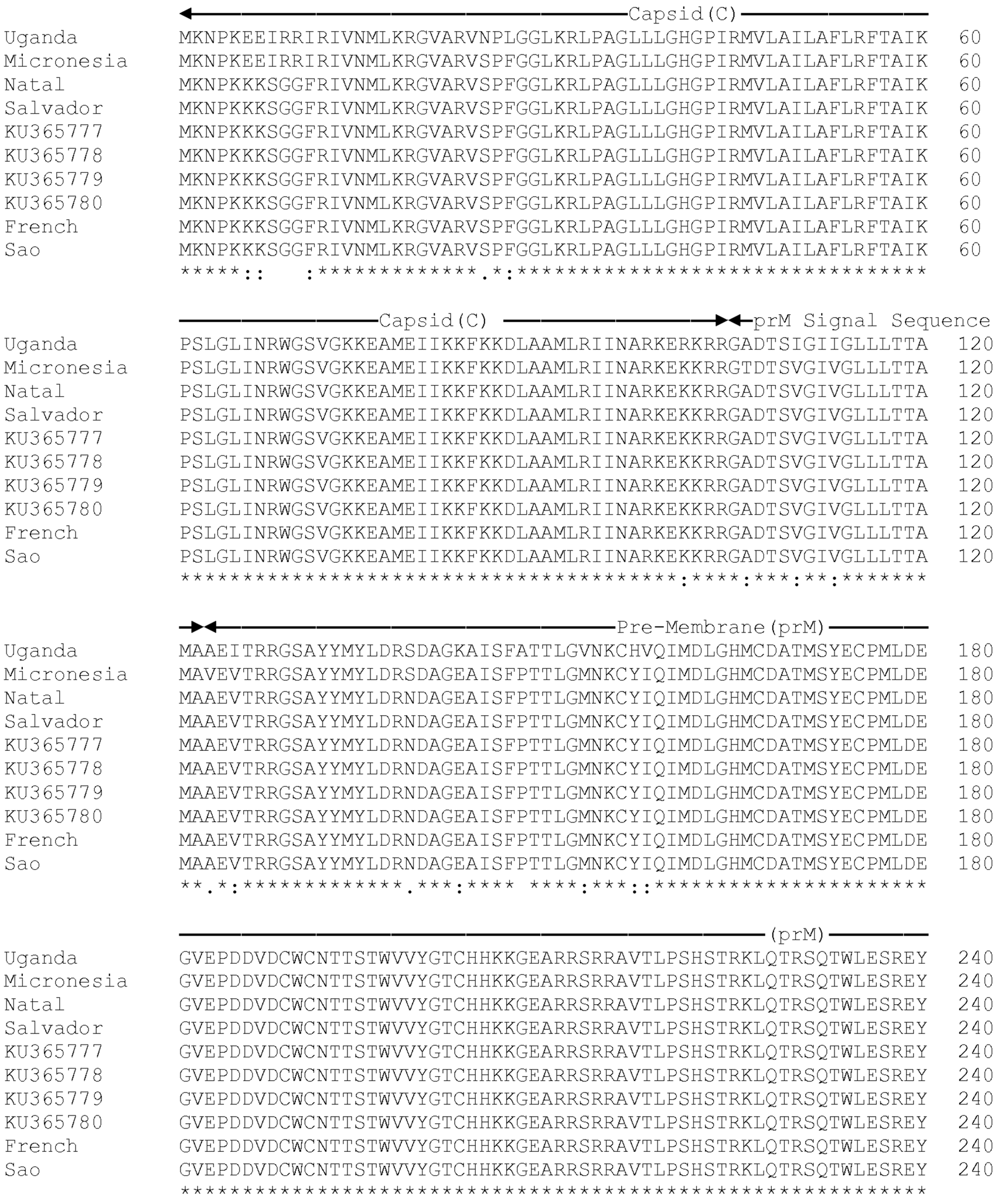

Secretion
5
(Virion)
(Virus-like particle)
3
4
E
prM
C
RNA
prM
E
NS1
NS4B
ER lumen
prM
E
NS2A
NS3
NS5
Cy
C
1
2

FIG.3A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Natal MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
Salvador MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
KU365777 MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
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French MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
Sao MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK 60
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Micronesia PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGTDTSVGIVGLLLTTA 120
Natal PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
Salvador PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
KU365777 PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
KU365778 PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
KU365779 PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
KU365780 PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
French PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
Sao PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA 120
Pre-Membrane(prM)
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Micronesia MAVEVTRRGSAYYMYLDRSDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
Natal MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
Salvador MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
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KU365779 MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
KU365780 MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
French MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
Sao MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE 180
(prM)
Uganda GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
Micronesia GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
Natal GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
Salvador GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
KU365777 GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
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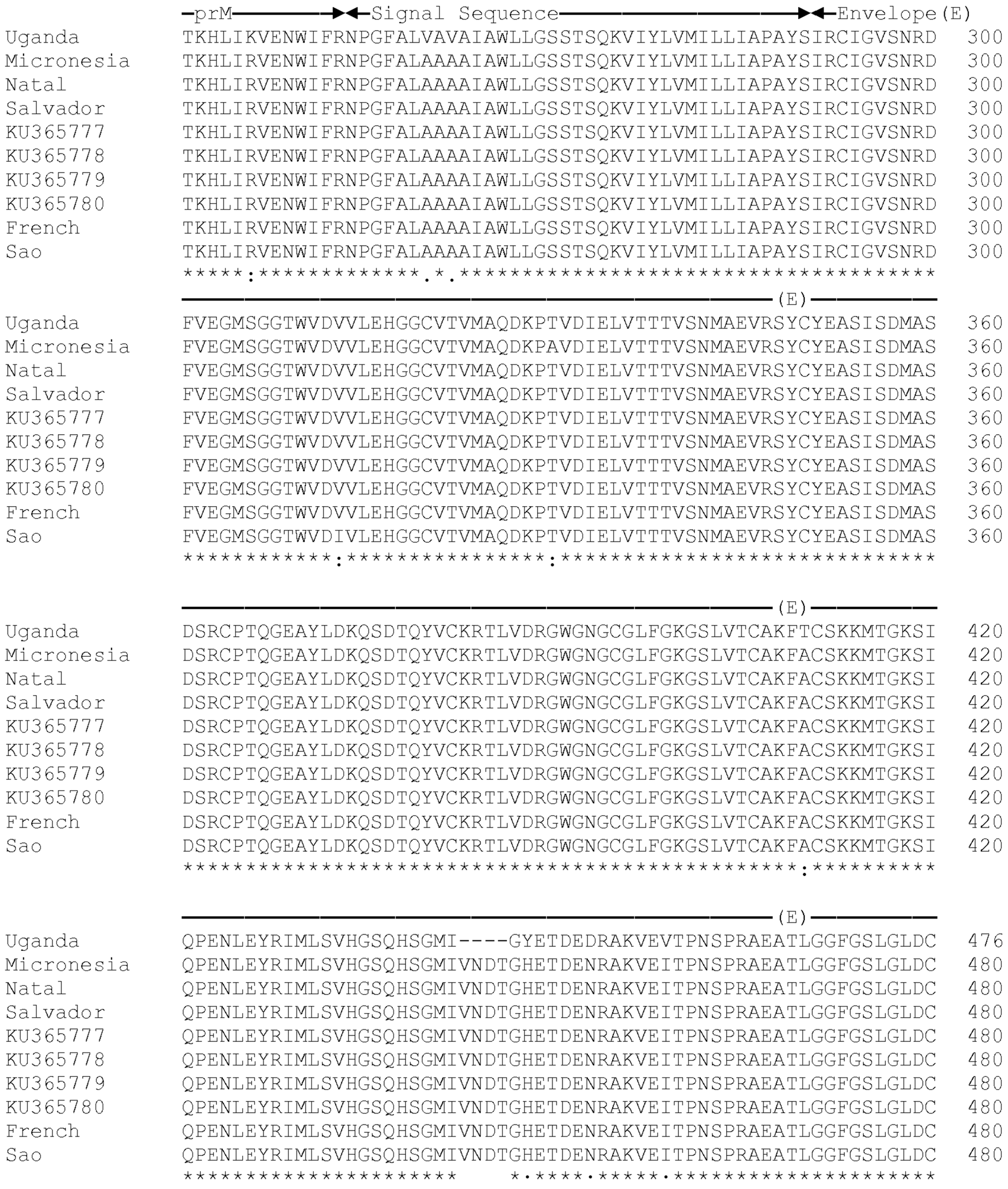
KU365780 GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
French GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240
Sao GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY 240

FIG.3B


prM
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Micronesia TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD 300
Natal TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD 300
Salvador TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD 300
KU365777 TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD 300
KU365778 TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD 300
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KU365780 TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD 300
French TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD 300
Sao TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD 300
(E)
Uganda FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
Micronesia FVEGMSGGTWVDVVLEHGGCVTVMAQDKPAVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
Natal FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
Salvador FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
KU365777 FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
KU365778 FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
KU365779 FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
KU365780 FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
French FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
Sao FVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS 360
(E)
Uganda DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFTCSKKMTGKSI 420
Micronesia DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI 420
Natal DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI 420
Salvador DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI 420
KU365777 DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI 420
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KU365780 DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI 420
French DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI 420
Sao DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI 420
(E)
Uganda QPENLEYRIMLSVHGSQHSGMI----GYETDEDRAKVEVTPNSPRAEATLGGFGSLGLDC 476
Micronesia QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC 480
Natal QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC 480
Salvador QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC 480
KU365777 QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC 480
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KU365779 QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC 480
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French QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC 480
Sao QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC 480

FIG.3C

```
                                                                (E)
Uganda          EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA  536
Micronesia      EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA  540
Natal           EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA  540
Salvador        EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA  540
KU365777        EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA  540
KU365778        EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA  540
KU365779        EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA  540
KU365780        EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA  540
French          EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA  540
Sao             EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA  540
                ************************************************************

                                                                (E)
Uganda          KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLFSGHLKCRLKMDKLRLKGVSYSLCTA  596
Micronesia      KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA  600
Natal           KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA  600
Salvador        KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA  600
KU365777        KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA  600
KU365778        KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA  600
KU365779        KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA  600
KU365780        KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA  600
French          KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA  600
Sao             KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA  600
                ********************************* ************************

                                                                (E)
Uganda          AFTFTKVPAETLHGTVTVEVQYAGTDGPCKIPVQMAVDMQTLTPVGRLITANPVITESTE  656
Micronesia      AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE  660
Natal           AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE  660
Salvador        AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE  660
KU365777        AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE  660
KU365778        AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE  660
KU365779        AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE  660
KU365780        AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE  660
French          AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE  660
Sao             AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE  660
                ******:************************:*.*****************************

                                                                (E)
Uganda          NSKMMLELDPPFGDSYIVIGVGDKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD  716
Micronesia      NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD  720
Natal           NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD  720
Salvador        NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD  720
KU365777        NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD  720
KU365778        NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD  720
KU365779        NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD  720
KU365780        NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD  720
French          NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD  720
Sao             NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD  720
                **********************.*************************************
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FIG.3D

```
                                                                  (E)
Uganda          FGSVGGVFNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLA  776
Micronesia      FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLVWLGLNTKNGSISLTCLA  780
Natal           FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA  780
Salvador        FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA  780
KU365777        FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA  780
KU365778        FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA  780
KU365779        FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA  780
KU365780        FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA  780
French          FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA  780
Sao             FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA  780
                ******.:**********************************:************* ***

                ——(E)——►
Uganda          LGGVMIFLSTAVSAD    791
Micronesia      LGGVLIFLSTAVSAD    795
Natal           LGGVLIFLSTAVSAD    795
Salvador        LGGVLIFLSTAVSAD    795
KU365777        LGGVLIFLSTAVSAD    795
KU365778        LGGVLIFLSTAVSAD    795
KU365779        LGGVLIFLSTAVSAD    795
KU365780        LGGVLIFLSTAVSAD    795
French          LGGVLIFLSTAVSAD    795
Sao             LGGVLIFLSTAVSAD    795
                ****.**********
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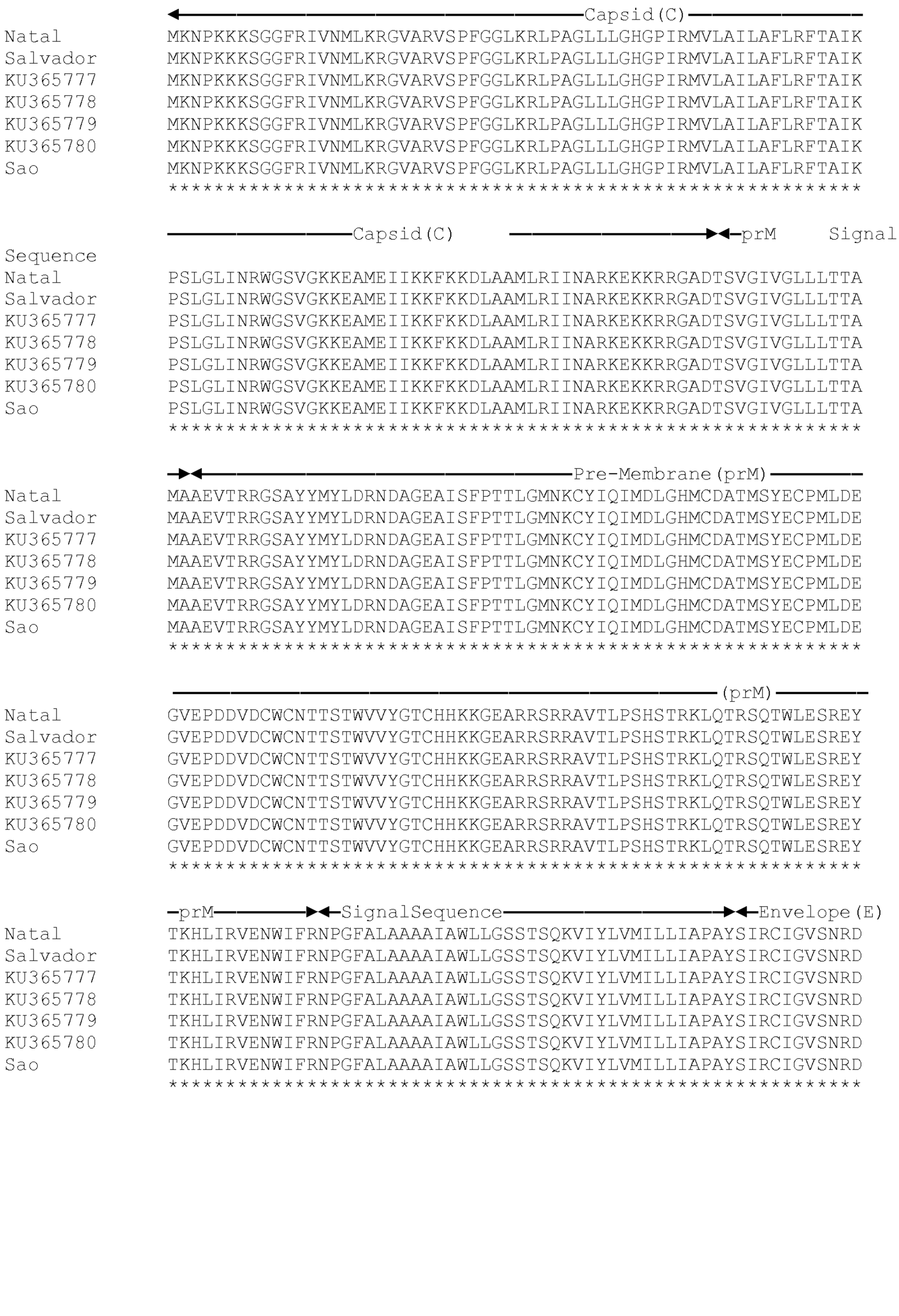
FIG.4A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KU365777 MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK
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KU365780 MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK
Sao MKNPKKKSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVLAILAFLRFTAIK
Capsid(C) prM Signal
Sequence
Natal PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA
Salvador PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA
KU365777 PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA
KU365778 PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA
KU365779 PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA
KU365780 PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA
Sao PSLGLINRWGSVGKKEAMEIIKKFKKDLAAMLRIINARKEKKRRGADTSVGIVGLLLTTA
Pre-Membrane(prM)
Natal MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE
Salvador MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE
KU365777 MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE
KU365778 MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE
KU365779 MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE
KU365780 MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE
Sao MAAEVTRRGSAYYMYLDRNDAGEAISFPTTLGMNKCYIQIMDLGHMCDATMSYECPMLDE
(prM)
Natal GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
Salvador GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
KU365777 GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
KU365778 GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
KU365779 GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
KU365780 GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
Sao GVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRRAVTLPSHSTRKLQTRSQTWLESREY
prM SignalSequence Envelope(E)
Natal TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD
Salvador TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD
KU365777 TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD
KU365778 TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD
KU365779 TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD
KU365780 TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD
Sao TKHLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMILLIAPAYSIRCIGVSNRD

FIG.4B

```
                       ------------------------------------------------------(E)-----------
Natal         FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS
Salvador      FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS
KU365777      FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS
KU365778      FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS
KU365779      FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS
KU365780      FVEGMSGGTWVDVVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS
Sao           FVEGMSGGTWVDIVLEHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMAS
              ************:***********************************************

                       ------------------------------------------------------(E)-----------
Natal         DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI
Salvador      DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI
KU365777      DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI
KU365778      DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI
KU365779      DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI
KU365780      DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI
Sao           DSRCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNGCGLFGKGSLVTCAKFACSKKMTGKSI
              ************************************************************

                       ------------------------------------------------------(E)-----------
Natal         QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC
Salvador      QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC
KU365777      QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC
KU365778      QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC
KU365779      QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC
KU365780      QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC
Sao           QPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDC
              ************************************************************

                       ------------------------------------------------------(E)-----------
Natal         EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA
Salvador      EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA
KU365777      EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA
KU365778      EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA
KU365779      EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA
KU365780      EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA
Sao           EPRTGLDFSDLYYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWNNKEALVEFKDAHA
              ************************************************************

                       ------------------------------------------------------(E)-----------
Natal         KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA
Salvador      KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA
KU365777      KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA
KU365778      KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA
KU365779      KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA
KU365780      KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA
Sao           KRQTVVVLGSQEGAVHTALAGALEAEMDGAKGRLSSGHLKCRLKMDKLRLKGVSYSLCTA
              ************************************************************
```

FIG.4C


(E)
Natal AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE
Salvador AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE
KU365777 AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE
KU365778 AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE
KU365779 AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE
KU365780 AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE
Sao AFTFTKIPAETLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTE
(E)
Natal NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
Salvador NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
KU365777 NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
KU365778 NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
KU365779 NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
KU365780 NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
Sao NSKMMLELDPPFGDSYIVIGVGEKKITHHWHRSGSTIGKAFEATVRGAKRMAVLGDTAWD
(E)
Natal FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
Salvador FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
KU365777 FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
KU365778 FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
KU365779 FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
KU365780 FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
Sao FGSVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMWLGLNTKNGSISLMCLA
(E)
Natal LGGVLIFLSTAVSAD
Salvador LGGVLIFLSTAVSAD
KU365777 LGGVLIFLSTAVSAD
KU365778 LGGVLIFLSTAVSAD
KU365779 LGGVLIFLSTAVSAD
KU365780 LGGVLIFLSTAVSAD
Sao LGGVLIFLSTAVSAD

FIG.5

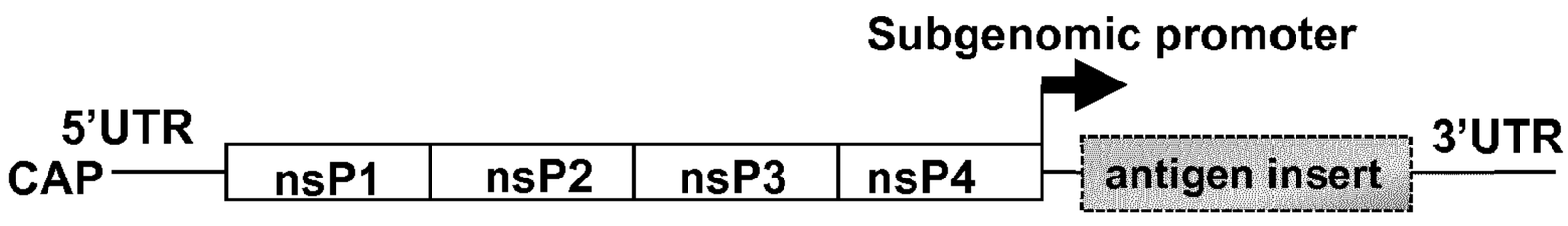

Subgenomic promoter
5'UTR
CAP
nsP1
nsP2
nsP3
nsP4
antigen insert
3'UTR

FIG.6

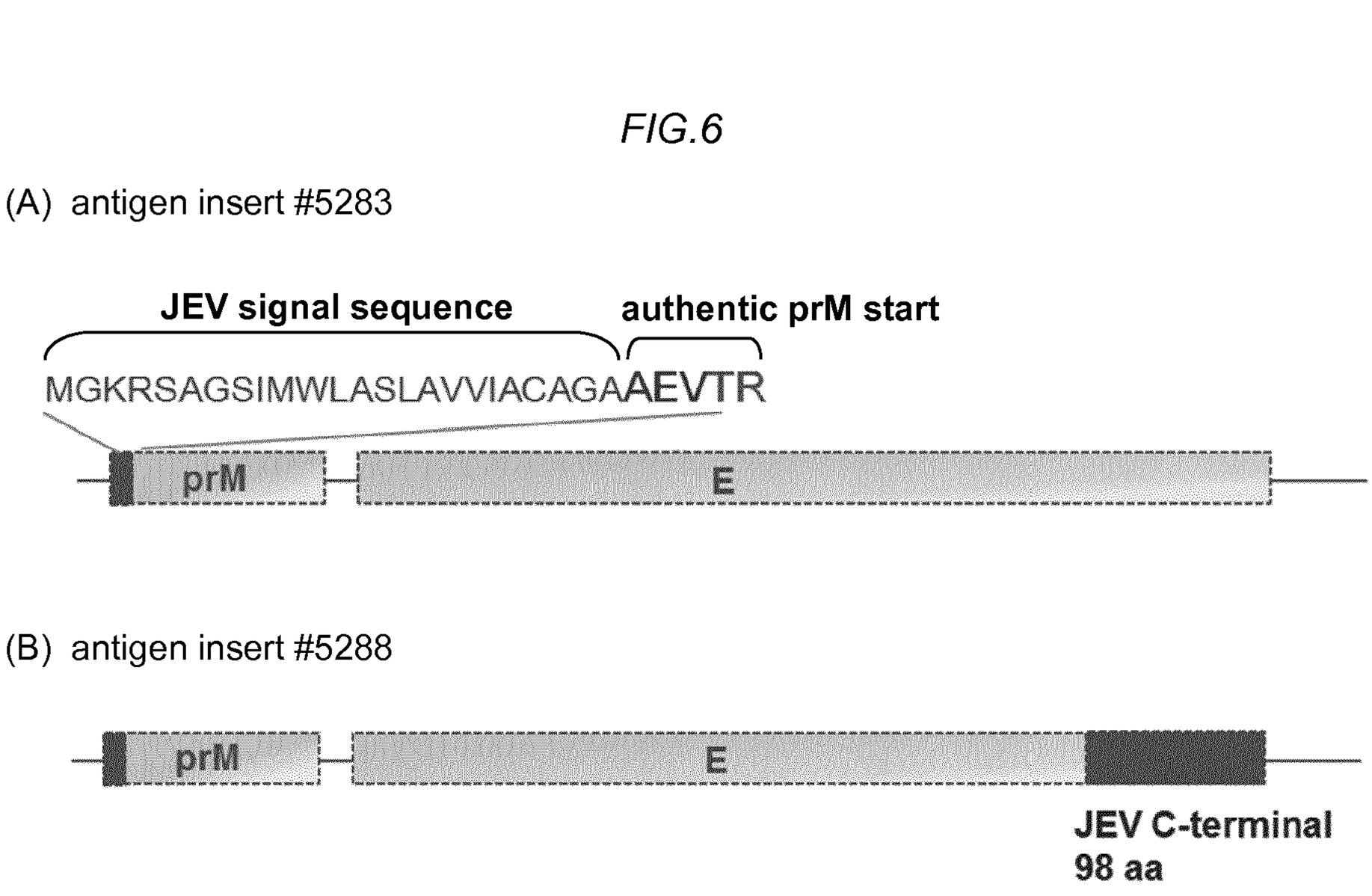

(A) antigen insert #5283
JEV signal sequence
authentic prM start
MGKRSAGSIMWLASLAVVIACAGAAEVTR
prM
E
(B) antigen insert #5288
prM
E
JEV C-terminal
98 aa

FIG.7

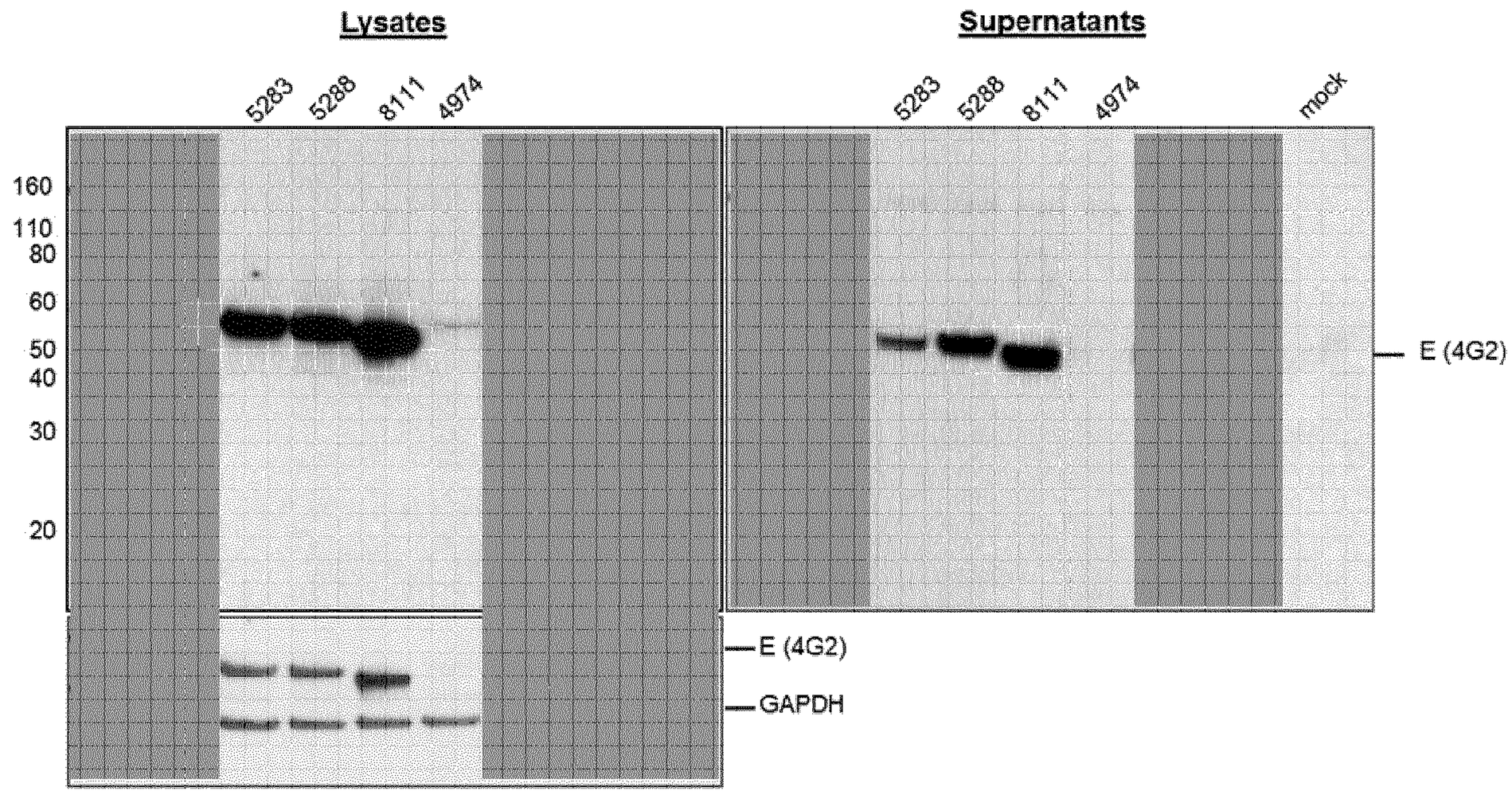

Lysates
Supernatants
5283
5288
8111
4974
5283
5288
8111
4974
mock
160
110
80
60
50
40
30
20
E (4G2)
E (4G2)
GAPDH

FIG.8

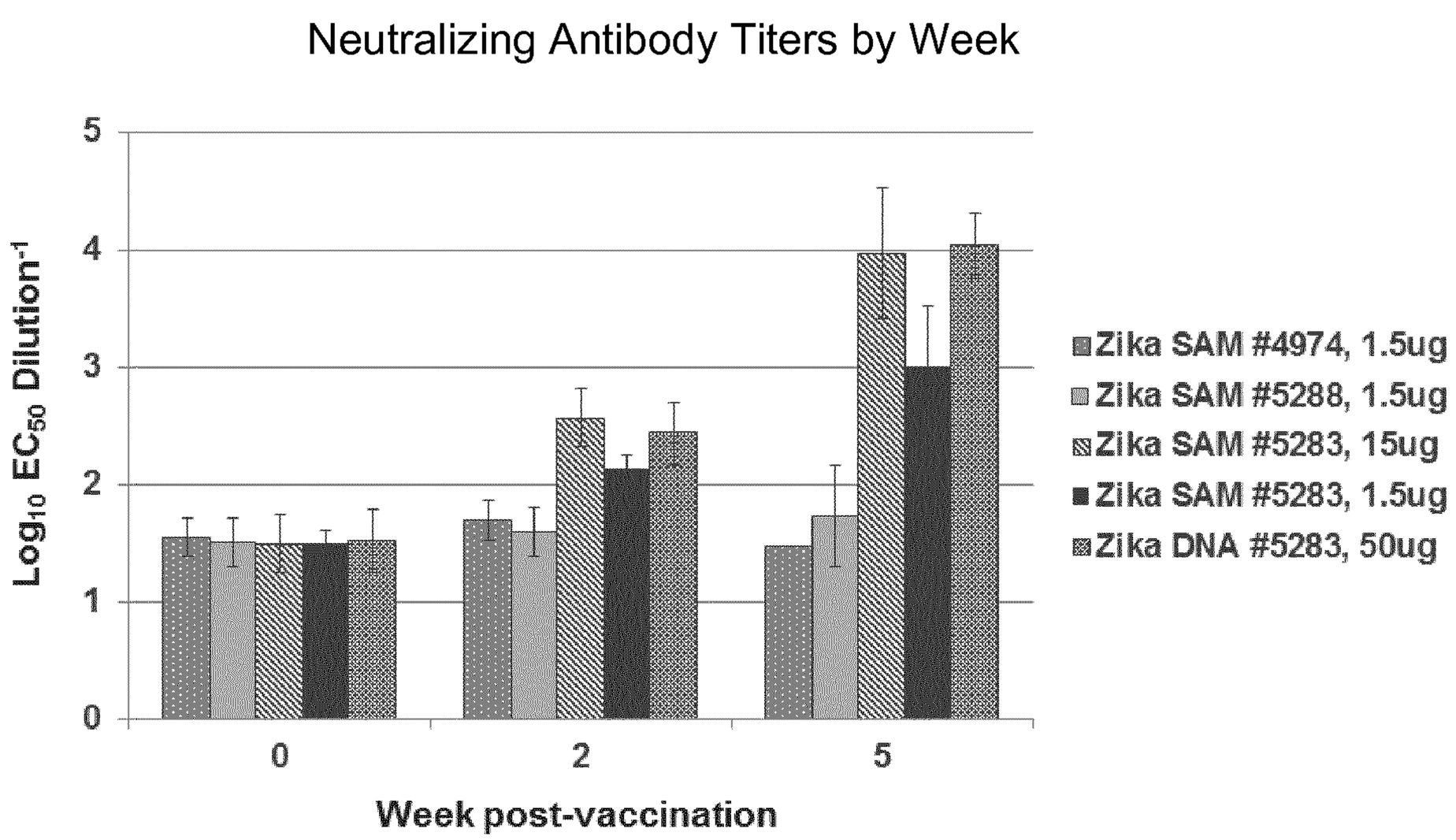

Neutralizing Antibody Titers by Week
Log10 EC50 Dilution-1
5
4
3
2
1
0
0
2
5
Week post-vaccination
Zika SAM #4974, 1.5ug
Zika SAM #5288, 1.5ug
Zika SAM #5283, 15ug
Zika SAM #5283, 1.5ug
Zika DNA #5283, 50ug

FIG.9

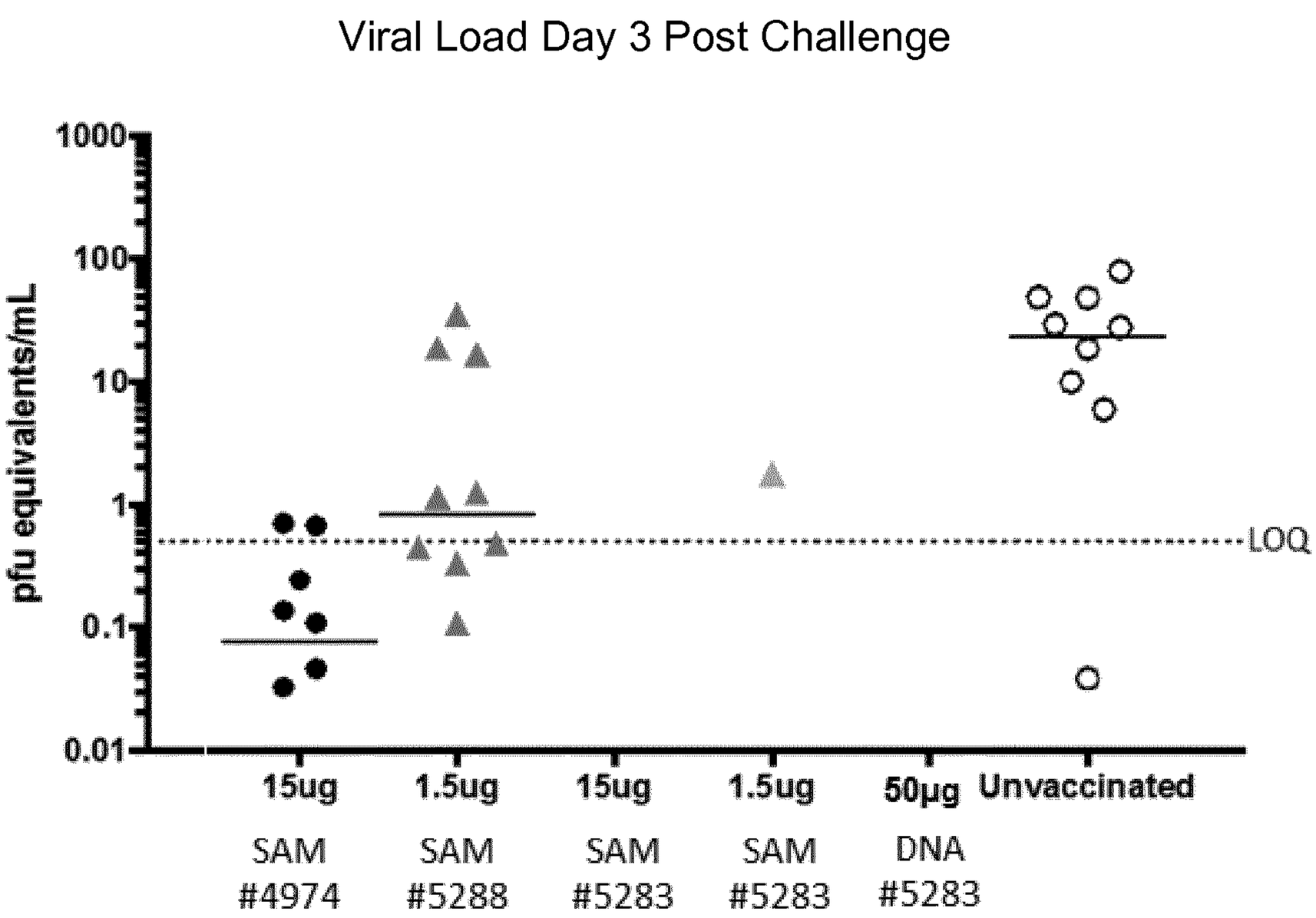

Viral Load Day 3 Post Challenge
pfu equivalents/mL
1000
100
10
1
0.1
0.01
LOQ
15ug
1.5ug
15ug
1.5ug
50µg
Unvaccinated
SAM #4974
SAM #5288
SAM #5283
SAM #5283
DNA #5283

FIG.10

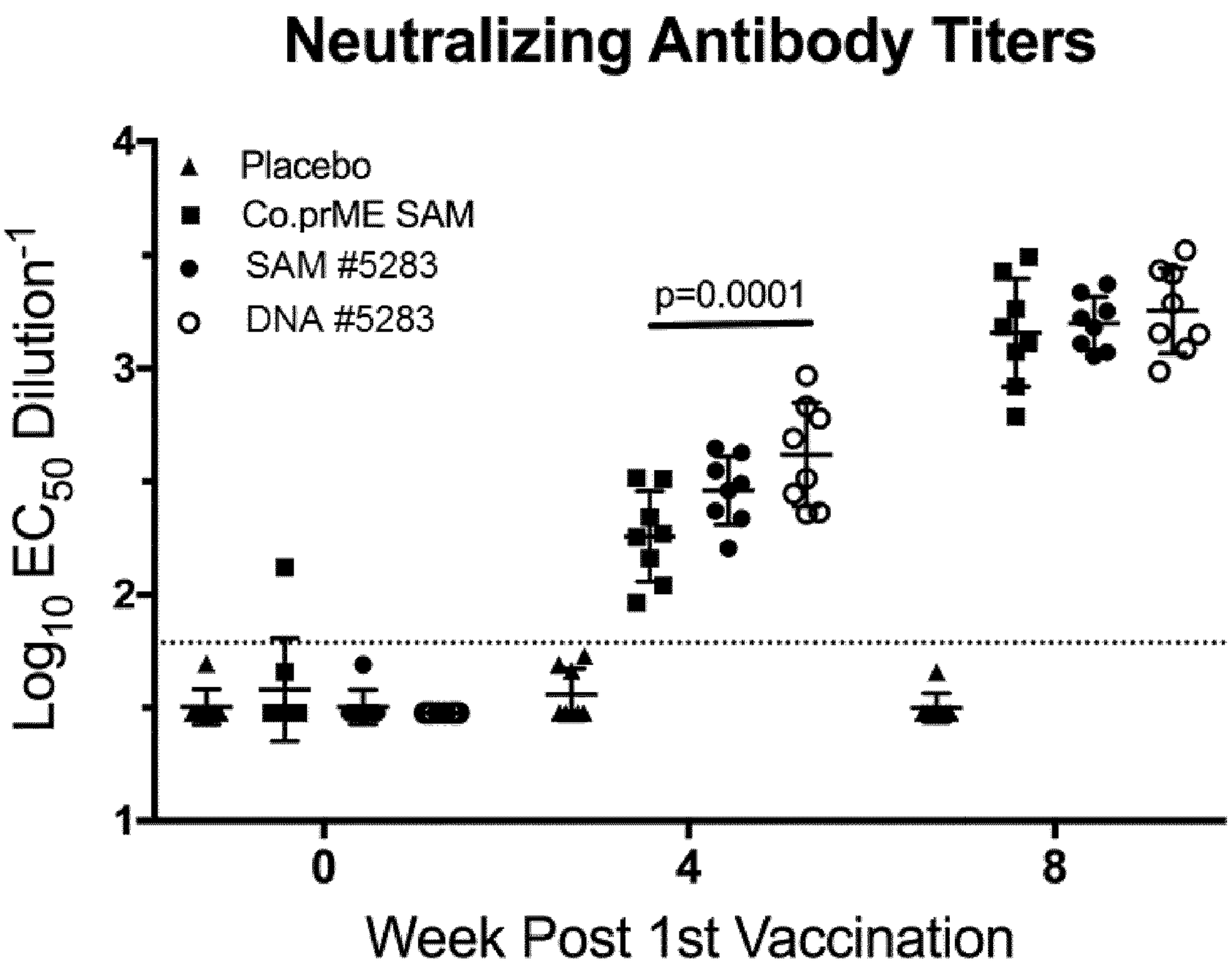

Neutralizing Antibody Titers
Placebo
Co.prME SAM
SAM #5283
DNA #5283
p=0.0001
Log10 EC50 Dilution-1
4
3
2
1
0
4
8
Week Post 1st Vaccination

FIG.11

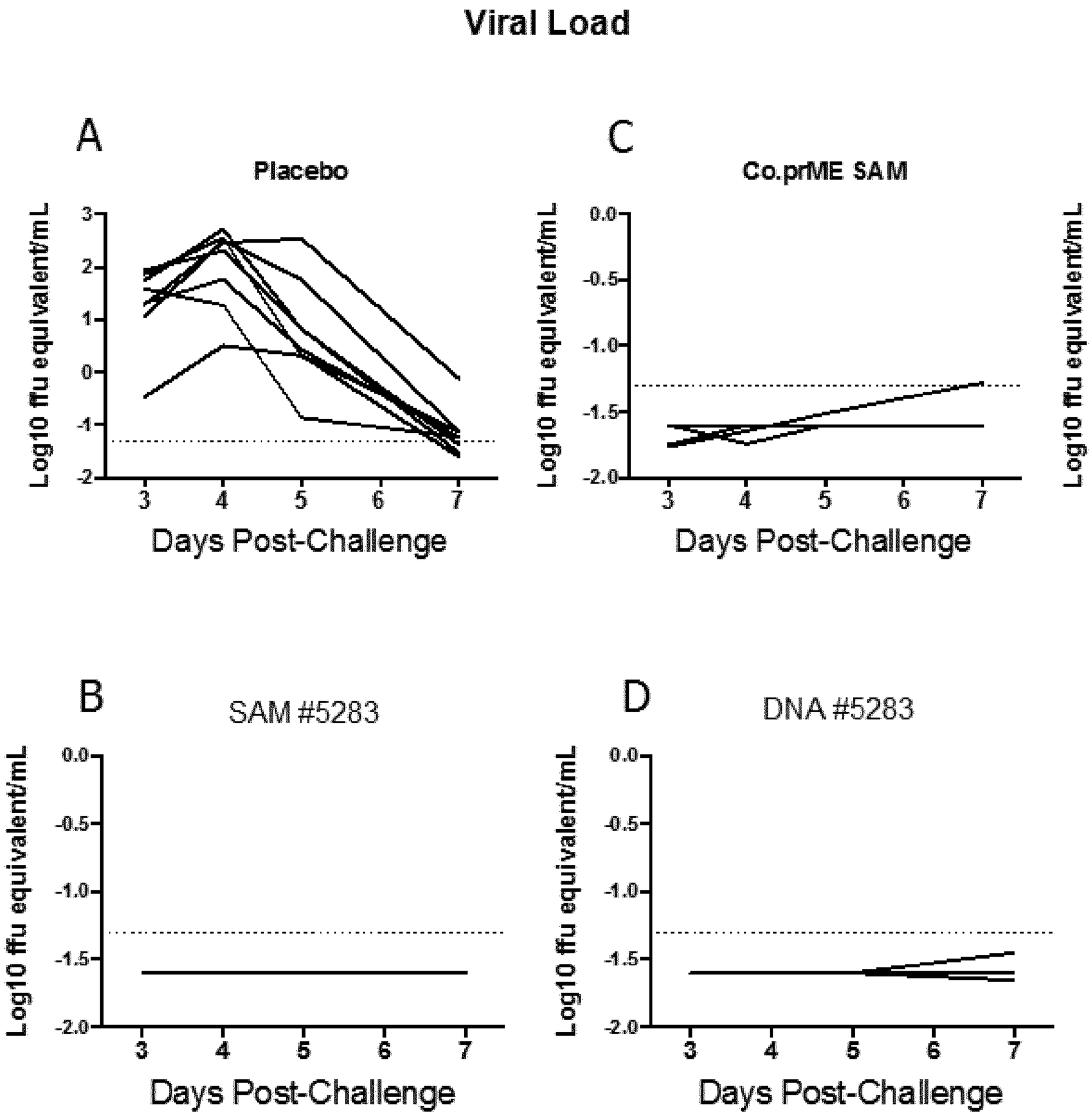
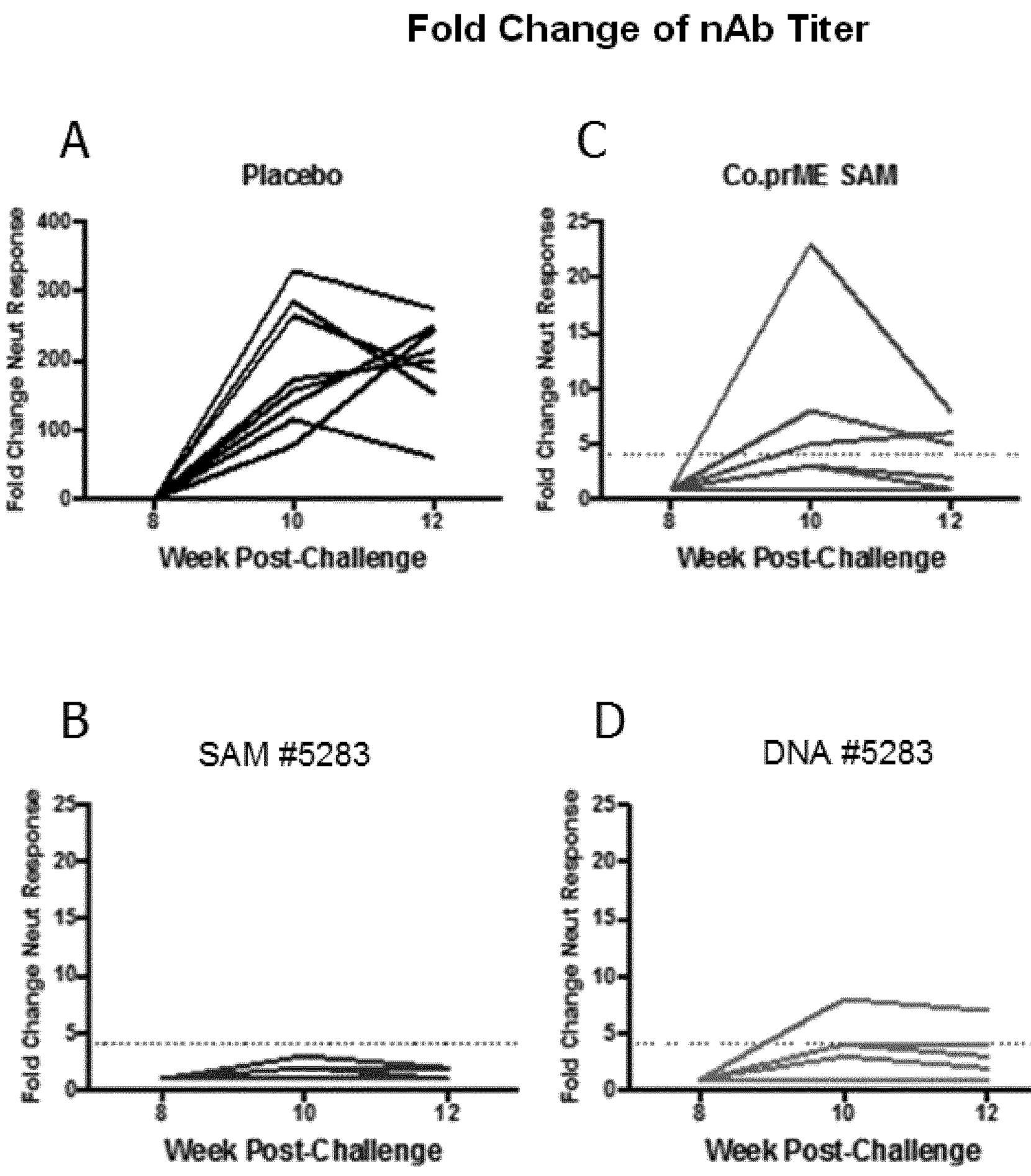

Viral Load
A
Placebo
Log10 ffu equivalent/mL
3
2
1
0
-1
-2
3
4
5
6
7
Days Post-Challenge
C
Co.prME SAM
Log10 ffu equivalent/mL
0.0
-0.5
-1.0
-1.5
-2.0
3
4
5
6
7
Days Post-Challenge
Log10 ffu equivalent/mL
B
SAM #5283
Log10 ffu equivalent/mL
0.0
-0.5
-1.0
-1.5
-2.0
3
4
5
6
7
Days Post-Challenge
D
DNA #5283
Log10 ffu equivalent/mL
0.0
-0.5
-1.0
-1.5
-2.0
3
4
5
6
7
Days Post-Challenge

FIG.12


Fold Change of nAb Titer
A
Placebo
Fold Change Neut Response
400
300
200
100
0
8
10
12
Week Post-Challenge
C
Co.prIME SAM
Fold Change Neut Response
25
20
15
10
5
0
8
10
12
Week Post-Challenge
B
SAM #5283
Fold Change Neut Response
25
20
15
10
5
0
8
10
12
Week Post-Challenge
D
DNA #5283
Fold Change Neut Response
25
20
15
10
5
0
8
10
12
Week Post-Challenge

ZIKA VIRAL ANTIGEN CONSTRUCTS

STATEMENT OF U.S. GOVERNMENT INTEREST

[0001] This invention was created in the performance of a Cooperative Research and Development Agreement with the National Institutes of Health, an Agency of the Department of Health and Human Services. The Government of the United States has certain rights in this invention.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted electronically in .XML format and is hereby incorporated by reference in its entirety. Said .XML copy, created on Sep. 15, 2022, is named "2801-0317PUS2.xml" and is 153,900 bytes in size. The sequence listing contained in this .XML file is part of the specification and is hereby incorporated by reference herein in its entirety.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0003] The present application is a 37 C.F.R. § 1.53(b) continuation of U.S. Application No. 16/461,503 filed on May 16, 2019 which is the National Phase of PCT International Application No. PCT/EP2017/079343, filed on Nov. 15, 2017, which claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Nos. 62/568,559 filed on Oct. 5, 2017; 62/485,090 filed on Apr. 13, 2017; and 62/423,398 filed on Nov. 17, 2016, all of which are hereby expressly incorporated by reference into the present application.

FIELD OF THE INVENTION

[0004] This invention is in the field of treating and preventing viral infections. In particular, the present invention relates to nucleic acid-based vaccine constructs encoding Zika viral antigens and the use of Zika viral antigens for treating and preventing Zika infections.

BACKGROUND TO THE INVENTION

[0005] Zika virus was first identified in Uganda in 1947 in rhesus monkeys through a monitoring network of sylvatic yellow fever. It was subsequently identified in humans in 1952 in Uganda and the United Republic of Tanzania. Outbreaks of Zika virus disease have been recorded in Africa, the Americas, Asia and the Pacific. Zika virus belongs to the genus *flavivirus*. Its reservoir is unknown.

[0006] Zika virus is a plus-strand RNA virus belonging to the family Flaviviridae. Zika virus disease is caused by a virus transmitted primarily by Aedes mosquitoes. People with Zika virus disease can have symptoms that can include mild fever, skin rash, conjunctivitis, muscle and joint pain, malaise or headache. These symptoms normally last for 2-7 days.

[0007] The Zika virus is known to circulate in Africa, the Americas, Asia and the Pacific. Transmitted by Aedes mosquitos, the virus has been known to cause either asymptomatic infection (in the majority of people infected) or a self-limiting illness with descending rash, conjunctivitis and low grade fever. However, during the ongoing Zika virus outbreak in the Americas an alarming increase in the number of babies born with microcephaly, as well as an increase in the incidence of Guillain-Barré syndrome has been reported. In addition to microcephaly, other fetal malformations and neurological disorders have been described.

[0008] Dowd et al. (Science, Vol. 354 Issue 6309, pp. 237-40 (2016) recently reported that DNA vaccines expressing the premembrane and envelope proteins of Zika virus were immunogenic in mice and nonhuman primates when administered by electroporation or needle-free injection; and that protection against viremia after Zika virus challenge correlated with serum neutralizing activity.

[0009] Chahal et al. (Scientific Reports, 7:252, pp. 1-9 (2017)) describe an alphavirus RNA vector encoding Zika virus structural antigens. When formulated with a modified dendrimer nanomaterial and administered to mice intramuscularly, the vaccine was found to be immunogenic.

[0010] Richner et al. (Cell, 168, pp. 1-12, (2017) describe a modified mRNA vaccine encoding wild-type or mutant Zika structural proteins. When encapsulated in lipid nanoparticles and administered intramuscularly to mice, the mRNA vaccine elicited high neutralizing antibody titers and protection from viral challenge.

[0011] Given the concerning disease burden and the potential for rapid dissemination, there is an urgent need for the development of components for use in a Zika virus immunogenic or vaccine composition.

SUMMARY OF THE INVENTION

[0012] The present inventors provide constructs useful as components of immunogenic compositions for the induction of an immune response in a subject against Zika viral infection, methods for their use in treatment, and processes for their manufacture.

[0013] In some embodiments, a nucleic acid-based vaccine construct encoding a polypeptide comprising a full-length Zika virus pre-M-E antigen (prME), or an immunogenic fragment thereof is provided.

[0014] In some embodiments, a vector comprising the construct as described is provided.

[0015] In some embodiments, a self-replicating RNA molecule (also referred to herein as a self-amplifying mRNA, or SAM molecule) comprising the construct as described is provided.

[0016] In some embodiments, a composition comprising an immunologically effective amount of one or more of the constructs, vectors, or self-replicating RNA molecules as described above is provided.

[0017] In some embodiments, a composition as described above is provided wherein the composition comprises an RNA-based vaccine.

[0018] In some embodiments, a composition as described above is provided wherein the composition comprises one or more constructs, vectors, or self-replicating RNA molecules as described above complexed with a particle of a cationic oil-in-water emulsion.

[0019] In some embodiments, a composition as described above for use in inducing an immune response against a Zika virus infection in a subject in need thereof is provided.

[0020] In some embodiments, a method is provided for inducing an immune response against a Zika virus infection in a subject in need thereof, which comprises administering to said subject an immunologically effective amount of a composition comprising one or more of the constructs, vectors, or self-replicating RNA molecules as described above.

[0021] In some embodiments, a method as described above is provided wherein the composition comprises one

or more constructs, vectors, or self-replicating RNA molecules as described above complexed with a particle of a cationic oil-in-water emulsion.

[0022] In some embodiments, a process is provided for producing an RNA-based vaccine comprising a step of transcribing a vector or DNA molecule encoding a self-replicating RNA molecule described above to produce an RNA comprising a coding region for the antigen.

[0023] In some embodiments, a method of preparing a composition as described above is provided wherein the method comprises 1) preparing a cationic oil-in-water emulsion; 2) preparing one or more constructs, vectors, or self-replicating RNA molecules as described above; and 3) adding the one or more constructs, vectors, or self-replicating RNA molecules to the cationic oil-in-water emulsion so that the construct, vector, or self-replicating RNA molecule complexes with the emulsion.

[0024] In some embodiments, a composition produced by the process described above is provided.

[0025] In some embodiments, a use of the construct, vector, self-replicating RNA molecule, or composition described above for inducing an immune response against a Zika virus infection in a subject is provided.

[0026] In some embodiments, a use of the construct, vector, self-replicating RNA molecule, or composition described above in the manufacture of a medicament that induces an immune response against a Zika virus infection in a subject is provided.

[0027] In some embodiments, a construct, vector, self-replicating RNA molecule, or composition described above for use in therapy is provided.

[0028] In some embodiments, a construct, vector, self-replicating RNA molecule, or composition described above for use in a method of inducing an immune response to a Zika virus infection in a subject is provided.

DESCRIPTION OF DRAWINGS/FIGURES

[0029] FIG. **1**: The organization of the Flavivirus genome, showing the polyprotein that is cleaved into structural and nonstructural proteins by a combination of viral and cellular proteases.

[0030] FIG. **2**: Formation of Flavivirus virions and subviral particles. (1) In natural infections, *flavivirus* proteins are produced by the processing of a polyprotein translated from the viral genomic RNA and inserted co-translationally into the endoplasmic reticulum (ER) membrane. Horizontal arrows indicate polyprotein cleavages by signal peptidase and arrow heads indicate cleavage by the viral NS2B-3 protease. The open arrow indicates a signalase cleavage which is inefficient unless cytoplasmic capsid (C) cleavage has occurred. (2) The minimal requirement for production of subviral particles is the precursor membrane (prM) and envelope (E) proteins. (3) Flavivirus particles are formed by budding on the ER membrane driven by the prM and E proteins independent of the C protein or preformed nucleocapsids. Virus infection results predominantly in the formation of virions. (4) Nucleocapsid-free virus-like particles are efficiently produced by recombinant expression of the prM and E proteins and are a by-product of *flavivirus* infection. (5) Virions and virus-like particles follow the exocytic pathway for secretion from infected/transfected cells. 'Cy' denotes the cytoplasmic side of the ER membrane.

[0031] FIGS. **3**A-D: CLUSTAL O(1.2.1) multiple sequence alignment of CprME proteins of Zika virus strains: Uganda (SEQ ID NO:10); Micronesia (SEQ ID NO:11); Natal (SEQ ID NO:2); Salvador (SEQ ID NO:8); Genbank Accession No. KU365777 (SEQ ID NO:13); Genbank Accession No. KU365778 (SEQ ID NO:14); Genbank Accession No. KU365779 (SEQ ID NO:15); Genbank Accession No. KU365780 (SEQ ID NO:16); French Polynesia ("French") (SEQ ID NO:12); and Sao Paolo ("Sao") (SEQ ID NO:9). An "*" (asterisk) indicates positions which have a single, fully conserved residue. A ":" (colon) indicates conservation between groups of strongly similar properties. A "." (period) indicates conservation between groups of weakly similar properties. The above SEQ ID NOS include the entire sequences shown in FIGS. **3**A-D for each strain except that SEQ ID NO: 2 does not include the capsid (C) portion of the Natal strain. However, the entire corresponding sequence for the Natal strain is shown in FIGS. **3**A-**3**D.

TABLE 1

Zika virus strains, year, and Genbank reference

STRAIN	YEAR	GENBANK NUMBER
Uganda		NC_012532
Micronesia	2007	EU545988.1
Natal (Brazil)	2016	KU527068
Salvador (Brazil)	2016	KU707826.1
Sao Paulo (Brazil)	2016	KU321639
French Polynesia	2013	KJ776791

[0032] FIGS. **4**A-C: CLUSTAL O(1.2.1) multiple sequence alignment of CprME proteins from Brazilian strains of Zika virus: Natal (SEQ ID NO:2); Salvador (SEQ ID NO:8); Genbank Accession No. KU365777 (SEQ ID NO:13); Genbank Accession No. KU365778 (SEQ ID NO:14); Genbank Accession No. KU365779 (SEQ ID NO:15); Genbank Accession No. KU365780 (SEQ ID NO:16); and Sao Paolo ("Sao") (SEQ ID NO:9). See Table 1 for Zika virus strains, year, and Genbank reference.

[0033] FIG. **5**: A SAM-Zika construct. The self-amplifying mRNA (SAM) background consists of VEE TC-83 replicon encoding the viral nonstructural proteins 1-4 (nsP1-4), followed by the subgenomic promoter, and an insert encoding a Zika antigen. The empty vector is shown in SEQ ID NO:17; the insert starts immediately after nucleotide 7561.

[0034] FIG. **6**: Design of antigen inserts for Zika-SAM constructs.

[0035] Antigen insert #5283, shown in (A), comprises a Japanese Encephalitis Virus (JEV) signal sequence followed by Zika prME, including the wild type prM start sequence ("AEVTR"). The full length amino acid sequence of antigen insert #5283 is shown in SEQ ID NO:19, including the JEV signal sequence (SEQ ID NO:5), followed by the Zika pr region (SEQ ID NO:20), M protein (SEQ ID NO:21) and E protein (SEQ ID NO:22). The DNA and RNA sequences encoding the antigen insert shown in (A) are presented in SEQ ID NOs:18 and 39, respectively.

[0036] Antigen insert #5288, shown in (B), is identical to the insert of (A), except that the last 98 amino acids of the Zika E protein are replaced with the last 98 amino acids from the C-terminus of the JEV E protein (Genbank Accession No. AFV52311.1; SEQ ID NO:7). Chang et al. (2003)

Virol. 306:170-180. The full length amino acid sequence of antigen insert #5288 is shown in SEQ ID NO:24, including the JEV signal sequence (SEQ ID NO:5), followed by the Zika pr region and M protein (SEQ ID NOS:25 and 26, respectively), and a hybrid Zika-JEV E protein (SEQ ID NO:27). The DNA and RNA sequences encoding the antigen insert shown in (B) are presented in SEQ ID NOs:23 and 40, respectively.

[0037] FIG. **7**: Analysis of expression and secretion of E protein from Zika-SAM constructs.

[0038] Expression and secretion of E protein was detected by immunoblot in cell lysates for self-amplifying mRNA (SAM) constructs encoding antigen inserts #5283 (JEV signal + Zika prME) and #5288 (JEV signal + hybrid Zika-JEV prME). Expression and secretion of E protein was also detected for a positive control antigen construct (#8111) but not for a negative control construct (#4974) or mock transfection ("Mock"). See Example 5.

[0039] FIG. **8**: Neutralizing Antibody Responses in Mice. Mice were found to have significant neutralizing Zika antibodies two weeks after a single vaccination with Zika-SAM construct #5283 (JEV signal + Zika prME), or with positive control Zika DNA construct #5283, as measured by reporter virus particle (RVP) neutralization assay. Neutralizing antibody titers were further increased two weeks after a second vaccination with the same Zika-SAM construct, or the positive control. Neutralizing Zika antibodies were below the limit of quantification before vaccination (Day 0), and after vaccination with negative control construct (#4974, 1.5 μg dose) or with #5288 (JEV signal + hybrid Zika-JEV prME, 1.5 μg dose). A dose-response effect was observed for SAM construct #5283, with 15 ug of RNA eliciting more neutralizing antibodies than 1.5 ug RNA.

[0040] FIG. **9**: Protection of Mice from Zika Challenge: Mice vaccinated on days 0 and 21 were challenged with live Zika virus on day 49. Viral load was measured 3 days post-challenge. Vaccination with SAM construct #5283 (at 1.5 μg and 15 μg doses), as well as the positive control (DNA #5283) were protective against Zika viremi. Unvaccinated mice and mice vaccinated with SAM construct #5288 (1.5 μg dose) or negative control construct #4974 were not protected in the Zika challenge. Dotted line indicates the limit of quantification (LOQ) of the assay.

[0041] FIG. **10**: Neutralizing Antibody Responses in Non-Human Primates (NHPs). Rhesus Macaques were immunized at weeks 0 and 4 with the Zika-SAM construct #5283 (75 μg/dose) or a codon-optimized SAM construct encoding the native Zika prME antigen (Co.prME SAM; 75 μg/dose). Control NHPs were administered placebo or immunized with Zika DNA construct #5283 (4 mg/dose). Zika neutralizing antibodies were significantly elevated four weeks after the first immunization of Zika SAM construct #5283 or Zika DNA #5283 as compared to placebo, with titers being further increased 4 weeks after the second immunization. Zika-SAM construct Co.prME SAM produced significantly less neutralizing antibodies compared to #5283 DNA after a single dose, but similar titers after two injections. Dotted line indicates the limit of quantification (LOQ) of the assay.

[0042] FIG. **11**: Protection of NHPs from Zika Challenge: Rhesus Macaques vaccinated as described in FIG. **10** were then challenged with live Zika virus at week 8. Viral load was measured daily from days 3-7 post-challenge. Placebo animals exhibited elevated viremia as early as 3 days post-challenge (A). Vaccination with Zika-SAM construct #5283 (B), Zika-SAM Co.prME (C), and DNA #5283 (D) were protective against Zika viremia, with Zika-SAM construct #5283 exhibiting a complete protection against Zika viremia. Dotted line indicates the limit of quantification (LOQ) of the assay.

[0043] FIG. **12**: Post-Challenge Serology in NHPs. Neutralizing antibody titers were determined 8, 10 and 12 weeks after Zika virus challenge, and are expressed as fold-change from pre-challenge levels. Placebo animals showed a sharp rise in neutralizing antibodies after Zika challenge, suggesting Zika infection in these animals. Two animals in the SAM Co.prME group, and one animal in the DNA #5283 group, showed elevated neutralizing antibody titers post-challenge, indicating that protection was not sterilizing in these animals. In contrast, animals in the Zika SAM #5283 group did not exhibit elevated neutralizing antibodies after Zika challenge, indicating that sterilizing protection was achieved in all subjects. Dotted line in (B), (C) and (D) indicates a 4-fold change in neutralizing titers, which is considered non-sterilizing in the flavivirus field.

DETAILED DESCRIPTION OF THE INVENTION

Antigens; Variants; Fragments; and Constructs

[0044] The present inventors provide constructs useful as components of immunogenic compositions for the induction of an immune response in a subject against Zika viral infection constructs useful for the expression of antigens, methods for their use in treatment, and processes for their manufacture. By construct is intended a nucleic acid that encodes polypeptide sequences described herein, and may comprise DNA, RNA, or non-naturally occurring nucleic acid monomers. The nucleic acid components of constructs are described more fully in the Nucleic Acids section herein.

[0045] In some embodiments, the constructs disclosed herein encode wild-type polypeptide sequences of a Zika virus, or a variant, or a fragment thereof. The constructs may further encode a polypeptide sequence heterologous to the polypeptide sequences of a Zika virus. In some embodiments, the constructs encode wild-type polypeptide sequences of a Brazilian strain Zika virus, or a variant, or a fragment thereof. By "Brazilian strain Zika virus" is intended any strain of Zika virus denoted as "Brazilian" in Table 1. Unless indicated otherwise, descriptions of the wild-type prME antigen are made by reference to the Natal strain (Brazil), GenBank number KU527068.1, as depicted in the SEQ ID NO:1 (nucleic acid) and SEQ ID NO:2 (polypeptide), and as depicted in FIGS. **3**A-D, and FIGS. **4**A-C.

[0046] A "variant" of a polypeptide sequence includes amino acid sequences having one or more amino acid substitutions, insertions and/or deletions when compared to the reference sequence. The variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type polypeptide, for example, to a polypeptide according to SEQ ID NO:2. Alternatively, or in addition, a fragment of a polypeptide may comprise an immunogenic fragment (i.e. an epitope-containing fragment) of the full-length polypeptide which may comprise a contiguous amino acid sequence of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least

16, at least 17, at least 18, at least 19, or more amino acids which is identical to a contiguous amino acid sequence of the full-length polypeptide.

[0047] A fragment of a polypeptide may comprise N- and/or C-terminal deletions when compared to a full-length polypeptide, for example SEQ ID NO:2, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids from the N-terminus, the C-terminus, or both the N-terminus and C-terminus of the full-length sequence. It may be specified that the deletions are of consecutive amino acids.

[0048] As used herein, the term "antigen" refers to a molecule containing one or more epitopes (e.g., linear, conformational or both) that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific immunological response (i.e. an immune response which specifically recognizes an antigen polypeptide). An "epitope" is that portion of an antigen that determines its immunological specificity.

[0049] T- and B-cell epitopes can be identified empirically (e.g. using PEPSCAN™ or similar methods). See the following references: Geysen et al. (1984) *PNAS USA* 81 :3998-4002; Carter (1994) *Methods Mol Biol* 36:207-23. They can be predicted (e.g. using the Jameson- Wolf antigenic index (see Jameson et al. (1988) *CABIOS* 4(1): 181-186), matrix-based approaches (see Raddrizzani & Hammer (2000) *Brief Bioinform* 1(2): 179-89), TEPITOPE™ (see De Lalla et al. (1999) *J. Immunol.* 163: 1725-29), neural networks (see Brusic et al. (1998) *Bioinformatics* 14(2): 121-30), OptiMer & EpiMer (see Meister et al. (1995) *Vaccine* 13(6):581-91; see Roberts et al. (1996) *AIDS Res Hum Retroviruses* 12(7):593-610), ADEPT™ (see Maksyutov & Zagrebelnaya (1993) *Comput Appl Biosci* 9(3):291-7), Tsites (see Feller & de la Cruz (1991) *Nature* 349(6311):720-1), hydrophilicity (see Hopp (1993) *Peptide Research* 6:183-190), antigenic index (see Welling et al. (1985) *FEBS Lett.* 188:215-218) or the methods disclosed in reference Davenport et al. (1995) *Immunogenetics* 42:392-297, etc.).

[0050] In some embodiments, the constructs herein encode a Zika virus prME antigen. By "Zika virus prME antigen" is intended the amino acid sequence, or a nucleotide sequence encoding the amino acid sequence, of a wild-type Zika virus structural protein prME, a variant, or a fragment thereof. FIG. **3** and FIG. **4** identify the amino acid sequence of several full-length wild-type Zika virus prME structural protein variants. The sequence identifier numbers for each are set forth in the Sequence Listing herein. See SEQ ID NOS:2 and 8-16.

[0051] Thus, where a Zika virus prME antigen is a variant of a wild-type prME polypeptide, the variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type polypeptide, for example, to a polypeptide according to SEQ ID NOS:2 and 8-16. Alternatively, or in addition, a fragment of a polypeptide may comprise an immunogenic fragment (i.e. an epitope-containing fragment) of the full-length polypeptide which may comprise a contiguous amino acid sequence of at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or more amino acids which is identical to a contiguous amino acid sequence of the full-length polypeptide.

[0052] Afragmentof a Zika virus prME polypeptide may comprise N- and/or C-terminal deletions when compared to a full-length polypeptide, for example SEQ ID NOS:2 and 8-16, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids from the N-terminus, the C-terminus, or both the N-terminus and C-terminus of the full-length sequence. It may be specified that the deletions are of consecutive amino acids. In some embodiments, the Zika virus prME polypeptide comprises a fragment selected from the group consisting of amino acids 1 to 692 of SEQ ID NO:2 and amino acids 21 to 692 of SEQ ID NO:2.

[0053] In some embodiments, an immunogenic fragment of a prME antigen comprises the full-length of the Zika virus M antigen. By "Zika virus M antigen" is intended the amino acid sequence, or a nucleotide sequence encoding the amino acid sequence, of SEQ ID NO:21. Where a Zika virus M antigen is a variant of a wild-type M polypeptide, the variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type polypeptide, for example, to a polypeptide according to SEQ ID NO:21.

[0054] A fragment of a Zika virus M antigen may comprise N- and/or C-terminal deletions when compared to a full-length polypeptide, for example SEQ ID NO:21, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids from the N-terminus, the C-terminus, or both the N-terminus and C-terminus of the full-length sequence. It may be specified that the deletions are of consecutive amino acids.

[0055] In some embodiments, an immunogenic fragment of a prME antigen comprises the full-length of the Zika virus E antigen. By "Zika virus E antigen" is intended the amino acid sequence, or a nucleotide sequence encoding the amino acid sequence, of SEQ ID NO:22. Where a Zika virus E antigen is a variant of a wild-type E polypeptide, the variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type polypeptide, for example, to a polypeptide according to SEQ ID NO:22. In some embodiments, a variant Zika virus E antigen may be a hybrid E antigen comprising an N-terminal fragment of a Zika E protein fused to a C-terminal fragment of a heterologous viral E protein. In some embodiments, the heterologous viral E protein is derived from a heterologous *flavivirus*, such as Japanese Encephalitis Virus (JEV).

[0056] A fragment of a Zika virus E antigen may comprise N- and/or C-terminal deletions when compared to a full-length polypeptide, for example SEQ ID NO:22, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 amino acids from the N-terminus, the C-terminus, or both the N-terminus and C-terminus of the full-length sequence. It may be specified that the deletions are of consecutive amino acids.

[0057] In some embodiments, a variant Zika virus prME antigen comprises a hybrid Zika-JEV E protein. A hybrid Zika-JEV E antigen may comprise an N-terminal fragment of a Zika E protein comprising at least amino acids (1 + x) to (422 ± y) of SEQ ID NO:22, where x is a whole number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20; and y is a whole number selected

from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In one embodiment, the N terminal fragment of a Zika E protein comprises amino acids 1 to 422 of SEQ ID NO:22.

[0058] A hybrid E antigen may comprise a C-terminal fragment of a JEV E protein comprising at least amino acids (205 ± x) to (302 ± y) of SEQ ID NO:7, where x is a whole number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20; and y is a whole number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In one embodiment, the C-terminal fragment of a JEV E protein comprises amino acids 205 to 302 of SEQ ID NO:7.

[0059] In one embodiment, a hybrid E antigen comprises the amino acid sequence of SEQ ID NO:27, or a fragment or variant thereof. A variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the polypeptide according to SEQ ID NO:27. Alternatively, or in addition, a fragment of a polypeptide may comprise an immunogenic fragment (i.e. an epitope-containing fragment) of the full-length polypeptide, such as a contiguous amino acid sequence of at least 50, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500 or more amino acids which is identical to a contiguous amino acid sequence of SEQ ID NO:27.

[0060] As noted elsewhere herein, the Zika virus RNA is translated as a polyprotein comprising a prM signal sequence. The prM signal sequence is located N-terminal to the prM antigen sequence. Cleavage occurs in the ER lumen by a cellular signal peptidase and generates the N terminus of prM. Where the polyprotein comprises a wild-type amino acid sequence, the polyprotein comprises a native prM signal sequence, SEQ ID NO:3. By "native prM signal sequence" is intended the amino acid sequence, or a nucleotide sequence encoding the amino acid sequence, of a signal sequence of a wild-type viral prME, SEQ ID NO:3. FIGS. **3**A-B and FIG. **4**A identify the amino acid sequence of several full-length native prM signal sequence variants from various Zika virus strains.

[0061] In some embodiments, the constructs encode a native prM signal sequence. Where the prM signal sequence is a variant of a native prM signal sequence, the variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the full-length polypeptide according to SEQ ID NO:3. Alternatively, or in addition, a fragment of a polypeptide may comprise a functional fragment (i.e., containing the sequence recognized and cleaved by the protease) of the full-length polypeptide which may comprise a contiguous amino acid sequence of at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17 amino acids of SEQ ID NO:3 which is identical to a contiguous amino acid sequence of full-length polypeptide.

[0062] In some embodiments, the construct encodes a heterologous, non-Zika signal sequence. In some embodiments, the construct encodes a Japanese Encephalitis Virus (JEV) signal sequence, a variant, or a fragment thereof, in place of the Zika prM signal sequence. By "JEV signal sequence" is intended the amino acid sequence as set forth in FIG. **6**: MGKRSAGSIMWLASLAWIACAGA (SEQ ID NO:5). A variant may comprise an amino acid sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a full-length wild-type polypeptide, for example, to a polypeptide according to SEQ ID NO:5. Alternatively, or in addition, a fragment of a polypeptide may comprise a functional fragment (i.e., containing the sequence recognized and cleaved by the protease) of the full-length polypeptide which may comprise a contiguous amino acid sequence of at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22 at least 23 amino acids, which is identical to a contiguous amino acid sequence of the full-length polypeptide.

[0063] In some embodiments, the construct encodes a polypeptide comprising from the N-terminal portion to the C-terminal portion: a JEV signal sequence and a Zika prME antigen, variant, or immunogenic fragment thereof. In some embodiments, the construct encodes a polypeptide comprising from the N-terminal portion to the C-terminal portion: a JEV signal sequence, a Zika prM antigen, and a hybrid E antigen comprising an N-terminal fragment of a Zika E protein fused to a C-terminal fragment of a JEV E protein.

[0064] In some embodiments, a construct encodes each component of the polypeptide, if present, juxtaposed immediately next to the adjacent component, i.e., without any intervening amino acids. In some embodiments, a linker group of 1, 2, 3, 4, or 5 amino acids is present between one or more of the components.

[0065] In some embodiments, the construct encodes a polypeptide having a sequence selected from the group consisting of SEQ ID NO:19 and SEQ ID NO:24. In some embodiments, the construct encodes a polypeptide which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:19 and SEQ ID NO:24. In some embodiments, the construct encodes a polypeptide which comprises a fragment of a full-length sequence selected from the group consisting of SEQ ID NO:19 and SEQ ID NO:24., wherein the fragment comprises a contiguous stretch of the amino acid sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids shorter than the full-length sequence.

[0066] In some embodiments, the construct comprises a DNA sequence selected from the group consisting of SEQ ID NO:18 and SEQ ID NO:23. In some embodiments, the construct comprises a DNA sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:18 and SEQ ID NO:23. In some embodiments, the construct comprises a DNA sequence which comprises a fragment of a full-length sequence selected from the group consisting of SEQ ID NO:18 and SEQ ID NO:23 wherein the fragment comprises a contiguous stretch of the DNA sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 nucleic acids shorter than the full-length sequence.

[0067] In some embodiments, the construct comprises an RNA sequence selected from the group consisting of SEQ ID NO:39 and SEQ ID NO:40. In some embodiments, the construct comprises an RNA sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least

98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:39 and SEQ ID NO:40. In some embodiments, the construct comprises an RNA sequence which comprises a fragment of a full-length sequence selected from the group consisting of SEQ ID NO:39 and SEQ ID NO:40 wherein the fragment comprises a contiguous stretch of the RNA sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 nucleic acids shorter than the full-length sequence.

[0068] In some embodiments, the construct comprises a fragment of a full-length RNA sequence selected from the group consisting of SEQ ID NO:39 and SEQ ID NO:40, wherein the fragment comprises a deletion of up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 nucleic acids from the 5′ end, the 3′ end, or both the 5′ and 3′ ends of the full-length sequence.

Polypeptides

[0069] In some embodiments, a polypeptide herein is in a non-naturally occurring form (e.g. a recombinant or modified form).

[0070] For example, polypeptides (e.g. antigens) disclosed herein can be prepared by chemical synthesis (in whole or in part), by digesting longer polypeptides using proteases, by translation from RNA, by purification from cell culture (e.g. from recombinant expression), from the organism itself, etc. An exemplary method for production of peptides <40 amino acids long involves in vitro chemical synthesis, see the following references: Bodanszky (1993) Principles of Peptide Synthesis (ISBN: 0387564314); and Fields et al. (1997) Meth Enzymol 289: Solid-Phase Peptide Synthesis. ISBN: 0121821900. Solid-phase peptide synthesis techniques, such as methods based on tBoc or Fmoc chemistry, are known in the art, see the following reference: Chan & White (2000) Fmoc Solid Phase Peptide Synthesis. ISBN: 0199637245. Enzymatic synthesis may also be used in part or in full, see the following reference: Kullmann (1987) Enzymatic Peptide Synthesis. ISBN: 0849368413. As an alternative to chemical synthesis, biological synthesis may be used e.g. the polypeptides may be produced by translation. This may be carried out in vitro or in vivo. Biological methods are in general restricted to the production of polypeptides based on L-amino acids, but manipulation of translation machinery (e.g. of aminoacyl tRNA molecules) can be used to allow the introduction of D-amino acids (or of other non- natural amino acids, such as iodotyrosine or methylphenylalanine, azidohomoalanine, etc.), see the following reference: Kullmann (1987) Enzymatic Peptide Synthesis. ISBN: 0849368413. Where D-amino acids are included, however, it is preferred to use chemical synthesis. Polypeptides of the disclosure may have covalent modifications at the C-terminus and/or N-terminus. They can also take various forms (e.g. native, fusions, glycosylated, non-glycosylated, lipidated, non-lipidated, phosphorylated, non-phosphorylated, myristoylated, non-myristoylated, monomeric, multimeric, particulate, denatured, etc.). The polypeptides can be naturally or non- naturally glycosylated (i.e. the polypeptide may have a glycosylation pattern that differs from the glycosylation pattern found in the corresponding naturally occurring polypeptide).

[0071] Non-naturally occurring forms of polypeptides herein may comprise one or more heterologous amino acid sequences (e.g. another antigen sequence, another signal sequence, a detectable tag, or the like) in addition to a Zika virus prME antigen sequence or a chimeric Zika virus prME sequence. For example, a polypeptide herein may be a fusion protein. Alternatively, or in addition, the amino acid sequence or chemical structure of the polypeptide may be modified (e.g. with one or more non-natural amino acids, by covalent modification, and/or or by having a different glycosylation pattern, for example, by the removal or addition of one or more glycosyl groups) compared to a naturally-occurring polypeptide sequence.

[0072] Polypeptides (e.g. antigens) disclosed herein are preferably provided in purified or substantially purified form i.e. substantially free from other polypeptides (e.g. free from naturally-occurring polypeptides), particularly from other Zika virus or host cell polypeptides; for example, at least about 50% pure (by weight), at least about 60% pure (by weight), at least about 70% pure (by weight), at least about 80% pure (by weight), or at least about 90% pure, etc. Alternatively, less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 10%, or less than about 5% of a composition is made up of other expressed polypeptides.

Nucleic Acids

[0073] The present inventors disclose herein nucleic acid molecules comprising a sequence which encodes a Zika virus prME antigen. Nucleic acids as disclosed herein can take various forms (e.g. single-stranded, double-stranded, vectors etc.). Nucleic acids may be circular or branched, but will generally be linear.

[0074] The nucleic acids used herein are preferably provided in purified or substantially purified form i.e. substantially free from other nucleic acids (e.g. free from naturally-occurring nucleic acids), particularly from other Zika virus or host cell nucleic acids, generally being at least about 50% pure (by weight), at least about 60% pure (by weight), at least about 70% pure (by weight), at least about 80% pure (by weight), and usually at least about 90% pure (by weight).

[0075] Nucleic acids may be prepared in many ways e.g. by chemical synthesis (e.g. phosphoramidite synthesis of DNA) in whole or in part, by digesting longer nucleic acids using nucleases (e.g. restriction enzymes), by joining shorter nucleic acids or nucleotides (e.g. using ligases or polymerases), from genomic or cDNA libraries, etc.

[0076] The term "nucleic acid" in general means a polymeric form of nucleotides of any length, which contain deoxyribonucleotides, ribonucleotides, and/or their analogs. It includes DNA, RNA, DNA/RNA hybrids. It also includes DNA or RNA analogs, such as those containing modified backbones (e.g. peptide nucleic acids (PNAs) or phosphorothioates) or modified bases. Thus the nucleic acid of the disclosure includes mRNA, DNA, cDNA, recombinant nucleic acids, branched nucleic acids, plasmids, vectors, etc. Where the nucleic acid takes the form of RNA, it may or may not have a 5′ cap.

[0077] The nucleic acids herein comprise a sequence which encodes at least one Zika virus prME antigen. Typically, the nucleic acids of the invention will be in recombinant form, i.e. a form which does not occur in nature. For example, the nucleic acid may comprise one or more heterologous nucleic acid sequences (e.g. a sequence encoding another antigen and/or a control sequence such as a promo-

ter or an internal ribosome entry site) in addition to the sequence encoding at least one Zika virus prME antigen. The nucleic acid may be part of a vector i.e. part of a nucleic acid construct designed for transduction/transfection of one or more cell types. Vectors may be, for example, "expression vectors" which are designed for expression of a nucleotide sequence in a host cell, or "viral vectors" which are designed to result in the production of a recombinant virus or virus-like particle.

[0078] Alternatively, or in addition, the sequence or chemical structure of the nucleic acid may be modified compared to a naturally-occurring sequence which encodes a Zika virus prME antigen. The sequence of the nucleic acid molecule may be modified, e.g. to increase the efficacy of expression or replication of the nucleic acid, or to provide additional stability or resistance to degradation.

[0079] The nucleic acid encoding the polypeptides described above may be codon optimized. By "codon optimized" is intended modification with respect to codon usage that may increase translation efficacy and/or half- life of the nucleic acid. A poly A tail (e.g., of about 30 adenosine residues or more) may be attached to the 3′ end of the RNA to increase its half-life. The 5′ end of the RNA may be capped with a modified ribonucleotide with the structure m7G (5′) ppp (5′) N (cap 0 structure) or a derivative thereof, which can be incorporated during RNA synthesis or can be enzymatically engineered after RNA transcription (e.g., by using Vaccinia Virus Capping Enzyme (VCE) consisting of mRNA triphosphatase, guanylyl- transferase and guanine-7-methytransferase, which catalyzes the construction of N7-monomethylated cap 0 structures). Cap 0 structure plays an important role in maintaining the stability and translational efficacy of the RNA molecule. The 5′ cap of the RNA molecule may be further modified by a 2′-O-Methyltransferase which results in the generation of a cap 1 structure (m7Gppp [m2 ′-O] N), which may further increase translation efficacy.

[0080] The nucleic acids may comprise one or more nucleotide analogs or modified nucleotides. As used herein, "nucleotide analog" or "modified nucleotide" refers to a nucleotide that contains one or more chemical modifications (e.g., substitutions) in or on the nitrogenous base of the nucleoside (e.g., cytosine (C), thymine (T) or uracil (U)), adenine (A) or guanine (G)). A nucleotide analog can contain further chemical modifications in or on the sugar moiety of the nucleoside (e.g., ribose, deoxyribose, modified ribose, modified deoxyribose, six-membered sugar analog, or open-chain sugar analog), or the phosphate. The preparation of nucleotides and modified nucleotides and nucleosides are well-known in the art, see the following references: US Pat. Nos. 4373071, 4458066, 4500707, 4668777, 4973679, 5047524, 5132418, 5153319, 5262530, 5700642. Many modified nucleosides and modified nucleotides are commercially available.

[0081] Modified nucleobases which can be incorporated into modified nucleosides and nucleotides and be present in the RNA molecules include: m5C (5-methylcytidine), m5U (5-methyluridine), m6A (N6-methyladenosine), s2U (2-thiouridine), Um (2′-0-methyluridine), mlA (1-methyladenosine); m2A (2-methyladenosine); Am (2-1-0-methyladenosine); ms2m6A (2-methylthio-N6-methyladenosine); i6A (N6-isopentenyladenosine); ms2i6A (2-methylthio-N6-isopentenyladenosine); io6A (N6-(cis-hydroxyisopentenyl)adenosine); ms2io6A (2-methylthio-N6-(cis-hydroxyisopentenyl) adenosine); g6A (N6-glycinylcarbamoyladenosine); t6A (N6-threonyl carbamoyladenosine); ms2t6A (2-methylthio-N6-threonyl carbamoyladenosine); m6t6A (N6-methyl-N6-threonylcarbamoyladenosine); hn6A(N6-hydroxynorvalylcarbamoyl adenosine); ms2hn6A (2-methylthio-N6-hydroxynorvalyl carbamoyladenosine); Ar(p) (2′-0-ribosyladenosine (phosphate)); I (inosine); mil (1-methylinosine); m′lm (I,2′-0-dimethylinosine); m3C (3-methylcytidine); Cm (2T-0-methylcytidine); s2C (2-thiocytidine); ac4C (N4-acetylcytidine); 5FC (5-fonnylcytidine); m5Cm (5,2-O-dimethylcytidine); ac4Cm (N4acetyl2TOmethylcytidine); k2C (lysidine); mlG (1-methylguanosine); m2G (N2-methylguanosine); m7G (7-methylguanosine); Gm (2′-0-methylguanosine); m22G (N2,N2-dimethylguanosine); m2Gm (N2,2′-0-dimethylguanosine); m22Gm (N2,N2,2′-0-trimethylguanosine); Gr(p) (2′-0-ribosylguanosine (phosphate)); yW (wybutosine); o2yW (peroxywybutosine); OHyW (hydroxywybutosine); OHyW* (undermodified hydroxywybutosine); imG (wyosine); mimG (methylguanosine); Q (queuosine); oQ (epoxyqueuosine); galQ (galtactosyl-queuosine); manQ (mannosyl-queuosine); preQo (7-cyano-7-deazaguanosine); preQi (7-aminomethyl-7-deazaguanosine); G* (archaeosine); D (dihydrouridine); m5Um (5,2′-0-dimethyluridine); s4U (4-thiouridine); m5s2U (5-methyl-2-thiouridine); s2Um (2-thio-2′-0-methyluridine); acp3U (3-(3-amino-3-carboxypropyl)uridine); ho5U (5-hydroxyuridine); mo5U (5-methoxyuridine); cmo5U (uridine 5-oxyacetic acid); mcmo5U (uridine 5-oxyacetic acid methyl ester); chm5U (5-(carboxyhydroxymethyl)uridine)); mchm5U (5-(carboxyhydroxymethyl)uridine methyl ester); mcm5U (5-methoxycarbonyl methyluridine); mcm5Um (S-methoxycarbonylmethyl-2-O-methyluridine); mcm5s2U (5-methoxycarbonylmethyl-2-thiouridine); nm5s2U (5-aminomethyl-2-thiouridine); mnm5U (5-methylaminomethyluridine); mnm5s2U (5-methylaminomethyl-2-thiouridine); mnm5se2U (5-methylaminomethyl-2-selenouridine); ncm5U (5-carbamoylmethyl uridine); ncm5Um (5-carbamoylmethyl-2′-0-methyluridine); cmnm5U (5-carboxymethylaminomethyluridine); cnmm5Um (5-carboxymethy 1 aminomethyl-2-L-Omethyl uridine); cmnm5s2U (5-carboxymethylaminomethyl-2-thiouridine); m62A (N6,N6-dimethyladenosine); Tm (2′-0-methylinosine); m4C (N4-methylcytidine); m4Cm (N4,2-0-dimethylcytidine); hm5C (5-hydroxymethylcytidine); m3U (3-methyluridine); cm5U (5-carboxymethyluridine); m6Am (N6,T-0-dimethyladenosine); rn62Am (N6,N6,0-2-trimethyladenosine); m2′7G (N2,7-dimethylguanosine); m2′2′7G (N2,N2,7-trimethylguanosine); m3Um (3,2T-0-dimethyluridine); m5D (5-methyldihydrouridine); £5Cm (5-formyl-2′-0-methylcytidine); mlGm (I,2′-0-dimethylguanosine); m′Am (1,2-O-dimethyl adenosine) irinomethyluridine); tm5s2U (S-taurinomethyl-2-thiouridine)); iniG-14 (4-demethyl guanosine); imG2 (isoguanosine); ac6A (N6-acetyladenosine), hypoxanthine, inosine, 8-oxo-adenine, 7-substituted derivatives thereof, dihydrouracil, pseudouracil, 2-thiouracil, 4-thiouracil, 5-aminouracil, 5-(Ci-Ce)-alkyluracil, 5-methyluracil, 5-(C2-C6)-alkenyluracil, 5-(C2-Ce)-alkynyluracil, 5-(hydroxymethyl)uracil, 5-chlorouracil, 5-fluorouracil, 5-bromouracil, 5-hydroxycytosine, 5-(Ci-C6)-alkylcytosine, 5-methylcytosine, 5-(C2-C6)-alkenylcytosine, 5-(C2-C6)-alkynylcytosine, 5-chlorocytosine, 5-fluorocytosine, 5-bromocytosine, N2-dimethylguanine, 7-deazaguanine, 8-azaguanine, 7-deaza-7-substituted guanine, 7-deaza-7-(C2-C6)alkynylguanine, 7-deaza-8-substituted

guanine, 8-hydroxyguanine, 6-thioguanine, 8-oxoguanine, 2-aminopurine, 2-amino-6-chloropurine, 2,4-diaminopurine, 2,6-diaminopurine, 8-azapurine, substituted 7-deazapurine, 7-deaza-7-substituted purine, 7-deaza-8-substituted purine, hydrogen (abasic residue), m5C, m5U, m6A, s2U, W, or 2′-0-methyl-U. Many of these modified nucleobases and their corresponding ribonucleosides are available from commercial suppliers.

Nucleic Acid-based Vaccines

[0082] The present inventors disclose compositions comprising a nucleic acid sequence which encodes a polypeptide comprising a Zika virus antigen, variant or fragment thereof. Such compositions may be a nucleic acid-based vaccine. A further composition comprising a nucleic acid sequence which encodes one or more additional (e.g., a second, third, fourth, fifth or sixth) Zika virus antigens may also be provided as a nucleic acid-based vaccine. In some embodiments, a composition comprises a nucleic acid sequence encoding a Zika virus prME antigen from a first Zika virus strain and an additional nucleic acid sequence encoding an additional Zika virus prME antigen from one or more other strains of Zika virus. In some embodiments, a composition comprises a nucleic acid sequence encoding a Zika virus prME antigen and one or more additional (e.g., a second, third, fourth, fifth or sixth) Zika virus antigen. Alternatively, one or more additional non-Zika virus antigens may be encoded.

[0083] The nucleic acid may, for example, be RNA (i.e. an RNA-based vaccine) or DNA (i.e. a DNA-based vaccine, such as a plasmid DNA vaccine). In certain embodiments, the nucleic acid-based vaccine is an RNA-based vaccine. In certain embodiments, the RNA-based vaccine comprises a self-replicating RNA molecule, also referred to herein as a self-amplifying mRNA (SAM) molecule. The self-replicating RNA molecule may be an alphavirus-derived RNA replicon.

[0084] Self-replicating RNA molecules are well known in the art and can be produced by using replication elements derived from, e.g., alphaviruses, and substituting the structural viral proteins with a nucleotide sequence encoding a protein of interest. A self-replicating RNA molecule is typically a +-strand molecule which can be directly translated after delivery to a cell, and this translation provides a RNA-dependent RNA polymerase which then produces both antisense and sense transcripts from the delivered RNA. Thus, the delivered RNA leads to the production of multiple daughter RNAs. These daughter RNAs, as well as collinear subgenomic transcripts, may be translated themselves to provide in situ expression of an encoded antigen (i.e. a Zika virus prM-F antigen), or may be transcribed to provide further transcripts with the same sense as the delivered RNA which are translated to provide in situ expression of the antigen. The overall result of this sequence of transcriptions is a huge amplification in the number of the introduced replicon RNAs and so the encoded antigen becomes a major polypeptide product of the cells.

[0085] One suitable system for achieving self-replication in this manner is to use an alphavirus-based replicon. These replicons are +-stranded (positive sense-stranded) RNAs which lead to translation of a replicase (or replicase-transcriptase) after delivery to a cell. The replicase is translated as a polyprotein which auto-cleaves to provide a replication complex which creates genomic-strand copies of the +-strand delivered RNA. These negative (-)-stranded transcripts can themselves be transcribed to give further copies of the +-stranded parent RNA and also to give a subgenomic transcript which encodes the antigen. Translation of the subgenomic transcript thus leads to in situ expression of the antigen by the infected cell. Suitable alphavirus replicons can use a replicase from a Sindbis virus, a Semliki forest virus, an eastern equine encephalitis virus, a Venezuelan equine encephalitis virus, etc. Mutant or wild-type virus sequences can be used e.g. the attenuated TC83 mutant of VEEV has been used in replicons, see the following reference: WO2005/113782, the context of which is incorporated by reference.

[0086] In certain embodiments, the self-replicating RNA molecule described herein encodes (i) a RNA-dependent RNA polymerase which can transcribe RNA from the self-replicating RNA molecule and (ii) a Zika virus prME antigen. The polymerase can be an alphavirus replicase e.g. comprising one or more of alphavirus proteins nsPI, nsP2, nsP3 and nsP4.

[0087] Whereas natural alphavirus genomes encode structural virion proteins in addition to the non-structural replicase polyprotein, in certain embodiments, the self-replicating RNA molecules do not encode alphavirus structural proteins. Thus, the self-replicating RNA can lead to the production of genomic RNA copies of itself in a cell, but not to the production of RNA-containing virions. The inability to produce these virions means that, unlike a wild-type alphavirus, the self-replicating RNA molecule cannot perpetuate itself in infectious form. The alphavirus structural proteins which are necessary for perpetuation in wild- type viruses are absent from self-replicating RNAs of the present disclosure and their place is taken by gene(s) encoding the immunogen of interest, such that the subgenomic transcript encodes the immunogen rather than the structural alphavirus virion proteins.

[0088] Thus, a self-replicating RNA molecule useful with the invention may have two open reading frames. The first (5′) open reading frame encodes a replicase; the second (3′) open reading frame encodes an antigen. In some embodiments, the RNA may have additional (e.g. downstream) open reading frames e.g. to encode further antigens or to encode accessory polypeptides.

[0089] In certain embodiments, the self-replicating RNA molecule disclosed herein has a 5′ cap (e.g. a 7-methylguanosine). This cap can enhance in vivo translation of the RNA. In some embodiments, the 5′ sequence of the self-replicating RNA molecule must be selected to ensure compatibility with the encoded replicase.

[0090] A self-replicating RNA molecule may have a 3′ poly-A tail. It may also include a poly- A polymerase recognition sequence (e.g. AAUAAA) near its 3′ end.

[0091] Self-replicating RNA molecules can have various lengths but they are typically 5000-25000 nucleotides long. Self-replicating RNA molecules will typically be single-stranded. Single-stranded RNAs can generally initiate an adjuvant effect by binding to TLR7, TLR8, RNA helicases and/or PKR. RNA delivered in double-stranded form (dsRNA) can bind to TLR3, and this receptor can also be triggered by dsRNA which is formed either during replication of a single-stranded RNA or within the secondary structure of a single-stranded RNA.

[0092] The self-replicating RNA can conveniently be prepared by in vitro transcription (IVT). IVT can use a (cDNA) template created and propagated in plasmid form in bacteria, or created synthetically (for example by gene synthesis and/or polymerase chain-reaction (PCR) engineering methods). For instance, a DNA-dependent RNA polymerase (such as the bacteriophage T7, T3 or SP6 RNA polymerases) can be used to transcribe the self-replicating RNA from a DNA template. Appropriate capping and poly-A addition reactions can be used as required (although the replicon's poly-A is usually encoded within the DNA template). These RNA polymerases can have stringent requirements for the transcribed 5′ nucleotide(s) and in some embodiments these requirements must be matched with the requirements of the encoded replicase, to ensure that the IVT-transcribed RNA can function efficiently as a substrate for its self-encoded replicase.

[0093] A self-replicating RNA can include (in addition to any 5′ cap structure) one or more nucleotides having a modified nucleobase. A RNA used with the invention ideally includes only phosphodiester linkages between nucleosides, but in some embodiments, it can contain phosphoramidate, phosphorothioate, and/or methylphosphonate linkages.

[0094] The self-replicating RNA molecule may encode a single heterologous polypeptide antigen (i.e. a Zika virus prME antigen) or, optionally, two or more heterologous polypeptide antigens linked together in a way that each of the sequences retains its identity (e.g., linked in series) when expressed as an amino acid sequence. The heterologous polypeptides generated from the self-replicating RNA may then be produced as a fusion polypeptide or engineered in such a manner to result in separate polypeptide or peptide sequences.

[0095] The self-replicating RNA molecules described herein may be engineered to express multiple nucleotide sequences, from two or more open reading frames, thereby allowing co-expression of proteins, such as one, two or more Zika virus antigens (e.g. one, two or more Zika virus prME antigens) together with cytokines or other immunomodulators, which can enhance the generation of an immune response. Such a self-replicating RNA molecule might be particularly useful, for example, in the production of various gene products (e.g., proteins) at the same time, for example, as a bivalent or multivalent vaccine.

[0096] If desired, the self-replicating RNA molecules can be screened or analyzed to confirm their therapeutic and prophylactic properties using various in vitro or in vivo testing methods that are known to those of skill in the art. For example, vaccines comprising self-replicating RNA molecule can be tested for their effect on induction of proliferation or effector function of the particular lymphocyte type of interest, e.g., B cells, T cells, T cell lines, and T cell clones. For example, spleen cells from immunized mice can be isolated and the capacity of cytotoxic T lymphocytes to lyse autologous target cells that contain a self-replicating RNA molecule that encodes a Zika virus prME antigen. In addition, T helper cell differentiation can be analyzed by measuring proliferation or production of TH1 (IL-2 and IFN-γ) and /or TH2 (IL-4 and IL-5) cytokines by ELISA or directly in CD4+ T cells by cytoplasmic cytokine staining and flow cytometry.

[0097] Self-replicating RNA molecules that encode a Zika virus prME antigen can also be tested for ability to induce humoral immune responses, as evidenced, for example, by induction of B cell production of antibodies specific for a Zika virus prME antigen of interest. These assays can be conducted using, for example, peripheral B lymphocytes from immunized individuals. Such assay methods are known to those of skill in the art. Other assays that can be used to characterize the self-replicating RNA molecules can involve detecting expression of the encoded Zika virus prME antigen by the target cells. For example, FACS can be used to detect antigen expression on the cell surface or intracellularly. Another advantage of FACS selection is that one can sort for different levels of expression; sometimes-lower expression may be desired. Other suitable method for identifying cells which express a particular antigen involve panning using monoclonal antibodies on a plate or capture using magnetic beads coated with monoclonal antibodies.

[0098] In some embodiments, the self-replicating RNA molecules comprise a sequence selected from the group consisting of SEQ ID NO:36 and SEQ ID NO:37. In some embodiments, the self-replicating RNA molecules comprise a sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:36 and SEQ ID NO:37. In some embodiments, the self-replicating RNA molecule comprises a fragment of a full-length sequence selected from the group consisting of SEQ ID NO:36 and SEQ ID NO:37 wherein the fragment comprises a contiguous stretch of the nucleic acid sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 nucleic acids shorter than full-length sequence.

[0099] In some embodiments, a DNA sequence encoding a self-replicating RNA molecule is provided, said DNA sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:34. In some embodiments, DNA sequence encoding a self-replicating RNA molecule comprises a sequence which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:34. In some embodiments, the DNA sequence encoding a self-replicating RNA molecule comprises a fragment of a full-length sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:34 wherein the fragment comprises a contiguous stretch of the nucleic acid sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 nucleic acids shorter than full-length sequence.

[0100] The nucleic acid-based vaccine may comprise a viral or a non-viral delivery system. The delivery system (also referred to herein as a delivery vehicle) may have adjuvant effects which enhance the immunogenicity of the encoded Zika virus prME antigen. For example, the nucleic acid molecule may be encapsulated in liposomes, non-toxic biodegradable polymeric microparticles or viral replicon particles (VRPs), or complexed with particles of a cationic oil-in-water emulsion. In some embodiments, the nucleic acid-based vaccine comprises a cationic nano-emulsion (CNE) delivery system or a lipid nanoparticle (LNP) delivery system. In some embodiments, the nucleic acid-based vaccine comprises a non-viral delivery system, i.e., the nucleic acid-based vaccine is substantially free of viral capsid. Alternatively, the nucleic acid-based vaccine may comprise viral replicon particles. In other embodiments, the nucleic acid-based vaccine may comprise a naked nucleic

acid, such as naked RNA (e.g. mRNA), but delivery via CNEs or LNPs is preferred.

[0101] In certain embodiments, the nucleic acid-based vaccine comprises a cationic nano-emulsion (CNE) delivery system. CNE delivery systems and methods for their preparation are described in the following reference: WO2012/006380. In a CNE delivery system, the nucleic acid molecule (e.g. RNA) which encodes the antigen is complexed with a particle of a cationic oil-in-water emulsion. Cationic oil-in-water emulsions can be used to deliver negatively charged molecules, such as an RNA molecule to cells. The emulsion particles comprise an oil core and a cationic lipid. The cationic lipid can interact with the negatively charged molecule thereby anchoring the molecule to the emulsion particles. Further details of useful CNEs can be found in the following references: WO2012/006380; WO2013/006834; and WO2013/006837 (the contents of each of which are incorporated herein in their entirety).

[0102] Thus, in a nucleic acid-based vaccine of the invention, an RNA molecule encoding a Zika virus prME antigen may be complexed with a particle of a cationic oil-in-water emulsion. The particles typically comprise an oil core (e.g. a plant oil or squalene) that is in liquid phase at 25° C., a cationic lipid (e.g. phospholipid) and, optionally, a surfactant (e.g. sorbitan trioleate, polysorbate 80); polyethylene glycol can also be included. In some embodiments, the CNE comprises squalene and a cationic lipid, such as 1,2-dioleoyloxy-3-(trimethylammonio)propane (DOTAP). In some preferred embodiments, the delivery system is a non-viral delivery system, such as CNE, and the nucleic acid-based vaccine comprises a self-replicating RNA (mRNA). This may be particularly effective in eliciting humoral and cellular immune responses. Advantages also include the absence of a limiting anti-vector immune response and a lack of risk of genomic integration.

[0103] In some embodiments, an RNA molecule encoding a Zika virus prME antigen may be complexed with a submicron cationic oil-in-water emulsion. In some embodiments, the cationic oil-in-water emulsion is characterized by an average particle size of from about 80 nm to 180 nm in diameter (or alternatively from about 80 to about 150 nm; from about 80 to 130 nm; or from about 100 nm). In some embodiments, the concentration of DOTAP in said emulsion, before RNA complexation, is at least about 2.5 mM, or from about 2.5 mM to about 8 mM. In a particular embodiment, the concentration of DOTAP in said emulsion is about 4 mg/ml (5.73 mM). The oil can be squalene or squalane.

[0104] In some embodiments, an RNA molecule encoding a Zika virus prME antigen is complexed to a cationic oil-in-water emulsion comprising DOTAP, squalene, sorbitan trioleate and polysorbate 80 in citrate buffer. Cationic oil-in-water emulsions suitable for delivery of an RNA molecule encoding a Zika virus prME antigen may contain about 2 mg/ml to 7 mg/ml DOTAP; about 3 mg/ml to 6 mg/ml Span 85; about 3 mg/ml to 6 mg/ml TWEEN™ 80; and about 30 mg/ml to 50 mg/ml squalene. In certain embodiments, the cationic oil-in-water emulsion, before complexing with RNA, contains about 4.3% w/v squalene, 0.5% TWEEN™ 80, 0.5% SPAN85, and 4 mg/mL DOTAP.

[0105] Also provided is a method of preparing a composition comprising an RNA molecule encoding a Zika virus prME antigen complexed to a cationic oil-in-water emulsion, the method comprising: (i) providing an oil-in-water emulsion as described herein; (ii) providing an aqueous solution comprising the RNA molecule; and (iii) combining the aqueous solution of (ii) and the oil-in-water emulsion of (i), thereby preparing the composition. If desired, the aqueous solution comprising the RNA molecule may be a buffer. The buffer may comprise one or more salt, buffer, saccharide, or polymer. In a preferred embodiment, the buffer comprises 560 mM sucrose, 20 mM NaCl, and 10 mM citrate, which can be mixed with a cationic oil in water emulsion described herein to produce a final aqueous phase that comprises 280 mM sucrose, 10 mM NaCl and 10 mM citrate.

[0106] LNP delivery systems and non-toxic biodegradable polymeric microparticles, and methods for their preparation are described in the following references: WO2012/006376 (LNP and microparticle delivery systems); Geall et al. (2012) PNAS USA. Sep 4; 109(36): 14604-9 (LNP delivery system); and WO2012/006359 (microparticle delivery systems). LNPs are non- virion liposome particles in which a nucleic acid molecule (e.g. RNA) can be encapsulated. The particles can include some external RNA (e.g. on the surface of the particles), but at least half of the RNA (and ideally all of it) is encapsulated. Liposomal particles can, for example, be formed of a mixture of zwitterionic, cationic and anionic lipids which can be saturated or unsaturated, for example; DSPC (zwitterionic, saturated), DlinDMA (cationic, unsaturated), and/or DMG (anionic, saturated). Preferred LNPs for use with the invention include an amphiphilic lipid which can form liposomes, optionally in combination with at least one cationic lipid (such as DOTAP, DSDMA, DODMA, DLinDMA, DLenDMA, etc.). A mixture of DSPC, DlinDMA, PEG-DMG and cholesterol is particularly effective. Other useful LNPs are described in the following references: WO2012/006376; WO2012/030901; WO2012/031046; WO2012/031043; WO2012/006378; WO2011/076807; WO2013/033563; WO2013/006825; WO2014/136086; WO2015/095340; WO2015/095346; WO2016/037053. In some embodiments, the LNPs are RV01 liposomes, see the following references: WO2012/006376 and Geall et al. (2012) PNAS USA. Sep 4; 109(36): 14604-9.

Pharmaceutical Compositions; Immunogenic Compositions

[0107] The disclosure provides compositions comprising a nucleic acid comprising a sequence which encodes a Zika virus polypeptide, for example a Zika virus prME antigen. The composition may be a pharmaceutical composition, e.g., an immunogenic composition or a vaccine composition. Accordingly, the composition may also comprise a pharmaceutically acceptable carrier. In some embodiments, the Zika virus is a Brazilian strain Zika virus.

[0108] A "pharmaceutically acceptable carrier" includes any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, sucrose, trehalose, lactose, and lipid aggregates (such as oil droplets or liposomes). Such carriers are well known to those of ordinary skill in the art. The compositions may also contain a pharmaceutically acceptable diluent, such as water, saline, glycerol, etc. Additionally,

auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present. Sterile pyrogen-free, phosphate-buffered physiologic saline is a typical carrier.

[0109] Pharmaceutical compositions may include the constructs, nucleic acid sequences, and/or polypeptide sequences described elsewhere herein in plain water (e.g. "w.f.i.") or in a buffer e.g. a phosphate buffer, a Tris buffer, a borate buffer, a succinate buffer, a histidine buffer, or a citrate buffer. Buffer salts will typically be included in the 5-20 mM range. Pharmaceutical compositions may have a pH between 5.0 and 9.5 e.g. between 6.0 and 8.0. Compositions may include sodium salts (e.g. sodium chloride) to give tonicity. A concentration of 10±2 mg/mL NaCl is typical, e.g. about 9 mg/mL. Compositions may include metal ion chelators. These can prolong RNA stability by removing ions which can accelerate phosphodiester hydrolysis. Thus a composition may include one or more of EDTA, EGTA, BAPTA, pentetic acid, etc. Such chelators are typically present at between 10-500 μM e.g. 0.1 mM. A citrate salt, such as sodium citrate, can also act as a chelator, while advantageously also providing buffering activity. Pharmaceutical compositions may have an osmolality of between 200 mOsm/kg and 400 mOsm/kg, e.g. between 240-360 mOsm/kg, or between 290-310 mOsm/kg. Pharmaceutical compositions may include one or more preservatives, such as thiomersal or 2-phenoxyethanol. Mercury-free compositions are preferred, and preservative-free vaccines can be prepared. Pharmaceutical compositions may be aseptic or sterile. Pharmaceutical compositions may be non-pyrogenic e.g. containing <1 EU (endotoxin unit, a standard measure) per dose, and preferably <0.1 EU per dose. Pharmaceutical compositions may be gluten free. Pharmaceutical compositions may be prepared in unit dose form. In some embodiments, a unit dose may have a volume of between 0.1-1.0 mL e.g. about 0.5 mL.

[0110] In some embodiments, the compositions disclosed herein are immunogenic composition that, when administered to a subject, induce a humoral and/or cellular antigen-specific immune response (i.e. an immune response which specifically recognizes a naturally occurring Zika virus polypeptide). For example, an immunogenic composition may induce a memory T and/or B cell population relative to an untreated subject following Zika virus infection, particularly in those embodiments where the composition comprises a nucleic acid comprising a sequence which encodes a Zika virus prME antigen or comprises a Zika virus antigen. In some embodiments, the subject is a vertebrate, such as a mammal e.g. a human or a veterinary mammal.

[0111] The compositions of the invention can be formulated as vaccine compositions. The vaccine will comprise an immunologically effective amount of antigen. By "an immunologically effective amount" is intended that the administration of that amount to a subject, either in a single dose or as part of a series, is effective for inducing a measurable immune response against Zika virus in the subject. This amount varies depending upon the health and physical condition of the individual to be treated, age, the taxonomic group of individual to be treated (e.g. human, non-human primate, etc.), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the composition or vaccine, the treating doctor's assessment of the medical situation, the severity of the disease, the potency of the compound administered, the mode of administration, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. Vaccines as disclosed herein may either be prophylactic (i.e. to prevent infection) or therapeutic (i.e. to treat infection), but will typically be prophylactic. In some embodiments, the vaccine compositions disclosed herein may induce an effective immune response against a Zika virus infection, i.e., a response sufficient for treatment or prevention of a Zika virus infection.

[0112] In some embodiments, the composition further comprises an additional antigen. In some embodiments, the composition is administered to a subject in combination with a further composition which comprises an additional antigen.

[0113] A composition of the present disclosure may also comprise, or be administered in conjunction with, one or more adjuvants (e.g. vaccine adjuvants), in particular where the composition comprises an immunologically effective amount of a nucleic acid encoding a Zika virus prME antigen or a Zika virus prME antigen. By adjuvant is intended that is capable of increasing an immune response against an antigen compared to administration of said antigen alone. In some aspects, adjuvant compositions as disclosed herein further comprise one or more immunostimulants, for example, a saponin such as QS21.

[0114] Adjuvants which may be used in compositions of the invention include, but are not limited to: (A) Mineral-containing compositions, for example aluminum and calcium salts, such as aluminum phosphates. (B) Oil emulsions, for example squalene-in-water emulsions, such as MF59 or AS03. Complete Freund's adjuvant (CFA) and incomplete Freund's adjuvant (IF A) may also be used. (C) Saponin formulations. (D) Virosomes and virus-like particles (VLPs). (E) Bacterial or microbial derivatives such as non-toxic derivatives of enterobacterial lipopolysaccharide (LPS), Lipid A derivatives, immunostimulatory oligonucleotides and ADP-ribosylating toxins and detoxified derivatives thereof. (F) Human immunomodulators, for example cytokines, such as interleukins, interferons, macrophage colony stimulating factor, and tumor necrosis factor. (G) Bioadhesives and mucoadhesives, such as esterified hyaluronic acid microspheres, cross-linked derivatives of poly(acrylic acid), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides and carboxymethylcellulose. (H) Microparticles, for example particles of ~100 nm to ~150 μm in diameter, more preferably ~200 nm to ~30 μm in diameter, and most preferably ~500 nm to ~10 μm in diameter) formed from materials that are biodegradable and non-toxic (e.g. a poly(a-hydroxy acid), a polyhydroxybutyric acid, a polyorthoester, a polyanhydride, a polycaprolactone, etc.), with poly(lactide-co-glycolide) are preferred, optionally treated to have a negatively-charged surface (e.g. with SDS) or a positively-charged surface (e.g. with a cationic detergent, such as CTAB). (I) Liposomes. (J) Polyoxyethylene ether and polyoxyethylene ester formulations. (K) Polyphosphazene (PCPP). (L) Muramyl peptides. (M) Imidazoquinolone compounds, for example Imiquamod and its homologues.

[0115] Combinations of one or more of the adjuvants identified above may also be used with the invention.

Methods of Use/Uses

[0116] In some embodiments are provided methods for inducing an immune response against a Zika virus infection in a subject in need thereof comprising a step of administering an immunologically effective amount of a construct or composition as disclosed herein. In some embodiments are provided the use of the constructs or compositions disclosed herein for inducing an immune response to a Zika virus prME antigen in a subject in need thereof. In some embodiments are provided the use of the constructs or compositions disclosed herein for inducing an immune response against a Zika virus infection in a subject. In some embodiments are provided use of the construct or composition as disclosed herein in the manufacture of a medicament that induces an immune response to a Zika virus infection in a subject. By "subject" is intended a vertebrate, such as a mammal e.g. a human or a veterinary mammal. In some embodiments, the subject is human. By "immune response" is intended a humoral and/or cellular antigen-specific immunological response (i.e. an immune response which specifically recognizes an antigen polypeptide) that can be demonstrated to neutralize Zika virus in vitro or control/reduce/eliminate Zika virus infection in vivo.

[0117] In some embodiments, the immune response is characterized by immunological memory against the Zika virus and/or an effective Zika virus-responsive memory T cell population.

[0118] In some embodiments the composition comprises an RNA molecule encoding a polypeptide selected from the group consisting of SEQ ID NO:19 and SEQ ID NO:24. In some embodiments, the composition comprises an RNA molecule encoding a polypeptide which is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a sequence selected from the group consisting of SEQ ID NO:19 and SEQ ID NO:24. In some embodiments, the composition comprises an RNA molecule encoding a polypeptide which comprises a fragment of a full-length sequence selected from the group consisting of SEQ ID NO:19 and SEQ ID NO:24, wherein the fragment comprises a contiguous stretch of the amino acid sequence of the full-length sequence up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids shorter than full-length sequence.

[0119] In some embodiments is provided a construct, a vector, a self-replicating RNA and/or molecule as described herein for use in therapy or medicine. In some embodiments, the compositions disclosed herein are for use in therapy or medicine. In preferred embodiment, the therapy is a vaccine therapy. Preferably the therapy is vaccine to prevent Zika virus infection.

[0120] In some embodiments is provided a construct, a vector, a self-replicating RNA and/or molecule as described herein for use in preventing or treating Zika virus infection in a subject in need thereof.

[0121] In some embodiments, the compositions disclosed herein are for use in preventing or treating Zika virus infection in a subject in need thereof.

[0122] In some embodiments, the compositions disclosed herein are for use in inducing an immune response against a Zika virus infection in a subject in need thereof.

[0123] In some embodiments is provided a construct, a vector, a self-replicating RNA molecule, and/or a composition as described herein for use in a method of inducing an immune response to a Zika virus infection in a subject in need thereof.

[0124] In some embodiments, methods are provided for preventing or shortening Zika virus infection and/or reducing or preventing the clinical symptoms upon Zika virus infection in a subject in need thereof, which comprises administering to said subject an immunologically effective amount of an immunogenic composition as provided herein.

[0125] In some embodiments is provided use of a construct or composition disclosed herein in the manufacture of an immunogenic composition for preventing or shortening Zika virus infection in a subject and/or reducing or prevent the clinical symptoms upon Zika virus infection in a subject.

[0126] In some embodiments, methods are provided for preventing or reducing transmission of a Zika virus infection from one subject to another. In specific embodiments, methods are provided for preventing or reducing transmission of a Zika virus infection to a fetus across the placental barrier. In some embodiments, a composition as described herein is administered to a woman in an amount effective to prevent transmission of a Zika virus infection across the placental barrier.

[0127] In some embodiments is provided use of a construct or composition disclosed herein in the manufacture of an immunogenic composition for preventing or reducing transmission of a Zika virus infection to a fetus across the placental barrier.

[0128] In some embodiments is provided a construct, a vector, a self-replicating RNA molecule, and/or a composition as described herein for use in a method of preventing or reducing transmission of a Zika virus infection to a fetus across the placental barrier.

[0129] In some embodiments, the subject is a human subject. In specific embodiments, the human subject has been exposed, or is at risk of being exposed, to a Zika virus infection.

Routes of Administration/Dosages

[0130] Compositions disclosed herein will generally be administered directly to a subject. Direct delivery may be accomplished by parenteral injection {e.g. subcutaneously, intraperitoneally, intravenously, intramuscularly, intradermally, or to the interstitial space of a tissue). Alternative delivery routes include rectal, oral (e.g. tablet, spray), buccal, sublingual, vaginal, topical, transdermal or transcutaneous, intranasal, ocular, aural, pulmonary or other mucosal administration. Intradermal and intramuscular administration are two preferred routes. Injection may be via a needle (e.g. a hypodermic needle), but needle-free injection may alternatively be used. A typical human intramuscular dose volume is 0.5 ml.

[0131] A dose of a nucleic acid (e.g. a nucleic acid-based vaccine, such as a Zika SAM vaccine) may have about 50 μg to about 100 μg nucleic acid. In one embodiment, a Zika SAM vaccine dose contains 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 μg RNA. In other embodiments, a dose of a Zika SAM vaccine may have <10 μg nucleic acid; e.g. from 1-10 μg, such as about 1 μg, 2.5 μg, 5 μg, 7.5 μg or 10 μg, but expression can be seen at much lower levels; e.g. using <1 μg/dose, <100 ng/dose, <10 ng/dose, <1 ng/dose, etc. Similarly, a dose of a protein antigen may have <10 μg

protein; e.g. from 1-10 μg, such as about 1 μg, 2.5 μg, 5 μg, 7.5 μg or 10 μg.

[0132] In preferred embodiments, a Zika SAM vaccine or vaccine composition is administered to a subject at an effective dose, meaning a dose sufficient to achieve a desired immune response, such as induction of neutralizing antibodies to Zika virus and/or protection against Zika virus infection.

[0133] In some embodiments, a Zika SAM vaccine described herein has an effective dose that is less than or equal to 50%, 40%, 30%, 20% or 10% of the effective dose of a DNA vaccine or vaccine composition encoding the same antigen. In some embodiments, a Zika SAM vaccine described herein has an effective dose that is one third or less of the effective dose of a DNA vaccine or vaccine composition encoding the same antigen.

Processes of Manufacture/Formulation

[0134] Processes for the manufacture of self-replicating RNA are provided herein. In some embodiments, the process of manufacturing a self-replicating RNA comprises a step of in vitro transcription (IVT) as described elsewhere herein. In some embodiments, the process of manufacturing a self-replicating RNA comprises a step of IVT to produce a RNA, and further comprises a step of combining the RNA with a non-viral delivery system as described elsewhere herein. In some embodiments, the process of manufacturing a self-replicating RNA comprises a step of IVT to produce a RNA, and further comprises a step of combining the RNA with a CNE delivery system as described elsewhere herein.

Sequence Identity

[0135] Identity or homology with respect to an amino acid sequence is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the reference amino acid sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Identity or homology with respect to a nucleic acid sequence is defined herein as the percentage of nucleotides in the candidate sequence that are identical with the reference nucleic acid sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity.

[0136] Sequence identity can be determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. Using a computer program such as BLAST, two polypeptides are aligned for optimal matching of their respective amino acids (either along the full length of one or both sequences or along a pre-determined portion of one or both sequences). The programs provide a default opening penalty and a default gap penalty, and a scoring matrix such as PAM 250 [a standard scoring matrix; see Dayhoff et al., in Atlas of Protein Sequence and Structure, vol. 5, supp. 3 (1978)] can be used in conjunction with the computer program. For example, the percent identity can then be calculated as: the total number of identical matches multiplied by 100 and then divided by the sum of the length of the longer sequence within the matched span and the number of gaps introduced into the shorter sequences in order to align the two sequences. The same methods used to compare polypeptides can also be used to calculate the percent identity of two polynucleotide sequences.

[0137] Where the present disclosure refers to a sequence by reference to a UniProt or Genbank accession code, the sequence referred to is the current version at the filing date of the present application.

General

[0138] Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. The term "plurality" refers to two or more. Additionally, numerical limitations given with respect to concentrations or levels of a substance, such as solution component concentrations or ratios thereof, and reaction conditions such as temperatures, pressures and cycle times are intended to be approximate. The term "about" used herein is intended to mean the amount ±10%.

[0139] The term "comprises" means "includes." Thus, unless the context requires otherwise, the word "comprises," and variations such as "comprise" and "comprising" will be understood to imply the inclusion of a stated compound or composition (e.g., nucleic acid, polypeptide, antigen) or step, or group of compounds or steps, but not to the exclusion of any other compounds, composition, steps, or groups thereof. Embodiments described as comprising certain components are intended to include embodiments consisting of the indicated components.

[0140] The abbreviation, "e.g." is derived from the Latin exempli gratia, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example."

[0141] The invention will be further described by reference to the following, non-limiting, figures and examples.

EXAMPLES

Example 1. Project Summary

[0142] The present inventors initiated work on a Zika vaccine using the SAM platform - synthetic, self-amplifying mRNA (SAM) derived from the alphavirus genome, expressing antigens of interest. The SAM constructs are evaluated for robust antigen production and antigenicity and further tested for their immunogenicity and efficacy using in vivo models.

Methods

[0143] The SAM vector VEE TC-83 was used as the background construct for cloning in the Examples. See SEQ ID NO:17.

Example 2. Selection of Antigen

[0144] The Flavivirus genome consists of capped single-stranded RNA of positive polarity of approximately 11.3 kb in length (FIG. **1**). The 5′ proximal quarter of the genome encodes the structural proteins capsid (C), pre-membrane (prM), and envelope (E). The nonstructural proteins NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 are involved

in viral RNA replication. The coding region is flanked by 5′ and 3′ untranslated regions (5′ and 3′ UTRs) of approximately 100 and 600 nucleotides in length, respectively. Translation of the viral genome yields a single polypeptide that is processed into the individual proteins by a combination of cellular proteases and a viral protease consisting of a catalytic subunit, NS3, and its cofactor, NS2B.

[0145] The structural proteins prM and E are cotranslationally inserted into the endoplasmic reticulum (ER) membrane and processed by signal peptidases, producing proteins that encapsidate C together with the viral RNA, by budding into the ER lumen (FIG. **2**). At a later step in viral maturation, prM on these particles is cleaved into mature M protein by a cellular furin protease prior to release from the cell. This prM cleavage is required for infectivity of the released virions. In addition to infectious virions, *flavivirus*-infected cells release sub-viral particles (SVPs) (FIG. **2**). These particles are smaller than virions, but contain the antigenically important E protein and the prM/M protein, which is essential for correct folding and incorporation of the E protein into SVPs and viral particles. However, unlike virions, SVPs do not contain either the C protein or the viral genome, and are thus non-infectious. SVPs can be produced in a variety of systems by co-expression of the prM and E proteins, and SVPs share properties with wild-type viruses, such as fusogenic activity and induction of a neutralizing immune response and have repeatedly been shown to stimulate protective immune responses against a number of *flavivirus* diseases. The present inventors selected structural proteins of Zika virus, namely- prM and E, for further experimentation.

Example 3. Strain Selection

[0146] The amino acid sequences of the C-prME proteins from Zika virus strains (available from NCBI/Genbank) from Zika outbreaks around the world from 2007 onwards were aligned to look for similarities and differences (FIG. **3**). These included the original African lineage strain from Uganda, Micronesia (2007), French Polynesia (2013), and the Brazilian strains from 2016 (FIG. **3**). In addition, seven strains of Zika virus from various regions in Brazil, were also compared for amino acid differences in the C-prME region (FIG. **4**). A high conservation was observed across the strains from different outbreaks, with the Brazilian strains almost identical in the CprME region. The Natal, Bahia strain (KU527068) was chosen as the representative strain. KU527068 was one of the first strains to be isolated from the brain of a fetus showing microcephaly.

Example 4. Design of Constructs

[0147] The design of Zika-SAM constructs of FIG. **6** includes cloning the sequence encoding the Zika virus (Natal, Brazil strain) structural pre-membrane (prM) and envelope (E) proteins under the subgenomic promoter in a SAM vector. A series of modifications to the SAM-prME constructs were made (Table 1, FIG. **6**). These include:

[0148] i. Substitution of the native prM signal peptide with the JEV signal peptide (FIGS. **6**A-B, antigen inserts #5283 and #5288).

[0149] ii. Substitution of the native C-terminus of Zika E protein with the C-terminus of JEV E protein (FIG. **6**B, antigen insert #5288).

[0150] iii. Truncation of the wild-type Zika prM start sequence to impair expression and secretion of the prME antigen (antigen insert #4974). Antigen insert #4974 was used as a negative control. The amino acid sequence of antigen insert #4974 is shown in SEQ ID NO:29, including the JEV signal sequence (SEQ ID NO:5), followed by a truncated Zika pr region (SEQ ID NO:30), Zika M protein (SEQ ID NO:31) and Zika E protein (SEQ ID NO:32). The DNA and RNA sequences encoding antigen insert #4974 are presented in SEQ ID NOs:28 and 41, respectively.

[0151] iv. A non-Zika antigen construct, #8111, was designed and used as a positive control.

Evaluation/Study Design

[0152] The constructs are evaluated in mammalian cells following electroporation of Zika-SAM RNA into BHK cells using the following methods:

[0153] a. SAM RNA replication- potency of the SAM-Zika constructs is tested by using antibodies against dsRNA and FACS.

[0154] b. Antigen expression is determined by immunoblots and immunofluorescence assays, to investigate cleaved prM and E protein in cell lysates and cell supernatant.

[0155] c. The production of SVPs is tested in mammalian cells by using established procedures for SVP isolation from cell supernatant.

[0156] Following identification of the most efficient candidate constructs formulation into LNP/CNE based-delivery systems is carried out and testing for antigenicity and immunogenicity is carried out in vivo.

Example 5. Expression and Secretion of Zika-SAM Constructs

[0157] The ability of cells to express and secrete Zika E protein and hybrid Zika-JEV E protein from the Zika-SAM constructs described above was evaluated according to the following methods.

[0158] On Day 0, BH K cells were plated at 8×10^6 in T225 flasks in Growth Media. For trypsinization, media was removed and cells were washed with 5 mls of PBS. The PBS wash was removed, and 5 mls of pre-warmed trypsin was added and spread thoroughly across the plate. Trypsin was removed and plates were kept at 37 deg C for 1-2 mins. Cells were then resuspended in 10 mls of growth media (5% FBS). Cells were counted and plated at required concentration into a new flask. The cells were then incubated at 37° C., 5% CO_2 for about 20 hours.

[0159] On Day 1, plates were prepared by adding 2 ml DMEM + 1% FBS + P/S (outgrowth media) to each well of a 6-well plate (one well per electroporation). Plates were kept warm in a 37° C. incubator. The electroporator was prepared to deliver 120 V, 25 ms pulse, 0.0 pulse interval, 1 pulse for a 2 mm cuvette. Cuvettes were labeled and kept on ice. Cells in growth phase were harvested as normal into BHK media (growth) and counted using a hemocytometer. Cells were trypsinized following the same trypsinization protocol as above. Standards and negative control electroporations were also prepared.

[0160] Cells were centrifuged at 1500 rpm (462x g) for 5 mins. Media was aspirated, and cells were washed once with 20 ml cold Opti-MEM media. Cells were again centrifuged at 1500 rpm (462x g) for 5 mins. Media was aspirated,

and the cells were resuspended in Opti-MEM media to 0.25 ml per electroporation.

[0161] For each sample, 4000 ng of RNA was mixed with 250 ul cells, and the mixture was pipetted gently 4-5 times. The cells and RNA mixture were transferred to 2 mm cuvettes and subjected to one pulse of electroporation using the parameters described above. Cells were allowed to rest at room temperature for 10 mins. Cells from one cuvette were added to one well of a pre-warmed 6-well plate, and the plate was tipped front and back and then side to side at a 45° angle to distribute cells evenly.

[0162] On Day 2 (30 h post-electroporation), the supernatant was collected. An aliquot of 75 ul was removed for Western blot, 25 ul 4X NuPAGE buffer was added to the aliquot (no reducing agent), and the aliquot was stored at -20° C. The rest of the supernatant was stored at -80° C.

[0163] Cells were washed once with ice cold PBS, and then scraped into 200 ul of RIPA buffer containing protease inhibitor cocktail (1 tablet in 10 ml) while keeping the plate on ice. The buffer containing cells was collected in microcentrifuge tubes, and subjected to two rounds of freeze thawing on dry ice. Samples were vortexed briefly, and pelleted at 8000 rpm for 5 min. Pellets were discarded and the supernatants were retained. 25 ul 4X NuPAGE buffer was added to a 75 ul aliquot of the lysates for Western blotting. Aliquots were stored at -20° C. The rest of the lysates were stored at -80° C.

Immunoblotting

[0164] 15 ul of the cell culture supernatants and 15 ul of the cell lysates were run on a 4-12% SDS PAGE gel (Bis-Tris) in 1X MOPS running buffer. The separated samples were transferred onto nitrocellulose membranes. Membranes were blocked for 2-3 hours in PBS-Tween 20 +5% milk. The *flavivirus* 4G2 primary antibody was added at 1:120 dilution in PBS-T-Milk and membranes were incubated overnight at 4° C. Membranes were then washed 3 times for 10 minutes each in PBS-T. Anti-mouse secondary (Odyssey® anti-Mouse 800CW-green (LI-COR, Inc., Lincoln, NE) at 1:5000) in LI-COR blocking buffer was then added, and the membranes were incubated for 1 hour. Membranes were washed three times for 2 minutes each, and then scanned on LI-COR Odyssey® imager (LI-COR, Inc., Lincoln, NE) at 800 channel, medium intensity.

Results

[0165] Zika E protein expression and secretion was detectable by immunoblot in lysates from cells electroporated with Zika-SAM constructs #5283 (JEV signal + wild type Zika prME), #5288 (JEV signal + hybrid Zika-JEV prME) and #8111 (positive control antigen). (FIG. **7**). Expression and secretion of Zika E protein were not detected in the N-terminally truncated construct (#4974) or Mock controls.

Example 6: Cationic Oil-in-Water Emulsions

[0166] Cationic nanoemulsions (CNEs) were prepared essentially according to the methods described in Brito et al., Molecular Therapy, Vol. 22, No. 12, pp. 2118-29 (2014) and International Patent Publication WO2013006834.

[0167] Briefly, squalene (Sigma, St. Louis, MO) was heated to 37° C., and DOTAP (Lipoid, Ludwigshafen Germany) was dissolved directly in squalene in the presence of sorbitan trioleate (SPAN 85; Sigma, St. Louis, MO). The resulting oil phase was then combined with the aqueous phase (TWEEN™; Sigma, St. Louis, MO, in citrate buffer) and immediately homogenized for 2 min using an T25 homogenizer (IKA, Wilmington, NC) at 24K RPM to produce a primary emulsion. The primary emulsions were passed three to five times through a M-110S Microfluidizer or a M-110P Microfluidizer (Microfluidics, Newton, MA) with an ice bath cooling coil at a homogenization pressure of approximately 15K-20K PSI. The batch samples were removed from the unit and stored at 4° C. The CNE formulation used in the present examples contains 4 mg/ml DOTAP; 4.7 mg/ml Span 85; 4.7 mg/ml TWEEN™; and 39 mg/ml squalene.

Example 7. Preparation of RNA-CNE Complexes

1. RNA Synthesis

[0168] Zika SAM constructs contain a bacteriophage T7 promoter located upstream of the alphavirus cDNA to facilitate the synthesis of the replicon RNA in vitro. SAM-Zika RNA for constructs #5283 (encoding JEV signal + Zika prME) and #5288 (encoding JEV signal + hybrid Zika-JEV prME), as well as negative control construct #4974, were synthesized using standard molecular biology techniques. Briefly, plasmid DNA encoding Zika-SAM constructs were linearized by endonuclease digestion a unique site located at the 3′ end of the replicon sequence. The linearized DNA was then transcribed into RNA by in vitro synthesis using a T7 RNA polymerase in the presence of the template DNA and nucleoside triphosphates (ATP, CTP, GTP and UTP). Following transcription, DNA template was digested with DNase, and the RNA transcripts were purified by LiCI precipitation and reconstituted in nuclease-free water. RNA was then capped using the Vaccinia Capping System (New England BioLabs, Ipswich, MA) and purified by LiCI precipitation. RNA concentration in each reaction was determined by spectrophotometry. Prior to RNA complexation, RNA was diluted to a concentration of 300 μg/ml in citrate buffer (10 mM citrate pH 6.2, 20 mM NaCl, 560 mM sucrose).

2. RNA Complexation

[0169] Zika SAM RNA was complexed with cationic nanoemulsion (CNE) particles essentially as described in Brito et al., Molecular Therapy, Vol. 22, No. 12, pp. 2118-29 (2014). Briefly, Zika SAM RNA (300 μg/ml in citrate buffer) was added to an equal volume of the CNE produced in Example 6, mixed, and allowed to complex on ice for 30 minutes to 2 hours. The final concentration of CNE-complexed RNA was 150 μg/ml.

[0170] The ratio of RNA to cationic lipid can be expressed as an N/P ratio, defined as the amount (moles) of protonatable nitrogen (N) atoms in the cationic lipid divided by the amount (moles) of phosphates (P) on the RNA. DOTAP for example has one nitrogen that can be protonated per molecule. The RNA concentration was used to calculate the amount of phosphate in solution using an estimate of 3 nmols of phosphate per microgram of RNA. The CNE formulations described above have an N/P ratio of 6.3:1.

Example 8. In Vivo Immunogenicity and Protection of Zika SAM CNE Formulations

[0171] Immunogenicity and protection of Zika SAM CNE formulations were examined in mice and non-human primates (NHP).

I. Mouse Study

[0172] Female BALB/c mice (6-12 weeks old; The Jackson Laboratory), were housed and bred in the animal facility of the Vaccine Research Center, NIAID, NIH, Bethesda, MD. All animal experiments were reviewed and approved by the Animal Care and Use Committee of the VRC, NIAID, NIH. All animals were housed and cared for in accordance with local, state, federal, and institutional policies in an American Association for Accreditation of Laboratory Animal Care-accredited facility at the NIH.

[0173] Mice were immunized twice according to the study design shown in Table 2. Briefly, groups of 10 mice each were administered CNE formulations containing the Zika SAM RNA constructs #5288 or #5283, or the negative control RNA construct #4974. Another group of mice received 50 μg of a DNA vaccine encoding construct #5283 by intramuscular electroporation, as a positive control. See Dowd et al., Science, Vol. 354 Issue 6309, pp. 237-40 (2016). All mice were challenged by intraperitoneal (i.p.) injection of live Zika virus on day 49.

TABLE 2

Mouse study design

Group	n	Delivery	Construct	Immunization Day 0	Immunization Day 21	Challenge Day 49
1	10	CNE56 / RNA	4974	1.5 μg	1.5 μg	100 PFU, IP
2	10	CNE56 / RNA	5288	1.5 μg	1.5 μg	100 PFU, IP
3	10	CNE56 / RNA	5283	15 μg	15 μg	100 PFU, IP
4	10	CNE56 / RNA	5283	1.5 μg	1.5 μg	100 PFU, IP
5	10	Electroporation/ DNA	5283	50 μg	50 μg	100 PFU, IP

[0174] Blood sera were collected on day 0, as well as 2 weeks after the first immunization, 2 weeks after the second immunization, and three days after the Zika virus challenge.

[0175] Zika neutralizing antibody titers were measured by reporter virus particle (RVP) neutralization assay according to methods described in Dowd, KA et al. Cell Rep. 16(6):1485-9 (2016). Results are shown in FIG. **8**. Two weeks after the first immunization with Zika SAM construct #5283, or the positive control DNA construct, there were significant levels of Zika neutralizing antibodies detected in the sera of immunized mice. Zika neutralizing antibody levels were even higher two weeks after the second immunization with the SAM construct #5283 or the positive control DNA construct.

[0176] A dose-dependent effect was observed, with the 15 μg dose of Zika SAM construct #5283 producing higher levels of neutralizing antibodies than the 1.5 μg dose. Notably, the 15 μg dose of Zika SAM construct #5283 produced a neutralizing antibody response that was comparable to the 50 μg dose of the DNA format of the same vaccine construct (DNA #5283). These results indicate that Zika SAM construct #5283 is capable of inducing a significant neutralizing antibody response to Zika virus.

[0177] On day 49 of the study, mice were challenged with intraperitoneal injections of live Zika virus (strain PRVABC57) at a dose of 100 plaque forming units (PFU). Serum samples were taken three days after challenge, and viral loads were determined by real time quantitative PCR (qPCR) of the Zika virus capsid gene.

[0178] As shown in FIG. **9**, mice vaccinated with Zika SAM construct #5283 (1.5 or 15 μg doses) or the positive control construct (DNA #5283) showed little or no detectable Zika virus in the serum. In contrast, significant Zika viral load was detected in unvaccinated animals, as well as in animals vaccinated with Zika SAM construct 5288 or negative control construct #4974. These results indicate that Zika SAM construct #5283 is capable of generating a protective immune response against Zika virus infection.

II. Non-Human Primate (NHP) Study

[0179] Immunogenicity of Zika SAM constructs was evaluated in non-human primates (NHPs). Rhesus macaques were immunized twice according to the study design shown in Table 3. Briefly, SAM-Zika RNA-CNE formulations were prepared as described in Example 7. Groups of 8 NHPs each were administered either placebo (phosphate buffered saline) or a CNE formulation containing a codon-optimized SAM construct, Co.prME, encoding the native Zika prME antigen (Group 2, 75 ug × 2; as described in PCT/IB2017/053242, filed Jun. 1, 2017); or Zika SAM construct #5283 (Group 3; 75 ug × 2). Another group of NHPs (Group 4; n=8) received two immunizations (4 mg each) of a DNA vaccine encoding construct #5283 intramuscularly by a needle-free injection device (PharmaJet), as a positive control. See Dowd et al., Science, Vol. 354 Issue 6309, pp. 237-40 (2016). All animals were challenged by intraperitoneal (i.p.) injection of live Zika virus (1000 PFU) 8 weeks after the first immunization.

TABLE 3

Group	Vaccine	Group size	Week 0 Vaccination	Week 4 Boost	Week 8 Challenge
1	Placebo	8	PBS	PBS	1000 PFU ZIKV
2	Co.prME SAM	8	75 μg	75 μg	1000 PFU ZIKV
3	SAM #5283	8	75 μg	75 μg	1000 PFU ZIKV
4	DNA #5283	8	4 mg	4 mg	1000 PFU ZIKV

[0180] Blood sera were collected on day 0, as well as 4 and 8 weeks after the first immunization; at each of days 3-7 after the Zika virus challenge; and at weeks 8, 10 and 12 after Zika virus challenge. Zika neutralizing antibody titers were measured by reporter virus particle (RVP) neutralization assay according to methods described in Dowd, KA et al. Cell Rep. 16(6):1485-9 (2016).

[0181] The immunogenicity results shown in FIG. **10** demonstrate that Zika neutralizing antibodies were significantly elevated four weeks after the first immunization of Zika SAM construct #5283 as compared to placebo, with titers being further increased 4 weeks after the second immunization. Zika DNA #5283 elicited similar neutralizing antibody titers at 4 and 8 weeks. Zika-SAM construct Co.prME SAM produced significantly less neutralizing antibodies compared to DNA #5283 after a single dose, but similar titers after two injections.

[0182] In week 8 of the study, NHPs were challenged with intraperitoneal injections of live Zika virus (strain PRVABC57) at a dose of 1000 plaque forming units (PFU). Viral loads after challenge were determined by real time quantitative PCR (qPCR) of the Zika virus capsid gene.

[0183] As shown in FIG. **11**, placebo animals exhibited elevated viremia as early as 3 days post-challenge (A). Vaccination with Zika-SAM construct #5283 (B), as well as positive controls Zika-SAM Co.prME (C) and DNA #5283 (D), were protective against Zika viremia, with Zika-SAM construct #5283 exhibiting a complete protection against Zika viremia.

[0184] Consistent with the viremia results, placebo animals showed a sharp rise in neutralizing antibodies after Zika challenge, further confirming Zika infection in these animals (FIG. **12**). Two animals in the SAM Co.prME group, and one animal in the DNA #5283 group, also showed elevated neutralizing antibody titers post-challenge, indicating that protection was not sterilizing in these animals. In contrast, animals in the Zika SAM #5283 group did not exhibit elevated neutralizing antibodies after Zika challenge, indicating that sterilizing protection was achieved in all subjects.

SEQUENCE LISTING

Sequence total quantity: 41
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FEATURE Location/Qualifiers
source 1..2079
mol_type = genomic DNA
organism = Zika virus

SEQ ID NO: 1

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gccatcgctt ggcttttggg aagctcaacg agccaaaaag tcatatactt ggtcatgata  540
ctgctgattg ccccggcata cagcatcagg tgcataggag tcagcaatag ggactttgtg  600
gaaggtatgt caggtgggac ttgggttgat gttgtcttgg aacatggagg ttgtgtcacc  660
gtaatggcac aggacaaacc gactgtcgac atagagctgg ttacaacaac agtcagcaac  720
atggcggagg taagatccta ctgctatgag gcatcaatat cagacatggc ttcggacagc  780
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gagaatctgg agtaccggat aatgctgtca gttcatggct cccagcacag tgggatgatc  1020
gttaatgaca caggacatga aactgatgag aatagagcga aggttgagat aacgcccaat  1080
tcaccaagag ccgaagccac cctgggggt tttggaagcc taggacttga ttgtgaaccg  1140
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atgtggttgg gtctgaacac aaagaatgga tctatttccc ttatgtgctt ggccttaggg  2040
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mol_type = protein
organism = Zika virus

SEQ ID NO: 2

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STRKLQTRSQ TWLESREYTK HLIRVENWIF RNPGFALAAA AIAWLLGSST SQKVIYLVMI  180
LLIAPAYSIR CIGVSNRDFV EGMSGGTWVD VVLEHGGCVT VMAQDKPTVD IELVTTTVSN  240
MAEVRSYCYE ASISDMASDS RCPTQGEAYL DKQSDTQYVC KRTLVDRGWG NGCGLFGKGS  300
LVTCAKFACS KKMTGKSIQP ENLEYRIMLS VHGSQHSGMI VNDTGHETDE NRAKVEITPN  360
SPRAEATLGG FGSLGLDCEP RTGLDFSDLY YLTMNNKHWL VHKEWFHDIP LPWHAGADTG  420
TPHWNNKEAL VEFKDAHAKR QTVVVLGSQE GAVHTALAGA LEAEMDGAKG RLSSGHLKCR  480
LKMDKLRLKG VSYSLCTAAF TFTKIPAETL HGTVTVEVQY AGTDGPCKVP AQMAVDMQTL  540
TPVGRLITAN PVITESTENS KMMLELDPPF GDSYIVIGVG EKKITHHWHR SGSTIGKAFE  600
ATVRGAKRMA VLGDTAWDFG SVGGALNSLG KGIHQIFGAA FKSLFGGMSW FSQILIGTLL  660
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SEQ ID NO: 3 moltype = AA length = 18
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source 1..18
mol_type = protein
organism = Zika virus

SEQ ID NO: 3

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SEQ ID NO: 4 moltype = DNA length = 72
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source 1..72
mol_type = genomic DNA
organism = Japanese encephalitis virus

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SEQ ID NO: 5 moltype = AA length = 24
FEATURE Location/Qualifiers
source 1..24
mol_type = protein
organism = Japanese encephalitis virus

SEQ ID NO: 5

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SEQ ID NO: 6 moltype = DNA length = 1008
FEATURE Location/Qualifiers
source 1..1008
mol_type = genomic DNA
organism = Japanese encephalitis virus

SEQ ID NO: 6

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ctcatggaat ttgaagaggc acatgccaca aaacaatctg ttgtagctct tgggtcacag  180
gagggaggcc tccatcaagc gttggcagga gccatcgtgg tggagtactc gagctcagtg  240
aagttgacat caggtcacct gaaatgcagg ctaaaaatgg acaaactggc tctgaagggc  300
acgacttatg gcatgtgtac agagaaattc tcgttcgcga aaaatccagc ggacacaggc  360
catggaacag ttgtcattga gctcacatac tctggaagtg atggtccctg taaaattccg  420
attgtctcag tcgcgagctt aaacgacatg acccctgtgg ggaggctggt aacagtaaac  480
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ggagactctt atatcgtggt tggaagaggg gacaagcaga ttaaccatca ctggcacaaa  600
gctggaagca cgctgggtaa agccttctca acaactttga aaggggctca gagactagca  660
gcgctaggcg acacagcctg ggatttcggc tccattggag gggtattcaa ctccataggg  720
aaagctgttc accaagtatt tggcggtgca ttcagaacgc tctttggggg aatgtcttgg  780
atcacacaag gactaatggg ggccttactt ctttggatgg gtgtcaacgc acgagaccgg  840
tcaatcgccc tggcttttct ggccacggga ggtgtgctcg tgtttttagc gaccaatgtg  900
catgctgaca ctggctgtgc cattgacatc acaagaaaag agatgaggtg tggaagtggc  960
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source 1..336

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HGTVVIELTY SGSDGPCKIP IVSVASLNDM TPVGRLVTVN PFVATSSSNS KVLVEMEPPF  180
GDSYIVVGRG DKQINHHWHK AGSTLGKAFS TTLKGAQRLA ALGDTAWDFG SIGGVFNSIG  240
KAVHQVFGGA FRTLFGGMSW ITQGLMGALL LWMGVNARDR SIALAFLATG GVLVFLATNV  300
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source                1..795
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                      organism = Zika virus
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GVEPDDVDCW CNTTSTWVVY GTCHHKKGEA RRSRRAVTLP SHSTRKLQTR SQTWLESREY  240
TKHLIRVENW IFRNPGFALA AAAIAWLLGS STSQKVIYLV MILLIAPAYS IRCIGVSNRD  300
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QPENLEYRIM LSVHGSQHSG MIVNDTGHET DENRAKVEIT PNSPRAEATL GGFGSLGLDC  480
EPRTGLDFSD LYYLTMNNKH WLVHKEWFHD IPLPWHAGAD TGTPHWNNKE ALVEFKDAHA  540
KRQTVVVLGS QEGAVHTALA GALEAEMDGA KGRLSSGHLK CRLKMDKLRL KGVSYSLCTA  600
AFTFTKIPAE TLHGTVTVEV QYAGTDGPCK VPAQMAVDMQ TLTPVGRLIT ANPVITESTE  660
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SEQ ID NO: 9          moltype = AA  length = 795
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                      organism = Zika virus
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GVEPDDVDCW CNTTSTWVVY GTCHHKKGEA RRSRRAVTLP SHSTRKLQTR SQTWLESREY  240
TKHLIRVENW IFRNPGFALA AAAIAWLLGS STSQKVIYLV MILLIAPAYS IRCIGVSNRD  300
FVEGMSGGTW VDIVLEHGGC VTVMAQDKPT VDIELVTTTV SNMAEVRSYC YEASISDMAS  360
DSRCPTQGEA YLDKQSDTQY VCKRTLVDRG WGNGCGLFGK GSLVTCAKFA CSKKMTGKSI  420
QPENLEYRIM LSVHGSQHSG MIVNDTGHET DENRAKVEIT PNSPRAEATL GGFGSLGLDC  480
EPRTGLDFSD LYYLTMNNKH WLVHKEWFHD IPLPWHAGAD TGTPHWNNKE ALVEFKDAHA  540
KRQTVVVLGS QEGAVHTALA GALEAEMDGA KGRLSSGHLK CRLKMDKLRL KGVSYSLCTA  600
AFTFTKIPAE TLHGTVTVEV QYAGTDGPCK VPAQMAVDMQ TLTPVGRLIT ANPVITESTE  660
NSKMMLELDP PFGDSYIVIG VGEKKITHHW HRSGSTIGKA FEATVRGAKR MAVLGDTAWD  720
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                      mol_type = protein
                      organism = Zika virus
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MAAEITRRGS AYYMYLDRSD AGKAISFATT LGVNKCHVQI MDLGHMCDAT MSYECPMLDE  180
GVEPDDVDCW CNTTSTWVVY GTCHHKKGEA RRSRRAVTLP SHSTRKLQTR SQTWLESREY  240
TKHLIKVENW IFRNPGFALV AVAIAWLLGS STSQKVIYLV MILLIAPAYS IRCIGVSNRD  300
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TKVPAETLHG TVTVEVQYAG TDGPCKIPVQ MAVDMQTLTP VGRLITANPV ITESTENSKM  660
MLELDPPFGD SYIVIGVGDK KITHHWHRSG STIGKAFEAT VRGAKRMAVL GDTAWDFGSV  720
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MIFLSTAVSA D 791

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FEATURE Location/Qualifiers

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mol_type = protein

organism = Zika virus

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QPENLEYRIM LSVHGSQHSG MIVNDTGHET DENRAKVEIT PNSPRAEATL GGFGSLGLDC 480

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KRQTVVVLGS QEGAVHTALA GALEAEMDGA KGRLSSGHLK CRLKMDKLRL KGVSYSLCTA 600

AFTFTKIPAE TLHGTVTVEV QYAGTDGPCK VPAQMAVDMQ TLTPVGRLIT ANPVITESTE 660

NSKMMLELDP PFGDSYIVIG VGEKKITHHW HRSGSTIGKA FEATVRGAKR MAVLGDTAWD 720

FGSVGGALNS LGKGIHQIFG AAFKSLFGGM SWFSQILIGT LLVWLGLNTK NGSISLTCLA 780

LGGVLIFLST AVSAD 795

SEQ ID NO: 12 moltype = AA length = 795

FEATURE Location/Qualifiers

source 1..795

mol_type = protein

organism = Zika virus

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GVEPDDVDCW CNTTSTWVVY GTCHHKKGEA RRSRRAVTLP SHSTRKLQTR SQTWLESREY 240

TKHLIRVENW IFRNPGFALA AAAIAWLLGS STSQKVIYLV MILLIAPAYS IRCIGVSNRD 300

FVEGMSGGTW VDVVLEHGGC VTVMAQDKPT VDIELVTTTV SNMAEVRSYC YEASISDMAS 360

DSRCPTQGEA YLDKQSDTQY VCKRTLVDRG WGNGCGLFGK GSLVTCAKFA CSKKMTGKSI 420

QPENLEYRIM LSVHGSQHSG MIVNDTGHET DENRAKVEIT PNSPRAEATL GGFGSLGLDC 480

EPRTGLDFSD LYYLTMNNKH WLVHKEWFHD IPLPWHAGAD TGTPHWNNKE ALVEFKDAHA 540

KRQTVVVLGS QEGAVHTALA GALEAEMDGA KGRLSSGHLK CRLKMDKLRL KGVSYSLCTA 600

AFTFTKIPAE TLHGTVTVEV QYAGTDGPCK VPAQMAVDMQ TLTPVGRLIT ANPVITESTE 660

NSKMMLELDP PFGDSYIVIG VGEKKITHHW HRSGSTIGKA FEATVRGAKR MAVLGDTAWD 720

FGSVGGALNS LGKGIHQIFG AAFKSLFGGM SWFSQILIGT LLMWLGLNTK NGSISLMCLA 780

LGGVLIFLST AVSAD 795

SEQ ID NO: 13 moltype = AA length = 795

FEATURE Location/Qualifiers

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mol_type = protein

organism = Zika virus

SEQ ID NO: 13

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MAAEVTRRGS AYYMYLDRND AGEAISFPTT LGMNKCYIQI MDLGHMCDAT MSYECPMLDE 180

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DSRCPTQGEA YLDKQSDTQY VCKRTLVDRG WGNGCGLFGK GSLVTCAKFA CSKKMTGKSI 420

QPENLEYRIM LSVHGSQHSG MIVNDTGHET DENRAKVEIT PNSPRAEATL GGFGSLGLDC 480

EPRTGLDFSD LYYLTMNNKH WLVHKEWFHD IPLPWHAGAD TGTPHWNNKE ALVEFKDAHA 540

KRQTVVVLGS QEGAVHTALA GALEAEMDGA KGRLSSGHLK CRLKMDKLRL KGVSYSLCTA 600

AFTFTKIPAE TLHGTVTVEV QYAGTDGPCK VPAQMAVDMQ TLTPVGRLIT ANPVITESTE 660

NSKMMLELDP PFGDSYIVIG VGEKKITHHW HRSGSTIGKA FEATVRGAKR MAVLGDTAWD 720

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LGGVLIFLST AVSAD 795

SEQ ID NO: 14 moltype = AA length = 795

FEATURE Location/Qualifiers

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mol_type = protein

organism = Zika virus

SEQ ID NO: 14

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DSRCPTQGEA YLDKQSDTQY VCKRTLVDRG WGNGCGLFGK GSLVTCAKFA CSKKMTGKSI 420
QPENLEYRIM LSVHGSQHSG MIVNDTGHET DENRAKVEIT PNSPRAEATL GGFGSLGLDC 480
EPRTGLDFSD LYYLTMNNKH WLVHKEWFHD IPLPWHAGAD TGTPHWNNKE ALVEFKDAHA 540
KRQTVVVLGS QEGAVHTALA GALEAEMDGA KGRLSSGHLK CRLKMDKLRL KGVSYSLCTA 600
AFTFTKIPAE TLHGTVTVEV QYAGTDGPCK VPAQMAVDMQ TLTPVGRLIT ANPVITESTE 660
NSKMMLELDP PFGDSYIVIG VGEKKITHHW HRSGSTIGKA FEATVRGAKR MAVLGDTAWD 720
FGSVGGALNS LGKGIHQIFG AAFKSLFGGM SWFSQILIGT LLMWLGLNTK NGSISLMCLA 780
LGGVLIFLST AVSAD 795

SEQ ID NO: 15 moltype = AA length = 795
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mol_type = protein
organism = Zika virus

SEQ ID NO: 15
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GVEPDDVDCW CNTTSTWVVY GTCHHKKGEA RRSRRAVTLP SHSTRKLQTR SQTWLESREY 240
TKHLIRVENW IFRNPGFALA AAAIAWLLGS STSQKVIYLV MILLIAPAYS IRCIGVSNRD 300
FVEGMSGGTW VDVVLEHGGC VTVMAQDKPT VDIELVTTTV SNMAEVRSYC YEASISDMAS 360
DSRCPTQGEA YLDKQSDTQY VCKRTLVDRG WGNGCGLFGK GSLVTCAKFA CSKKMTGKSI 420
QPENLEYRIM LSVHGSQHSG MIVNDTGHET DENRAKVEIT PNSPRAEATL GGFGSLGLDC 480
EPRTGLDFSD LYYLTMNNKH WLVHKEWFHD IPLPWHAGAD TGTPHWNNKE ALVEFKDAHA 540
KRQTVVVLGS QEGAVHTALA GALEAEMDGA KGRLSSGHLK CRLKMDKLRL KGVSYSLCTA 600
AFTFTKIPAE TLHGTVTVEV QYAGTDGPCK VPAQMAVDMQ TLTPVGRLIT ANPVITESTE 660
NSKMMLELDP PFGDSYIVIG VGEKKITHHW HRSGSTIGKA FEATVRGAKR MAVLGDTAWD 720
FGSVGGALNS LGKGIHQIFG AAFKSLFGGM SWFSQILIGT LLMWLGLNTK NGSISLMCLA 780
LGGVLIFLST AVSAD 795

SEQ ID NO: 16 moltype = AA length = 795
FEATURE Location/Qualifiers
source 1..795
mol_type = protein
organism = Zika virus

SEQ ID NO: 16
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LVMILLIAPA YSIRCIGVSN RDFVEGMSGG TWVDVVLEHG GCVTVMAQDK PTVDIELVTT  240
TVSNMAEVRS YCYEASISDM ASDSRCPTQG EAYLDKQSDT QYVCKRTLVD RGWGNGCGLF  300
GKGSLVTCAK FACSKKMTGK SIQPENLEYR IMLSVHGSQH SGMIVNDTGH ETDENRAKVE  360
ITPNSPRAEA TLGGFGSLGL DCEPRTGLDF SDLYYLTMNN KHWLVHKEWF HDIPLPWHAG  420
ADTGTPHWNN KEALVEFKDA HAKRQTVVVL GSQEGAVHTA LAGALEAEMD GAKGRLSSGH  480
LKCRLKMDKL RLKGVSYSLC TAAFTFTKIP AETLHGTVTV EVQYAGTDGP CKVPAQMAVD  540
MQTLTPVGRL ITANPVITES TENSKMMLEL DPPFGDSYIV IGVGEKKITH HWHRSGSTIG  600
KAFEATVRGA KRMAVLGDTA WDFGSVGGAL NSLGKGIHQI FGAAFKSLFG GMSWFSQILI  660
GTLLMWLGLN TKNGSISLMC LALGGVLIFL STAVSA                             696

SEQ ID NO: 20          moltype = AA  length = 92
FEATURE                Location/Qualifiers
source                 1..92
                       mol_type = protein
                       organism = Zika virus
SEQ ID NO: 20
AEVTRRGSAY YMYLDRNDAG EAISFPTTLG MNKCYIQIMD LGHMCDATMS YECPMLDEGV  60
EPDDVDCWCN TTSTWVVYGT CHHKKGEARR SR                                92

SEQ ID NO: 21          moltype = AA  length = 60
FEATURE                Location/Qualifiers
source                 1..60
                       mol_type = protein
                       organism = Zika virus
SEQ ID NO: 21
RAVTLPSHST RKLQTRSQTW LESREYTKHL IRVENWIFRN PGFALAAAAI AWLLGSSTSQ  60

SEQ ID NO: 22          moltype = AA  length = 520
FEATURE                Location/Qualifiers
source                 1..520
                       mol_type = protein
                       organism = Zika virus
SEQ ID NO: 22
KVIYLVMILL IAPAYSIRCI GVSNRDFVEG MSGGTWVDVV LEHGGCVTVM AQDKPTVDIE  60
LVTTTVSNMA EVRSYCYEAS ISDMASDSRC PTQGEAYLDK QSDTQYVCKR TLVDRGWGNG  120
CGLFGKGSLV TCAKFACSKK MTGKSIQPEN LEYRIMLSVH GSQHSGMIVN DTGHETDENR  180
AKVEITPNSP RAEATLGGFG SLGLDCEPRT GLDFSDLYYL TMNNKHWLVH KEWFHDIPLP  240
WHAGADTGTP HWNNKEALVE FKDAHAKRQT VVVLGSQEGA VHTALAGALE AEMDGAKGRL  300
SSGHLKCRLK MDKLRLKGVS YSLCTAAFTF TKIPAETLHG TVTVEVQYAG TDGPCKVPAQ  360
MAVDMQTLTP VGRLITANPV ITESTENSKM MLELDPPFGD SYIVIGVGEK KITHHWHRSG  420
STIGKAFEAT VRGAKRMAVL GDTAWDFGSV GGALNSLGKG IHQIFGAAFK SLFGGMSWFS  480
QILIGTLLMW LGLNTKNGSI SLMCLALGGV LIFLSTAVSA                        520

SEQ ID NO: 23          moltype = DNA  length = 2091
FEATURE                Location/Qualifiers
misc_feature           1..2091
                       note = recombinant Zika virus antigen construct
source                 1..2091
                       mol_type = other DNA
                       organism = synthetic construct
SEQ ID NO: 23
atgggcaagc ggtccgccgg ctctatcatg tggctggcct ctctggccgt ggtcatcgca  60
tgcgcaggag cagcggaggt caccaggaga ggcagcgcct actatatgta cctggacaga  120
aatgatgccg gcgaggccat cagctttccc accacactgg gcatgaacaa gtgttacatc  180
cagatcatgg acctgggcca catgtgcgat gccaccatgt cctatgagtg tccaatgctg  240
gacgagggcg tggagcccga cgatgtggat tgctggtgta ataccacatc cacatgggtg  300
gtgtacggca cctgccacca caagaaggga gaggcaaggc gctctcggag agcagtgaca  360
ctgccttccc actctacccg gaagctgcag acaagatctc agacctggct ggagagccgg  420
gagtatacaa agcacctgat ccgggtggag aactggatct ttagaaatcc aggattcgca  480
ctggcagcag cagcaatcgc atggctgctg ggcagctcca cctcccagaa agtgatctac  540
ctggtcatga tcctgctgat cgcccctgcc tattccatca ggtgcatcgg cgtgtctaat  600
cgcgacttcg tggagggcat gtctggcggc acctgggtgg atgtggtgct ggagcacggc  660
ggctgcgtga cagtgatggc ccaggacaag ccaaccgtgg acatcgagct ggtgaccaca  720
accgtgagca acatggccga ggtgcggtcc tactgctatg aggccagcat ctccgacatg  780
gcctctgata gcagatgtcc cacccagggc gaggcctacc tggacaagca gtctgataca  840
cagtacgtgt gcaagaggac cctggtggac aggggatggg gaaatggatg tggcctgttt  900
ggcaagggca gcctggtgac atgcgccaag ttcgcctgtt ctaagaagat gaccggcaag  960
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atcacaccta acagcccacg cgccgaggcc accctgggag gatttggctc cctgggcctg  1140
gactgcgagc ctagaacagg cctggacttc tccgatctgt actatctgac catgaacaat  1200
aagcactggc tggtgcacaa ggagtggttt cacgacatcc cactgccatg gcacgcagga  1260
gcagatacag gaaccccaca ctggaacaat aaggaggccc tggtggagtt caaggatgcc  1320
cacgccaaga ggcagacagt ggtggtgctg ggcagccagg agggagcagt gcacaccgcc  1380
ctggcaggcg ccctggaggc cgagatggac ggagcaaagg gccgcctgtc tagcggccac  1440
ctgaagtgcc ggctgaagat ggataagctg agactgaagg gcgtgtccta ctctctgtgc  1500
acagccgcct tcaccttcac caagatccct gccgagacac tgcacggcac agtgaccgtg  1560
gaggtgcagt atgccggcac agacggcccc tgtaaggtgc ctgcccagat ggccgtggat  1620
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accgagaact ctaagatgat gctggagctg gacccccctt ttggcgatag ctatatcgtg  1740
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aaggccttta gcaccaccct gaagggagca cagaggctgg ccgccctggg cgacaccgca  1860
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ttcggcggcg cctttcggac cctgttcggc ggcatgtctt ggatcaccca gggcctgatg  1980
ggcgccctgc tgctgtggat gggcgtgaac gcccgggacc ggtctatcgc cctggccttc  2040
ctggccaccg gaggcgtgct ggtgttcctg gccaccaacg tgcacgcctg a           2091

SEQ ID NO: 24          moltype = AA  length = 696
FEATURE                Location/Qualifiers
REGION                 1..696
                       note = recombinant Zika virus antigen construct
source                 1..696
                       mol_type = protein
                       organism = synthetic construct
SEQ ID NO: 24
MGKRSAGSIM WLASLAVVIA CAGAAEVTRR GSAYYMYLDR NDAGEAISFP TTLGMNKCYI  60
QIMDLGHMCD ATMSYECPML DEGVEPDDVD CWCNTTSTWV VYGTCHHKKG EARRSRRAVT  120
LPSHSTRKLQ TRSQTWLESR EYTKHLIRVE NWIFRNPGFA LAAAAIAWLL GSSTSQKVIY  180
LVMILLIAPA YSIRCIGVSN RDFVEGMSGG TWVDVVLEHG GCVTVMAQDK PTVDIELVTT  240
TVSNMAEVRS YCYEASISDM ASDSRCPTQG EAYLDKQSDT QYVCKRTLVD RGWGNGCGLF  300
GKGSLVTCAK FACSKKMTGK SIQPENLEYR IMLSVHGSQH SGMIVNDTGH ETDENRAKVE  360
ITPNSPRAEA TLGGFGSLGL DCEPRTGLDF SDLYYLTMNN KHWLVHKEWF HDIPLPWHAG  420
ADTGTPHWNN KEALVEFKDA HAKRQTVVVL GSQEGAVHTA LAGALEAEMD GAKGRLSSGH  480
LKCRLKMDKL RLKGVSYSLC TAAFTFTKIP AETLHGTVTV EVQYAGTDGP CKVPAQMAVD  540
MQTLTPVGRL ITANPVITES TENSKMMLEL DPPFGDSYIV IGVGEKKITH HWHRSGSTLG  600
KAFSTTLKGA QRLAALGDTA WDFGSIGGVF NSIGKAVHQV FGGAFRTLFG GMSWITQGLM  660
GALLLWMGVN ARDRSIALAF LATGGVLVFL ATNVHA                            696

SEQ ID NO: 25          moltype = AA  length = 92
FEATURE                Location/Qualifiers
source                 1..92
                       mol_type = protein
                       organism = Zika virus
SEQ ID NO: 25
AEVTRRGSAY YMYLDRNDAG EAISFPTTLG MNKCYIQIMD LGHMCDATMS YECPMLDEGV  60
EPDDVDCWCN TTSTWVVYGT CHHKKGEARR SR                                92

SEQ ID NO: 26          moltype = AA  length = 60
FEATURE                Location/Qualifiers
source                 1..60
                       mol_type = protein
                       organism = Zika virus
SEQ ID NO: 26
RAVTLPSHST RKLQTRSQTW LESREYTKHL IRVENWIFRN PGFALAAAAI AWLLGSSTSQ  60

SEQ ID NO: 27          moltype = AA  length = 520
FEATURE                Location/Qualifiers
REGION                 1..520
                       note = hybrid Zika-JEV E protein
source                 1..520
                       mol_type = protein
                       organism = synthetic construct
SEQ ID NO: 27
KVIYLVMILL IAPAYSIRCI GVSNRDFVEG MSGGTWVDVV LEHGGCVTVM AQDKPTVDIE  60
LVTTTVSNMA EVRSYCYEAS ISDMASDSRC PTQGEAYLDK QSDTQYVCKR TLVDRGWGNG  120
CGLFGKGSLV TCAKFACSKK MTGKSIQPEN LEYRIMLSVH GSQHSGMIVN DTGHETDENR  180
AKVEITPNSP RAEATLGGFG SLGLDCEPRT GLDFSDLYYL TMNNKHWLVH KEWFHDIPLP  240
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WHAGADTGTP HWNNKEALVE FKDAHAKRQT VVVLGSQEGA VHTALAGALE AEMDGAKGRL  300
SSGHLKCRLK MDKLRLKGVS YSLCTAAFTF TKIPAETLHG TVTVEVQYAG TDGPCKVPAQ  360
MAVDMQTLTP VGRLITANPV ITESTENSKM MLELDPPFGD SYIVIGVGEK KITHHWHRSG  420
STLGKAFSTT LKGAQRLAAL GDTAWDFGSI GGVFNSIGKA VHQVFGGAFR TLFGGMSWIT  480
QGLMGALLLW MGVNARDRSI ALAFLATGGV LVFLATNVHA                        520

SEQ ID NO: 28          moltype = DNA  length = 2082
FEATURE                Location/Qualifiers
misc_feature           1..2082
                       note = recombinant Zika virus antigen construct
source                 1..2082
                       mol_type = other DNA
                       organism = synthetic construct
SEQ ID NO: 28
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tgcgcaggag caaccaggag aggcagcgcc tactatatgt acctggacag aaatgatgcc  120
ggcgaggcca tcagctttcc caccacactg ggcatgaaca agtgttacat ccagatcatg  180
gacctgggcc acatgtgcga tgccaccatg tcctatgagt gtccaatgct ggacgagggc  240
gtggagcccg acgatgtgga ttgctggtgt aataccacat ccacatgggt ggtgtacggc  300
acctgccacc acaagaaggg agaggcaagg cgctctcgga gagcagtgac actgccttcc  360
cactctaccc ggaagctgca gacaagatct cagacctggc tggagagccg ggagtataca  420
aagcacctga tccgggtgga gaactggatc tttagaaatc caggattcgc actggcagca  480
gcagcaatcg catggctgct gggcagctcc acctcccaga aagtgatcta cctggtcatg  540
atcctgctga tcgcccctgc ctattccatc aggtgcatcg gcgtgtctaa tcgcgacttc  600
gtggagggca tgtctggcgg cacctgggtg gatgtggtgc tggagcacgg cggctgcgtg  660
acagtgatgg cccaggacaa gccaaccgtg gacatcgagc tggtgaccac aaccgtgagc  720
aacatggccg aggtgcggtc ctactgctat gaggccagca tctccgacat ggcctctgat  780
agcagatgtc ccacccaggg cgaggcctac ctggacaagc agtctgatac acagtacgtg  840
tgcaagagga ccctggtgga caggggatgg ggaaatggat gtggcctgtt tggcaagggc  900
agcctggtga catgcgccaa gttcgcctgt tctaagaaga tgaccggcaa gagcatccag  960
ccagagaacc tggagtaccg gatcatgctg agcgtgcacg gcagccagca ctccggcatg  1020
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aacagcccac gcgccgaggc caccctggga ggatttggct ccctgggcct ggactgcgag  1140
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ggaaccccac actggaacaa taaggaggcc ctggtggagt tcaaggatgc ccacgccaag  1320
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cggctgaaga tggataagct gagactgaag ggcgtgtcct actctctgtg cacagccgcc  1500
ttcaccttca ccaagatccc tgccgagaca ctgcacggca cagtgaccgt ggaggtgcag  1560
tatgccggca cagacggccc ctgtaaggtg cctgcccaga tggccgtgga tatgcagaca  1620
ctgacccctg tgggcaggct gatcaccgcc aatccagtga tcacagagtc caccgagaac  1680
tctaagatga tgctggagct ggacccccct tttggcgata gctatatcgt gatcggcgtg  1740
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gaggccaccg tgaggggagc aaagaggatg gccgtgctgg gcgacaccgc atgggatttc  1860
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ctgatgtggc tgggcctgaa caccaagaat ggctctatca gcctgatgtg cctggccctg  2040
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SEQ ID NO: 29          moltype = AA  length = 693
FEATURE                Location/Qualifiers
REGION                 1..693
                       note = recombinant Zika virus antigen construct
source                 1..693
                       mol_type = protein
                       organism = synthetic construct
SEQ ID NO: 29
MGKRSAGSIM WLASLAVVIA CAGATRRGSA YYMYLDRNDA GEAISFPTTL GMNKCYIQIM  60
DLGHMCDATM SYECPMLDEG VEPDDVDCWC NTTSTWVVYG TCHHKKGEAR RSRRAVTLPS  120
HSTRKLQTRS QTWLESREYT KHLIRVENWI FRNPGFALAA AAIAWLLGSS TSQKVIYLVM  180
ILLIAPAYSI RCIGVSNRDF VEGMSGGTWV DVVLEHGGCV TVMAQDKPTV DIELVTTTVS  240
NMAEVRSYCY EASISDMASD SRCPTQGEAY LDKQSDTQYV CKRTLVDRGW GNGCGLFGKG  300
SLVTCAKFAC SKKMTGKSIQ PENLEYRIML SVHGSQHSGM IVNDTGHETD ENRAKVEITP  360
NSPRAEATLG GFGSLGLDCE PRTGLDFSDL YYLTMNNKHW LVHKEWFHDI PLPWHAGADT  420
GTPHWNNKEA LVEFKDAHAK RQTVVVLGSQ EGAVHTALAG ALEAEMDGAK GRLSSGHLKC  480
RLKMDKLRLK GVSYSLCTAA FTFTKIPAET LHGTVTVEVQ YAGTDGPCKV PAQMAVDMQT  540
LTPVGRLITA NPVITESTEN SKMMLELDPP FGDSYIVIGV GEKKITHHWH RSGSTIGKAF  600
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EATVRGAKRM AVLGDTAWDF GSVGGALNSL GKGIHQIFGA AFKSLFGGMS WFSQILIGTL 660
LMWLGLNTKN GSISLMCLAL GGVLIFLSTA VSA 693

SEQ ID NO: 30 moltype = AA length = 89
FEATURE Location/Qualifiers
source 1..89
mol_type = protein
organism = Zika virus
SEQ ID NO: 30
TRRGSAYYMY LDRNDAGEAI SFPTTLGMNK CYIQIMDLGH MCDATMSYEC PMLDEGVEPD 60
DVDCWCNTTS TWVVYGTCHH KKGEARRSR 89

SEQ ID NO: 31 moltype = AA length = 60
FEATURE Location/Qualifiers
source 1..60
mol_type = protein
organism = Zika virus
SEQ ID NO: 31
RAVTLPSHST RKLQTRSQTW LESREYTKHL IRVENWIFRN PGFALAAAAI AWLLGSSTSQ 60

SEQ ID NO: 32 moltype = AA length = 520
FEATURE Location/Qualifiers
source 1..520
mol_type = protein
organism = Zika virus
SEQ ID NO: 32
KVIYLVMILL IAPAYSIRCI GVSNRDFVEG MSGGTWVDVV LEHGGCVTVM AQDKPTVDIE 60
LVTTTVSNMA EVRSYCYEAS ISDMASDSRC PTQGEAYLDK QSDTQYVCKR TLVDRGWGNG 120
CGLFGKGSLV TCAKFACSKK MTGKSIQPEN LEYRIMLSVH GSQHSGMIVN DTGHETDENR 180
AKVEITPNSP RAEATLGGFG SLGLDCEPRT GLDFSDLYYL TMNNKHWLVH KEWFHDIPLP 240
WHAGADTGTP HWNNKEALVE FKDAHAKRQT VVVLGSQEGA VHTALAGALE AEMDGAKGRL 300
SSGHLKCRLK MDKLRLKGVS YSLCTAAFTF TKIPAETLHG TVTVEVQYAG TDGPCKVPAQ 360
MAVDMQTLTP VGRLITANPV ITESTENSKM MLELDPPFGD SYIVIGVGEK KITHHWHRSG 420
STIGKAFEAT VRGAKRMAVL GDTAWDFGSV GGALNSLGKG IHQIFGAAFK SLFGGMSWFS 480
QILIGTLLMW LGLNTKNGSI SLMCLALGGV LIFLSTAVSA 520

SEQ ID NO: 33 moltype = DNA length = 12090
FEATURE Location/Qualifiers
misc_feature 1..12090
note = SAM vector backbone with ZIka antigen insert
source 1..12090
mol_type = other DNA
organism = synthetic construct
SEQ ID NO: 33
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caagaatcag gaaaatgtta gaggagcaca aggagccgtc acctctcatt accgccgagg 1500
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aggttaccag ctacgatggc gaggacaaga tcggctctta cgctgtgctt tctccgcagg  1740
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gttgcactaa atctgtgact tcggtcgtct caaccttgtt ttacgacaaa aaaatgagaa  2640
cgacgaatcc gaaagagact aagattgtga ttgacactac cggcagtacc aaacctaagc  2700
aggacgatct cattctcact tgtttcagag ggtgggtgaa gcagttgcaa atagattaca  2760
aaggcaacga aataatgacg gcagctgcct ctcaagggct gacccgtaaa ggtgtgtatg  2820
ccgttcggta caaggtgaat gaaaatcctc tgtacgcacc cacctcagaa catgtgaacg  2880
tcctactgac ccgcacggag gaccgcatcg tgtggaaaac actagccggc gacccatgga  2940
taaaaacact gactgccaag taccctggga atttcactgc cacgatagag gagtggcaag  3000
cagagcatga tgccatcatg aggcacatct tggagagacc ggaccctacc gacgtcttcc  3060
agaataaggc aaacgtgtgt tgggccaagg ctttagtgcc ggtgctgaag accgctggca  3120
tagacatgac cactgaacaa tggaacactg tggattattt tgaaacggac aaagctcact  3180
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gtctattttc tgcacccact gttccgttat ccattaggaa taatcactgg gataactccc  3300
cgtcgcctaa catgtacggg ctgaataaag aagtggtccg tcagctctct cgcaggtacc  3360
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                       note = recombinant Zika virus antigen construct
source                 1..2091
                       mol_type = other RNA
                       organism = synthetic construct
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SEQ ID NO: 40 moltype = RNA length = 2091
FEATURE Location/Qualifiers
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note = recombinant Zika virus antigen construct
source 1..2091
mol_type = other RNA
organism = synthetic construct

SEQ ID NO: 40

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SEQ ID NO: 41 moltype = RNA length = 2082
FEATURE Location/Qualifiers
misc_feature 1..2082
note = recombinant Zika virus antigen construct
source 1..2082
mol_type = other RNA
organism = synthetic construct

SEQ ID NO: 41

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```

We claim:

1. An RNA-based vaccine construct encoding a polypeptide comprising a Zika virus prME antigen or a variant thereof,

wherein the construct encodes a JEV signal sequence or a variant thereof in place of the Zika virus prME signal sequence, and

wherein said construct comprises a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence which is at least 95% identical to SEQ ID NO:19.

2. A construct according to claim **1** wherein said JEV signal sequence or variant thereof is juxtaposed immediately next to and N-terminal of the Zika virus prME antigen or variant thereof.

3. A construct according to claim **1** wherein said construct comprises a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence which is at least 98% identical to SEQ ID NO:19.

4. A composition comprising:

an immunologically effective amount of one or more of the constructs of claim **1**; and

a pharmaceutically acceptable carrier.

5. The composition according to claim **4**, wherein the composition comprises a non-viral delivery material.

6. The composition according to claim **5**, wherein non-viral delivery material comprises a submicron cationic oil-in-water emulsion which comprises an oil core, a cationic lipid, and a surfactant.

7. The composition according to claim **4** wherein the composition further comprises one or more nucleic acid sequences which encode one or more additional antigens and/or the composition further comprises one or more additional antigens.

8. The composition according to claim **4**, wherein the composition is pharmaceutically acceptable for administration to a subject in combination with a further composition which comprises a nucleic acid comprising a sequence which encodes an additional antigen; and/or the composition is pharmaceutically acceptable for administration to the subject in combination with a further composition which comprises an additional antigen.

9. The composition according to claim **4** wherein the composition comprises one or more adjuvants.

10. The construct of claim **1**; which is suitable for use in therapy.

11. A method for inducing an immune response to a Zika virus infection in a subject which comprises administering the construct of claim **1** to a subject.

12. A construct according to claim **2**, wherein said construct comprises a nucleic acid sequence encoding a polypeptide comprising an amino acid sequence which is at least 98% identical to SEQ ID NO: 19.

13. A composition comprising:

an immunologically effective amount of one or more of the constructs of claim **2**; and

a pharmaceutically acceptable carrier.

14. A composition comprising:

an immunologically effective amount of one or more of the constructs of claim **3**; and

a pharmaceutically acceptable carrier.

15. The composition according to claim **4**, wherein the composition comprises a non-viral delivery material.

16. The composition according to claim **5**, wherein said non-viral delivery material comprises a submicron cationic

oil-in-water emulsion; a liposome; or a biodegradable polymeric microparticle delivery system.

17. The composition according to claim **15**, wherein said non-viral delivery material comprises a submicron cationic oil-in-water emulsion; a liposome; or a biodegradable polymeric microparticle delivery system.

18. The composition according to claim **15**, wherein said non-viral delivery material comprises a submicron cationic oil-in-water emulsion comprises an oil core, a cationic lipid, and a surfactant.

19. The composition of any one of claim **4** which is suitable for use in therapy.

20. A method for inducing an immune response to a Zika virus infection in a subject which comprises administering the composition of claim **4** to a subject.

* * * * *