

ALPHAVIRUS NSP MUTANTS AS VACCINES

TECHNICAL FIELD

[0001] The disclosure generally relates to the field of immunology and virology. The present disclosure relates to polypeptides, polynucleotides, expression vectors, infectious clones, virus particles, and immunogenic compositions of recombinant alphaviruses, for use in these fields, in particular as vaccines against alphavirus infection. The present disclosure also relates to methods for eliciting an immune response against alphavirus infection using the immunogenic compositions of the present disclosure.

BACKGROUND

[0002] Alphaviruses are a genus of enveloped, RNA viruses that cause diseases in humans and animals, with symptoms such as fever, rash and arthritis. The alphavirus genome encodes nonstructural (nsP) and structural proteins. More specifically, the genome encodes four nsPs which are involved in virus replication and pathogenesis, and five structural proteins that make up the virion. Substitutions of amino acids at functionally coupled sites in the nsPs were previously shown to affect polypeptide processing and controlled the virulence of the alphavirus Semliki Forest Virus (SFV).

[0003] Another alphavirus is Chikungunya virus (CHIKV), an arthropod-borne alphavirus that causes acute febrile illness and chronic debilitating polyarthralgia. The interferon- α/β receptor (IFNAR) dependent Type 1 interferon (IFN) signaling pathway has been shown to be critical in CHIKV induced anti-viral mechanisms. Yet another alphavirus is O'nyong'nyong Virus (ONNV), which is also an arthropod-borne alphavirus that causes symptoms such as fever, rash, headache and arthralgia.

[0004] There are presently no known commercial vaccines to prevent such alphavirus infections, such as CHIKV and ONNV infections, or medicine for treating such infections. Therefore, there is a need to provide vaccines against such alphavirus infections, and methods for eliciting an immune response against the alphavirus infections.

SUMMARY

[0005] In one example, there is provided a recombinant polypeptide comprising an amino acid SEQ ID NO: 1, or a variant thereof, comprising one or more mutations selected from the group consisting of: (a) a mutation at a position equivalent to amino acid position 532 of SEQ ID NO: 1, and (b) a mutation at a position equivalent to amino acid position 1050 of SEQ ID NO: 1. In one aspect, the present disclosure refers to a recombinant polypeptide comprising an amino acid sequence SEQ ID NO: 1, or a variant thereof comprising at least 80% identity to the amino acid sequence SEQ ID NO: 1, comprising one or more mutations selected from the group consisting of: (a) substitution of R with H at a position equivalent to amino acid position 532 of SEQ ID NO: 1, and (b) substitution of E with V at a position equivalent to amino acid position 1050 of SEQ ID NO: 1, wherein the mutations are attenuating mutations.

[0006] Advantageously, mutation at a position equivalent to amino acid position 532 of SEQ ID NO: 1 alone, results in higher Type 1 IFN immune response in primary mouse fibroblasts in murine

ex-vivo and *in vivo* infection models. Additionally, when the mutation at position 532 occurs simultaneously in combination with a mutation at position 1050 of SEQ ID NO: 1, both sites being functionally coupled, not only is the Type 1 IFN immune response significantly enhanced, but alphavirus harboring these nsP mutations also exhibited lower infectivity.

5 [0007] In another aspect, there is provided a polynucleotide encoding the polypeptide described herein.

[0008] In another aspect, there is provided an expression vector comprising the polynucleotide sequence described herein.

10 [0009] In another aspect, there is provided an infectious clone comprising the polynucleotide sequence described herein.

[0010] In another aspect, there is provided a recombinant virus particle comprising the polypeptide described herein.

[0011] In another aspect, there is provided a recombinant virus particle comprising the polynucleotide described herein.

15 [0012] In another aspect, there is provided a virus particle derived from the infectious clone described herein.

[0013] In another aspect, there is provided an immunogenic composition comprising an infectious clone, a recombinant virus particle and/or a virus particle described herein.

20 [0014] In another example, there is provided a method of eliciting an immune response against alphavirus infection in a subject comprising administering an immunogenic composition described herein to the subject. In one aspect, the present disclosure refers to use of an immunogenic composition as disclosed herein in the manufacture of a medicament for eliciting an immune response against alphavirus infection in a subject. Advantageously, the method is capable of providing protection to subjects administered with the immunogenic composition against wild-type (WT) alphavirus infection, as well as re-infection upon subsequent exposure to alphavirus.

DEFINITION OF TERMS

[0015] The following words and terms used herein shall have the meaning indicated:

30 [0016] The term "polypeptide" refers to any polymer of amino acids (dipeptide or greater) linked through peptide bonds or modified peptide bonds, whether produced naturally or synthetically.

[0017] The term "polynucleotide" includes a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues of natural nucleotides that hybridize to nucleic acids in a manner similar to naturally occurring nucleotides. The terms "polynucleotide", "nucleic acid", "nucleic acid molecule", and "nucleic acid sequence" are used interchangeably herein unless the context indicates otherwise.

35 [0018] The term "recombinant" when used in reference to a polypeptide, polynucleotide or virus refers to a polypeptide, polynucleotide or virus that is not naturally occurring, or was made

artificially. A recombinant virus refers to a virus that may carry a recombinant polynucleotide or a virus that expresses a recombinant polypeptide.

[0019] The term “equivalent”, when used in reference to the position of an amino acid in a polypeptide sequence or the position of a nucleic acid in a polynucleotide sequence, refers to a position of the amino acid or nucleic acid in the sequence of a given polypeptide or polynucleotide, which corresponds in position (in either primary or tertiary structure) to a position of the amino acid in SEQ ID NO: 1, or its corresponding polynucleotide sequence, as the case may be. Such equivalent positions in a particular sequence can be determined using methods known in the art, for example based on sequence alignment against the reference sequence or by comparing experimentally revealed or predicted 3D-structures of corresponding proteins. For example, amino acid positions 1185-1187 of the non-structural protein of SFV are equivalent to amino acid positions 1183-1185 of the non-structural protein of CHIKV; amino acid positions 532 and 1050 of the non-structural protein P1234 of O'nyong'nyong Virus are equivalent to amino acid positions 532 and 1050 of the non-structural protein P1234 of CHIKV, respectively; amino acid positions 531 and 1048 of the non-structural protein P1234 of Ross River Virus are equivalent to amino acid positions 532 and 1050 of the non-structural protein P1234 of CHIKV, respectively; amino acid positions 530 and 1048 of the non-structural protein P1234 of Barmah Forest Virus are equivalent to amino acid positions 532 and 1050 of the non-structural protein P1234 of CHIKV, respectively; and amino acid positions 543 and 1059 of the non-structural protein P1234 of Venezuelan Equine Encephalitis Virus are equivalent to amino acid positions 532 and 1050 of the non-structural protein P1234 of Chikungunya Virus, respectively.

[0020] The term “variant” as used herein includes a reference to substantially similar sequences. These sequence variants may have at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to a reference polypeptide or polynucleotide sequence, or to a section within the polypeptide or polynucleotide reference sequence.

The reference sequence may be any one of SEQ IN NOs: 1 to 7. For example, a variant of any one of SEQ IN NOs: 1 to 7 may have at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% identity to SEQ ID NOs: 1 to 7. In one example, where the variant sequence includes a mutation, the differences that make up the variants are expected to occur outside of the section where the mutation is located. That is to say that the differences that constitute the variant sequences are outside the location of the mutation. By way of an example, if a sequence comprises a mutation at amino acid residue 515, then the differences that make up the variants are found at any location within the variant sequence other than at amino acid residue 515. In another

example, if a sequence comprises a mutation at base pair 1500, the differences that make up the variants are found at any location within the variant sequence other than at base pair 1500.

[0021] A polypeptide variant may be a biological variant, which displays substantially the same biological activity as a reference polypeptide. The reference polypeptide may be any one of SEQ ID NO: 1 to SEQ ID NO: 4.

[0022] A polypeptide variant may also, or alternatively, be a functional variant, which is a variant having at least 80% (e.g. 80%, 85%, 90%, 95% or 99%) identity to the reference polypeptide sequence, and possessing the same activity as the reference polypeptide sequence. The reference polypeptide may be any one of SEQ ID NO: 1 to SEQ ID NO: 4. For example, SEQ ID NO: 2 is a functional variant of SEQ ID NO: 1.

[0023] As used herein, the term "missense mutation" refers to a point mutation in which a single nucleotide change results in a codon that codes for a different amino acid. Missense mutations may or may not affect the activity of a polypeptide. In some cases, missense mutations may render the resulting polypeptide to be nonfunctional.

[0024] As used herein, the term "substitution" refers to a mutation in which one or more nucleotides changes result in a codon that codes for a different amino acid. Substitution of an amino acid may or may not affect the activity of a polypeptide. In some cases, substitution of an amino acid may render the resulting polypeptide to be nonfunctional.

[0025] As used herein, the term "attenuated" virus refers to a virus which is infectious but not pathogenic; or an infectious virus which may or may not be pathogenic, but which either produces defective particles during each round of replication or produces fewer progeny virions than does the corresponding wild type virus during replication. Pathogenic viruses which are engineered to produce defective particles or a reduced number of progeny virions may be "attenuated" in that even though the virus is capable of causing disease, the titers of virus obtained in a vaccinated individual will provide only subclinical levels of infection.

[0026] A "virus particle" may be a complete, incomplete or empty particle. A complete "virus particle" is typically composed of the viral genetic material (DNA or RNA), a protein coat, and in some cases an envelope of lipids that surrounds the protein coat. On the other hand, an incomplete particles or empty "virus particle" typically contains protein, but not the genetic material.

[0027] As used herein, the term "infectious clone" may refer to a double-stranded DNA or cDNA copy of a viral genome that is carried on a bacterial plasmid. The DNA (or RNAs which are produced from the DNA) can be introduced into cells (such as C6/36 *Aedes albopictus* cell line, BHK-21 cell line, and VeroE6 cell line) via transfection to produce infectious viruses.

[0028] As used herein, the term "expression" may refer to the expression of a polypeptide from a gene. Thus, an "expression vector" may refer to a recombinant polynucleotide capable of expressing

polypeptides. Generally, such expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleotide sequence encoding the polypeptide that is to be expressed.

[0029] The term “immunogenic composition” as used herein refers to a composition which is capable of stimulating the immune system of a subject. In this way, immune protection may be provided against an antigen not recognized as a self-antigen by the immune system.

[0030] The term “administering” and variations of that term including “administer” and “administration”, includes contacting, applying, delivering or providing a composition of the disclosure to subject by any appropriate means.

[0031] “Immune response” refers to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, and can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defence systems.

[0032] The term “subject” refers to patients of human or other mammals, and includes any individual it is desired to be treated using the immunogenic compositions and methods of the disclosure. However, it will be understood that “subject” does not imply that symptoms are present. Suitable mammals that fall within the scope of the disclosure include, but are not restricted to, primates, livestock animals (e.g. sheep, cows, horses, donkeys, pigs), laboratory test animals (e.g. rabbits, mice, rats, guinea pigs, hamsters), companion animals (e.g. cats, dogs) and captive wild animals (e.g. foxes, deer, dingoes).

[0033] Unless specified otherwise, the terms “comprising” and “comprise”, and grammatical variants thereof, are intended to represent “open” or “inclusive” language such that they include recited elements but also permit inclusion of additional, unrecited elements.

[0034] As used herein, the term “about”, in the context of, but not limited to, concentrations of DNA, chemicals, chemical solutions, enzymes or components of a buffer, typically means $\pm 5\%$ of the stated value, more typically $\pm 4\%$ of the stated value, more typically $\pm 3\%$ of the stated value, more typically, $\pm 2\%$ of the stated value, even more typically $\pm 1\%$ of the stated value, and even more typically $\pm 0.5\%$ of the stated value.

[0035] Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from one to six should be considered to have specifically disclosed sub-ranges such as from one to three, from one to four, from one to five, from two to four, from two to six, from three to six

etc., as well as individual numbers within that range, for example, one, two, three, four, five, and six. This applies regardless of the breadth of the range.

[0036] Certain embodiments may also be described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0037] The disclosure illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the disclosure claimed. Thus, it should be understood that although the present disclosure has been specifically disclosed by preferred embodiments and optional features, modification and variation of the disclosures embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this disclosure.

DETAILED DISCLOSURE OF THE EMBODIMENTS

[0038] CHIKV infection causes Chikungunya viral disease. The disease may present symptoms such as fever and severe joint pain which is debilitating and can vary in duration. Currently, there is no cure for the disease. The present disclosure provides alphavirus nonstructural protein (nsP) mutants as vaccines against CHIKV. These mutants may be generated by mutagenizing the nsP region of an alphavirus. For example, mutagenesis can be targeted to one or more specific sites of the nsP region of an alphavirus. The resulting alphavirus nsP mutant and/or the recombinant products can be used as vaccines and/or for production of immunogenic compositions for providing immunity to a particular alphavirus strain. For example, the alphavirus can be, but is not limited to, CHIKV, SFV, O'nyong'nyong virus, Ross River virus or Venezuelan Equine Encephalitis virus.

[0039] The nsP region in the CHIKV genome encodes four non-structural proteins which have various functions in virus replication and pathogenesis. In one example, the inventors have identified two specific mutations in the nsP region in CHIKV which are located at amino acid positions 532 of SEQ ID NO: 1 and 1050 of SEQ ID NO: 1 which can be useful in preparing recombinant alphavirus to be used as vaccines. In this example, the amino acid at position 532 of SEQ ID NO: 1 is mutated from arginine (R) to histidine (H), while the amino acid at position 1050 of SEQ ID NO: 1 is mutated from glutamic acid (E) to valine (V).

[0040] Therefore, in one aspect, there is provided a recombinant polypeptide comprising an amino acid SEQ ID NO: 1, or a variant thereof, comprising one or more mutations selected from the group consisting of: (a) a mutation at a position equivalent to amino acid position 532 of SEQ ID NO: 1, and (b) a mutation at a position equivalent to amino acid position 1050 of SEQ ID NO: 1. In one example, the recombinant polypeptide comprises an amino acid SEQ ID NO: 1, or a variant thereof, with a mutation at a position equivalent to amino acid position 532 of SEQ ID NO: 1. In another example, the recombinant polypeptide comprises an amino acid SEQ ID NO: 1, or a variant thereof, with a mutation at a position equivalent to amino acid position 1050 of SEQ ID NO: 1. In yet another example, the recombinant polypeptide comprises an amino acid SEQ ID NO: 1, or a variant thereof, with a mutation at a position equivalent to amino acid position 532 of SEQ ID NO: 1, and a mutation at a position equivalent to amino acid position 1050 of SEQ ID NO: 1.

[0041] SEQ ID NO: 1 is the wild-type polypeptide sequence of the nsP region of CHIKV LR2006 OPY1 strain. A recombinant polypeptide may be generated from SEQ ID NO: 1 through mutagenesis to introduce suitable amino acid substitutions at these nsP positions. Mutagenesis at specific sites, or site-directed mutagenesis, may be conducted using methods known in the art, such as, but not limited to polymerase incomplete primer extension (PIPE) cloning method, primer extension using mutagenic oligonucleotides and inverse PCR. Similar mutations can be introduced into positions equivalent to the amino acid positions 532 and 1050 of SEQ ID NO: 1 in the nsP region of other alphavirus such as, but not limited to, SFV, O'nyong'nyong virus, Ross River virus and Venezuelan Equine Encephalitis virus. The positions "equivalent" to the amino acid positions 532 and 1050 of SEQ ID NO: 1 refer to amino acid positions in a polypeptide sequence which correspond in position to the positions 532 and 1050 of sequence of SEQ ID NO: 1. The position may refer to a position of the amino acids in the primary or tertiary structure of the polypeptides. Such positions may be determined using methods known in the art, for example based on sequence alignment against SEQ ID NO: 1 (such as the sequence alignment shown in Figure 11).

[0042] In one example, the amino acid SEQ ID NO: 1 is derived from a CHIKV strain LR2006 OPY1. The amino acid SEQ ID NO: 1 can also be derived from, but is not limited to, CHIKV strains CNR20235, SGP007, SG011 and IND91.

[0043] In one example, the recombinant polypeptide comprises a variant of SEQ ID NO: 1. The variant may be a biological variant that is derived from the nsP polypeptide region of another alphavirus or a functional variant that is derived from any one of SEQ ID NOs: 1 to 4. The variant may be at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 1. Variants of SEQ ID NO: 1 of the present disclosure may have at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity or at least 99% sequence identity to SEQ ID NO: 1. The variants may have about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about

89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% sequence identity to SEQ ID NO: 1. The variants may also have at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO: 1.

[0044] In another example, the recombinant polypeptide described herein comprises a polypeptide selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and variants thereof. SEQ ID NO: 2 is a polypeptide sequence of SEQ ID NO: 1 wherein the R at position 532 is mutated to H. SEQ ID NO: 3 is a polypeptide sequence of SEQ ID NO: 1 wherein the E at position 1050 is mutated to V. SEQ ID NO: 4 is a polypeptide sequence of SEQ ID NO: 1 wherein the R at position 532 is mutated to H and the E at position 1050 is mutated to V. Variants of SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4 of the present disclosure may have at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity or at least 99% sequence identity to any one of SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4. The variants may have about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% sequence identity to any one of SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4. The variants may also have at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.

[0045] In one example, the mutation comprises a missense mutation and/or an amino acid substitution. In some examples of the amino acid substitutions as disclosed herein, the amino acid substitution is a conservative amino acid substitution. In other examples, the amino acid substitution is a non-conservative substitution. The terms “conservative amino acid substitution” and “non-conservative amino acid substitution” are used consistently with their meanings in the art. For example, “conservative amino acid substitution” as used herein refers to a substitution or replacement of one amino acid for another amino acid with similar properties within a polypeptide chain. However, it is also known in the art that some amino acids can substitute for each other even though they belong to different groups.

[0046] In one example, the substitution at position 532 comprises substitution of R with H.

[0047] In another example, the substitution at position 1050 comprises substitution of E with V.

[0048] In another example, the substitutions at positions 532 and 1050, comprise substitutions of R with H and E with V, respectively.

[0049] As demonstrated in the Examples below, CHIKV nsP mutant which contains the amino acid substitution from R to H at position 532 of SEQ ID NO: 1 (herein referred to as RH CHIKV) show reduced viral infectivity (Figure 4) and replicative potential in mouse tail fibroblasts (MTFs) (Figure 1). Simultaneous mutations to CHIKV nsP region from R to H at position 532 and also from E to V at position 1050 of SEQ ID NO: 1 (herein referred to as RHEV CHIKV) similarly show reduced viral infectivity and replicative potential in MTFs (Figure 1).

[0050] Infection of MTFs with RH CHIKV and RHEV CHIKV also resulted in a higher Type 1 IFN immune response (Figure 2). Figure 3A shows that mice infected with RH CHIKV and RHEV CHIKV display faster clearance of viremia compared to mice infected with wild-type CHIKV (herein referred to as WT CHIKV). Attenuation of the Chikungunya disease pathology, measured through joint inflammation, is also found to occur in mice infected with RH CHIKV and RHEV CHIKV (Figure 3B).

[0051] Infection with RH CHIKV is found to provide protection from subsequent WT CHIKV infection (Figure 5). Similarly, infection with RHEV CHIKV is also found to provide protection from subsequent WT CHIKV infection (Figure 5).

[0052] In another aspect, there is provided a polynucleotide encoding the polypeptide described herein.

[0053] In one example, the polynucleotide is DNA. In another example, the polynucleotide is RNA.

[0054] In one example, the polynucleotide is selected from the group consisting of SEQ ID NO: 5 (which is the polynucleotide sequence encoding SEQ ID NO: 2), SEQ ID NO: 6 (which is the polynucleotide sequence encoding SEQ ID NO: 3), SEQ ID NO: 7 (which is the polynucleotide sequence encoding SEQ ID NO: 4), and variants thereof. In one example, the variant is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7. Variants of SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7 of the present disclosure may have at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity or at least 99% sequence identity to any one of SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7. The variants may have about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98% or about 99% sequence identity to any one of SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7. The variants may also have at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to any one of SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7.

[0055] In another aspect, there is provided an expression vector comprising the polynucleotide sequence described herein. Examples of suitable expression vectors are, but not limited to, adeno-associated virus expression vectors, baculovirus expression vector and hybrid vectors.

[0056] In another aspect, there is provided an infectious clone comprising the polynucleotide sequence described herein. The infectious clone can be constructed by inserting the genome (in DNA or cDNA form) of an infectious virus, such as the polynucleotide sequence described herein, or a genome comprising the polynucleotide described herein, into a plasmid for infection into a host cell, such as an epithelial cell, an endothelial cell, a primary myeloid cell and a lymphoid cell. Exemplary uses of the infectious clones are, but not limited to, use for infectivity assays or for inducing an immune response in the host cell. Plasmids which can be used as the backbone to generate the infectious clones include, but are not limited to, pBluescript II KS and pBR322. These infectious clones may be specifically engineered to not cause mortality but yet still be immunogenic in a subject, such as but not limited to human. Therefore, such infectious clones may be suitable for use as vaccines.

[0057] In another aspect, there is provided a recombinant virus particle comprising the polypeptide described herein.

[0058] In another aspect, there is provided a recombinant virus particle comprising the polynucleotide described herein.

[0059] In one example of the recombinant virus particle, the virus is an alphavirus. Exemplary alphaviruses may be such as, but not limited to, CHIKV, SFV, O'nyong'nyong virus, Ross River virus and Venezuelan Equine Encephalitis virus.

[0060] In one example, the alphavirus is derived from a CHIKV strain. In one example, the CHIKV strain is LR2006 OPY1.

[0061] In one example, the recombinant virus particle is a live attenuated virus. The inventors have demonstrated in Figure 3B that the exemplary mutant viruses of the disclosure, specifically the RH CHIKV and RHEV CHIKV nsP mutants, are attenuated as they do not induce the typically higher fold change in inflammation to the footpad of mice compared to WT CHIKV. Instead, the mutants exhibit much lower fold change in inflammation to the foot pad of infected mice compared to WT CHIKV. The difference in joint inflammation severity between the nsP mutants and WT CHIKV can be determined using statistical analysis such as Mann-Whitney U-test, Student's t-test and one-way analysis of variance (ANOVA).

[0062] In another aspect, there is provided a virus particle derived from the infectious clone described herein.

[0063] In another aspect, there is provided an immunogenic composition comprising an infectious clone, a recombinant virus particle and/or a virus particle described herein. In general, suitable

compositions may be prepared according to methods which are known to those of ordinary skill in the art and accordingly may include a pharmaceutically acceptable carrier, diluent and/or adjuvant. The carriers, diluents and adjuvants must be "acceptable" in terms of being compatible with the other ingredients of the composition, and not deleterious to the recipient thereof.

5 [0064] One skilled in the art would be able, by routine experimentation, to determine an effective and safe amount of the immunogenic composition for administration to achieve the desired immunogenic response.

[0065] Generally, an effective dosage to achieve the desired immunogenic response is expected to be in the range of about 0.0001mg to about 1000mg per kg body weight per 24 hours; typically, about
10 0.001mg to about 750mg per kg body weight per 24 hours; about 0.01mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 250mg per kg body weight per 24 hours; about 1.0mg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range about 1.0mg to about 200mg per kg body weight per 24 hours; about 1.0mg to about 100mg per kg body weight per 24
15 hours; about 1.0mg to about 50mg per kg body weight per 24 hours; about 1.0mg to about 25mg per kg body weight per 24 hours; about 5.0mg to about 50mg per kg body weight per 24 hours; about 5.0mg to about 20mg per kg body weight per 24 hours; about 5.0mg to about 15mg per kg body weight per 24 hours.

[0066] Alternatively, an effective dosage to achieve the desired immunogenic response may be up
20 to about 500mg/m². Generally, an effective dosage is expected to be in the range of about 25 to about 500mg/m², preferably about 25 to about 350mg/m², more preferably about 25 to about 300mg/m², still more preferably about 25 to about 250mg/m², even more preferably about 50 to about 250mg/m², and still even more preferably about 75 to about 150mg/m².

[0067] In another example, the amount of vaccine administered to elicit the desired immunogenic
25 response is quantified based on the number of viruses. The number of viruses can be determined using methods known in the art, such as, but not limited to plaque assay, focus forming assay and endpoint dilution assay. The number of viruses to achieve the desired immunogenic response is expected to be in the range of about 10 to 10 million plaque forming units (PFU).

[0068] Generally, an effective PFU to achieve the desired immunogenic response may be about
30 10 to about 1.5 million PFU, about 1 million to about 2.5 million PFU, about 2 million to about 3.5 million PFU, about 3 million to about 4.5 million PFU, about 4 million to about 5.5 million PFU, about 5 million to about 6.5 million PFU, about 6 million to about 7.5 million PFU, about 7 million to about 8.5 million PFU, about 8 million to about 9.5 million PFU, or about 9 million to about 10 million PFU.

[0069] Alternatively, an effective PFU to achieve the desired immunogenic response may be about 100,000 to about 1 million PFU, about 900,000 to about 2 million PFU, about 1.9 million PFU to about 3 million PFU, about 2.9 million PFU to about 4 million PFU, about 3.9 million to about 5 million PFU, about 4.9 million PFU to about 6 million PFU, about 5.9 million PFU to about 7 million PFU, about 6.9 million PFU to about 7 million PFU, about 7.9 million PFU to about 9 million PFU, or about 8.9 million PFU to about 10 million PFU. In one example, the number of nsP mutants administered to elicit the desired immunogenic response may be about 1 million PFU as determined using plaque assay.

[0070] In one example of the immunogenic composition, the recombinant virus is a live attenuated CHIKV. In one example, the immunogenic composition further comprises an adjuvant, a preservative, a stabilizer and/or a pharmaceutically acceptable carrier. In one example, the adjuvant is selected from the group consisting of (a) aluminum hydroxide, (b) aluminum phosphate, (c) gamma inulin, (d) alammulin (a combination of aluminum hydroxide and gamma inulin), (e) cholecalciferol in oil, (f) an oil in water emulsion OWEM1, containing squalene, tween-80, Span-85 in 10 mM phosphate-citrate buffer, (f) oil in water emulsion OWEM2 containing squalene, tween-80, Span-85, alpha tocopherol in phosphate- citrate buffer, and (g) an oil in water emulsion OWEM3 containing squalene, tween-80, Span-85, cholecalciferol in phosphate-citrate buffer. In another example, the immunogenic composition may further comprise an expression vector described herein.

[0071] In another aspect, there is provided a method of eliciting an immune response against alphavirus infection in a subject comprising administering an immunogenic composition described herein to the subject. As demonstrated in the Examples below, the exemplary RH CHIKV and RHEV CHIKV nsP mutants are capable of inducing the immune response (Figure 3), which shows that infection with these mutants results in higher levels of Type 1 IFN response compared to infection with WT CHIKV. The exemplary RH CHIKV and RHEV CHIKV nsP mutants are capable of further providing protection from subsequent re-infection with WT CHIKV (Figure 5).

[0072] The immunogenic composition may be administered to the subject by a route selected from the group consisting of intramuscular, intradermal, subcutaneous, intravenous, oral, and intranasal administration. Thus, the immunogenic compositions of the disclosure may be in a form suitable for parenteral administration (that is, subcutaneous, intramuscular or intravenous injection), in the form of a formulation suitable for oral ingestion (such as capsules, tablets, caplets, elixirs, for example), or in an aerosol form suitable for administration by inhalation (such as by intranasal inhalation or oral inhalation).

[0073] For administration as an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2 propylene glycol.

[0074] For oral administration, suitable carriers, diluents, excipients and adjuvants include peanut oil, liquid paraffin, sodium carboxymethylcellulose, methylcellulose, sodium alginate, gum acacia, gum tragacanth, dextrose, sucrose, sorbitol, mannitol, gelatine and lecithin. In addition these oral formulations may contain suitable flavouring and colourings agents. When used in capsule form the capsules may be coated with compounds such as glyceryl monostearate or glyceryl distearate which delay disintegration.

[0075] Solid forms for oral administration may contain binders acceptable in human and veterinary pharmaceutical practice, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatine, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, guar gum, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, dextrose, kaolin, cellulose, calcium carbonate, calcium silicate or dicalcium phosphate. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

[0076] Liquid forms for oral administration may contain, in addition to the above agents, a liquid carrier. Suitable liquid carriers include water, oils such as olive oil, peanut oil, sesame oil, sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides or mixtures thereof.

[0077] Suspensions for oral administration may further comprise dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, poly-vinyl-pyrrolidone, sodium alginate or acetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters of fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or -laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or -laurate and the like.

[0078] The emulsions for oral administration may further comprise one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above or natural gums such as guar gum, gum acacia or gum tragacanth.

[0079] Drops for oral administration according to the present disclosure may comprise sterile aqueous or oily solutions or suspensions. These may be prepared by dissolving the immunogenic agent in an aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may then be clarified by filtration, transferred to a suitable container and sterilised. Sterilisation may be achieved by: autoclaving or maintaining at 90°C-100°C for half an hour, or by filtration, followed by transfer to a container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

[0080] The composition may incorporate any suitable surfactant such as an anionic, cationic or non-ionic surfactant such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also be included.

[0081] The compositions may also be administered in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances, and are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolisable lipid capable of forming liposomes can be used. The compositions in liposome form may contain stabilisers, preservatives, excipients and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic.

[0082] In one example, the subject to be administered the immunogenic composition is a human at risk of CHIKV infection, such as subjects living in areas (or in close proximity to areas) with a Chikungunya outbreak. The human subjects can be either adults or children. In another example, the subject to be administered the immunogenic composition is a human at risk of O'nyong'nyong virus infection, such as subjects living in areas (or in close proximity to areas) with an O'nyong'nyong outbreak. The method of the disclosure can also be used on other subjects at risk of CHIKV infection such as, but are not limited to, non-human primates, livestock animals (eg. sheep, cows, horses, donkeys, pigs), laboratory test animals (eg. rabbits, mice, rats, guinea pigs, hamsters), companion animals (eg. cats, dogs) and captive wild animals (eg. foxes, deer, dingoes).

[0083] The development of the alphavirus nsP mutants of the present disclosure may potentially be manipulated further not only as vaccine for alphavirus, but also as alternative vaccine candidates for all alphavirus because a similar effect of attenuation could be created using other mutations that affect the processing of nsP1/2 site in these viruses.

BRIEF DESCRIPTION OF THE DRAWINGS

[0084] The disclosure will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

5 [0085] **Fig. 1** shows that mutations in the CHIKV nsPs reduce viral infectivity and replicative potential in mouse tail fibroblasts (MTFs). Primary MTFs were isolated from C57BL/6 mice. MTFs were infected with ZsGreen (ZsG)-tagged wild-type (WT) CHIKV, CHIKV with R to H amino acid substitution (RH) at position 532, CHIKV with E to V amino acid substitution (EV) at position 1050 and CHIKV with both R to H and E to V amino acid mutations (RHEV) at both positions 532 and
10 1050, respectively. Subsequent analysis of infected MTF population was done by flow cytometry.

The graph in (A) shows the proportions of live MTFs that are infected at various time points. Statistical analysis was done using unpaired t-test ($*p<0.05$).

The graph in (B) shows the viral load of the MTFs at different time points.

The graph in (C) shows the 50% Tissue Culture Infective Dose (TCID₅₀) at 12hpi. The data are
15 representative of three independent experiments and are presented as mean $\pm$ SD.

The graph in (D) shows the proportions of live MTFs that are infected at 12hpi. Statistical analysis was done using unpaired t-test ($*p<0.05$). The graph in (D) is adapted from the 12 hpi time point for graph (A) with additional data for EV CHIKV.

The graph in (E) shows the viral load of the MTFs at 12 hpi. The data are representative of three
20 independent experiments and are presented as mean $\pm$ SD. The graph in (E) is adapted from the 12 hpi time point for graph (B) with additional data for EV CHIKV.

[0086] **Fig. 2** shows that mutations in the CHIKV nsPs result in more robust Type 1 IFN response. CHIKV-infected MTFs were harvested at 12 hours post infection (hpi), and concentrations of Type 1 IFN were analysed using Luminex® screening assay.

25 The graph in (A) shows the IFN- α concentration in CHIKV-infected MTFs at 12hpi.

The graph in (B) shows the IFN- β concentration in CHIKV-infected MTFs at 12hpi. The data are representative of three independent experiments and are presented as mean $\pm$ SD.

[0087] **Fig. 3** shows that mutations in the CHIKV nsPs results in faster clearance of viremia and less severe virus-induced joint inflammation. WT C57BL/6 mice were infected with ZsG-tagged WT
30 CHIKV, RH CHIKV, EV CHIKV and RHEV CHIKV at the metatarsal region of the footpad.

The graph in (A) shows viremia progression in virus-infected mice which were monitored over 2 weeks.

The graph in (B) shows joint inflammation of the mice which were monitored over 2 weeks. The data are representative of two independent experiments and are presented as mean $\pm$ SD (n=11).”Statistical

analysis was performed across all CHIKV-infected groups using one-way ANOVA, followed by Dunnett's post test comparing WT CHIKV (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

[0088] **Fig. 4** shows that mutations in the CHIKV nsPs result in lower infectivity at the site of inflammation. WT C57BL/6 mice were infected subcutaneously with ZsG-tagged WT CHIKV, RH CHIKV, EV CHIKV and RHEV CHIKV at the metatarsal region of the footpad.

The graph in (A) shows the level of leukocytes isolated from the footpad at 3 days post infection (dpi) and 6dpi using WT CHIKV, RH CHIKV, EV CHIKV and RHEV CHIKV, analysed using flow cytometry (G).

Infections with WT CHIKV, RH CHIKV and RHEV CHIKV, in various leukocyte subsets, were assessed, including in (B) monocytes and macrophages, (C) neutrophils, (D) NK cells, (E) CD4+ T cells, and (F) CD8+ T cells.

The data are representative of two independent experiments and are presented as mean $\pm$ SD (n=8). Statistical analysis was performed using two-tailed Mann Whitney U test (* $p < 0.05$).

[0089] **Fig. 5** shows that mice infected with CHIKV nsP mutants are protected from virus infection. WT C57BL/6 mice were re-infected with WT CHIKV 90 dpi after the first infection (with WT CHIKV, RH CHIKV and RHEV CHIKV) at the metatarsal region of the footpad. Joint inflammation of the mice was monitored over 2 weeks.

[0090] **Fig. 6** shows that the reduction in joint inflammation and absence of viremia upon CHIKV re-infection in vaccinated mice is mediated by CHIKV neutralizing antibody response. WT C57BL/6 mice were vaccinated subcutaneously with WT CHIKV or CHIKV nsP mutants (RH CHIKV, EV CHIKV and RHEV CHIKV) at the metatarsal region of the footpad. Re-infection with WT CHIKV infection was performed via the same route at 3 months post-infection.

The graph in (A) shows the severity of joint inflammation.

The graph in (B) shows viremia of the mice which were monitored over 2 weeks. The data are presented as mean $\pm$ SD (n=6).

The graph in (C) shows presence of CHIKV-specific antibodies in the pooled sera of the re-infected mice.

The graph in (D) shows pooled sera from re-infected mice were neutralizing against CHIKV infection in an in vitro neutralization assay. Percentage infection was normalized to virus-only infection. All data are presented as mean $\pm$ SD.

[0091] **Fig. 7** shows vaccination with attenuated CHIKVs protect against CHIKV-induced joint pathology.

Panel (A) shows representative hematoxylin and eosin (H&E) images of inflamed joint footpad on 6 dpi. "Ed" marks region of edema; the arrow in the "Mock-vaccinated + WT CHIKV" column marks synovitis; the arrow in the "WT CHIKV-vaccinated + WT CHIKV" column marks normal synovial

membrane; the arrow in the “RH CHIKV-vaccinated + WT CHIKV” column marks mild synovial hyperplasia; “*” marks infiltration of mononuclear cells; “D” marks degeneration of muscle; “N” marks necrosis of muscle; and “R” marks regeneration of muscle.

Panel (B) shows the histopathological scoring of edema, inflammation in different regions of the joint footpad and muscle pathology of CHIKV infected animals (n=5 per group) on 6 dpi.

Scoring was done on three sections from each joint footpad, and data were expressed as means $\pm$ SD. All data were analysed by one-way ANOVA with Tukey post-test (*P <0.05, **P <0.01, and ***P <0.001).

[0092] **Fig. 8** shows vaccination with WT and attenuated CHIKV do not induce protective antibodies in μ MT mice. μ MT mice were vaccinated subcutaneously with WT CHIKV or attenuated CHIKV (RH CHIKV) at the metatarsal region of the footpad. Re-infection with WT CHIKV infection was performed via the same route at 3 months post-infection.

The graph in (A) shows joint inflammation of re-infected μ MT mice.

The graph in (B) shows viremia quantification in mice.

The graph in (C) shows CHIKV-specific antibody IgG titer in μ MT mice.

The graph in (D) shows the neutralization capacity of the antibodies in μ MT peripheral blood determined using neutralization assay as described above.

Data are presented in mean $\pm$ standard error of the mean (SEM) and are representative of 2 independent experiments (n = 8).

[0093] **Fig. 9** shows high levels of viremia are detected in RAG-1^{-/-} mice vaccinated with WT and RH CHIKV. RAG-1^{-/-} mice were vaccinated subcutaneously with WT CHIKV or RH CHIKV at the metatarsal region of the footpad. Re-infection with WT CHIKV infection was performed via the same route at 3 months post-infection.

The graph in (A) shows joint inflammation of RAG-1^{-/-} and (B) viremia quantification in RAG-1^{-/-} mice upon vaccination.

The graph in (C) shows joint inflammation of RAG-1^{-/-} and (D) viremia quantification in RAG-1^{-/-} mice upon re-infection with WT CHIKV.

Data are presented in mean $\pm$ standard deviation (SD) (n = 5).

[0094] **Fig. 10** shows vaccination with RH CHIKV protects mice against WT ONNV infection. WT C57BL/6 mice were vaccinated subcutaneously with WT CHIKV or RH CHIKV at the metatarsal region of the footpad. Re-infection with WT ONNV was performed via the same route at 3 months post-infection.

The graph in (A) shows the severity of joint inflammation and (B) viremia of the mice monitored over 2 weeks. The data are presented as mean $\pm$ SD (n=5). All data are presented as mean $\pm$ SD. Data were analysed by Mann-Whitney *U* two-tailed analysis (*P <0.05, **P <0.01).

[0095] Fig. 11 shows the alignment of P1234 polypeptides of seven alphaviruses using multiple sequence alignment. “VEEV” indicates Venezuelan equine encephalitis virus; “BFV” indicates Barmah Forest virus; “ONNV” indicates O’nyong’nyong virus; “CHIKV” indicates Chikungunya virus; “RRV” indicates Ross River virus; “SFV” indicates Semliki Forest virus; and “MAYV” indicates Mayaro virus. An “*” (asterisk) indicates positions which have a single, fully conserved residue. A “:” (colon) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix. A “.” (period) indicates conservation between groups of weakly similar properties - scoring ≤ 0.5 in the Gonnet PAM 250 matrix. The amino acid residues with grey background indicate the amino acid residues at a position equivalent to position 532 of SEQ ID NO.: 1. The amino acids residues which are in bold, underlined and with grey background indicate the amino acid residues at a position equivalent to position 1050 of SEQ ID NO.: 1.

TABLES

[0096] Table 1: List of polypeptide sequences of the disclosure.

Polypeptide	Sequence
SEQ ID NO: 1 Polypeptide sequence of wild-type CHIKV LR2006 OPY1 non-structural polypeptide. The amino acid residues at positions 532 (R) and 1050 (E) are underlined and in bold.	MDPVYVDIDADSAFLKALQRAYPMFEVEPRQVTPNDHANA RAFSLAIKLIEQEIDPDSTILDIGSAPARRMMSDRKYHCVCP MRS AEDPERLANYARKLASAAGKVLDRNISGKIGDLQAVMA VPDTETPTFCLHTDVSCRQRADVAIYQDVYAVHAPTSLYHQ AIKGV RVAYWVGFDTPPFMYNAMAGAYPSYSTNWADEQV LKAKNIGLCSTDLTEGRRGKLSIMRGKKLKPCDRVLFSVGST LYPESRKLKSWHLPSVFHLKGKLSFTCRCDTVVSCEGYVV KRITMSPGLYGKTTGYAVTHHADGFLMCKTTDTVDGERMSF SVCTYVPATICDQMTGILATEVTPEDAQKLLVGLNQRI VNG RTQRNTNTMKNYLLPVVAQAFSKWAKECRKDMEDEKLLGV RERTLTCCCLWAFKKQKTHTVYKRPDTQSIQKVQAEFDSFV VPSLWSSGLSIPLRTRIKWLLSKVPKTDLPYSGDAREARDAE KEAEEEREAE LTREALPPLQAAQEDVQVEIDVEQLED R AGA GIIETPRGAIKVTAQPTDHVVGEYLVLSPTVLR SQKLSLIHA LAEQVKTC THNGRAGRYAVEAYDGRVLVPSGYAISPEDFQS LSESATMVYNEREFVNRKLHHIAMHG PALNTDEESYELVRA ERTEHEYVDVDQRRCKKEEAAGLV LVGDLTNPPYHEFAY EGLKIRPACPYKIAVIGVFGVPGSGKSAIKNLVTRQDLVTSG KKENCQEITTDV MRQRGLEISARTVDSLLLNGCNR PVDVLY VDEAFACHSGTLLALIALVRPRQKVVL CGDPKQCGFFNMMQ MKVNYNHNICTQVYHKSISR RCTLPVTAIVSSLHYEGKMRTT NEYNKPIVVDTTGSTKPDGDLVLT CFRGWVKQLQIDYRGY EVMTAAASQGLTRKGVYAVRQKVNENPLYASTSEHVNLL TRTEGKLVWKTLSGDPWIKTLQNPPKGNFKATIKEW EVEHA SIMAGICSHQMTFDTFQNKANVCWAKSLVPILETAGIKL NDR QWSQIIQAFKEDKAYSP E VALNEICTRMYGVDLDSGLFSKPL

	VSVYYADNHWDNRPGGKMFGFNPEAASILERKYPFTKGKW NINKQICVTTTRIEDFNPTTNIPANRRLPHSLVAEHRPVKGER MEWLVNKINGHHVLLVSGYNLALPTKRVTWVAPLGVRGAD YTYNLELGLPATLGRYDLVVINIHTPFRIHHYQQCVDHAMKL QMLGGDSLRLLPKPGGSLLIRAYGYADRTSERVICVLGRKFERS SRALKPPCVTSNTEMFFLFSNFDNGRRNFTTHVMNNQLNAA FVGQVTRAGCAPSYRVKRMDIAKNDEECVVNAANPRGLPG GGVCKAVYKKWPESFKNSATPVGTAKTVMCGTYPVIHAVG PNFSNYSESEGDRELAAYREVAKVTRLGVNSVAIPLLSTG VYSGGKDRLTQSLNHLFTAMDSTDADVVIYCRDKEWEKKIS EAIQMRTQVELLDEHISIDCDIVRVHPDSSLAGRKGYSTTEGA LYSYLEGTRFHQTAVDMAEIHTMWPKQTEANEQVCLYALG ESIESIRQKCPVDDADASSPPKTVPCLCRYAMTPERVTRLRM NHVTSIIVCSSFPLPKYKIEGVQKVCKSKVMLFDHNVPSRVSP REYRSSQESAQEASTITSLTHSQFDLSVDGEILPVPSDLDDADA PALEPALDDGATHTLPTSTGNLAAVSDWVMSTVPVAPPRRR RGRNLTVTCDEREGNITPMASVRFFRAELCPVVQETAETRDT AMSLQAPPSTATEPNHPPISFGASSETFPITFGDFNEGEIESLSS ELLTFGDFLPGEVDDLTDSDWSTCSDTDDELRLDRAGGYIFS SDTGPGHLQQKSVRQSVLPVNTLEEVHEEKCYPPKLDEAKE QLLLKKLQESASMANRSRYQSRKVENMKAAIIQRLKRGCRLL YLMSETPKVPTYRTTYPAPVYSPPINVRLSNPESAVAACNEFL ARNYPTVSSYQITDEYDAYLDMVDGSESCLDRAFNP SKLRS YPKQHAYHAPSIRSAVPSPFQNTLQNVLAAATKRNCNVTQM RELPTLDSAVFNVECFKKFACNQEYWEEFAASPIRITTENLAT YVTKLKGPKAAALFAKTHNLLPLQEVPMDFRTVDMKRDVK VTPGTHKTEERPKVQVIQAAEPLATAYLCGIHRELVRRLNAV LLPNVHTLFDMSAEDFDAIIAAHFKPGDTVLETDIASFDKSQD DSLALTALMLLEDLGVHDHSLDLIEAAFGEISSCHLPTGTRFK FGAMMKSGMFLTLFVNTLLNITIASRVLEDRLTKSACAAFIG DDNIIHGVVSDELMAARCATWMNMEVKIIDAVVSLKAPYFC GGFILHDTVGTACRVADPLKRLFKLGKPLAAGDEQDEDRR RALADEVIRWQRTGLIDELEKAVYSRYEVQGISVVVMSMAT FASSRSNFELRGPVITLYGGPK
SEQ ID NO: 2 Polypeptide sequence of CHIKV LR2006 OPY1 non-structural polyprotein with mutation at position 532. The amino acid residue at position 532 (R mutated to H) is underlined and in bold.	MDPVYVDIDADSAFLKALQRAYPMFEVEPRQVTPNDHANA RAFSHLAIKLIEQEIDPDSTILDIGSAPARRMMSDRKYHCVCP MRSAEDPERLANYARKLASAAGKVLDRNISGKIGDLQAVMA VPDTETPTFCLHTDVSCRQRADVAIYQDVYAVHAPTSLYHQ AIKGVRVAYWVGFDTPPFMYNAMAGAYPSYSTNWADEQV LKAKNIGLCSTDLTEGRRGKLSIMRGKKLKPCDRVLFSVGST LYPESRKLLKSWHLPSVFHLKGKLSFTCRCDTVVSCEGYVV KRITMSPGLYGKTTGYAVTHHADGFLMCKTTDTVDGERMSF SVCTYVPATICDQMTGILATEVTPEDAQKLLVGLNQRIVVNG RTQRNTNTMKNYLLPVVAQAFSKWAKECRKDMEDEKLLGV RERTLTCCCLWAFKKQKTHTVYKRPDTQSIQKVQAEFDSFV VPSLWSSGLSIPLRTRIKWLLSKVPKTDLPYSGDAREARDAE

	KEAEEEREAEALTREALPPLQAAQEDVQVEIDVEQLEDHAGA GIIETPRGAIKVTAQPTDHVVGEYLVLSPQTVLRSQKLSLIHA LAEQVKTCTHNGRAGRYAVEAYDGRVLVPSGYAISPEDFQS LSESATMVYNEREFVNRKLHHIAMHGPALNTDEESYELVRA ERTEHEYVYDVDQRRCKKEEAAGLVLVGDLTNPPYHEFAY EGLKIRPACPYKIAVIGVFGVPGSGKSAAIKNLVTRQDLVTSG KKENCQEITTDVMRQRGLEISARTVDSLLLNGCNRPVLDVLY VDEAFACHSGTLLALIALVRPRQKVVLCDGPKQCGFFNMMQ MKVNYNHNICTQVYHKSISRCTLPTAIVSSLHYEGKMRTT NEYNKPIVDDTTGSTKPDGDLVLTFCRGWVKQLQIDYRGY EVMTAAASQGLTRKGVYAVRQKVNENPLYASTSEHVNLL TRTEGKLVWKTLSGDPWIKTLQNPFGNFKATIKEWEEHA SIMAGICSHQMTFDTFQNKANVCWAKSLVPILETAGIKLNDR QWSQIIQAFKEDKAYSPEVALNEICTRMYGVDLDSGLFSKPL VSVYYADNHWDNRPGGKMFGFNPEAASILERKYPFTKGKW NINKQICVTTTRIEDFNPTTNIPANRRLPHSLVAEHRPVKGER MEWLVNKGHHVLLVSGYNLALPTKRVTWVAPLGVRGAD YTYNLELGLPATLGRYDLVVINIHTPFRIHHYQQCVDHAMKL QMLGGDSLRLKPGGSLLIRAYGYADRTSERVICVLGRKFRS SRALKPPCVTSNTEMFFLFSNFDNGRRNFTTHVMNNQLNAA FVGQVTRAGCAPSYRVKRMDIAKNDEECVVNAANPRGLPG GGVCKAVYKKWPESFKNSATPVGTAKTVMCGTYPVIAVG PNFSNYSESEGDRELAAAYREVAKVTRLGVNSVAIPLLSTG VYSGGKDRLTQSLNHLFTAMDSTDADVVIYCRDKEWEKKIS EAIQMRTQVELLDEHISIDCDIVRVHPDSSLAGRKGYSTTEGA LYSYLEGTRFHQTAVDMAEIHTMWPKQTEANEQVCLYALG ESIESIRQKCPVDDADASSPPKTPVCLCRYAMTPERVTRLRM NHVTSIIVCSSFPLPKYKIEGVQKVCKSKVMLFDHNVPSRVSP REYRSSQESAQEASTITSLTHSQFDLSVDGEILPVPSDLADA PALEPALDDGATHTLPTTGNLAAVSDWVMSTVPVAPRRR RGRNLTVTCDEREGNITPMASVRFFRAELCPVVQETAETRDT AMSLQAPPSTATEPNHPPISFGASSETFPITFGDFNEGEIESLSS ELLTFGDFLPGEVDDLTDSDWSTCSDTDDELRLDRAGGYIFS SDTGPGHLQQKSVRQSVLPVNTLEEVEHEKCYPPKLDEAKE QLLLKKLQESASMANRSRYQSRKVENMKAAIIQRLKRGCR YLMSETPKVPTYRTTYPAPVYSPPINVRLSNPESAVAACNEFL ARNYPTVSSYQITDEYDAYLDMVDGSESCDRATFNPSKLR YPKQHAYHAPSIRSAVPSPFQNTLQNVLAAATKRNCNVTQM RELPTLDSAVFNVECFKKFACNQEYWEFEAASPIRITTENLAT YVTKLKGPKAAALFAKTHNLLPLQEVPMDFRTVDMKRDVK VTPGKHTTEERPKVQVIQAAEPLATAYLCGIHRELVRRLNAV LLPNVHTLFDMSAEDFDAIIAAHFKPGDTVLETDIASFDKSQD DSLALTALMLLEDLGVHDHSLDLIEAAFGEISSCHLPTGTRFK FGAMMKSGMFLTLFVNTLLNITIASRVLEDRLTKSACAAFIG DDNIIHGVSDELMAARCATWMNMEVKIIDAVVSLKAPYFC GGFILHDTVGTACRVADPLKRLFKLGKPLAAGDEQDEDRR
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	RALADEVIRWQRTGLIDELEKAVYSRYEVQGISVVVMSMAT FASSRSNFEKLRGPVITLYGGPK
SEQ ID NO: 3 Polypeptide sequence of CHIKV LR2006 OPY1 non-structural polyprotein with mutation at position 1050. The amino acid residue at position 1050 (E mutated to V) is underlined and in bold.	MDPVYVDIDADSAFLKALQRAYPMFEVEPRQVTPNDHANA RAFSHLAIKLIEQEIDPDSTILDIGSAPARRMMSDRKYHCVC MRSAEDPERLANYARKLASAAGKVLDRNISGKIGDLQAVMA VPDTETPTFCLHTDVSCRQRADVAIYQDVYAVHAPTSLYHQ AIKGV RVAYWVGFDTPFMYNAMAGAYPSYSTNWADEQV LKAKNIGLCSTDLTEGRRGKLSIMRGKKLKPCDRVLFSVGST LYPESRKLKSWHLPSVFHLKGKLSFTCRCDTVVSCEGYVV KRITMSPGLYGKTTGYAVTHHADGFLMCKTTDTVDGERMSF SVCTYVPATICDQMTGILATEVTPEDAQKLLVGLNQRIVNG RTQRNTNTMKNYLLPVVAQAFSKWAKECRKDMEDEKLLGV RERTLTCCCLWAFKKQKTHTVYKRPDTQSIQKVQAEFDSFV VPSLWSSGLSIPLRTRIKWLLSKVPKTDLPYSGDAREARDAE KEAEEEREAEALTREALPPLQAAQEDVQVEIDVEQLEDRAGA GIIETPRGAIKVTAQPTDHVVGEYLVLSPTVLR SQKLSLIHA LAEQVKTCTHNGRAGRYAVEAYDGRVLVPSGYAISPEDFQS LSESATMVYNEREFVNRKLHHIAMHG PALNTDEESYELVRA ERTEHEYVYDQRRCKKEEAAGLVLVGDLTNPPYHEFAY EGLKIRPACPYKIAVIGVFGVPGSGKSAIKNLVTRQDLVTSG KKENCQEITTDV MRQRGLEISARTVDSL LNGCNR PVDVLY VDEAFACHSGTLLALIALVRPRQKVVL CGDPKQCGFFNMMQ MKVNYNHNICTQVYHKSISRCTL PVT AIVSSLHYEGKMRTT NEYNKPIVDDTTGSTKPDGDLVLT CFRGWVKQLQIDYRGY EVMTAAASQGLTRKGVYAVRQKVNENPLYASTSEHVNVL TRTEGKLVWKTLSGDPWIKTLQNPPKGNFKATIKEWVEHA SIMAGICSHQMTFDTFQNKANVCWAKSLVPILETAGIKLND RQWSQIIQAFKEDKAYSP<u>V</u>ALNEICTRMYGVDLDSGLFSKPL VSVYYADNHWDNRPGGKMFGFNPEAASILERKYPFTKGKW NINKQICVTTTRIEDFNPTTNIPANRRLPHSLVAEHRPVKGER MEWLVNKINGHHVLLVSGYNLALPTKRVTWVAPLGVRGAD YTYNLELGLPATLGRYDLV VINIHTPFRIHHYQQCVDHAMKL QMLGGDSLRL LKPGGSLLIRAYGYADRTSERVICVLGRKF RSRALKPPCVTSNTEMFFLFSNFDNGRRNFTTHVMNNQLNAA FVGQVTRAGCAPSYRVKRMDIAKNDEECVVNAANPRGLPG GGVCKAVYKKWPESFKNSATPVGTAKTVMCGTYPVIHVG PNFSNYSESEGDRELAAYREVAKVTRLGVNSVAIPLLSTG VYSGGKDRLTQSLNHLFTAMDSTDADVVIYCRDKEWEKKIS EAIQMRTQVELLDEHISIDCDIVRVHPDSSLAGRKGYSTTEGA LYSYLEGTRFHQTAVDMAEIHTMWPKQTEANEQVCLYALG ESIESIRQKCPVDDADASSPPKTPVCLCRYAMTPERVTRLRM NHVTSIIVCSSFPLPKYKIEGVQKV KCSKVMLFDHNVPSRVSP REYRSSQESAQEASTITSLTHSQFDLSVDGEILPVPSDLDA DAPALEPALDDGATHTL PSTTGNLAAVSDWVMSTVPVAPPRRR RGRNLTVTCDEREGNITPMASVRFFRAELCPVVQETAETRDT AMSLQAPPSTATEPNHPPISFGASSETFPITFGDFNEGEIESLSS

	ELLTFGDFLPGEVDDLTDSDWSTCSDTDDELRLDRAGGYIFS SDTGPGLHQQKSVRQSVLPVNTLEEVHEEKCYPPKLDEAKE QLLLKKLQESASMANRSRYQSRKVENMKAIIQRLKRGCR YLMSETPKVPTYRTTYPAPVYSPPINVRLSNPESAVAACNEFL ARNYPTVSSYQITDEYDAYLDMVDGSESCDRATFNPSKLR YPKQHAYHAPSIRSAVPSPFQNTLQNVLAAATKRNCNVTQM RELPTLDSAVFNVECFKKFACNQEYWEEFAASPIRITTENLAT YVTKLKGPKAAALFAKTHNLLPLQEVPMDFRTVDMKRDVK VTPGTHTEERPKVQVIQAAEPLATAYLCGIHRELVRRLNAV LLPNVHTLFDMSAEDFDAIIAAHFKPGDTVLETDIASFDKSQD DSLALTALMLLEDLGVHDHSLDLIEAAFGEISSCHLPTGTRFK FGAMMKSGMFLTLFVNTLLNITIASRVLEDRLTKSACAAFIG DDNIIHGVVSDELMAARCATWMNMEVKIIDAVVSLKAPYFC GGFILHDTVGTACRVADPLKRLFKLGKPLAAGDEQDEDRR RALADEVIRWQRTGLIDELEKAVYSRYEVQGISVVVMSMAT FASSRSNFEKLRGPVITLYGGPK
SEQ ID NO: 4 Polypeptide sequence of CHIKV LR2006 OPY1 non-structural polyprotein with mutations at 532 and 1050. The amino acid residues at positions 532 (R mutated to H) and 1050 (E mutated to V) are underlined and in bold.	MDPVYVDIDADSAFLKALQRAYPMFEVEPRQVTPNDHANA RAFSHLAIKLIEQEIDPDSTILDIGSAPARRMMSDRKYHCVCP MRS AEDPERLAN YARKLASAAGKVLDNRNISKIGDLQAVMA VPDTETPTFCLHTDVSCRQRA DVAIYQDVYAVHAPTSLYHQ AIKGV RVAYWVGFDTPFMYNAMAGAYPSYSTNWADEQV LKAKNIGLCSTDLTEGRRGKLSIMRGKKLKPCDRVLFSVGST LYPESRKLLKSWHLPSVFHLKGKLSFTCRCDTVVSCEGYVV KRITMSPGLY GKT TGYAVTHHADGFLMCKTTDTVDGERMSF SVCTYVPATICDQMTGILATEVTPEDAQKLLVGLNQRI VNG RTQRNTNTMKNYLLPVVAQA FSKWAKECRKDMEDEKLLGV RERTLTCCCLWAFKKQKTHTVYKRPDTQSIQKVQAEFDSFV VPSLWSSGLSIPLRTRIKWLLSKVPKTDLPYSGDAREARDAE KEAEEEREAE LTREALPPLQAAQEDVQVEIDVEQLED HAGA GIIETPRGAIKVTAQPTDHVVGEYLVLSPTVLRSQKLSLIHA LAEQVKTC THNGRAGRYAVEAYDGRVLVPSGYAISPEDFQS LSESATMVYN EREFVNRKLHHIAMHGPALNTDEESYELVRA ERTEHEYVYD V DQRRCKKEEAAGLVLVGDLTNPPYHEFAY EGLKIRPACPYKIAVIGVFGVPGSGKSAIKNLVTRQDLVTSG KKENCQEITTDVMRQRGLEISARTVDSLLLNGCNRPV D VLY VDEAFACHSGTLLALIALVRPRQKVVL CGDPKQCGFFNMMQ MKVNYNHNICTQVYHKSISRRCTLPVTAIVSSLHYEGKMRTT NEYNKPIVVDTTGSTKPDPGDLVLT C FRGWVKQLQIDYRGY EVMTAAASQGLTRKGVYAVRQKVNENPLYASTSEHVNVL TRTEGKLVWKTLSGDPWIKTLQNP PKGNFKATIKEW E V EHA SIMAGICSHQMTFDTFQNKANVCWAKSLVPILETAGIKLNDR QWSQIIQAFKEDKAYSP V VALNEICTRMYGVDLDSGLFSKPL VSVYYADNHWDNRPGGKMFGFNPEAASILERKYPFTKGKW NINKQICVTTRIEDFNPTTNIIPANRRLPHSLVAEHRPVKGER MEWL VNKINGHHVLLVSGYNLALPTKRVTVVAPLGVRGAD YTYNLELGLPATLGRYDLV VINIHTPFRIHHYQQCVDHAMKL

	QMLGGDSLRLLPKPGSLLIRAYGYADRTSERVICVLGRKFRS SRALKPPCVTSNTEMFFLFSNFDNGRRNFTTHVMNNQLNAA FVGQVTRAGCAPSYRVKRMDIAKNDEECVVNAANPRGLPG GGVCKAVYKKWPESFKNSATPVGTAKTVMCGTYPVIHAVG PNFSNYSESEGDRELAAYREVAKVTRLGVNSVAIPLLSTG VYSGGKDRLTQSLNHLFTAMDSTDADVVIYCRDKEWEKKIS EAIQMRTQVELLDEHISIDCDIVRVHPDSSLAGRKGYSTTEGA LYSYLEGTRFHQTAVDMAEIHTMWPKQTEANEQVCLYALG ESIESIRQKCPVDDADASSPPKTVPCLCRYAMTPERVTRLRM NHVTSIIVCSSFPLPKYKIEGVQKVKCSKVMLFDHNVPSRVSP REYRSSQESAQEASTITSLTHSQFDLSVDGEILPVPSDLDDADA PALEPALDDGATHTLPTTGNLAAVSDWVMSTVPVAPRRR RGRNLTVTCDEREGNITPMASVRFFRAELCPVVQETAETRD AMSLQAPPSTATEPNHPPISFGASSETFPITFGDFNEGEIESLSS ELLTFGDFLPGEVDDLTDSDWSTCSDTDDELRLDRAGGYIFS SDTGPGHLQKQSVRQSVLPVNTLEEVHEEKCYPPKLDEAKE QLLLKKLQESASMANRSRYQSRKVENMKAIIQRLKRGCR YLMSETPKVPTYRTTYPAPVYSPPINVRLSNPESAVAACNEFL ARNYPTVSSYQITDEYDAYLDMVDGSESCLD RATFNPSKLRS YPKQHAYHAPSIRSAVPSPFQNTLQNVLAAATKRNCNVTQM RELPTLDSAVFNVECFKKFACNQEYWEEFAASPIRITTENLAT YVTKLKGPKAAALFAKTHNLLPLQEVPMDFRTVDMKRDVK VTPGTHTEERPKVQVIQAAEPLATAYLCGIHRELVRRLNAV LLPNVHTLFDMSAEDFDAIIAAHFKPGDTVLETDIASFDKSQD DSLALTALMLLEDLGVHDHSLDLIEAAFGEISSCHLPTGTRFK FGAMMKSGMFLTLFVNTLLNITIASRVLEDRLTKSACAAFIG DDNIIHGVSDELMAARCATWMNMEVKIIDAVVSLKAPYFC GGFILHDTVGTACRVADPLKRLFKLGKPLAAGDEQDEDRR RALADEVIRWQRTGLIDELEKAVYSRYEVQGISVVVMSMAT FASSRSNFEKLRGPVITLYGGPK
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[0097] Table 2: List of polynucleotide sequences of the disclosure.

Polynucleotide	Sequence
SEQ ID NO: 5 Polynucleotide sequence of CHIKV LR2006 OPY1 non-structural polyprotein with mutation at position 532 (CHIKV-R532H). The amino acid at position 532 is mutated from R to H via mutagenesis of nucleotide sequences from AGA to CAC (underlined and in bold).	ATGGCTGCGTGAGACACACGTAGCCTACCAGTTTCTTAC TGCTCTACTCTGCAAAGCAAGAGATTAATAACCCATCAT GGATCCTGTGTACGTGGACATAGACGCTGACAGCGCCTT TTTGAAGGCCCTGCAACGTGCGTACCCCATGTTTGAGGT GGAACCAAGGCAGGTCACACCGAATGACCATGCTAATGC TAGAGCGTTCTCGCATCTAGCTATAAACTAATAGAGCA GGAAATTGACCCCGACTCAACCATCCTGGATATCGGCAG TGCGCCAGCAAGGAGGATGATGTCGGACAGGAAGTACC ACTGCGTCTGCCCCGATGCGCAGTGCAGGAAGATCCCGAGA GACTCGCCAATTATGCGAGAAAGCTAGCATCTGCCGCAG GAAAAGTCCTGGACAGAAACATCTCTGGAAAGATCGGG GACTTACAAGCAGTAATGGCCGTGCCAGACACGGAGAC

	GCCAACATTCTGCTTACACACAGACGTCTCATGTAGACA GAGAGCAGACGTCGCTATATACCAAGACGTCTATGCTGT ACACGCACCCACGTCGCTATACCACCAGGCGATTAAAGG GGTCCGAGTGGCGTACTGGGTGTTGGGTTCGACACAACCC GTTTCATGTACAATGCCATGGCGGGTGCCTACCCCTCATA CTCGACAAACTGGGCAGATGAGCAGGTAAGGCTA AGAACATAGGATTATGTTCAACAGACCTGACGGAAGGTA GACGAGGCAAGTTGTCTATTATGAGAGGGAAAAAGCTA AAACCGTGCGACCGTGTGCTGTTCTCAGTAGGGTCAACG CTCTACCCGGAAGCCGCAAGCTACTTAAGAGCTGGCAC CTGCCATCGGTGTTCCATTTAAAGGGCAAACCTCAGCTTC ACATGCCGCTGTGATACAGTGGTTTCGTGTGAGGGCTAC GTCGTTAAGAGAATAACGATGAGCCCAGGCCTTTATGGA AAAACCACAGGGTATGCGGTAACCCACCACGCAGACGG ATTCCTGATGTGCAAGACTACCGACACGGTTGACGGCGA AAGAATGTCATTCTCGGTGTGCACATACGTGCCGGCGAC CATTTGTGATCAAATGACCGGCATCCTTGCTACAGAAGT CACGCCGGAGGATGCACAGAAGCTGTTGGTGGGGCTGA ACCAGAGAATAGTGGTTAACGGCAGAACGCAACGGAAT ACGAACACCATGAAAAATTATCTGCTTCCCGTGGTCCG CAAGCCTTCAGTAAGTGGGCAAAGGAGTGCCGGAAAGA CATGGAAGATGAAAACTCCTGGGGGTCAGAGAAAGAA CACTGACCTGCTGCTGTCTATGGGCATTCAAGAAGCAGA AAACACACACGGTCTACAAGAGGCCTGATACCCAGTCAA TTCAGAAGGTTCAAGGCCGAGTTTGACAGCTTTGTGGTAC CGAGTCTGTGGTCGTCCGGGTTGTCAATCCCTTTGAGGAC TAGAATCAAATGGTTGTAAAGCAAGGTGCCAAAAACCGA CCTGATCCCATACAGCGGAGACGCCCAGAAAGCCCGGG ACGCAGAAAAAGAAGCAGAGGAAGAACGAGAAGCAGA ACTGACTCGCGAAGCCCTACCACCTCTACAGGCAGCACA GGAAGATGTTTCAGGTCGAAATCGACGTGGAACAGCTTGA GGAC<u>CAC</u>GCGGGCGCAGGAATAATAGAGACTCCGAGAG GAGCTATCAAAGTTACTGCCCAACCAACAGACCACGTCTG TGGGAGAGTACCTGGTACTCTCCCCGCAGACCGTACTAC GTAGCCAGAAGCTCAGTCTGATTCACGCTTTGGCGGAGC AAGTGAAGACGTGCACGCACAACGGACGAGCAGGGAGG TATGCGGTCTGAAGCGTACGACGGCCGAGTCCTAGTGCCC TCAGGCTATGCAATCTCGCCTGAAGACTTCCAGAGTCTA AGCGAAAGCGCAACGATGGTGTATAACGAAAGAGAGTT CGTAAACAGAAAGCTACACCATATTGCGATGCACGGACC AGCCCTGAACACCGACGAAGAGTCGTATGAGCTGGTGAG GGCAGAGAGGACAGAACACGAGTACGTCTACGACGTGG ATCAGAGAAGATGCTGTAAGAAGGAAGAAGCCGCAGGA CTGGTACTGGTGGGCGACTTGACTAATCCGCCCTACCAC GAATTTCGCATATGAAGGGCTAAAAATCCGCCCTGCCTGC CCATACAAAATTGCAGTCATAGGAGTCTTCGGAGTACCG
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	GGATCTGGCAAGTCAGCTATTATCAAGAACCTAGTTACC AGGCAGGACCTGGTGA CTAGCGGAAAGAAAGAAA ACTG CCAAGAAATCACCACCGACGTGATGAGACAGAGAGGTC TAGAGATATCTGCACGTACGGTTGACTCGCTGCTCTTGA ATGGATGCAACAGACCAGTCGACGTGTTGTACGTAGACG AGGCGTTTGC GTGCCACTCTGGAACGCTACTTGCTTTGAT CGCCTTGGTGAGACCAAGGCAGAAAGTTGTACTTTGTGG TGACCCGAAGCAGTGCGGCTTCTTCAATATGATGCAGAT GAAAGTCAACTATAATCACAACATCTGCACCCAAGTGTA CCACAAAAGTATCTCCAGGCGGTGTACACTGCCTGTGAC CGCCATTGTGTCATCGTTGCATTACGAAGGCAAAATGCG CACTACGAATGAGTACAACAAGCCGATTGTAGTGAGACAC TACAGGCTCAACAAAACCTGACCCTGGAGACCTCGTGTT AACGTGCTTCAGAGGGTGGGTAAACA ACTGCAAATTGA CTATCGTGGATACGAGGTCATGACAGCAGCCGCATCCCA AGGGTTAACCAGAAAAGGAGTTTACGCAGTTAGACAAA AAGTTAATGAAAACCCGCTCTATGCATCAACGTCAGAGC ACGTCAACGTACTCCTAACGCGTACGGAAGGTAAACTGG TATGGAAGACACTTTCCGGCGACCCGTGGATAAAGACGC TGCAGAACCCACCGAAAGGAACTTCAAAGCAACTATTA AGGAGTGGGAGGTGGAGCATGCATCAATAATGGCGGGC ATCTGCAGTCACCAAATGACCTTCGATACATTCCAAAAT AAAGCCAACGTTTGTGGGCTAAGAGCTTGGTCCCTATC CTCGAAACAGCGGGGATAAACTAAATGATAGGCAGTG GTCTCAGATAATTCAAGCCTTCAAAGAAGACAAAGCATA CTCACCTGAAGTAGCCCTGAATGAAATATGTACGCGCAT GTATGGGGTGGATCTAGACAGCGGGCTATTTTCTAAACC GTTGGTGTCTGTGTATTACGCGGATAACCACTGGGATAA TAGGCCTGGAGGGAAATGTTTCGGATTTAACCCCGAGGC AGCATCCATTCTAGAAAGAAAGTATCCATTCACAAAAGG GAAGTGGAACATCAACAAGCAGATCTGCGTGACTACCAG GAGGATAGAAGACTTTAACCCTACCACCAACATCATACC GGCCAACAGGAGACTACCACACTCATTAGTGGCCGAACA CCGCCCAGTAAAAGGGGAAAGAATGGAATGGCTGGTTA ACAAGATAAACGGCCACCACGTGCTCCTGGTCAGTGGCT ATAACCTTGCACTGCCTACTAAGAGAGTCACTTGGGTAG CGCCGTTAGGTGTCCGCGGAGCGGACTACACATAACAACC TAGAGTTGGGTCTGCCAGCAACGCTTGGTAGGTATGACC TAGTGGTCATAAACATCCACACACCTTTTCGCATACACC ATTACCAACAGTGCGTCGACCACGCAATGAACTGCAAAA TGCTCGGGGGTGACTCATTGAGACTGCTCAAACCGGGCG GCTCTCTATTGATCAGAGCATATGGTTACGCAGATAGAA CCAGTGAACGAGTCATCTGCGTATTGGGACGCAAGTTTA GATCGTCTAGAGCGTTGAAACCACCATGTGTCACCAGCA ACACTGAGATGTTTTTCCTATTCAGCAACTTTGACAATGG CAGAAGGAATTCACAACCTCATGTCATGAACAATCAACT
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	GAATGCAGCCTTCGTAGGACAGGTCACCCGAGCAGGATG TGCACCGTTCGTACCGGGTAAAACGCATGGACATCGCGAA GAACGATGAAGAGTGCCTAGTCAACGCCGCTAACCCCTCG CGGGTTACCGGGTGGCGGTGTTTGCAAGGCAGTATACAA AAAATGGCCGGAGTCTTTAAGAACAGTGCAACACCAGT GGGAACCGCAAAAACAGTTATGTGCGGTACGTATCCAGT AATCCACGCTGTTGGACCAAACCTTCTCTAATTATTCGGAG TCTGAAGGGGACCGGGAATTGGCAGCTGCCTATCGAGAA GTCGCAAAGGAAGTAACTAGGCTGGGAGTAAATAGTGT AGCTATACCTCTCCTCTCCACAGGTGTATACTCAGGAGG GAAAGACAGGCTGACCCAGTCACTGAACCACCTCTTTAC AGCCATGGACTCGACGGATGCAGACGTGGTCATCTACTG CCGCGACAAAGAATGGGAGAAGAAAATATCTGAGGCCA TACAGATGCGGACCCAAGTAGAGCTGCTGGATGAGCACA TCTCCATAGACTGCGATATTGTTTCGCGTGCACCCTGACAG CAGCTTGGCAGGCAGAAAAGGATACAGCACCACGGAAG GCGCACTGTACTCATATCTAGAAGGGACCCGTTTTCATC AGACGGCTGTGGATATGGCGGAGATACATACTATGTGGC CAAAGCAAACAGAGGCCAATGAGCAAGTCTGCCTATATG CCCTGGGGGAAAGTATTGAATCGATCAGGCAGAAATGCC CGGTGGATGATGCAGACGCATCATCTCCCCCAAACCTG TCCCGTGCCTTTGCCGTTACGCTATGACTCCAGAACGCGT CACCCGGCTTCGCATGAACCACGTCACAAGCATAATTGT GTGTTCTTCGTTTCCCCTCCCAAAGTACAAAATAGAAGG AGTGCAAAAAGTCAAATGCTCTAAGGTAATGCTATTTGA CCACAACGTGCCATCGCGCGTAAGTCCAAGGGAATATAG ATCTTCCCAGGAGTCTGCACAGGAGGCGAGTACAATCAC GTCCTGACGCATAGTCAATTTCGACCTAAGCGTTGATGG CGAGATACTGCCCCGTCCCGTCAGACCTGGATGCTGACGC CCCAGCCCTAGAACCAGCACTAGACGACGGGGCGACAC ACACGCTGCCATCCACAACCGGAAACCTTGCGGGCCGTGT CTGATTGGGTAAATGAGCACCGTACCTGTGCGCGCCGCCA GAAGAAGGCGAGGGAGAAACCTGACTGTGACATGTGAC GAGAGAGAAGGGAATATAACACCCATGGCTAGCGTCCG ATTCTTTAGGGCAGAGCTGTGTCCGGTCGTACAAGAAAC AGCGGAGACGCGTGACACAGCAATGTCTCTTCAGGCACC ACCGAGTACCGCCACGGAACCGAATCATCCGCCGATCTC CTTCGGAGCATCAAGCGAGACGTTCCCCATTACATTTGG GGACTTCAACGAAGGAGAAATCGAAAGCTTGTCTTCTGA GCTACTAACTTTCGGAGACTTCTTACCAGGAGAAGTGGA TGACTTGACAGACAGCGACTGGTCCACGTGCTCAGACAC GGACGACGAGTTAAGACTAGACAGGGCAGGTGGGTATA TATTCTCGTCGGACACCGGTCCAGGTCATTTACAACAGA AGTCAGTACGCCAGTCAGTGCTGCCGGTGAACACCCTGG AGGAAGTCCACGAGGAGAAGTGTTACCCACCTAAGCTGG ATGAAGCAAAGGAGCAACTATTACTTAAGAACTCCAGG
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AGAGTGCATCCATGGCCAACAGAAGCAGGTATCAGTCGC GCAAAGTAGAAAACATGAAAGCAGCAATCATCCAGAGA CTAAAGAGAGGGCTGTAGACTATACTTAATGTCAGAGACC CCAAAAGTCCCTACTTACCGGACTACATATCCGGCGCCT GTGTACTCGCCTCCGATCAACGTCCGATTGTCCAATCCCG AGTCCGCAGTGGCAGCATGCAATGAGTTCTTAGCTAGAA ACTATCCAACGTCTCATCATACCAAATTACCGACGAGT ATGATGCATATCTAGACATGGTGGACGGGTCCGAGAGTT GCCTGGACCGAGCGACATTCAATCCGTCAAAACTCAGGA GCTACCCGAAACAGCACGCTTACCACGCGCCCTCCATCA GAAGCGCTGTACCGTCCCCATTCCAGAACACACTACAGA ATGTACTGGCAGCAGCCACGAAAAGAACTGCAACGTC ACACAGATGAGGGAATTACCCACTTTGGACTCAGCAGTA TTCAACGTGGAGTGTTTCAAAAAATTTCGCATGCAACCAA GAATACTGGGAAGAATTTGCTGCCAGCCCTATTAGGATA ACAACTGAGAATTTAGCAACCTATGTTACTAACTAAAA GGGCCAAAAGCAGCAGCGCTATTCGCAAAAACCCATAAT CTACTGCCACTACAGGAAGTACCAATGGATAGGTTTACA GTAGATATGAAAAGGGACGTAAAGGTGACTCCTGGTACA AAGCATACAGAGGAAAGACCTAAGGTGCAGGTTATACA GGCGGCTGAACCCTTGGCGACAGCATACCTATGTGGGAT TCACAGAGAGCTGGTTAGGAGGCTGAACGCCGTCTCTCT ACCCAATGTACATACTATTTGACATGTCTGCCGAGGA TTTCGATGCCATCATAGCCGCACACTTTAAGCCAGGAGA CACTGTTTTGGAAACGGACATAGCCTCCTTTGATAAGAG CCAAGATGATTCACCTTGCCTTACTGCTTTGATGCTGTTA GAGGATTTAGGGGTGGATCACTCCCTGCTGGACTTGATA GAGGCTGCTTTCGGAGAGATTTCCAGCTGTCACCTACCG ACAGGTACGCGCTTCAAGTTCGGCGCCATGATGAAATCA GGTATGTTCTAACTCTGTTTCGTCAACACATTGTTAAACA TCACCATCGCCAGCCGAGTGCTGGAAGATCGTCTGACAA AATCCGCGTGCGCGGCCTTCATCGGCGACGACAACATAA TACATGGAGTCGTCTCCGATGAATTGATGGCAGCCAGAT GTGCCACTTGGATGAACATGGAAGTGAAGATCATAGATG CAGTTGTATCCTTGAAAGCCCCTTACTTTTGTGGAGGGTT TATACTGCACGATACTGTGACAGGAACAGCTTGCAGAGT GGCAGACCCGCTAAAAAGGCTTTTTTAACTGGGCAAACC GCTAGCGGCAGGTGACGAACAAGATGAAGATAGAAGAC GAGCGCTGGCTGACGAAGTGATCAGATGGCAACGAACA GGGCTAATTGATGAGCTGGAGAAAGCGGTATACTCTAGG TACGAAGTGCAGGGTATATCAGTTGTGGTAATGTCCATG GCCACCTTTGCAAGCTCCAGATCCAACTTCGAGAAGCTC AGAGGACCCGTCATAACTTTGTACGGCGGTCTAAATAG GTACGCACTACAGCTACCTATTTTGCAGAAGCCGACAGC AAGTATCTAAACACTAATCAGCTACAATGGAGTTCATCC CAACCCAACTTTTTACAATAGGAGGTACCAGCCTCGAC
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CCTGGACTCCGCGCCCTACTATCCAAGTCATCAGGCCCA GACCGCGCCCTCAGAGGCAAGCTGGGCAACTTGCCCAGC TGATCTCAGCAGTTAATAAACTGACAATGCGCGCGGTAC CACAACAGAAGCCACGCAGGAATCGGAAGAATAAGAAG CAAAAGCAAAAACAACAGGCGCCACAAAACAACAAAA TCAAAAGAAGCAGCCACCTAAAAAGAAACCGGCTCAAA AGAAAAAGAAGCCGGGCCGCAGAGAGAGGATGTGCATG AAAATCGAAAATGATTGTATTTTCGAAGTCAAGCACGAA GGTAAGGTAACAGGTTACGCGTGCCTGGTGGGGGACAA AGTAATGAAACCAGCACACGTAAAGGGGACCATCGATA ACGCGGACCTGGCCAACTGGCCTTTAAGCGGTCATCTA AGTATGACCTTGAATGCGCGCAGATACCCGTGCACATGA AGTCCGACGCTTCGAAGTTCACCCATGAGAAACCGGAGG GGTACTACAACTGGCACCACGGAGCAGTACAGTACTCAG GAGGCCGGTTCACCATCCCTACAGGTGCTGGCAAACCAG GGGACAGCGGCAGACCGATCTTCGACAACAAGGGACGC GTGGTGGCCATAGTCTTAGGAGGAGCTAATGAAGGAGCC CGTACAGCCCTCTCGGTGGTGACCTGGAATAAAGACATT GTCACTAAAATCACCCCGAGGGGGCCGAAGAGTGGAG TCTTGCCATCCCAGTTATGTGCCTGTTGGCAAACACCACG TTCCCCTGCTCCCAGCCCCCTTGACGCCCCTGCTGCTACG AAAAGGAACCGGAGGAAACCCTACGCATGCTTGAGGAC AACGTCATGAGACCTGGGTACTATCAGCTGCTACAAGCA TCCTTAACATGTTCTCCCCACCGCCAGCGACGCAGCACC AAGGACAACCTCAATGTCTATAAAGCCACAAGACCATAC TTAGCTCACTGTCCCGACTGTGGAGAAGGGCACTCGTGC CATAGTCCCGTAGCACTAGAACGCATCAGAAATGAAGCG ACAGACGGGACGCTGAAAATCCAGGTCTCCTTGCAAATC GGAATAAAGACGGATGACAGCCACGATTGGACCAAGCT GCGTTATATGGACAACCACATGCCAGCAGACGCAGAGA GGGCGGGGCTATTTGTAAGAACATCAGCACCGTGTACGA TTACTGGAACAATGGGACACTTCATCCTGGCCCCGATGTC CAAAAGGGGAAACTCTGACGGTGGGATTCACTGACAGTA GGAAGATTAGTCACTCATGTACGCACCCATTTACCACG ACCCTCCTGTGATAGGTCGGGAAAAATTCCATTCCCGAC CGCAGCACGGTAAAGAGCTACCTTGACGACGTACGTGC AGAGCACCGCCGCAACTACCGAGGAGATAGAGGTACAC ATGCCCCCAGACACCCCTGATCGCACATTAATGTCACAA CAGTCCGGCAACGTAAAGATCACAGTCAATGGCCAGACG GTGCGGTACAAGTGTAATTGCGGTGGCTCAAATGAAGGA CTAACAACCTACAGACAAAGTGATTAATAACTGCAAGGTT GATCAATGTCATGCCGCGGTACCAATCACAAAAAGTGG CAGTATAACTCCCCTCTGGTCCCGCGTAATGCTGAACTTG GGGACCGAAAAGGAAAAATTACATCCCGTTTCCGCTGG CAAATGTAACATGCAGGGTGCCTAAAGCAAGGAACCCC ACCGTGACGTACGGGAAAAACCAAGTCATCATGCTACTG

	TATCCTGACCACCCAACACTCCTGTCCTACCGGAATATG GGAGAAGAACCAAACACTATCAAGAAGAGTGGGTGATGCA TAAGAAGGAAGTCGTGCTAACCGTGCCGACTGAAGGGCT CGAGGTCACGTGGGGCAACAACGAGCCGTATAAGTATTG GCCGCAGTTATCTACAAACGGTACAGCCCATGGCCACCC GCATGAGATAATTCTGTATTATTATGAGCTGTACCCCACT ATGACTGTAGTAGTTGTGTCAGTGGCCACGTTTCATACTCC TGTCGATGGTGGGTATGGCAGCGGGGATGTGCATGTGTG CACGACGCAGATGCATCACACCGTATGAACTGACACCAG GAGCTACCGTCCCTTTTCCTGCTTAGCCTAATATGCTGCAT CAGAACAGCTAAAGCGGCCACATACCAAGAGGCTGCGA TATACCTGTGGAACGAGCAGCAACCTTTGTTTTGGCTAC AAGCCCTTATTCCGCTGGCAGCCCTGATTGTTCTATGCAA CTGTCTGAGACTCTTACCATGCTGCTGTAAAACGTTGGCT TTTTTAGCCGTAATGAGCGTCGGTGCCCACTGTGAGC GCGTACGAACACGTAACAGTGATCCCGAACACGGTGGG AGTACCGTATAAGACTCTAGTCAATAGACCTGGCTACAG CCCCATGGTATTGGAGATGGAACTACTGTCAGTCACTTT GGAGCCAACACTATCGCTTGATTACATCACGTGCGAGTA CAAAACCGTCATCCCGTCTCCGTACGTGAAGTGCTGCGG TACAGCAGAGTGCAAGGACAAAAACCTACCTGACTACA GCTGTAAGGTCTTCACCGGCGTCTACCCATTTATGTGGGG CGGCGCCTACTGCTTCTGCGACGCTGAAAACACGCAGTT GAGCGAAGCACACGTGGAGAAGTCCGAATCATGCAAAA CAGAATTTGCATCAGCATAACAGGGCTCATACCGCATCTG CATCAGCTAAGCTCCGCGTCCTTTACCAAGGAAATAACA TCACTGTAACCTGCCTATGCAAACGGCGACCATGCCGTCA CAGTTAAGGACGCCAAATTCATTGTGGGGCCAATGTCTT CAGCCTGGACACCTTTCGACAACAAAATTGTGGTGTACA AAGGTGACGTCTATAACATGGACTACCCGCCCTTTGGCG CAGGAAGACCAGGACAATTTGGCGATATCCAAAGTCGCA CACCTGAGAGTAAAGACGTCTATGCTAATACACAACCTGG TACTGCAGAGACCGGCTGTGGGTACGGTACACGTGCCAT ACTCTCAGGCACCATCTGGCTTTAAGTATTGGCTAAAAG AACGCGGGGCGTCGCTGCAGCACACAGCACCATTTGGCT GCCAAATAGCAACAAACCCGGTAAGAGCGGTGAACTGC GCCGTAGGGAACATGCCCATCTCCATCGACATACCGGAA GCGGCCTTCACTAGGGTCGTCGACGCGCCCTCTTTAACG GACATGTCGTGCGAGGTACCAGCCTGCACCCATTCCTCA GACTTTGGGGGCGTCGCCATTATTAAATATGCAGCCAGC AAGAAAGGCAAGTGTGCGGTGCATTTCGATGACTAACGCC GTCATATTTCGGGAAGCTGAGATAGAAGTTGAAGGGAAT TCTCAGCTGCAAATCTCTTTCTCGACGGCCTTAGCCAGCG CCGAATTCGCGGTACAAGTCTGTTCTACACAAGTACACT GTGCAGCCGAGTGCCACCCCCGAAGGACCACATAGTCA ACTACCCGGCGTCACATACCACCCTCGGGGTCCAGGACA
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	TCTCCGCTACGGCGATGTCATGGGTGCAGAAGATCACGG GAGGTGTGGGACTGGTTGTTGCTGTTGCCGCACTGATTCT AATCGTGGTGCTATGCGTGTCGTTACGACAGGCACTAACT TGACAATTAAGTATGAAGGTATATGTGTCCCCTAAGAGA CACACTGTACATAGCAAATAATCTATAGATCAAAGGGCT ACGCAACCCCTGAATAGTAACAAAATACAAAATCACTAA AAATTATAAAAACAGAAAAATACATAAATAGGTATACGT GTCCCCTAAGAGACACATTGTATGTAGGTGATAAGTATA GATCAAAGGGCCGAATAACCCCTGAATAGTAACAAAAT ATGAAAATCAATAAAAATCATAAAATAGAAAAACCATA AACAGAAGTAGTTCAAAGGGCTATAAAACCCCTGAATAG TAACAAAACATAAAAATTAATAAAAATCAAATGAATACCA TAATTGGCAAACGGAAGAGATGTAGGTACTTAAGCTTCC TAAAAGCAGCCGAACCTCACTTTGAGAAGTAGGCATAGCA TACCGAACTCTTCCACGATTCTCCGAACCCACAGGGACG TAGGAGATGTTATTTTGTGTTTTAATATTTCAAAAAAAAAA AAAAAAAAAAAAAAAA
SEQ ID NO: 6 Polynucleotide sequence of CHIKV LR2006 OPY1 non- structural polyprotein with mutation at position 1050 (CHIKV-E1050V). The amino acid at position 1050 is mutated from E to V via mutagenesis of nucleotide sequences from GAA to GTG (underlined and in bold).	ATGGCTGCGTGAGACACACGTAGCCTACCAGTTTCTTAC TGCTCTACTCTGCAAAGCAAGAGATTAATAACCCATCAT GGATCCTGTGTACGTGGACATAGACGCTGACAGCGCCTT TTTGAAGGCCCTGCAACGTGCGTACCCCATGTTTGAGGT GGAACCAAGGCAGGTCACACCGAATGACCATGCTAATGC TAGAGCGTTCTCGCATCTAGCTATAAACTAATAGAGCA GGAAATTGACCCCGACTCAACCATCCTGGATATCGGCAG TGCGCCAGCAAGGAGGATGATGTCGGACAGGAAGTACC ACTGCGTCTGCCCAGTGCGCAGTGCGGAAGATCCCGAGA GACTCGCCAATTATGCGAGAAAGCTAGCATCTGCCGCAG GAAAAGTCCTGGACAGAAACATCTCTGGAAAGATCGGG GACTTACAAGCAGTAATGGCCGTGCCAGACACGGAGAC GCCAACATTCTGCTTACACACAGACGTCTCATGTAGACA GAGAGCAGACGTCGCTATATACCAAGACGTCTATGCTGT ACACGCACCCACGTCGCTATACCAACAGGCGATTAAAGG GGTCCGAGTGCGTACTGGGTGTTGTTGACACAACCCC GTTTCATGTACAATGCCATGGCGGGTGCCTACCCCTCATA CTCGACAACTGGGCAGATGAGCAGGTACTGAAGGCTA AGAACATAGGATTATGTTCAACAGACCTGACGGAAGGTA GACGAGGCAAGTTGTCTATTATGAGAGGGAAAAAGCTA AAACCGTGCGACCGTGTGCTGTTCTCAGTAGGGTCAACG CTCTACCCGGAAAGCCGCAAGCTACTTAAGAGCTGGCAC CTGCCATCGGTGTTCCATTTAAAGGGCAAACCTCAGCTTC ACATGCCGCTGTGATACAGTGGTTTCGTGTGAGGGCTAC GTCGTTAAGAGAATAACGATGAGCCCAGGCCTTTATGGA AAAACCACAGGGTATGCGGTAACCCACCACGCAGACGG ATTCCTGATGTGCAAGACTACCGACACGGTTGACGGCGA AAGAATGTCATTCTCGGTGTGCACATACGTGCCGGCGAC CATTTGTGATCAAATGACCGGCATCCTTGCTACAGAAGT

	CACGCCGGAGGATGCACAGAAGCTGTTGGTGGGGCTGA ACCAGAGAATAGTGGTTAACGGCAGAACGCAACGGAAT ACGAACACCATGAAAAATTATCTGCTTCCCGTGGTCCG CAAGCCTTCAGTAAGTGGGCAAAGGAGTGCCGGAAGA CATGGAAGATGAAAACTCCTGGGGGTCAGAGAAAGAA CACTGACCTGCTGCTGTCTATGGGCATTCAAGAAGCAGA AAACACACACGGTCTACAAGAGGCCTGATACCCAGTCAA TTCAGAAGGTTCAAGGCCGAGTTTGACAGCTTTGTGGTAC CGAGTCTGTGGTCGTCCGGGTTGTCAATCCCTTTGAGGAC TAGAATCAAATGGTTGTTAAGCAAGGTGCCAAAAACCGA CCTGATCCCATACAGCGGAGACGCCCCGAGAAGCCCGGG ACGCAGAAAAAGAAGCAGAGGAAGAACGAGAAGCAGA ACTGACTCGCGAAGCCCTACCACCTCTACAGGCAGCACA GGAAGATGTTCAAGTTCGAAATCGACGTGGAACAGCTTGA GGACAGAGCGGGCGCAGGAATAATAGAGACTCCGAGAG GAGCTATCAAAGTTACTGCCCAACCAACAGACCACGTCG TGGGAGAGTACCTGGTACTCTCCCCGCAGACCGTACTAC GTAGCCAGAAGCTCAGTCTGATTCACGCTTTGGCGGAGC AAGTGAAGACGTGCACGCACAACGGACGAGCAGGGAGG TATGCGGTCTGAAGCGTACGACGGCCGAGTCCTAGTGCCC TCAGGCTATGCAATCTCGCCTGAAGACTTCCAGAGTCTA AGCGAAAGCGCAACGATGGTGTATAACGAAAGAGAGTT CGTAAACAGAAAGCTACACCATATTGCGATGCACGGACC AGCCCTGAACACCGACGAAGAGTCGTATGAGCTGGTGAG GGCAGAGAGGACAGAACACGAGTACGTCTACGACGTGG ATCAGAGAAGATGCTGTAAGAAGGAAGAAGCCGCAGGA CTGGTACTGGTGGGCGACTTGACTAATCCGCCCTACCAC GAATTTCGCATATGAAGGGCTAAAAATCCGCCCTGCCTGC CCATACAAAATTGCAGTCATAGGAGTCTTCGGAGTACCG GGATCTGGCAAGTCAGCTATTATCAAGAACCTAGTTACC AGGCAGGACCTGGTGACTIONAGCGGAAAGAAAGAAAACCTG CCAAGAAATCACACCGACGTGATGAGACAGAGAGGTC TAGAGATATCTGCACGTACGGTTGACTCGCTGCTCTTGA ATGGATGCAACAGACCAGTCGACGTGTTGTACGTAGACG AGGCGTTTGCGTGCCACTCTGGAACGCTACTTGCTTTGAT CGCCTTGGTGAGACCAAGGCAGAAAGTTGTACTTTGTGG TGACCCGAAGCAGTGCGGCTTCTTCAATATGATGCAGAT GAAAGTCAACTATAATCACAACATCTGCACCCAAGTGTA CCACAAAAGTATCTCCAGGCGGTGTACACTGCCTGTGAC CGCCATTGTGTCATCGTTGCATTACGAAGGCAAAATGCG CACTACGAATGAGTACAACAAGCCGATTGTAGTGACAC TACAGGCTCAACAAAACCTGACCCTGGAGACCTCGTGTT AACGTGCTTCAGAGGGTGGGTAAACAACCTGCAAATTGA CTATCGTGGATACGAGGTCATGACAGCAGCCGCATCCCA AGGGTTAACCAGAAAAGGAGTTTACGCAGTTAGACAAA AAGTTAATGAAAACCCGCTCTATGCATCAACGTCAGAGC
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ACGTCAACGTACTCCTAACGCGTACGGAAGGTAAACTGG
TATGGAAGACACTTTCCGGCGACCCGTGGATAAAGACGC
TGCAGAACCCACCGAAAGGAACTTCAAAGCAACTATTA
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AAAGCCAACGTTTGTGGGCTAAGAGCTTGGTCCCTATC
CTCGAAACAGCGGGGATAAACTAAATGATAGGCAGTG
GTCTCAGATAATTCAAGCCTTCAAAGAAGACAAAGCATA
CTCACCT**GTG**GTAGCCCTGAATGAAATATGTACGCGCAT
GTATGGGGTGGATCTAGACAGCGGGCTATTTTCTAAACC
GTTGGTGTCTGTGTATTACGCGGATAACCACTGGGATAA
TAGGCCTGGAGGGAAAATGTTTCGGATTTAACCCCGAGGC
AGCATCCATTCTAGAAAGAAAGTATCCATTACAAAAGG
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GAGGATAGAAGACTTTAACCCTACCACCAACATCATACC
GGCCAACAGGAGACTACCACACTCATTAGTGGCCGAACA
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ACAAGATAAACGGCCACCACGTGCTCCTGGTCAGTGGCT
ATAACCTTGCACTGCCTACTAAGAGAGTCACTTGGGTAG
CGCCGTTAGGTGTCCGCGGAGCGGACTACACATACAACC
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CGGGTTACCGGGTGGCGGTGTTTGCAAGGCAGTATACAA
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GGGAACCGCAAAAACAGTTATGTGCGGTACGTATCCAGT
AATCCACGCTGTTGGACCAAACCTTCTCTAATTATTCGGAG
TCTGAAGGGGACCGGGAATTGGCAGCTGCCTATCGAGAA
GTCGCAAAGGAAGTAACTAGGCTGGGAGTAAATAGTGT
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TACAGATGCGGACCCAAGTAGAGCTGCTGGATGAGCACA
TCTCCATAGACTGCGATATTGTTTCGCGTGCACCCTGACAG
CAGCTTGGCAGGCAGAAAAGGATACAGCACCACGGAAG
GCGCACTGTACTCATATCTAGAAGGGACCCGTTTTTCATC

	AGACGGCTGTGGATATGGCGGAGATACATACTATGTGGC CAAAGCAAACAGAGGCCAATGAGCAAGTCTGCCTATATG CCCTGGGGGAAAGTATTGAATCGATCAGGCAGAAATGCC CGGTGGATGATGCAGACGCATCATCTCCCCCAAACCTG TCCCGTGCCTTTGCCGTTACGCTATGACTCCAGAACGCGT CACCCGGCTTCGCATGAACCACGTCACAAGCATAATTGT GTGTTCTTCGTTTCCCCTCCCAAAGTACAAAATAGAAGG AGTGCAAAAAGTCAAATGCTCTAAGGTAATGCTATTTGA CCACAACGTGCCATCGCGCGTAAGTCCAAGGGAATATAG ATCTTCCCAGGAGTCTGCACAGGAGGCGAGTACAATCAC GTCCTGACGCATAGTCAATTTCGACCTAAGCGTTGATGG CGAGATACTGCCCCGTCCCGTCAGACCTGGATGCTGACGC CCCAGCCCTAGAACCAGCACTAGACGACGGGGCGACAC ACACGCTGCCATCCACAACCGGAAACCTTGCGGGCCGTGT CTGATTGGGTAAATGAGCACCGTACCTGTGCGCGCCGCCA GAAGAAGGCGAGGGAGAAACCTGACTGTGACATGTGAC GAGAGAGAAGGGAATATAACACCCATGGCTAGCGTCCG ATTCTTTAGGGCAGAGCTGTGTCCGGTCGTACAAGAAAC AGCGGAGACGCGTGACACAGCAATGTCTCTTCAGGCACC ACCGAGTACCGCCACGGAACCGAATCATCCGCCGATCTC CTTCGGAGCATCAAGCGAGACGTTCCCCATTACATTTGG GGACTTCAACGAAGGAGAAATCGAAAGCTTGTCTTCTGA GCTACTAACTTTCGGAGACTTCTTACCAGGAGAAGTGGA TGACTTGACAGACAGCGACTGGTCCACGTGCTCAGACAC GGACGACGAGTTAAGACTAGACAGGGCAGGTGGGTATA TATTCTCGTCGGACACCGGTCCAGGTCATTTACAACAGA AGTCAGTACGCCAGTCAGTGCTGCCGGTGAACACCCTGG AGGAAGTCCACGAGGAGAAGTGTTACCCACCTAAGCTGG ATGAAGCAAAGGAGCAACTATTACTTAAGAACTCCAGG AGAGTGCATCCATGGCCAACAGAAGCAGGTATCAGTCGC GCAAAGTAGAAAACATGAAAGCAGCAATCATCCAGAGA CTAAAGAGAGGCTGTAGACTATACTTAATGTCAGAGACC CCAAAAGTCCCTACTTACCGGACTACATATCCGGCGCCT GTGTACTCGCCTCCGATCAACGTCCGATTGTCCAATCCCG AGTCCGCAGTGGCAGCATGCAATGAGTTCTTAGCTAGAA ACTATCCAACGTCTCATCATACCAAATTACCGACGAGT ATGATGCATATCTAGACATGGTGGACGGGTCCGAGAGTT GCCTGGACCGAGCGACATTCAATCCGTCAAAACTCAGGA GCTACCCGAAACAGCACGCTTACCACGCGCCCTCCATCA GAAGCGCTGTACCGTCCCCATTCCAGAACACACTACAGA ATGTACTGGCAGCAGCCACGAAAAGAACTGCAACGTC ACACAGATGAGGGAATTACCCACTTTGGACTCAGCAGTA TTCAACGTGGAGTGTTTCAAAAAATTTCGCATGCAACCAA GAATACTGGGAAGAATTTGCTGCCAGCCCTATTAGGATA ACAACTGAGAATTTAGCAACCTATGTTACTAACTAAAA GGGCCAAAAGCAGCAGCGCTATTCGCAAAAACCCATAAT
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CTACTGCCACTACAGGAAGTACCAATGGATAGGTTTACA GTAGATATGAAAAGGGACGTAAAGGTGACTCCTGGTACA AAGCATACAGAGGAAAGACCTAAGGTGCAGGTTATACA GGCGGCTGAACCCTTGGCGACAGCATACCTATGTGGGAT TCACAGAGAGCTGGTTAGGAGGCTGAACGCCGTCCTCCT ACCCAATGTACATACTATTTGACATGTCTGCCGAGGA TTTCGATGCCATCATAGCCGCACACTTTAAGCCAGGAGA CACTGTTTTGGAAACGGACATAGCCTCCTTTGATAAGAG CCAAGATGATTCACTTGGCCTTACTGCTTTGATGCTGTTA GAGGATTTAGGGGTGGATCACTCCCTGCTGGACTTGATA GAGGCTGCTTTCGGAGAGATTTCCAGCTGTCACCTACCG ACAGGTACGCGCTTCAAGTTCGGCGCCATGATGAAATCA GGTATGTTCCCTAACTCTGTTTCGTCAACACATTGTAAACA TCACCATCGCCAGCCGAGTGCTGGAAGATCGTCTGACAA AATCCGCGTGCGCGGCCCTTCATCGGCGACGACAACATAA TACATGGAGTCGTCTCCGATGAATTGATGGCAGCCAGAT GTGCCACTTGATGAACATGGAAGTGAAGATCATAGATG CAGTTGTATCCTTGAAAGCCCCTTACTTTTGTGGAGGGTT TATACTGCACGATACTGTGACAGGAACAGCTTGCAGAGT GGCAGACCCGCTAAAAAGGCTTTTTAACTGGGCAAACC GCTAGCGGCAGGTGACGAACAAGATGAAGATAGAAGAC GAGCGCTGGCTGACGAAGTGATCAGATGGCAACGAACA GGGCTAATTGATGAGCTGGAGAAAGCGGTATACTCTAGG TACGAAGTGCAGGGTATATCAGTTGTGGTAATGTCCATG GCCACCTTTGCAAGCTCCAGATCCAACCTTCGAGAAGCTC AGAGGACCCGTCATAACTTTGTACGGCGGTCTAAATAG GTACGCACTACAGCTACCTATTTTGCAGAAGCCGACAGC AAGTATCTAAACACTAATCAGCTACAATGGAGTTCATCC CAACCCAACTTTTTACAATAGGAGGTACCAGCCTCGAC CCTGGACTCCGCGCCCTACTATCCAAGTCATCAGGCCCA GACCGCGCCCTCAGAGGCAAGCTGGGCAACTTGCCCAGC TGATCTCAGCAGTTAATAAACTGACAATGCGCGCGGTAC CACAAACAGAAGCCACGCAGGAATCGGAAGAATAAGAAG CAAAAGCAAAAACAACAGGCGCCACAAAACAACAAAA TCAAAAGAAGCAGCCACCTAAAAAGAAACCGGCTCAAA AGAAAAAGAAGCCGGGCCGAGAGAGAGGATGTGCATG AAAATCGAAAATGATTGTATTTTCGAAGTCAAGCACGAA GGTAAGGTAACAGGTTACGCGTGCCTGGTGGGGGACAA AGTAATGAAACCAGCACACGTAAAGGGGACCATCGATA ACGCGGACCTGGCCAACTGGCCTTTAAGCGGTATCTA AGTATGACCTTGAATGCGCGCAGATACCCGTGCACATGA AGTCCGACGCTTCGAAGTTCACCCATGAGAAACCGGAGG GGTACTACAACTGGCACCACGGAGCAGTACAGTACTCAG GAGGCCGGTTCACCATCCCTACAGGTGCTGGCAAACCAG GGGACAGCGGCAGACCGATCTTCGACAACAAGGGACGC GTGGTGGCCATAGTCTTAGGAGGAGCTAATGAAGGAGCC

	CGTACAGCCCTCTCGGTGGTGACCTGGAATAAAGACATT GTCATAAAATCACCCCCGAGGGGGCCGAAGAGTGGAG TCTTGCCATCCCAGTTATGTGCCTGTTGGCAAACACCACG TTCCCCTGCTCCCAGCCCCCTTGACGCCCCTGCTGCTACG AAAAGGAACCGGAGGAAACCCTACGCATGCTTGAGGAC AACGTCATGAGACCTGGGTACTATCAGCTGCTACAAGCA TCCTTAACATGTTCTCCCCACCGCCAGCGACGCAGCACC AAGGACAACCTCAATGTCTATAAAGCCACAAGACCATAC TTAGCTCACTGTCCCGACTGTGGAGAAGGGCACTCGTGC CATAGTCCCGTAGCACTAGAACGCATCAGAAATGAAGCG ACAGACGGGACGCTGAAAATCCAGGTCTCCTTGCAAATC GGAATAAAGACGGATGACAGCCACGATTGGACCAAGCT GCGTTATATGGACAACCACATGCCAGCAGACGCAGAGA GGGCGGGGCTATTTGTAAGAACATCAGCACCGTGTACGA TTACTGGAACAATGGGACACTTCATCCTGGCCCCGATGTC CAAAAGGGGAAACTCTGACGGTGGGATTCACTGACAGTA GGAAGATTAGTCACTCATGTACGCACCCATTTACCACG ACCCTCCTGTGATAGGTCTGGGAAAAATTCCATTCCCGAC CGCAGCACGGTAAAGAGCTACCTTGACGACGTCACGTGC AGAGCACCGCCGCAACTACCGAGGAGATAGAGGTACAC ATGCCCCCAGACACCCCTGATCGCACATTAATGTCACAA CAGTCCGGCAACGTAAAGATCACAGTCAATGGCCAGACG GTGCGGTACAAGTGTAATTGCGGTGGCTCAAATGAAGGA CTAACAACCTACAGACAAAGTGATTAATAACTGCAAGGTT GATCAATGTCATGCCGCGGTACCAATCACAAAAAGTGG CAGTATAACTCCCCTCTGGTCCCGCGTAATGCTGAACTTG GGGACCGAAAAGGAAAAATTACATCCCGTTTCCGCTGG CAAATGTAACATGCAGGGTGCCTAAAGCAAGGAACCCC ACCGTGACGTACGGGAAAAACCAAGTCATCATGCTACTG TATCCTGACCACCAACACTCCTGTCCTACCGGAATATG GGAGAAGAACCAAACTATCAAGAAGAGTGGGTGATGCA TAAGAAGGAAGTCGTGCTAACCGTGCCGACTGAAGGGCT CGAGGTACGTTGGGGCAACAACGAGCCGTATAAGTATTG GCCGCAGTTATCTACAAACGGTACAGCCCATGGCCACCC GCATGAGATAATTCTGTATTATTATGAGCTGTACCCCACT ATGACTGTAGTAGTTGTGTCAGTGGCCACGTTCACTACC TGTCGATGGTGGGTATGGCAGCGGGGATGTGCATGTGTG CACGACGCAGATGCATCACACCGTATGAACTGACACCAG GAGCTACCGTCCCTTTCCTGCTTAGCCTAATATGCTGCAT CAGAACAGCTAAAGCGGCCACATACCAAGAGGCTGCGA TATACCTGTGGAACGAGCAGCAACCTTTGTTTTGGCTAC AAGCCCTTATTCCGCTGGCAGCCCTGATTGTTCTATGCAA CTGTCTGAGACTCTTACCATGCTGCTGTAAAACGTTGGCT TTTTTAGCCGTAATGAGCGTCGGTGCCCACTGTGAGC GCGTACGAACACGTAACAGTGATCCCGAACACGGTGGG AGTACCGTATAAGACTCTAGTCAATAGACCTGGCTACAG
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	CCCCATGGTATTGGAGATGGAACACTGTGTCAGTCACTTT GGAGCCAACTACTATCGCTTGATTACATCACGTGCGAGTA CAAAACCGTCATCCCGTCTCCGTACGTGAAGTGCTGCGG TACAGCAGAGTGCAAGGACAAAAACCTACCTGACTACA GCTGTAAGGTCTTCACCGGCGTCTACCCATTTATGTGGGG CGGCGCCTACTGCTTCTGCGACGCTGAAAACACGCAGTT GAGCGAAGCACACGTGGAGAAGTCCGAATCATGCAAAA CAGAATTTGCATCAGCATACAGGGCTCATACCGCATCTG CATCAGCTAAGCTCCGCGTCCTTTACCAAGGAAATAACA TCACTGTAAGTGCCTATGCAAACGGCGACCATGCCGTCA CAGTTAAGGACGCCAAATTCATTGTGGGGCCAATGTCTT CAGCCTGGACACCTTTTCGACAACAAAATTGTGGTGTACA AAGGTGACGTCTATAACATGGACTACCCGCCCTTTGGCG CAGGAAGACCAGGACAATTTGGCGATATCCAAAGTCGCA CACCTGAGAGTAAAGACGTCTATGCTAATACACAACCTGG TACTGCAGAGACCGGCTGTGGGTACGGTACACGTGCCAT ACTCTCAGGCACCATCTGGCTTTAAGTATTGGCTAAAAG AACGCGGGGCGTCGCTGCAGCACACAGCACCATTTGGCT GCCAAATAGCAACAAACCCGGTAAGAGCGGTGAACTGC GCCGTAGGGAACATGCCCATCTCCATCGACATACCGGAA GCGGCCTTCACTAGGGTCGTCGACGCGCCCTCTTTAACG GACATGTCTGTGCGAGGTACCAGCCTGCACCCATTCTCA GACTTTGGGGGCGTCGCCATTATTAATATGCAGCCAGC AAGAAAGGCAAGTGTGCGGTGCATTTCGATGACTAACGCC GTCATATTTCGGGAAGCTGAGATAGAAGTTGAAGGGAAT TCTCAGCTGCAAATCTCTTTCTCGACGGCCTTAGCCAGCG CCGAATTCGCGGTACAAGTCTGTTCTACACAAGTACACT GTGCAGCCGAGTGCCACCCCCGAAGGACCACATAGTCA ACTACCCGGCGTCACATACCACCCTCGGGGTCCAGGACA TCTCCGCTACGGCGATGTCATGGGTGCAGAAGATCACGG GAGGTGTGGGACTGGTTGTTGCTGTTGCCGCACTGATTCT AATCGTGGTGCTATGCGTGTCTGTTACGACAGGCACTAACT TGACAATTAAGTATGAAGGTATATGTGTCCCCTAAGAGA CACACTGTACATAGCAAATAATCTATAGATCAAAGGGCT ACGCAACCCCTGAATAGTAACAAAATACAAAATCACTAA AAATTATAAAAACAGAAAAATACATAAATAGGTATACGT GTCCCCTAAGAGACACATTGTATGTAGGTGATAAGTATA GATCAAAGGGCCGAATAACCCCTGAATAGTAACAAAAT ATGAAAATCAATAAAAATCATAAAATAGAAAAACCATA AACAGAAGTAGTTCAAAGGGCTATAAAACCCCTGAATAG TAACAAAACATAAAATTAATAAAAATCAAATGAATACCA TAATTGGCAAACGGAAGAGATGTAGGTACTTAAGCTTCC TAAAAGCAGCCGAACCTCACTTTGAGAAGTAGGCATAGCA TACCGAACTCTTCCACGATTCTCCGAACCCACAGGGACG TAGGAGATGTTATTTTGTTTTTAATATTTCAAAAAAAAAA AAAAAAAAAAAAAA
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SEQ ID NO: 7

Polynucleotide sequence of CHIKV LR2006 OPY1 non-structural polyprotein with mutations at 532 and 1050 (CHIKV-R532H+E1050V).

The amino acids at positions 532 and 1050 are mutated from R to H and E to V, respectively, via mutagenesis of nucleotide sequences from AGA to CAC (underlined and in bold) and GAA to GTG (underlined and in bold), respectively.

ATGGCTGCGTGAGACACACGTAGCCTACCAGTTTCTTAC
TGCTCTACTCTGCAAAGCAAGAGATTAATAACCCATCAT
GGATCCTGTGTACGTGGACATAGACGCTGACAGCGCCTT
TTTGAAGGCCCTGCAACGTGCGTACCCCATGTTTGAGGT
GGAACCAAGGCAGGTCACACCGAATGACCATGCTAATGC
TAGAGCGTTCTCGCATCTAGCTATAAACTAATAGAGCA
GGAAATTGACCCCGACTCAACCATCCTGGATATCGGCAG
TGCGCCAGCAAGGAGGATGATGTCTGGACAGGAAGTACC
ACTGCGTCTGCCCAGTGCGCAGTGCGGAAGATCCCGAGA
GACTCGCCAATTATGCGAGAAAGCTAGCATCTGCCGCAG
GAAAAGTCCTGGACAGAAACATCTCTGGAAAGATCGGG
GACTTACAAGCAGTAATGGCCGTGCCAGACACGGAGAC
GCCAACATTCTGCTTACACACAGACGTCTCATGTAGACA
GAGAGCAGACGTCGCTATATACCAAGACGTCTATGCTGT
ACACGCACCCACGTCGCTATACCACCAGGCGATTAAAGG
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GTTTATGTACAATGCCATGGCGGGTGCCTACCCCTCATA
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GACGAGGCAAGTTGTCTATTATGAGAGGGAAAAAGCTA
AAACCGTGCGACCGTGTGCTGTTCTCAGTAGGGTCAACG
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EXAMPLES

Example 1: Materials and Methods

Cell lines and cell culture method

- 5 [0098] The cell lines used are African green monkey kidney epithelial cells (Vero-E6) and mouse tail fibroblasts (MTFs) were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco). *Aedes albopictus* monolayer (C6/36) cells were cultured in Leibovitz's medium (L-15) (Gibco) supplemented with 10% FBS. All cultures were incubated at 37 °C with 5% CO₂ supplied with the exception of C6/36 which was incubated at
- 10 28 °C with no CO₂ supplied. All media and reagents were tested negative for endotoxins.

Virus strains

- [0099] The virus strain used is the LR2006 OPY1 strain.

Method for generating mutations in CHIKV

[00100] PCR-based site directed mutagenesis and subcloning were performed using the Polymerase Incomplete Primer Extension (PIPE) cloning method to generate the mutations at amino acid positions 532 and 1050 in CHIKV. The method is adapted from Saul, et al. (2015).

5 Method for determining viral infectivity and replicative potential in MTFs

[00101] Primary MTFs were first isolated from C57BL/6 mice. The isolated MTFs were then infected with ZsGreen (ZsG)-tagged WT CHIKV, CHIKV with R to H amino acid substitution (RH) and CHIKV with both R to H and E to V amino acid mutations (RHEV). Flow cytometry was used for subsequent analysis of infected MTF populations.

- 10 [00102] Respective Zs-Green tagged CHIKV mutants were used to infect mouse tail fibroblasts (2×10^6 cells per infection) for 1.5 h in a 37 °C incubator, with atmosphere of 5% (v/v) CO₂. Virus overlay was removed and cells were washed once with appropriate serum-free medium before they were re-suspended in appropriate complete medium. Cells were further incubated at 37 °C, with atmosphere of 5% (v/v) CO₂, before being harvested at
- 15 indicated time points. During harvesting, 140 µl of infected cell suspension was aliquoted for viral RNA extraction. Cells were acquired using either BD FACS Calibur or BD FACS Canto II (BD Biosciences) to detect for ZsGreen signal in the FITC channel, to measure infectivity. Software used include BD FACSDiva software (for FACSCanto II) (BD Biosciences). A total of 30,000–50,000 cells were acquired and results were analyzed with FlowJo (version
- 20 10) (Tree Star).

Method for determining concentration of Type 1 IFN in MTFs

- [00103] CHIKV-infected MTFs were harvested at 12 hours post infection (hpi), and the concentration of Type 1 IFN were measured using the Luminex® screening assay kit according to the manufacturer's protocol which may be found on
- 25 <https://www.thermofisher.com/order/catalog/product/EPX020-22187-901>. Three independent experiments were performed and the data were presented as mean ± SD.

Method for determining viremia

- [00104] Ten microliters of blood was obtained from the tail vein and re-suspended in 120µl of DPBS and 10µl of citrate-phosphate-dextrose solution (Sigma-Aldrich). Purification of viral RNA
- 30 from the blood samples was performed with QIAamp Viral RNA Kit (QIAGEN) following the manufacturer's instructions. Viral copies were quantified by quantitative Real-time polymerase chain reaction (qRT-PCR) using a QuantiTect Probe RT-PCR Kit (QIAGEN), with primers and probe specific for CHIKV nsP 1, and extrapolated from a standard curve generated using serial dilutions of CHIKV negative-sense nsP 1 RNA transcripts

Method for infecting mouse to induce joint inflammation

[00105] Mice were inoculated subcutaneously in the ventral side of the right hind footpad with 1×10^6 plaque forming units (PFU) of the respective virus in 30 μ l of Dulbecco's Phosphate-Buffered Saline (DPBS). Level of viremia was monitored daily from 1 day post-infection (dpi) until 8 dpi, and subsequently every alternate day until 14 dpi. Joint swelling of the virus-inoculated foot was measured daily from 0 dpi to 14 dpi. Height (thickness) and breadth measurements were done for the metatarsal region of the foot, and quantified as (height $\times$ breadth). The disease score was expressed as the relative fold change in foot size compared with pre-infected foot (0 dpi), using the following formula: $[(x - \text{day } 0)/\text{day } 0 \times 100]$, where x is the quantified joint inflammation for each respective day.

Method for isolation of leukocyte and measurement of the levels of subsets of leukocytes

[00106] For the hind feet joint cell analysis, mice were sacrificed, and footpads and ankles were removed at 6 dpi, deskinning, and placed immediately in 4 ml digestion medium containing dispase (2 U/ml; Invitrogen), Collagenase IV (20 μ g/ml; Sigma-Aldrich), and DNase I mix (50 μ g/ml; Roche Applied Science) in complete RPMI medium. Tissues were incubated in digestion medium for 4 h at 37°C, 5% CO₂ on a shaker. Digested tissues and digestion medium were deposited onto a 40- μ m cell strainer, and 3 ml fresh complete RPMI medium was added. Digested tissues were ground against the cell strainer with a 1-ml syringe plunger, using a circular motion to release a maximum number of cells into the medium. Cells were centrifuged at 500 $\times$ g, and RBCs were lysed in buffered ammonium chloride solution. Cells were washed once in complete RPMI medium, resuspended in 10 ml complete RPMI medium, and overlaid onto 10 ml 35% v/v Percoll/RPMI 1640 medium (Sigma-Aldrich). Cells were centrifuged at 2400 rpm for 20 min, resuspended, and washed once more with complete medium before being counted.

[00107] Appropriate number of cells was transferred into 96-well v-bottom plates (Greiner Bio-one, Germany) staining in preparation for flow cytometry acquisition. Cells were first incubated with 50 μ l of LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Life Technologies, USA) at room temperature for 30 minutes, followed by washing with 100 μ l of DPBS. Cells were then incubated with 1% rat and mouse serum blocking buffer (Sigma Aldrich, St. Louis, Missouri, USA) to prevent non-specific binding for 20 minutes. Antibodies were used at 1:200 dilutions for each sample. The cells were then incubated with the respective antibody master mix for 20 minutes, before washing with DPBS. The cells were then fixed with 150 μ l of IC Fixation Buffer (eBioscience, USA) for 5 minutes. The cells were washed with DPBS, and subsequently re-suspended in 150 μ l of DPBS. Samples were then acquired with BD LSRII™ 5 lasers flow cytometer using the FACSDiva™ software, and analyzed with FlowJo version 10.0.7.

Method for detecting CHIKV antibody and antibody neutralization assay

[00108] ELISA

[00109] Antibody (Ab) titers were assessed by a virion-based ELISA. CHIKV-coated (10^6 virions/well in 50 μ l dPBS) polystyrene 96-well MaxiSorp plates (Nunc) were blocked with PBS containing 0.05% Tween 20 (PBST) and 5% w/v nonfat milk for 1.5 hours at 37°C. Sera from normal and infected groups of animals were heat inactivated and serially diluted in Ab diluent (0.05% PBST + 2.5% w/v nonfat milk). One hundred microliters of diluted sera was added into each well and incubated for 1 hour at 37°C. HRP-conjugated goat anti-mouse IgG, IgG1, IgG2b, IgG2c, IgG3, and IgM Abs were used. Total IgG and IgM quantification assays were performed using sera from individual animals diluted at 1:2000 and 1:100, respectively. Pooled sera were used for antibody isotyping. All HRP-conjugated Abs were from Santa Cruz, except for IgG3 (Southern Biotech). ELISA assays were developed using TMB substrate (Sigma-Aldrich), and terminated by Stop reagent (Sigma-Aldrich). Absorbance was measured at 450 nm. CHIKV-specific Ab isotype Ab titers are defined as the lowest dilution required for a detectable signal above control naïve pooled sera.

[00110] Neutralization assay

Neutralizing activity of antibodies was tested using an immunofluorescence-based cell infection assay in HEK293T cells. WT CHIKV LR2006 OPY1 infectious clone expressing sub-genomic ZsGreen protein was incubated with heat-inactivated mouse sera, diluted with complete media, for 1 hour at 37°C with gentle rocking (160 rpm). Virus-Ab mixtures were added at multiplicity of infection (MOI) 5 to HEK293T cells seeded in a 96-well plate (3×10^4 cells/well) and incubated for 18 hrs. Subsequently, cells were harvested and fixed with 4% paraformaldehyde, followed by acquisition using the MACSQuant Analyzer (Miltenyi Biotec). Infected cells expressing ZsGreen were quantified with FlowJo v10.0.7 software (FlowJo, LLC). Percentage of infectivity was calculated according to this equation: % Infectivity = $100 \times (\% \text{ infection from neutralization group} / \% \text{ infection from virus infection group})$.

Histological analysis

[00111] Mice were euthanized on 6 dpi, and perfused with 10% neutral buffered formalin (NBF). The virus-inoculated joints were harvested from the respective mice, and fixed in 10% NBF for 24 hours at room temperature. The joints then underwent decalcification in 5% formic acid, and sectioned to three parts at 5mm interval. Sectioned tissues were routinely processed, stained with hematoxylin and eosin (H&E), and embedded in paraffin wax, before being sliced into 5- μ m films. Tissues were viewed under the Olympus BX53 upright microscope (Olympus Life Science) and images were taken with Olympus DP71 digital color camera using Olympus DP controller and DP manager software.

[00112] Histological assessments were performed by histo-pathologists in a blinded fashion, with pathological changes evaluated using a scoring method in each individual animal, based on the

presence of edema, inflammation, muscle necrosis, tendonitis, and synovitis. Severity grades were assigned to the following scale: 0 – no finding; 1 – minimal; 2 – mild; 3 – moderate; 4 – marked; 5 – severe.

5 Example 2: Mutations in the CHIKV nsPs reduce viral infectivity and replicative potential in mouse tail fibroblasts

[00113] To investigate if mutation in the CHIKV nsPs reduces the infectivity and replicative potential of CHIKV, primary MTFs were first isolated from C57BL/6 mice. The MTFs were then infected with ZsGreen (ZsG)-tagged WT CHIKV, CHIKV with R to H amino acid substitution (RH CHIKV), CHIKV with E to V amino acid substitution (EV CHIKV) and CHIKV with both R to H and E to V amino acid mutations (RHEV CHIKV) as described above. Flow cytometry was then used for subsequent analysis of infected MTF populations.

[00114] Lower percentage of CHIKV-infected MTFs were observed from day 6 to day 24 when infection was performed using CHIKV with the RH mutation at position 532 and RHEV mutations at positions 532 and 1050, compared to the WT CHIKV (Figure 1A). Therefore, RH and RHEV mutations resulted in reduced CHIKV infectivity. Lower percentage of EV CHIKV-infected MTFs were observed at 12 hpi (Figure 1D). Therefore, EV mutation resulted also in reduced CHIKV infectivity. Mock groups (“MK” in Figures 1A and 1B) are negative controls for the experiments. During the infection step of the experiments, the mock groups are treated with serum free DMEM media, which is the same media that the virus is re-suspended in.

[00115] To investigate if mutation in the CHIKV nsPs affects the replicative potential of CHIKV, the viral load in MTFs infected using the RH and RHEV CHIKV were determined using the method described above. Figure 1B shows that the viral loads in MTFs infected with RH and RHEV CHIKV mutants reduced from 12 hours post infection and extended at least up to 24 hours post infection compared to wild-type infected cells. However, Figure 1E shows no significant change to the viral load in MTFs infected with EV CHIKV-infected MTFs at 12 hpi compared to MTFs infected with ST CHIKV. Virus quantification was also performed at 12 hours post infection using the TCID₅₀ assay. Figure 1C shows that at the 12 hour post infection time point, the infectious virus titre for RH CHIKV and RHEV CHIKV were significantly lower than the wild-type. Therefore, RH and RHEV mutations resulted in reduced replicative potential of CHIKV in MTFs as observed in the viral load and TCID₅₀ assays.

Example 3: Mutations in the CHIKV nsPs result in more robust Type 1 IFN response

[00116] To investigate if mutation in the CHIKV nsP affects the Type 1 IFN response, the MTFs infected with the CHIKV constructs were harvested at 12 hours post infection. The concentrations of the Type 1 IFN were then analysed using the Luminex® screening assay as described above.

[00117] Figure 2A shows that infection with the RH CHIKV nsP mutant increased the concentration of IFN- α . Similarly, infection with RH CHIKV nsP mutant also increased the concentration of IFN- β (Figure 2B). RHEV CHIKV nsP mutant resulted in further increase in the concentrations of both IFN- α and IFN- β compared to WT CHIKV and the RH CHIKV nsP mutant (Figures 2A and 2B). Therefore, mutation in the CHIKV nsP results in increased Type 1 IFN response in infected MTF cells. Furthermore, it was shown that simultaneous RHEV CHIKV nsP mutant induced a more robust IFN response in MTF cells. However, EV CHIKV mutants did not increase the concentrations of IFN- α or IFN- β compared to WT CHIKV, RH CHIKV and RHEV CHIKV (Figures 2A and 2B).

Example 4: Mutations in the CHIKV nsPs result in faster clearance of viremia and less severe virus-induced joint inflammation

[00118] To investigate if the mutations in the CHIKV nsPs affect the clearance of the CHIKV from the bloodstream or viremia, WT C57BL/6 mice were infected with ZsG-tagged WT CHIKV, RH CHIKV, EV CHIKV and RHEV CHIKV nsP mutants at the metatarsal region of the footpad as described above.

[00119] The progression of viremia in the virus-infected mice was monitored over the course of two weeks as shown in Figure 3A. Both the RH CHIKV and RHEV CHIKV nsP mutants displayed faster clearance of viremia compared to the WT CHIKV, with the RHEV CHIKV nsP mutant showing the faster clearance rate among the four CHIKV tested. However, EV CHIKV displayed the highest viremia and slowest clearance rate among the CHIKV tested.

[00120] To investigate the severity of the joint inflammation induced by the different nsP mutants, the joint inflammation of mice infected with the WT CHIKV, RH CHIKV, EV CHIKV and the RHEV CHIKV nsP mutant were measured over the course of two weeks. Figure 3B shows that the mice infected with the CHIKV nsP mutants also displayed less severe joint inflammation compared to the WT CHIKV. However, the joint inflammation of mice infected with EV CHIKV was not reduced compared to the WT CHIKV.

Example 5: Mutations in the CHIKV nsPs result in lower infectivity at the site of inflammation

[00121] To investigate if mutation in the CHIKV nsPs affects viral infectivity at the site of inflammation, WT C57BL/6 mice were infected subcutaneously with ZsG-tagged WT CHIKV, RH CHIKV, EV CHIKV and RHEV CHIKV nsP mutant at the metatarsal region of the footpad.

Leukocytes were then isolated from the footpad at 3 and 6 days post infection. Infections in CD45+ leukocytes and various leukocyte subsets (specifically the monocytes and macrophages, neutrophils, NK cells, CD4+T cells and CD8+ T cells) were assessed using flow cytometry as explained above. Figure 4G shows the leukocytes isolated for each treatment as analysed using flow cytometry.

5 [00122] Figure 4A shows the RH, EV and RHEV mutations were able to reduce the viral infectivity of the CHIKV nsP mutants on CD45+ leukocytes.

[00123] Figure 4B-4F shows that both the RH and RHEV mutations were able to reduce the viral infectivity of the CHIKV nsP mutants on various subsets of leukocytes. It was also found that the RH mutation in the CHIKV drastically reduced the viral infectivity in these cells.

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Example 6: Mice infected with CHIKV nsP mutants are protected from virus infection

[00124] To investigate if infection with the RH and RHEV CHIKV nsP mutants results in protection of the infected mice from subsequent virus infection, WT C57BL/6 mice were re-infected at the metatarsal region of the footpad with WT CHIKV, RH CHIKV and RHEV CHIKV nsP mutants at 90 days after the first infection. Joint inflammation of the re-infected mice was monitored over 2 weeks.

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[00125] Figure 5A, 5C and 5D show that the relative fold increase in footpad size in mice infected with RH CHIKV and RHEV CHIKV nsP mutants were significantly decreased. Therefore, RH CHIKV and RHEV CHIKV nsP mutants are protected from subsequent WT CHIKV virus infection.

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Example 7: Antibody response to CHIKV nsP mutant infection

[00126] Figure 6A shows that there was no joint inflammation observed in the re-infection phase using WT CHIKV after the initial infection with RH CHIKV, EV CHIKV and the RHEV CHIKV. Therefore, vaccinated mice did not suffer from joint swelling upon WT CHIKV infection. Viremia was also not detected in any of the mice vaccinated with RH CHIKV, EV CHIKV and the RHEV CHIKV when re-infected with WT CHIKV (Figure 6B). Infection with WT CHIKV did not provide such protection against foot pad inflammation or viremia when re-infected with WT CHIKV.

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[00127] To investigate the antibody response of mice during the re-infection phase, presence of CHIKV-specific antibodies were measured as described above. Figure 6C shows the production of anti-CHIKV IgG antibody in the sera of re-infected mice is more robust in vaccinated mice compared to non-vaccinated mice.

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[00128] The neutralization capacities of the CHIKV-specific antibodies (that are present in the sera) produced by re-infected mice were also investigated using the neutralisation assay described above. Figure 6D shows that the sera elicited during the re-infection phase had higher virus neutralizing capacity.

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Example 8: Protection from CHIKV-induced edema formation and tissue damage

[00129] Histological assays were also performed as described above to visually observe if vaccination with the nsP mutants provide protection against CHIKV-induced edema formation and tissue damage. Figure 7A and 7B shows that vaccination with WT CHIKV and RH CHIKV protects mice from CHIKV-induced edema formation and tissue damage.

Example 9: Anti-CHIKV antibody production is important protection against joint inflammation and viremia

[00130] To investigate if antibodies are important for protection against joint inflammation and viremia, μ MT mice (mice which lack antibodies) were first vaccinated with WT CHIKV or RH CHIKV. The mice were then re-infected with WT CHIKV 3 months post-infection. Vaccination of μ MT mice did not protect the mice from the re-infection (Figure 8A). High level of viremia was also observed in the re-infected μ MT mice (Figure 8B). Virion-based ELISA, performed as described above, did not detect CHIKV-specific antibodies in the μ MT mice (Figure 8C). Similarly, the sera from the μ MT mice did not show neutralizing activity against CHIKV in a human cell-line in vitro infection model (Figure 8D). Thus, mature B lymphocytes and antibodies are important for protection against joint inflammation and viremia in the vaccinated mice. Without the mature B lymphocytes and antibodies, vaccinated μ MT mice stay unprotected against CHIKV infection. Therefore, anti-CHIKV antibody production is important protection against joint inflammation and viremia.

Example 10: B and T lymphocytes are important for clearing viremia during vaccination and re-infection

[00131] To investigate if mature B and T lymphocytes are important for protection against joint inflammation and viremia, RAG-1^{-/-} mice (which lack mature B and T lymphocytes) are first vaccinated with WT CHIKV or RH CHIKV. Reinfection with WT CHIKV did not result in joint inflammation (Figure 9C) even though joint inflammation was observed in the vaccination phase (Figures 9A). However, it was found that viremia was not cleared during the vaccination phase with RH CHIKV (Figure 9B). After 3 months from the vaccination, the mice were re-infected with WT-CHIKV. Vaccination of RAG-1^{-/-} mice did not stop the replication of CHIKV in the host cell (Figure 9D). However, no joint swelling were observed during the re-infected with WT-CHIKV (Figure 9D). Thus, mature B and T lymphocytes are important for protection against joint inflammation and viremia in the vaccinated mice. Without the mature B and T lymphocytes, vaccinated RAG-1^{-/-} mice stay unprotected against CHIKV infection. Therefore, B and T lymphocytes are important for clearing viremia during vaccination and re-infection.

Example 11: CHIKV nsP mutant protects against joint swelling from ONNV infection

[00132] To investigate if vaccination with CHIKV nsP mutants provides protection against WT ONNV infection, WT C57BL/6 mice were vaccinated subcutaneously with WT CHIKV or RH CHIKV at the metatarsal region of the footpad. The mice were then re-infected with WT ONNV 3 months post-infection with the CHIKV nsP mutants. Vaccinated mice did not suffer from joint swelling upon ONNV infection (Figure 10A). Low levels of viremia could be detected in the blood of both vaccinated and naïve mice infected with ONNV (Figure 10B). Therefore, vaccination with RH CHIKV did provide protection against joint swelling, but only a lower level of protection against viremia when infected with ONNV.

[00133] **References:**

Saul S, Ferguson M, Cordonin C, et al. Differences in Processing Determinants of Nonstructural Polyprotein and in the Sequence of Nonstructural Protein 3 Affect Neurovirulence of Semliki Forest Virus. Diamond MS, ed. Journal of Virology. 2015;89(21):11030-11045. doi:10.1128/JVI.01186-15.

CLAIMS

1. A recombinant polypeptide comprising an amino acid sequence SEQ ID NO: 1, or a variant thereof comprising at least 80% identity to the amino acid sequence SEQ ID NO: 1,
5 comprising one or more mutations selected from the group consisting of:
(a) a substitution of R with H at a position equivalent to amino acid position 532 of SEQ ID NO: 1, and
(b) a substitution of E with V at a position equivalent to amino acid position 1050 of SEQ ID NO: 1,
10 wherein the mutations are attenuating mutations.
2. The recombinant polypeptide of claim 1, wherein the amino acid SEQ ID NO: 1 is derived from a Chikungunya virus strain LR2006 OPY1.
- 15 3. The recombinant polypeptide of claim 1 or 2, comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and variants thereof.
4. A polynucleotide encoding the polypeptide of any one of claims 1 to 3.
- 20 5. The polynucleotide of claim 4, wherein the polynucleotide is DNA or RNA.
6. The polynucleotide of claim 4 or 5, wherein the polynucleotide is selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, and variants thereof.
- 25 7. The polynucleotide of claim 6, wherein the variant is at least 80%, 85%, 90%, or 95% identical to SEQ ID NO: 5, SEQ ID NO: 6 and SEQ ID NO: 7.
8. An expression vector comprising the polynucleotide sequence of any one of claims 4 to 7.
- 30 9. An infectious clone comprising the polynucleotide sequence of any one of claims 4 to 7.
10. A recombinant virus particle comprising the polypeptide of any one of claims 1 to 3.
- 35 11. A recombinant virus particle comprising the polynucleotide of any one of claims 4 to 7.

12. The recombinant virus particle of claim 10 or 11, wherein the virus is an alphavirus.
- 5 13. The recombinant virus particle of claim 12, wherein the alphavirus is derived from a Chikungunya virus strain.
14. The recombinant virus particle of claim 13, wherein the Chikungunya virus strain is LR2006 OPY1.
- 10 15. The recombinant virus particle of any one of claims 10 to 14, wherein the virus is a live attenuated virus.
16. A virus particle derived from the infectious clone of claim 9.
- 15 17. An immunogenic composition comprising an infectious clone of claim 9, a recombinant virus particle of any one of claims 10 to 15 and/or a virus particle of claim 16.
18. The immunogenic composition of claim 17, wherein the recombinant virus is a live attenuated Chikungunya virus.
- 20 19. The immunogenic composition of claim 17 or 18 further comprising an adjuvant, a preservative, a stabilizer and/or a pharmaceutically acceptable carrier.
- 25 20. The immunogenic composition of claim 19, wherein the adjuvant is selected from the group consisting of (a) aluminum hydroxide, (b) aluminum phosphate, (c) gamma inulin, (d) alammulin (a combination of aluminum hydroxide and gamma inulin), (e) cholecalciferol in oil, (f) an oil in water emulsion OWEM1, containing squalene, tween-80, Span-85 in 10 mM phosphate-citrate buffer, (f) oil in water emulsion OWEM2 containing squalene, tween-80, Span-85, alpha tocopherol in phosphate- citrate buffer, and (g) an oil in water emulsion
30 OWEM3 containing squalene, tween-80, Span-85, cholecalciferol in phosphate-citrate buffer.
21. The immunogenic composition of any one of claims 17 to 20 further comprising the expression vector of claim 8.

22. Use of an immunogenic composition of any one of claims 17 to 21 in the manufacture of a medicament for eliciting an immune response against alphavirus infection in a subject.
- 5 23. The use of claim 22, wherein the subject is a human or non-human primate at risk of Chikungunya virus infection.
24. The use of claim 22, wherein the subject is a human or non-human primate at risk of O'nyong'nyong virus infection.
- 10 25. The use any one of claims 22 to 24, wherein the immunogenic composition is to be administered by a route selected from the group consisting of intramuscular, intradermal, subcutaneous, intravenous, oral, and intranasal administration.