



(51) International Patent Classification:

A61K 31/7105 (2006.01) C12N 15/38 (2006.01)

A61K 39/25 (2006.01) A61P 31/22 (2006.01)

(21) International Application Number:

PCT/US2018/022643

(22) International Filing Date:

15 March 2018 (15.03.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/471,809 15 March 2017 (15.03.2017) US

62/490,112 26 April 2017 (26.04.2017) US

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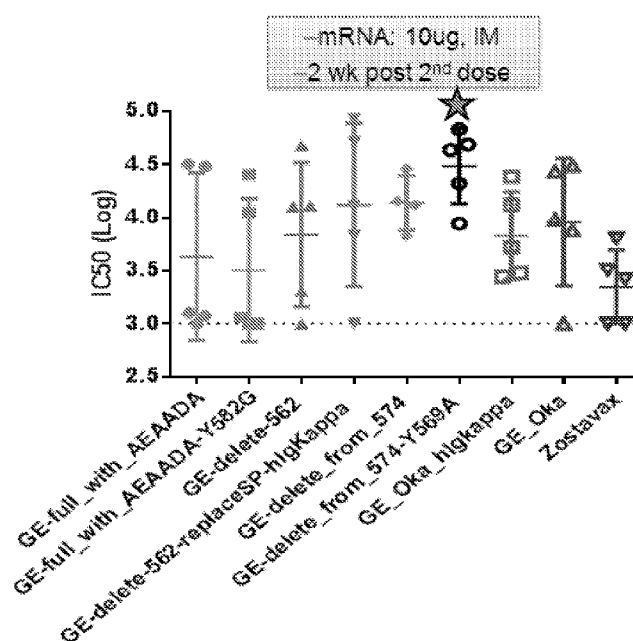
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: VARICELLA ZOSTER VIRUS (VZV) VACCINE

Fig. 13



(57) Abstract: Aspects of the disclosure relate to nucleic acid vaccines. The vaccines include at least one RNA polynucleotides having an open reading frame encoding at least one varicella zoster virus (VZV) antigen. Methods for preparing and using such vaccines are also described.



Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

VARICELLA ZOSTER VIRUS (VZV) VACCINE

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application number 62/471,809, filed March 15, 2017, and U.S. provisional application number 62/490,112, filed April 26, 2017, each of which is incorporated by reference herein in its entirety.

BACKGROUND

Varicella is an acute infectious disease caused by varicella zoster virus (VZV). Varicella zoster virus is one of eight herpesviruses known to infect humans and vertebrates. VZV is also known as chickenpox virus, varicella virus, zoster virus, and human herpesvirus type 3 (HHV-3). VZV only affects humans, and commonly causes chickenpox in children, teens and young adults and herpes zoster (shingles) in adults (rarely in children). The primary VZV infection, which results in chickenpox (varicella), may result in complications, including viral or secondary bacterial pneumonia. Even when the clinical symptoms of chickenpox have resolved, VZV remains dormant in the nervous system of the infected person (virus latency) in the trigeminal and dorsal root ganglia. In about 10–20% of cases, VZV reactivates later in life, travelling from the sensory ganglia back to the skin where it produces a disease (rash) known as shingles or herpes zoster. VZV can also cause a number of neurologic conditions ranging from aseptic meningitis to encephalitis. Other serious complications of VZV infection include postherpetic neuralgia, Mollaret's meningitis, zoster multiplex, thrombocytopenia, myocarditis, arthritis, and inflammation of arteries in the brain leading to stroke, myelitis, herpes ophthalmicus, or zoster sine herpette. In rare instances, VZV affects the geniculate ganglion, giving lesions that follow specific branches of the facial nerve. Symptoms may include painful blisters on the tongue and ear along with one sided facial weakness and hearing loss.

Varicella cases have declined 97% since 1995, mostly due to vaccination. However, an estimated 500,000 to 1 million episodes of herpes zoster (shingles) occur annually in just the United States. The lifetime risk of herpes zoster is estimated to be at least 32%, with increasing age and cellular immunosuppression being the most important risk factors. In fact, it is estimated that 50% of persons living until the age of 85 will develop herpes zoster.

A live attenuated VZV Oka strain vaccine is available and is marketed in the United States under the trade name VARIVAX[®] (Merck). A similar, but not identical, VZV vaccine is marketed globally as VARILRIX[®] (GlaxoSmithKline). Since its approval in 1995, it has

been added to the recommended vaccination schedules for children in Australia, the United States, and several other countries. In 2007, the Advisory Committee on Immunization Practices (ACIP) recommended a second dose of vaccine before school entry to ensure the maintenance of high levels of varicella immunity. In 2001-2005, outbreaks were reported in schools with high varicella vaccination coverage, indicating that even in settings where most children were vaccinated and the vaccine performed as expected, varicella outbreaks could not be prevented with the one-dose vaccination policy. As a result, two-dose vaccination is the adopted protocol; however, even with two doses of vaccine, there are reported incidences of breakthrough varicella. Furthermore, varicella vaccination has raised concerns that the immunity induced by the vaccine may not be lifelong, possibly leaving adults vulnerable to more severe disease as the immunity from their childhood immunization wanes.

In 2005, the FDA approved the combined live attenuated combination measles-mumps-rubella-varicella (MMRV) vaccine PROQUAD™ (Merck) for use in persons 12 months to 12 years in age. While the attenuated measles, mumps, and rubella vaccine viruses in MMRV are identical and of equal titer to those in the MMR vaccine, the titer of Oka/Merck VZV is higher in MMRV vaccine than in single-antigen varicella vaccine.

In 2006, the United States Food and Drug Administration approved ZOSTAVAX® (Merck) for the prevention of shingles (herpes zoster) in persons 60 years or older (currently 50 -59 years of age is approved). ZOSTAVAX® contains the same Oka/Merck varicella zoster virus used in the varicella and MMRV vaccines, but at a much higher titer (>10-fold higher viral dose) than that present in both of these vaccines, as the concentrated formulation is designed to elicit an immune response in older adults whose immunity to VZV wanes with advancing age.

Although the varicella vaccine has been shown to be safe in healthy individuals, there is evidence that immunity to VZV infection conferred by the vaccine wanes over time, rendering the vaccinated individuals susceptible to shingles, a more serious condition. In addition, there have been reports that individuals have developed chicken pox or shingles from the varicella vaccination. The vaccine may establish a latent infection in neural ganglia, which can then reactivate to cause herpes zoster.

Moreover, live attenuated virus is not suitable for all subjects, including pregnant women and persons with moderate or severe acute illnesses. Also, varicella is not suitable or approved for immunocompromised patients, including persons with immunosuppression due to leukemia, lymphoma, generalized malignancy, immune deficiency disease or immunosuppressive therapy. Likewise, persons with moderate or severe cellular immunodeficiency resulting from infection with human immunodeficiency virus (HIV)

5 There are one million cases of herpes zoster in the U.S. each year. An estimated \$1 billion is spent annually on direct medical costs for herpes zoster in the US and treatment for herpes zoster is not always effective or available.

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Various VZV amino acid sequences encompassed by the present disclosure are provided in Tables 1-9. RNA (*e.g.*, mRNA) vaccines as provided herein may include at least one RNA polynucleotide encoding at least one of the VZV glycoproteins provided in Table 1, or a fragment, homolog (*e.g.*, having at least 80%, 85%, 90%, 95%, 98% or 99% identity) or variant or derivative thereof.

Some embodiments of the present disclosure provide VZV vaccines that include at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding at least one VZV antigenic polypeptide. Some embodiments of the present disclosure provide VZV vaccines that include at least one RNA polynucleotide having an open reading frame encoding two or more VZV antigenic polypeptides. Some embodiments of the present disclosure provide VZV vaccines that include two or more RNA polynucleotides having an open reading frame encoding two or more VZV antigenic polypeptides.

In some embodiments, an antigenic polypeptide is a VZV glycoprotein. For example, a VZV glycoprotein may be VZV gE, gI, gB, gH, gK, gL, gC, gN, or gM. In some embodiments, the antigenic polypeptide is a VZV gE polypeptide. In some embodiments, the antigenic polypeptide is a VZV gI polypeptide. In some embodiments, the antigenic polypeptide is a VZV gB polypeptide. In some embodiments, the antigenic polypeptide is a VZV gH polypeptide. In some embodiments, the antigenic polypeptide is a VZV gK polypeptide. In some embodiments, the antigenic polypeptide is a VZV gL polypeptide. In some embodiments, the antigenic polypeptide is a VZV gC polypeptide. In some embodiments, the antigenic polypeptide is a VZV gN polypeptide. In some embodiments, the antigenic polypeptide is a VZV gM polypeptide. In some embodiments, the VZV glycoprotein is encoded by a nucleic acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

In some embodiments, the VZV glycoprotein is a variant gE polypeptide. In some embodiments, the variant VZV gE polypeptide is a truncated polypeptide lacking the anchor domain (ER retention domain). In some embodiments, the truncated VZV gE polypeptide comprises (or consists of, or consists essentially of) amino acids 1-561 of VZV gE polypeptide. In some embodiments, the truncated VZV gE polypeptide comprises (or consists of, or consists essentially of) amino acids 1-561 of SEQ ID NO: 10. In some embodiments, the truncated VZV gE polypeptide comprises (or consists of, or consists essentially of) amino acids 1-573 of SEQ ID NO: 18. In some embodiments, the truncated VZV gE polypeptide comprises (or consists of, or consists essentially of) amino acids 1-573 of SEQ ID NO: 10. In some embodiments, the variant VZV gE polypeptide is a truncated polypeptide lacking the carboxy terminal tail domain. In some embodiments, the truncated VZV gE polypeptide comprises (or consists of, or consists essentially of) amino acids 1-573

of VZV gE polypeptide. In some embodiments, the truncated VZV gE polypeptide comprises(or consists of, or consists essentially of) amino acids 1-573 of SEQ ID NO: 34.

In some embodiments, the variant VZV gE polypeptide has at least one mutation in one or more motif(s) associated with ER retention, wherein the mutation(s) in one or more motif(s) results in decreased retention of the VZV gE polypeptide in the ER and/or golgi. In some embodiments, the variant VZV gE polypeptide has at least one mutation in one or more motif(s) associated with targeting gE to the golgi or trans-golgi network (TGN), wherein the mutation(s) in one or more motif(s) results in decreased targeting or localization of the VZV gE polypeptide to the golgi or TGN. In some embodiments, the variant VZV gE polypeptide has at least one mutation in one or more motif(s) associated with the internalization of VZV gE or the endocytosis of gE, wherein the mutation(s) in one or more motif(s) results in decreased endocytosis of the VZV gE polypeptide. In some embodiments, the variant VZV gE polypeptide has at least one mutation in one or more phosphorylated acidic motif(s), such as SSTT (SEQ ID NO: 122). In some embodiments, the variant VZV gE polypeptide is a full-length VZV gE polypeptide having a Y582G mutation. In some embodiments, the variant VZV gE polypeptide is a full-length VZV gE polypeptide having a Y569A mutation. In some embodiments, the variant VZV gE polypeptide is a full-length VZV gE polypeptide having a Y582G mutation and a Y569A mutation. In some embodiments, the variant VZV gE polypeptide is an antigenic fragment comprising amino acids 1-573 of VZV gE and having a Y569A mutation. In some embodiments, the variant VZV gE polypeptide is an antigenic fragment comprising SEQ ID NO: 38.

In some embodiments, the variant VZV gE polypeptide is a full-length VZV gE polypeptide having an Igκ sequence. In some embodiments, the variant VZV gE polypeptide is SEQ ID NO: 14. In some embodiments, the variant VZV gE polypeptide is a full-length VZV gE polypeptide having an A-E-A-A-D-A sequence (SEQ ID NO: 58) that replaces SESTD T (SEQ ID NO: 59). This is a replacement of the Ser/Thr -rich "SSTT" (SEQ ID NO: 122) acidic cluster with an Ala-rich sequence. In some embodiments, the variant VZV gE polypeptide is SEQ ID NO: 26. In some embodiments in which the VZV gE polypeptide has an A-E-A-A-D-A sequence (SEQ ID NO: 58), the variant VZV gE polypeptide also has at least one mutation in one or more motif(s) associated with ER/golgi retention, TGN localization, or endocytosis (*e.g.*, has a Y582G mutation, a Y569A mutation, or both a Y582G mutation and a Y569A mutation) and/or has at least one mutation in one or more phosphorylated acidic motif(s), such as a SSTT (SEQ ID NO: 122) motif. In some embodiments, the variant VZV gE polypeptide is or comprises the amino acid sequence of SEQ ID NO: 30.

In some embodiments, the variant VZV gE polypeptide is a full-length VZV gE polypeptide having an additional sequence at the C-terminus that aids in secretion of the polypeptide or its localization to the cell membrane. In some embodiments, the variant VZV gE polypeptide is a full-length VZV gE polypeptide having an IgKappa sequence at the C-terminus. In some embodiments, the VZV gE polypeptide has additional sequence at the C-terminus that aids in secretion (*e.g.*, has an IgKappa sequence at the C-terminus) and has at least one mutation in one or more motif(s) associated with ER retention, TGN localization, or endocytosis (*e.g.*, has a Y582G mutation, a Y569A mutation, or both a Y582G mutation and a Y569A mutation) and/or has at least one mutation in one or more phosphorylated acidic motif(s), such as the SSTT (SEQ ID NO: 122) motif. In some embodiments, the variant VZV gE polypeptide is a truncated polypeptide lacking the anchor domain (ER retention domain) and having an additional sequence at the C-terminus that aids in secretion of the polypeptide (*e.g.*, an IgKappa sequence at the C-terminus). In some embodiments, the truncated VZV gE polypeptide comprises amino acids 1-561 and has an IgKappa sequence at the C-terminus. In some embodiments, the variant polypeptide is SEQ ID NO: 22. In some embodiments, the variant VZV gE polypeptide is a truncated polypeptide lacking the carboxy terminal tail domain and having an additional sequence at the C-terminus that aids in secretion of the polypeptide, for example, having an IgKappa sequence at the C-terminus. In some embodiments, the truncated VZV gE polypeptide comprises amino acids 1-573 and has an IgKappa sequence at the C-terminus.

In some embodiments, the antigenic polypeptide comprises two or more glycoproteins. In some embodiments, the two or more glycoproteins are encoded by a single RNA polynucleotide. In some embodiments, the two or more glycoproteins are encoded by two or more RNA polynucleotides, for example, each glycoprotein is encoded by a separate RNA polynucleotide. In some embodiments, the two or more glycoproteins can be any combination of VZV gE, gI, gB, gH, gK, gL, gC, gN, and gM polypeptides. In some embodiments, the two or more glycoproteins can be any combination of VZV gE and at least one of gI, gB, gH, gK, gL, gC, gN, and gM polypeptides. In some embodiments, the two or more glycoproteins can be any combination of VZV gI and at least one of gE, gB, gH, gK, gL, gC, gN, and gM polypeptides. In some embodiments, the two or more glycoproteins can be any combination of VZV gE, gI, and at least one of gB, gH, gK, gL, gC, gN, and gM polypeptides.

In some embodiments, the two or more VZV glycoproteins are gE and gI. In some embodiments, the two or more VZV glycoproteins are gE and gB. In some embodiments, the two or more VZV glycoproteins are gI and gB. In some embodiments, the two or more VZV

glycoproteins are gE, gI, and gB. In some embodiments, the two or more VZV glycoproteins are gE and gH. In some embodiments, the two or more VZV glycoproteins are gI and gH. In some embodiments, the two or more VZV glycoproteins are gE, gI, and gH. In some embodiments, the two or more VZV glycoproteins are gE and gK. In some embodiments, the two or more VZV glycoproteins are gI and gK. In some embodiments, the two or more VZV glycoproteins are gE, gI, and gK. In some embodiments, the two or more VZV glycoproteins are gE and gL. In some embodiments, the two or more VZV glycoproteins are gI and gL. In some embodiments, the two or more VZV glycoproteins are gE, gI, and gL. In some embodiments, the two or more VZV glycoproteins are gE and gC. In some embodiments, the two or more VZV glycoproteins are gI and gC. In some embodiments, the two or more VZV glycoproteins are gE, gI, and gC. In some embodiments, the two or more VZV glycoproteins are gE and gN. In some embodiments, the two or more VZV glycoproteins are gI and gN. In some embodiments, the two or more VZV glycoproteins are gE, gI, and gN. In some embodiments, the two or more VZV glycoproteins are gE and gM. In some embodiments, the two or more VZV glycoproteins are gI and gM. In some embodiments, the two or more VZV glycoproteins are gE, gI, and gM.

In some embodiments, the vaccine comprises any two or more VZV glycoproteins (*e.g.*, any of the variant VZV gE disclosed in the preceding paragraphs and in the Examples and Figures), and the VZV gE is a variant gE, such as any of the variant VZV gE glycoproteins disclosed herein, for example, any of the variant VZV gE disclosed in the preceding paragraphs and in the Examples and Figures.

In some embodiments, the VZV vaccine includes two or more RNA polynucleotides having an open reading frame encoding two or more VZV antigenic polypeptides (either encoded by a single RNA polynucleotide or encoded by two or more RNA polynucleotides, for example, each glycoprotein encoded by a separate RNA polynucleotide), and the two or more VZV glycoproteins are a variant gE (*e.g.*, any of the variant gE polypeptides disclosed herein in the preceding paragraphs) and a VZV glycoprotein selected from gI, gB, gH, gK, gL, gC, gN, and gM polypeptides. In some embodiments, the two or more VZV glycoproteins are a variant gE (*e.g.*, any of the variant gE polypeptides disclosed herein in the preceding paragraphs) and gI. In some embodiments, the glycoproteins are VZV gI and variant VZV gE, and the variant VZV gE polypeptide is a truncated polypeptide lacking the anchor domain (ER retention domain) (*e.g.*, a truncated VZV gE polypeptide comprising amino acids 1-561 of SEQ ID NO: 10). In some embodiments, the glycoproteins are VZV gI and variant VZV gE, and the variant VZV gE polypeptide is a truncated polypeptide lacking the carboxy terminal tail domain (*e.g.*, a truncated VZV gE polypeptide comprising amino

acids 1-573 of SEQ ID NO: 18). In some embodiments, the glycoproteins are VZV gI and variant VZV gE, and the variant VZV gE polypeptide has at least one mutation in one or more motif(s) associated with ER retention, TGN localization, and/or endocytosis (*e.g.*, the variant VZV gE has a Y582G mutation, a Y569A mutation, or both a Y582G mutation and a Y569A mutation) and/or has at least one mutation in one or more phosphorylated acidic motif(s), such as SSTT (SEQ ID NO: 122) motif. In some embodiments, the glycoproteins are VZV gI and variant VZV gE, and the variant VZV gE polypeptide is an antigenic fragment comprising amino acids 1-573 of VZV gE and having a Y569A mutation. In some embodiments, the glycoproteins are VZV gI and variant VZV gE, and the variant VZV gE polypeptide is a full-length VZV gE polypeptide having an A-E-A-A-D-A (SEQ ID NO: 58) sequence. In some embodiments, the glycoproteins are VZV gI and variant VZV gE, and the VZV gE polypeptide has an A-E-A-A-D-A (SEQ ID NO: 58) sequence and a Y582G mutation, a Y569A mutation, or both a Y582G mutation and a Y569A mutation. In some embodiments, the glycoproteins are VZV gI and variant VZV gE, and the VZV gE polypeptide is a full-length VZV gE polypeptide having an additional sequence at the C-terminus that aids in secretion of the polypeptide (*e.g.*, an IgKappa sequence). In some embodiments, the glycoproteins are VZV gI and variant VZV gE, and the VZV gE polypeptide is a full-length VZV gE polypeptide having an IgKappa sequence and a Y582G mutation, a Y569A mutation, or both a Y582G mutation and a Y569A mutation. In some embodiments, the glycoproteins are VZV gI and variant VZV gE, and the VZV gE polypeptide is a truncated VZV gE polypeptide lacking the anchor domain (ER retention domain) and having an IgKappa sequence. In some embodiments, the variant VZV gE polypeptide is a truncated polypeptide comprising amino acids 1-561 or amino acids 1-573 and having an IgKappa sequence at the C-terminus.

In any of the above-described embodiments, the VZV vaccine may further comprise a live attenuated VZV, a whole inactivated VZV, or a VZV virus-like particle (VLP). In some embodiments, the live attenuated VZV, whole inactivated VZV, or VZV VLP is selected from or derived from the following strains and genotypes: VZV E1 strain, genotypes E1_32_5, E1_Kel, E1_Dumas, E1_Russia 1999, E1_SD, E1_MSP, E1_36, E1_49, E1_BC, E1_NH29; VZV E2 strain, genotypes E2_03-500, E2_2, E2_11, E2_HJO; VZV J strain, genotype pOka; VZV M1 strain, genotype M1_CA123; VZV M2 strain, genotypes M2_8 and M2_DR; and VZV M4 strain, genotypes Spain 4242, France 4415, and Italy 4053.

Alternate RNA vaccines comprising RNA polynucleotides encoding other viral protein components of VZV, for example, tegument proteins are encompassed by the present disclosure. Thus, some embodiments of the present disclosure provide VZV vaccines that

include at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding at least one VZV tegument protein. Some embodiments of the present disclosure provide VZV vaccines that include at least one RNA polynucleotide having an open reading frame encoding at least one VZV tegument protein and at least one VZV glycoprotein. Some
5 embodiments of the present disclosure provide VZV vaccines that include at least one RNA polynucleotide having an open reading frame encoding at least one VZV tegument protein and at least one RNA polynucleotide having an open reading frame encoding at least one VZV glycoprotein. In some embodiments, RNA vaccines comprise RNA (*e.g.*, mRNA) polynucleotide(s) encoding one or more VZV tegument protein(s) and one or more VZV
10 glycoprotein(s) selected from VZV gE, gI, gB, gH, gK, gL, gC, gN, and gM polypeptides. In some embodiments, the VZV glycoprotein is a VZV gE polypeptide. In some embodiments, the VZV glycoprotein is a VZV gI polypeptide. In some embodiments, the VZV glycoprotein is a variant VZV gE polypeptide, such as any of the variant VZV gE polypeptides disclosed herein. In some embodiments, the VZV glycoproteins are VZV gE
15 glycoproteins and VZV gI glycoproteins.

In some embodiments, at least one RNA polynucleotide is encoded by at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 41 and homologs having at least 80% (*e.g.*, 85%, 90%, 95%, 98%, 99%) identity with a nucleic acid
20 sequence selected from SEQ ID NO: 1-8 and 41. In some embodiments, at least one RNA polynucleotide is encoded by at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 41 and homologs having at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.8% or 99.9%) identity with a nucleic acid
25 sequence selected from SEQ ID NO: 1-8 and 41. In some embodiments, at least one RNA polynucleotide is encoded by at least one fragment of a nucleic acid sequence (*e.g.*, a fragment having an antigenic sequence or at least one epitope) selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 41 and homologs having at least 80% (*e.g.*, 85%, 90%, 95%,
30 98%, 99%) identity with a nucleic acid sequence selected from SEQ ID NO: 1-8 and 41. In some embodiments, at least one RNA polynucleotide is encoded by at least one epitope of a nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 41.

In some embodiments, at least one RNA polynucleotide is a gE polypeptide encoded
35 by SEQ ID NO: 1. In some embodiments, at least one RNA polynucleotide is a gI

polypeptide encoded by SEQ ID NO: 2. In some embodiments, at least one RNA polynucleotide is a truncated gE polypeptide encoded by SEQ ID NO: 3. In some embodiments, at least one RNA polynucleotide is a truncated gE polypeptide encoded by SEQ ID NO: 5. In some embodiments, at least one RNA polynucleotide is a truncated gE polypeptide having Y569A mutation encoded by SEQ ID NO: 6. In some embodiments, at least one RNA polynucleotide is a gE polypeptide having an AEAADA sequence SEQ ID NO: 58 encoded by SEQ ID NO: 7. In some embodiments, at least one RNA polynucleotide is a gE polypeptide having a Y582G mutation and a AEAADA sequence (SEQ ID NO: 58) encoded by SEQ ID NO: 8. In some embodiments, at least one RNA polynucleotide is a gE polypeptide encoded by SEQ ID NO: 41.

In some embodiments, at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic polypeptide having at least 90% identity to the amino acid sequence of any one of SEQ ID NO: 10, 14, 18, 22, 26, 30, 34, 38, 42 and 45-55. In some embodiments, at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic polypeptide having at least 95% identity to the amino acid sequence of any one of SEQ ID NO: 10, 14, 18, 22, 26, 30, 34, 38, 42 and 45-55. In some embodiments, at least one RNA polynucleotide encodes an antigenic polypeptide having at least 96% identity to the amino acid sequence of any one of SEQ ID NO: 10, 14, 18, 22, 26, 30, 34, 38, 42 and 45-55. In some embodiments, at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic polypeptide having at least 97% identity to the amino acid sequence of any one of SEQ ID NO: 10, 14, 18, 22, 26, 30, 34, 38, 42 and 45-55. In some embodiments, at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic polypeptide having at least 98% identity to the amino acid sequence of any one of SEQ ID NO: 10, 14, 18, 22, 26, 30, 34, 38, 42 and 45-55. In some embodiments, at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic polypeptide having at least 99% identity to the amino acid sequence of any one of SEQ ID NO: 10, 14, 18, 22, 26, 30, 34, 38, 42 and 45-55.

In some embodiments, the open reading frame from which the VZV polypeptide is encoded is codon-optimized. In some embodiments, the at least one RNA polynucleotide encodes an antigenic protein of any one of SEQ ID NO: 10, 14, 18, 22, 26, 30, 34, 38, 42 and 45-55, and wherein the RNA polynucleotide is codon-optimized mRNA. In some embodiments, the at least one RNA polynucleotide comprises a mRNA sequence identified by any one of SEQ ID NO: 92-108. In some embodiments, the mRNA sequence identified by any one of SEQ ID NO: 92-108 is codon optimized to encode antigenic VZV polypeptides that are as immunogenic, or more immunogenic than, the antigenic VZV polypeptides encoded by any one of SEQ ID NO: 92-108.

In some embodiments, the at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic protein of SEQ ID NO: 10, wherein the RNA (*e.g.*, mRNA) polynucleotide has less than 80% identity to wild-type mRNA sequence. In some embodiments, the at least one RNA polynucleotide encodes an antigenic protein of SEQ ID NO: 10, wherein the RNA (*e.g.*, mRNA) polynucleotide has greater than 80% identity to wild-type mRNA sequence, but does not include wild-type mRNA sequence. In some embodiments, the at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic protein of SEQ ID NO: 42, wherein the RNA (*e.g.*, mRNA) polynucleotide has less than 80% identity to wild-type mRNA sequence. In some embodiments, the at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic protein of SEQ ID NO: 42, wherein the RNA polynucleotide has greater than 80% identity to wild-type mRNA sequence, but does not include wild-type mRNA sequence. In some embodiments, the at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic protein of SEQ ID NO: 14, wherein the RNA (*e.g.*, mRNA) polynucleotide has less than 80% identity to wild-type mRNA sequence. In some embodiments, the at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic protein of SEQ ID NO: 14, wherein the RNA (*e.g.*, mRNA) polynucleotide has greater than 80% identity to wild-type mRNA sequence, but does not include wild-type mRNA sequence. In some embodiments, the at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic protein of SEQ ID NO: 26, wherein the RNA polynucleotide has less than 80% identity to wild-type mRNA sequence. In some embodiments, the at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic protein of SEQ ID NO: 26, wherein the RNA (*e.g.*, mRNA) polynucleotide has greater than 80% identity to wild-type mRNA sequence, but does not include wild-type mRNA sequence. In some embodiments, the at least one RNA (*e.g.*, mRNA) polynucleotide encodes an antigenic protein of SEQ ID NO: 30, wherein the RNA polynucleotide has less than 80% identity to wild-type mRNA sequence. In some embodiments, the at least one RNA polynucleotide encodes an antigenic protein of SEQ ID NO: 30, wherein the RNA (*e.g.*, mRNA) polynucleotide has greater than 80% identity to wild-type mRNA sequence, but does not include wild-type mRNA sequence. In some embodiments, the at least one RNA (*e.g.*, mRNA) polynucleotide is encoded by a sequence selected from any one of SEQ ID NO: 1-8 and SEQ ID NO 41 and includes at least one chemical modification.

In some embodiments, the VZV vaccine is multivalent. In some embodiments, the RNA polynucleotide comprises a polynucleotide sequence derived from VZV E1 strain, including, for example, any one or more of genotypes E1_32_5, E1_Kel, E1_Dumas, E1_Russia 1999, E1_SD, E1_MSP, E1_36, E1_49, E1_BC, and E1_NH29. In some embodiments, the RNA (*e.g.*, mRNA) polynucleotide comprises a polynucleotide sequence

derived from VZV E2 strain, including, for example, any one or more of genotypes E2_03-500, E2_2, E2_11, and E2_HJO. In some embodiments, the RNA (*e.g.*, mRNA) polynucleotide comprises a polynucleotide sequence derived from VZV J strain, including, for example, genotype pOka. In some embodiments, the RNA (*e.g.*, mRNA) polynucleotide comprises a polynucleotide sequence derived from VZV M1 strain, including, for example, genotype M1_CA123. In some embodiments, the RNA (*e.g.*, mRNA) polynucleotide comprises a polynucleotide sequence derived from VZV M2 strain, including, for example, genotypes M2_8 and M2_DR. In some embodiments, the RNA (*e.g.*, mRNA) polynucleotide comprises a polynucleotide sequence derived from VZV M4 strain, including, for example, any one or more of genotypes Spain 4242, France 4415, and Italy 4053.

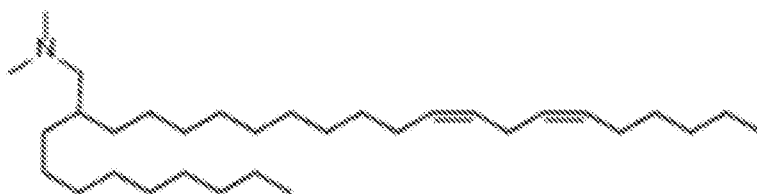
Some embodiments of the present disclosure provide a VZV vaccine that includes at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding at least one VZV antigenic polypeptide and at least one 5' terminal cap. In some embodiments, a 5' terminal cap is 7mG(5')ppp(5')NlmpNp. Some embodiments of the present disclosure provide a VZV vaccine that includes at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding at least one VZV antigenic polypeptide, wherein the at least one RNA (*e.g.*, mRNA) polynucleotide has at least one chemical modification. In some embodiments, the at least one RNA (*e.g.*, mRNA) polynucleotide further comprises a second chemical modification. In some embodiments, the at least one RNA (*e.g.*, mRNA) polynucleotide having at least one chemical modification has a 5' terminal cap. In some embodiments, the at least one chemical modification is selected from pseudouridine, N1-methylpseudouridine, N1-ethylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methoxyuridine and 2'-O-methyl uridine. In some embodiments, every (100%) of the uridines of the at least one RNA polynucleotide comprises a chemical modification, such as a N1-methylpseudouridine modification or a N1-ethylpseudouridine modification.

Some embodiments of the present disclosure provide a VZV vaccine that includes at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding at least one VZV antigenic polypeptide, wherein at least 80% (*e.g.*, 85%, 90%, 95%, 98%, 99%, 100%) of the uracil in the open reading frame have a chemical modification, optionally wherein the vaccine is formulated in a lipid nanoparticle. In some embodiments, 100% of the uracil in the open reading frame have a chemical modification. In some embodiments, a

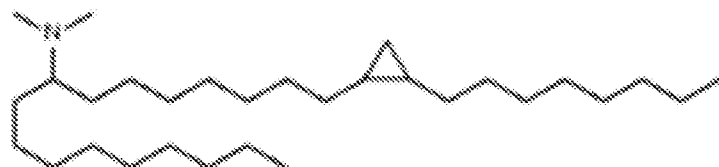
chemical modification is in the 5-position of the uracil. In some embodiments, a chemical modification is a N1-methyl pseudouridine. In some embodiments, 100% of the uracil in the open reading frame are modified to include N1-methyl pseudouridine.

Some embodiments of the present disclosure provide a VZV vaccine that is formulated within a cationic lipid nanoparticle. In some embodiments, the cationic lipid nanoparticle comprises a cationic lipid, a PEG-modified lipid, a sterol and a non-cationic lipid. In some embodiments, a cationic lipid is an ionizable cationic lipid and the non-cationic lipid is a neutral lipid, and the sterol is a cholesterol. In some embodiments, a cationic lipid is selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, (12Z,15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine, and N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine.

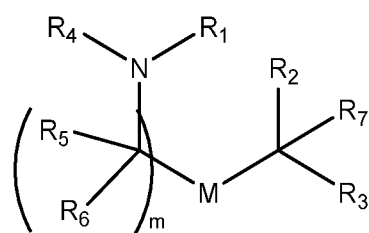
In some embodiments, the lipid is



In some embodiments, the lipid is



In some embodiments, at least one cationic lipid selected from compounds of Formula (I):



(I),

or a salt or isomer thereof, wherein:

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR,

- 5 -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a carbocycle, heterocycle, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -N(R)₂, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂,
10 -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(R)N(R)₂C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

- 15 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

- 20 R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

- 25 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl; each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl; each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

- 30 In some embodiments, a subset of compounds of Formula (I) includes those in which when R₄ is -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, or -CQ(R)₂, then (i) Q is not -N(R)₂ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

In some embodiments, a subset of compounds of Formula (I) includes those in which R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and

- 35 -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR,

- 5 -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, 10 -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, mono- or di-alkylamino, and C₁₋₃ alkyl, and each n is independently 15 selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,

- 20 and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

- 25 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,

-R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

- 30 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those in which

- 35 R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and

-R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

- 5 R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈,
 10 -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R₄ is -(CH₂)_nQ in which n is 1 or 2, or (ii) R₄
 15 is -(CH₂)_nCHQR in which n is 1, or (iii) R₄ is -CHQR, and -CQ(R)₂, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,

- 20 -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R,

- 25 -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

- 30 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

- 35 In some embodiments, a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those in which R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₂₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is -(CH₂)_nQ or -(CH₂)_nCHQR, where Q is -N(R)₂, and n is selected from 3, 4, and 5;

- 5 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

- 10 R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;
 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;
 15 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;
 each Y is independently a C₃₋₆ carbocycle;
 each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
 or salts or isomers thereof.

- 20 In some embodiments, a subset of compounds of Formula (I) includes those in which
 R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

- 25 R₄ is selected from the group consisting of -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, and -CQ(R)₂, where Q is -N(R)₂, and n is selected from 1, 2, 3, 4, and 5;
 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 30 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

- 35 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,

-R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;

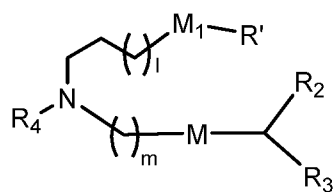
each Y is independently a C₃₋₆ carbocycle;

5 each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those of Formula (IA):



(IA),

or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M₁ is a bond or M'; R₄ is unsubstituted C₁₋₃ alkyl, or -(CH₂)_nQ, in

which Q is OH, -NHC(S)N(R)₂, -NHC(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)R₈, -NHC(=NR₉)N(R)₂, -NHC(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, heteroaryl or

15 heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R₂ and R₃ are

independently selected from the group consisting of H, C₁₋₁₄ alkyl, and C₂₋₁₄ alkenyl.

In some embodiments, the cationic lipid nanoparticle has a molar ratio of about 20-60% cationic lipid, about 5-25% non-cationic lipid, about 25-55% sterol, and about 0.5-15% PEG-modified lipid. In some embodiments, the nanoparticle has a polydispersity value of less than 0.4. In some embodiments, the nanoparticle has a net neutral charge at a neutral pH value. In some embodiments, the nanoparticle has a mean diameter of 50-200 nm.

Some embodiments of the present disclosure provide methods of inducing an antigen specific immune response in a subject, comprising administering to the subject a VZV RNA (e.g., mRNA) vaccine in an amount effective to produce an antigen specific immune response. In some embodiments, an antigen specific immune response comprises a T cell response or a B cell response. In some embodiments, an antigen specific immune response comprises a T cell response and a B cell response. In some embodiments, a method of producing an antigen specific immune response involves a single administration of the vaccine. In some embodiments, a method further includes administering to the subject a booster dose of the vaccine. In some embodiments, a vaccine is administered to the subject by intradermal or intramuscular injection.

Also provided herein are VZV RNA (*e.g.*, mRNA) vaccines for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering the vaccine to the subject in an amount effective to produce an antigen specific immune response.

5 Further provided herein are uses of VZV RNA (*e.g.*, mRNA) vaccines in the manufacture of a medicament for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering the vaccine to the subject in an amount effective to produce an antigen specific immune response.

10 Some aspects of the present disclosure provide methods of preventing or treating VZV infection comprising administering to a subject the VZV RNA (*e.g.*, mRNA) vaccine of the present disclosure. In some embodiments, the VZV RNA (*e.g.*, mRNA) vaccine is formulated in an effective amount to produce an antigen specific immune response in a subject.

15 Some embodiments of the present disclosure provide methods of inducing an antigen specific immune response in a subject, the methods comprising administering to a subject a VZV RNA (*e.g.*, mRNA) vaccine as provided herein in an effective amount to produce an antigen specific immune response in a subject.

20 In some embodiments, an anti-VZV antigenic polypeptide antibody titer produced in the subject is increased by at least 1 log relative to a control. In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a control.

25 In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in the subject is increased at least 2 times relative to a control. In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in the subject is increased at least 5 times relative to a control. In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in the subject is increased at least 10 times relative to a control. In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in the subject is increased 2-10 times relative to a control.

30 In some embodiments, the control is an anti-VZV antigenic polypeptide antibody titer produced in a subject who has not been administered VZV vaccine. In some embodiments, the control is an anti-VZV antigenic polypeptide antibody titer produced in a subject who has been administered a live attenuated or inactivated VZV vaccine. In some embodiments, the control is an anti-VZV antigenic polypeptide antibody titer produced in a subject who has been administered a recombinant or purified VZV protein vaccine. In some embodiments,

the control is an anti-VZV antigenic polypeptide antibody titer produced in a subject who has been administered an VZV virus-like particle (VLP) vaccine.

In some embodiments, the effective amount is a dose equivalent to at least a 2-fold reduction in the standard of care dose of a recombinant VZV protein vaccine, wherein an anti-VZV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-VZV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified VZV protein vaccine, a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine.

In some embodiments, the effective amount is a dose equivalent to at least a 4-fold reduction in the standard of care dose of a recombinant VZV protein vaccine, wherein an anti-VZV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-VZV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified VZV protein vaccine, a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine.

In some embodiments, the effective amount is a dose equivalent to at least a 10-fold reduction in the standard of care dose of a recombinant VZV protein vaccine, wherein an anti-VZV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-VZV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified VZV protein vaccine, a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine.

In some embodiments, the effective amount is a dose equivalent to at least a 100-fold reduction in the standard of care dose of a recombinant VZV protein vaccine, wherein an anti-VZV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-VZV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified VZV protein vaccine, a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine.

In some embodiments, the effective amount is a dose equivalent to at least a 1000-fold reduction in the standard of care dose of a recombinant VZV protein vaccine, wherein an anti-VZV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-VZV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified VZV protein vaccine, a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine.

In some embodiments, the effective amount is a dose equivalent to a 2-fold to 1000-fold reduction in the standard of care dose of a recombinant VZV protein vaccine, wherein an anti-VZV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-

VZV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified VZV protein vaccine, a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine.

In some embodiments, the effective amount is a total dose of 25 µg to 1000 µg, or 50 µg to 1000 µg or 25 to 200 µg. In some embodiments, the effective amount is a total dose of 50 µg, 100 µg, 200 µg, 400 µg, 800 µg, or 1000 µg. In some embodiments, the effective amount is a total dose of 200 µg. In some embodiments, the effective amount is a total dose of 50 µg to 400 µg. In some embodiments, the effective amount is a total dose of 50 µg, 100 µg, 150 µg, 200 µg, 250 µg, 300 µg, 350 µg or 400 µg. In some embodiments, the effective amount is a dose of 25 µg administered to the subject a total of two times. In some
embodiments, the effective amount is a dose of 50 µg administered to the subject a total of two times. In some embodiments, the effective amount is a dose of 100 µg administered to the subject a total of two times. In some embodiments, the effective amount is a dose of 200 µg administered to the subject a total of two times. In some
embodiments, the effective amount is a dose of 400 µg administered to the subject a total of two times. In some
embodiments, the effective amount is a dose of 500 µg administered to the subject a total of two times.

In some embodiments, the efficacy (or effectiveness) of the VZV RNA (*e.g.*, mRNA) vaccine against VZV is greater than 60%.

Vaccine efficacy may be assessed using standard analyses (*see, e.g.*, Weinberg *et al.*, *J Infect Dis.* 2010 Jun 1;201(11):1607-10). For example, vaccine efficacy may be measured by double-blind, randomized, clinical controlled trials. Vaccine efficacy may be expressed as a proportionate reduction in disease attack rate (AR) between the unvaccinated (ARU) and vaccinated (ARV) study cohorts and can be calculated from the relative risk (RR) of disease
among the vaccinated group with use of the following formulas:

$$\text{Efficacy} = (\text{ARU} - \text{ARV})/\text{ARU} \times 100; \text{ and}$$

$$\text{Efficacy} = (1 - \text{RR}) \times 100.$$

Likewise, vaccine effectiveness may be assessed using standard analyses (*see, e.g.*, Weinberg *et al.*, *J Infect Dis.* 2010 Jun 1;201(11):1607-10). Vaccine effectiveness is an
assessment of how a vaccine (which may have already proven to have high vaccine efficacy) reduces disease in a population. This measure can assess the net balance of benefits and adverse effects of a vaccination program, not just the vaccine itself, under natural field conditions rather than in a controlled clinical trial. Vaccine effectiveness is proportional to vaccine efficacy (potency) but is also affected by how well target groups in the population are
immunized, as well as by other non-vaccine-related factors that influence the ‘real-world’

outcomes of hospitalizations, ambulatory visits, or costs. For example, a retrospective case control analysis may be used, in which the rates of vaccination among a set of infected cases and appropriate controls are compared. Vaccine effectiveness may be expressed as a rate difference, with use of the odds ratio (OR) for developing infection despite vaccination:

5 Effectiveness = $(1 - \text{OR}) \times 100$.

In some embodiments, the efficacy (or effectiveness) of the VZV RNA (*e.g.*, mRNA) vaccine against VZV is greater than 65%. In some embodiments, the efficacy (or effectiveness) of the vaccine against VZV is greater than 70%. In some embodiments, the efficacy (or effectiveness) of the vaccine against VZV is greater than 75%. In some
10 embodiments, the efficacy (or effectiveness) of the vaccine against VZV is greater than 80%. In some embodiments, the efficacy (or effectiveness) of the vaccine against VZV is greater than 85%. In some embodiments, the efficacy (or effectiveness) of the vaccine against VZV is greater than 90%.

In some embodiments, the vaccine immunizes the subject against VZV up to 1 year
15 (*e.g.* for a single VZV season). In some embodiments, the vaccine immunizes the subject against VZV for up to 2 years. In some embodiments, the vaccine immunizes the subject against VZV for more than 2 years. In some embodiments, the vaccine immunizes the subject against VZV for more than 3 years. In some embodiments, the vaccine immunizes the subject against VZV for more than 4 years. In some embodiments, the vaccine
20 immunizes the subject against VZV for 5-10 years.

In some embodiments, the subject administered an VZV RNA (*e.g.*, mRNA) vaccine is between the ages of about 12 months old and about 10 years old (*e.g.*, about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 years old). In some embodiments, the subject administered an VZV RNA (*e.g.*, mRNA) vaccine is between the ages of about 12 months old and about 15 months old (*e.g.*,
25 about 12, 12.5, 13, 13.5, 14, 14.5 or 15 months old). In some embodiments, the subject administered an VZV RNA (*e.g.*, mRNA) vaccine is between the ages of about 4 years old and about 6 years old (*e.g.*, about 4, 4.5, 5, 5.6, or 6 years old).

In some embodiments, the subject is a young adult between the ages of about 20 years and about 50 years (*e.g.*, about 20, 25, 30, 35, 40, 45 or 50 years old).

30 In some embodiments, the subject is an elderly subject about 60 years old, about 70 years old, or older (*e.g.*, about 60, 65, 70, 75, 80, 85 or 90 years old).

In some embodiments, the subject has been exposed to VZV, is infected with (has) VZV, or is at risk of infection by VZV.

In some embodiments, the subject is immunocompromised (has an impaired immune
35 system, *e.g.*, has an immune disorder or autoimmune disorder).

Some aspects of the present disclosure provide varicella zoster virus (VZV) RNA (*e.g.*, mRNA) vaccines containing a signal peptide linked to a VZV antigenic polypeptide. Thus, in some embodiments, the VZV RNA (*e.g.*, mRNA) vaccines contain at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding a signal peptide linked to a VZV antigenic peptide. Also provided herein are nucleic acids encoding the VZV RNA (*e.g.*, mRNA) vaccines disclosed herein.

Other aspects of the present disclosure provide varicella zoster virus (VZV) vaccines containing a signal peptide linked to a VZV antigenic polypeptide. In some embodiments, the VZV antigenic polypeptide is a VZV glycoprotein. In some embodiments, the VZV glycoprotein is selected from VZV gE, gI, gB, gH, gK, gL, gC, gN, and gM. In some embodiments, the VZV glycoprotein is VZV gE or a variant VZV gE polypeptide.

In some embodiments, the signal peptide is a IgE signal peptide. In some embodiments, the signal peptide is an IgE HC (Ig heavy chain epsilon-1) signal peptide. In some embodiments, the signal peptide has the sequence MDWTWILFLVAAATRVHS (SEQ ID NO: 56). In some embodiments, the signal peptide is an IgGκ signal peptide. In some embodiments, the signal peptide has the sequence METPAQLLFLLLLWLPDTTG (SEQ ID NO: 57). In some embodiments, the signal peptide is selected from: a Japanese encephalitis PRM signal sequence (MLGSNSGQRVVFTILLLLVAPAYS; SEQ ID NO: 109), VSVg protein signal sequence (MKCLLYLAFLFIGVNCA; SEQ ID NO: 110) and Japanese encephalitis JEV signal sequence (MWLVSLAIVTACAGA; SEQ ID NO: 111).

Further provided herein are nucleic acids encoding VZV vaccines disclosed herein. Such VZV vaccines include at least one ribonucleic acid (RNA) (*e.g.*, mRNA) polynucleotide having an open reading frame encoding a signal peptide linked to a VZV antigenic polypeptide. In some embodiments, the VZV antigenic peptide is a VZV glycoprotein. In some embodiments, the VZV glycoprotein is selected from VZV gE, gI, gB, gH, gK, gL, gC, gN, and gM. In some embodiments, the VZV antigenic peptide is a VZV gE or a variant of the gE polypeptide.

In some embodiments, an effective amount of an VZV RNA (*e.g.*, mRNA) vaccine (*e.g.*, a single dose of the VZV vaccine) results in a 2 fold to 200 fold (*e.g.*, about 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 or 200 fold) increase in serum neutralizing antibodies against VZV, relative to a control. In some embodiments, a single dose of the VZV RNA (*e.g.*, mRNA) vaccine results in an about 5 fold, 50 fold, or 150 fold increase in serum neutralizing antibodies against VZV, relative to a control. In some embodiments, a single dose of the VZV RNA (*e.g.*, mRNA) vaccine

results in an about 2 fold to 10 fold, or an about 40 to 60 fold increase in serum neutralizing antibodies against VZV, relative to a control.

In some embodiments, efficacy of RNA vaccines RNA (*e.g.*, mRNA) can be significantly enhanced when combined with a flagellin adjuvant, in particular, when one or more antigen-encoding mRNAs is combined with an mRNA encoding flagellin.

RNA (*e.g.*, mRNA) vaccines combined with the flagellin adjuvant (*e.g.*, mRNA-encoded flagellin adjuvant) have superior properties in that they may produce much larger antibody titers and produce responses earlier than commercially available vaccine formulations. While not wishing to be bound by theory, it is believed that the RNA vaccines, for example, as mRNA polynucleotides, are better designed to produce the appropriate protein conformation upon translation, for both the antigen and the adjuvant, as the RNA (*e.g.*, mRNA) vaccines co-opt natural cellular machinery. Unlike traditional vaccines, which are manufactured *ex vivo* and may trigger unwanted cellular responses, RNA (*e.g.*, mRNA) vaccines are presented to the cellular system in a more native fashion.

Some embodiments of the present disclosure provide RNA (*e.g.*, mRNA) vaccines that include at least one RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding at least one antigenic polypeptide and at least one RNA (*e.g.*, mRNA polynucleotide) having an open reading frame encoding a flagellin adjuvant.

In some embodiments, at least one flagellin polypeptide (*e.g.*, encoded flagellin polypeptide) is a flagellin protein. In some embodiments, at least one flagellin polypeptide (*e.g.*, encoded flagellin polypeptide) is an immunogenic flagellin fragment. In some embodiments, at least one flagellin polypeptide and at least one antigenic polypeptide are encoded by a single RNA (*e.g.*, mRNA) polynucleotide. In other embodiments, at least one flagellin polypeptide and at least one antigenic polypeptide are each encoded by a different RNA polynucleotide.

In some embodiments at least one flagellin polypeptide has at least 80%, at least 85%, at least 90%, or at least 95% identity to a flagellin polypeptide having a sequence of any one of SEQ ID NO: 115-117.

In some embodiments the nucleic acid vaccines described herein are chemically modified. In other embodiments the nucleic acid vaccines are unmodified.

In some embodiments, the RNA polynucleotide is any one of SEQ ID NO: 1-8 and 41 and includes at least one chemical modification. In other embodiments, the RNA polynucleotide is any one of SEQ ID NO: 1-8 and 41 and does not include any nucleotide modifications, or is unmodified. In yet other embodiments, the at least one RNA polynucleotide encodes an antigenic protein of any one of SEQ ID NO: 10, 14, 18, 22, 26, 30,

34, 38, 42 and 45-55 and includes at least one chemical modification. In other embodiments, the RNA polynucleotide encodes an antigenic protein of any one of SEQ ID NO: 10, 14, 18, 22, 26, 30, 34, 38, 42 and 45-55 and does not include any nucleotide modifications, or is unmodified.

5 In some embodiments, the RNA polynucleotide comprises a sequence of any one of SEQ ID NO: 142-150. In some embodiments, the RNA polynucleotide comprises a sequence of any one of SEQ ID NO: 142-150 and includes at least one chemical modification. In other embodiments, the RNA polynucleotide comprises a sequence of any one of SEQ ID NO: 142-150 and does not include any nucleotide modifications, or is unmodified.

10 Yet other aspects provide compositions for and methods of vaccinating a subject comprising administering to the subject a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic VZV polypeptide, wherein the RNA polynucleotide does not include a stabilization element, and wherein an adjuvant is not co-formulated or co-administered with the vaccine.

15 In other aspects the invention is a composition for or method of vaccinating a subject comprising administering to the subject a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide wherein a dosage of between 10 µg/kg and 400 µg/kg of the nucleic acid vaccine is administered to the subject. In some embodiments the dosage of the RNA polynucleotide is 1-5 µg, 5-10 µg,
20 10-15 µg, 15-20 µg, 10-25 µg, 20-25 µg, 20-50 µg, 30-50 µg, 40-50 µg, 40-60 µg, 60-80 µg, 60-100 µg, 50-100 µg, 80-120 µg, 40-120 µg, 40-150 µg, 50-150 µg, 50-200 µg, 80-200 µg, 100-200 µg, 120-250 µg, 150-250 µg, 180-280 µg, 200-300 µg, 50-300 µg, 80-300 µg, 100-300 µg, 40-300 µg, 50-350 µg, 100-350 µg, 200-350 µg, 300-350 µg, 320-400 µg, 40-380 µg, 40-100 µg, 100-400 µg, 200-400 µg, or 300-400 µg per dose. In some embodiments, the
25 nucleic acid vaccine is administered to the subject by intradermal or intramuscular injection. In some embodiments, the nucleic acid vaccine is administered to the subject on day zero. In some embodiments, a second dose of the nucleic acid vaccine is administered to the subject on day twenty one.

In some embodiments, a dosage of 25 micrograms of the RNA polynucleotide is
30 included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 100 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 50 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some
35 embodiments, a dosage of 75 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 150

micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 400 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, a dosage of 200 micrograms of the RNA polynucleotide is included in the nucleic acid vaccine administered to the subject. In some embodiments, the RNA polynucleotide accumulates at a 100 fold higher level in the local lymph node in comparison with the distal lymph node. In other embodiments the nucleic acid vaccine is chemically modified and in other embodiments the nucleic acid vaccine is not chemically modified.

Aspects of the invention provide a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide does not include a stabilization element, and a pharmaceutically acceptable carrier or excipient, wherein an adjuvant is not included in the vaccine. In some embodiments, the stabilization element is a histone stem-loop. In some embodiments, the stabilization element is a nucleic acid sequence having increased GC content relative to wild type sequence.

Aspects of the invention provide nucleic acid vaccines comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide is present in the formulation for in vivo administration to a host, which confers an antibody titer superior to the criterion for seroprotection for the first antigen for an acceptable percentage of human subjects. In some embodiments, the antibody titer produced by the mRNA vaccines of the invention is a neutralizing antibody titer. In some embodiments the neutralizing antibody titer is greater than a protein vaccine. In other embodiments the neutralizing antibody titer produced by the mRNA vaccines of the invention is greater than an adjuvanted protein vaccine. In yet other embodiments the neutralizing antibody titer produced by the mRNA vaccines of the invention is 1,000-10,000, 1,200-10,000, 1,400-10,000, 1,500-10,000, 1,000-5,000, 1,000-4,000, 1,800-10,000, 2000-10,000, 2,000-5,000, 2,000-3,000, 2,000-4,000, 3,000-5,000, 3,000-4,000, or 2,000-2,500. A neutralization titer is typically expressed as the highest serum dilution required to achieve a 50% reduction in the number of plaques.

Also provided are nucleic acid vaccines comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide is present in a formulation for in vivo administration to a host for eliciting a longer lasting high antibody titer than an antibody titer elicited by an mRNA vaccine having a stabilizing element or formulated with an adjuvant and encoding the first antigenic polypeptide. In some embodiments, the RNA polynucleotide is formulated to

produce a neutralizing antibodies within one week of a single administration. In some embodiments, the adjuvant is selected from a cationic peptide and an immunostimulatory nucleic acid. In some embodiments, the cationic peptide is protamine.

Aspects provide nucleic acid vaccines comprising one or more RNA polynucleotides having an open reading frame comprising at least one chemical modification or optionally no chemical modification, the open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide is present in the formulation for in vivo administration to a host such that the level of antigen expression in the host significantly exceeds a level of antigen expression produced by an mRNA vaccine having a stabilizing element or formulated with an adjuvant and encoding the first antigenic polypeptide.

Other aspects provide nucleic acid vaccines comprising one or more RNA polynucleotides having an open reading frame comprising at least one chemical modification or optionally no chemical modification, the open reading frame encoding a first antigenic polypeptide, wherein the vaccine has at least 10 fold less RNA polynucleotide than is required for an unmodified mRNA vaccine to produce an equivalent antibody titer. In some embodiments, the RNA polynucleotide is present in a dosage of 25-100 micrograms.

Aspects of the invention also provide a unit of use vaccine, comprising between 10ug and 400 µg of one or more RNA polynucleotides having an open reading frame comprising at least one chemical modification or optionally no chemical modification, the open reading frame encoding a first antigenic polypeptide, and a pharmaceutically acceptable carrier or excipient, formulated for delivery to a human subject. In some embodiments, the vaccine further comprises a cationic lipid nanoparticle.

Aspects of the invention provide methods of creating, maintaining or restoring antigenic memory to a VZV strain in an individual or population of individuals comprising administering to said individual or population an antigenic memory booster nucleic acid vaccine comprising (a) at least one RNA polynucleotide, said polynucleotide comprising at least one chemical modification or optionally no chemical modification and two or more codon-optimized open reading frames, said open reading frames encoding a set of reference antigenic polypeptides, and (b) optionally a pharmaceutically acceptable carrier or excipient. In some embodiments, the vaccine is administered to the individual via a route selected from the group consisting of intramuscular administration, intradermal administration and subcutaneous administration. In some embodiments, the administering step comprises contacting a muscle tissue of the subject with a device suitable for injection of the composition. In some embodiments, the administering step comprises contacting a muscle

tissue of the subject with a device suitable for injection of the composition in combination with electroporation.

Aspects of the invention provide methods of vaccinating a subject comprising administering to the subject a single dosage of between 25 µg/kg and 400 µg/kg of a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a first antigenic polypeptide in an effective amount to vaccinate the subject.

Other aspects provide nucleic acid vaccines comprising one or more RNA polynucleotides having an open reading frame comprising at least one chemical modification, the open reading frame encoding a first antigenic polypeptide, wherein the vaccine has at least 10 fold less RNA polynucleotide than is required for an unmodified mRNA vaccine to produce an equivalent antibody titer. In some embodiments, the RNA polynucleotide is present in a dosage of 25-100 micrograms.

Other aspects provide nucleic acid vaccines comprising an LNP formulated RNA polynucleotide having an open reading frame comprising no modified nucleotides (unmodified), the open reading frame encoding a first antigenic polypeptide, wherein the vaccine has at least 10 fold less RNA polynucleotide than is required for an unmodified mRNA vaccine not formulated in a LNP to produce an equivalent antibody titer. In some embodiments, the RNA polynucleotide is present in a dosage of 25-100 micrograms.

The data presented in the Examples demonstrate significant enhanced immune responses using the formulations of the invention. The data demonstrated the effectiveness of both chemically modified and unmodified RNA vaccines of the invention. Surprisingly, in contrast to prior art reports that it was preferable to use chemically unmodified mRNA formulated in a carrier for the production of vaccines, it was discovered herein that chemically modified mRNA-LNP vaccines required a much lower effective mRNA dose than unmodified mRNA, i.e., tenfold less than unmodified mRNA when formulated in carriers other than LNP. Both the chemically modified and unmodified RNA vaccines of the invention produce better immune responses than mRNA vaccines formulated in a different lipid carrier.

In other aspects the invention encompasses a method of treating an elderly subject age 60 years or older comprising administering to the subject a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a VZV antigenic polypeptide in an effective amount to vaccinate the subject.

In other aspects the invention encompasses a method of treating a young subject age 17 years or younger comprising administering to the subject a nucleic acid vaccine

comprising one or more RNA polynucleotides having an open reading frame encoding a VZV antigenic polypeptide in an effective amount to vaccinate the subject.

In other aspects the invention encompasses a method of treating an adult subject comprising administering to the subject a nucleic acid vaccine comprising one or more RNA polynucleotides having an open reading frame encoding a VZV antigenic polypeptide in an effective amount to vaccinate the subject.

In preferred aspects, vaccines of the invention (e.g., LNP-encapsulated mRNA vaccines) produce prophylactically- and/or therapeutically- efficacious levels, concentrations and/or titers of antigen-specific antibodies in the blood or serum of a vaccinated subject. As defined herein, the term antibody titer refers to the amount of antigen-specific antibody produces in s subject, e.g., a human subject. In exemplary embodiments, antibody titer is expressed as the inverse of the greatest dilution (in a serial dilution) that still gives a positive result. In exemplary embodiments, antibody titer is determined or measured by enzyme-linked immunosorbent assay (ELISA). In exemplary embodiments, antibody titer is determined or measured by neutralization assay, e.g., by microneutralization assay. In certain aspects, antibody titer measurement is expressed as a ratio, such as 1:40, 1:100, etc.

In exemplary embodiments of the invention, an efficacious vaccine produces an antibody titer of greater than 1:40, greater that 1:100, greater than 1:400, greater than 1:1000, greater than 1:2000, greater than 1:3000, greater than 1:4000, greater than 1:500, greater than 1:6000, greater than 1:7500, greater than 1:10000. In exemplary embodiments, the antibody titer is produced or reached by 10 days following vaccination, by 20 days following vaccination, by 30 days following vaccination, by 40 days following vaccination, or by 50 or more days following vaccination. In exemplary embodiments, the titer is produced or reached following a single dose of vaccine administered to the subject. In other embodiments, the titer is produced or reached following multiple doses, e.g., following a first and a second dose (e.g., a booster dose.)

In exemplary aspects of the invention, antigen-specific antibodies are measured in units of $\mu\text{g/ml}$ or are measured in units of IU/L (International Units per liter) or mIU/ml (milli International Units per ml). In exemplary embodiments of the invention, an efficacious vaccine produces $>0.5 \mu\text{g/ml}$, $>0.1 \mu\text{g/ml}$, $>0.2 \mu\text{g/ml}$, $>0.35 \mu\text{g/ml}$, $>0.5 \mu\text{g/ml}$, $>1 \mu\text{g/ml}$, $>2 \mu\text{g/ml}$, $>5 \mu\text{g/ml}$ or $>10 \mu\text{g/ml}$. In exemplary embodiments of the invention, an efficacious vaccine produces $>10 \text{ mIU/ml}$, $>20 \text{ mIU/ml}$, $>50 \text{ mIU/ml}$, $>100 \text{ mIU/ml}$, $>200 \text{ mIU/ml}$, $>500 \text{ mIU/ml}$ or $>1000 \text{ mIU/ml}$. In exemplary embodiments, the antibody level or concentration is produced or reached by 10 days following vaccination, by 20 days following vaccination, by 30 days following vaccination, by 40 days following vaccination, or by 50 or

more days following vaccination. In exemplary embodiments, the level or concentration is produced or reached following a single dose of vaccine administered to the subject. In other embodiments, the level or concentration is produced or reached following multiple doses, e.g., following a first and a second dose (e.g., a booster dose.) In exemplary embodiments, antibody level or concentration is determined or measured by enzyme-linked immunosorbent assay (ELISA). In exemplary embodiments, antibody level or concentration is determined or measured by neutralization assay, e.g., by microneutralization assay.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention. This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways.

BRIEF DESCRIPTION OF DRAWINGS

The accompanying drawings are not intended to be drawn to scale. In the drawings, each identical or nearly identical component that is illustrated in various figures is represented by a like numeral. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

Fig. 1 is a schematic depicting a proposed Varicella zoster virus pathway.

Fig. 2 is a schematic of the constructs encoding VZV gE (strain Oka).

Fig. 3 depicts the study design and injection schedule for the immunization of BALB/C mice with MC3 formulated mRNA encoded VZV gE antigens.

Fig. 4 is a schematic showing various variant VZV gE antigens. This figure depicts SEQ ID NOs: 120, 132, and 58 from top to bottom, respectively.

Fig. 5 is a graph showing the results of an ELISA assay that shows the levels of anti-VZV gE IgG in the serum of mice vaccinated with various VZV gE mRNAs in comparison with VARIVAX[®] vaccine.

Fig. 6 is a graph showing the results of an ELISA assay indicating the levels of anti-VZV gE IgG in the serum of mice vaccinated with various VZV gE mRNAs in comparison with VARIVAX[®] vaccine.

Fig. 7 is a graph showing the results of an ELISA assay indicating the levels of anti-VZV gE IgG in the serum of mice vaccinated with various VZV gE mRNAs in comparison with VARIVAX[®] vaccine.

Figs. 8A and 8B show confocal microscopy of human melanoma (MeWo) cells stained with an antibodies to show the golgi apparatus.

Figs. 9A-9C show confocal microscopy of MeWo cells stained with antibodies against VZV gE to show VZV gE expression and trafficking. The sequence AEAADA depicted in Fig. 9C is SEQ ID NO: 58.

Figs. 10A and 10B are schematics depicting various VZV wildtype genotypes.

Figs. 11A and 11B are graphs showing the results of an ELISA assay, which shows the levels of anti-VZV gE IgG in the serum of mice vaccinated with VZV gE variant mRNAs in comparison with ZOSTAVAX[®] vaccine. The sequence AEAADA depicted throughout Figs. 11A and 11B is SEQ ID NO: 58.

Figs. 12A and 12B are graphs showing the results of an ELISA assay, which shows the levels of anti-VZV gE IgG in the serum of mice vaccinated with VZV gE variant mRNAs in comparison with ZOSTAVAX[®] vaccine. The sequence AEAADA depicted throughout Figs. 12A and 12B is SEQ ID NO: 58.

Fig. 13 is a graph showing the results of an ELISA assay measuring the antibody titer in the sera of mice immunized with VZV gE variant mRNA vaccines. Anti-VZV gE response induced by VZV gE variants mRNAs in mice are greater than that of ZOSTAVAX[®]. The gE variant mRNA for GE-delete_from_574-Y569A induced an immune response that is 1 log greater than ZOSTAVAX[®]. The sequence AEAADA depicted throughout Fig. 13 is SEQ ID NO: 58.

Fig. 14A is a graph showing the results of an ELISA assay, which shows the levels of anti-VZV gE IgG in the serum of mice vaccinated with VZV gE variant mRNAs after primary exposure with ZOSTAVAX[®] vaccine (groups 1-5) or VZV-gE-del_574_Y569A (group 6). The sequence AEAADA depicted throughout Fig. 14A is SEQ ID NO: 58. Fig. 14B is a graph showing the determination of EC50.

Fig. 15A and 15B are graphs showing the results of the T cell analysis of mice vaccinated with VZV gE variant mRNAs after primary exposure with ZOSTAVAX[®] vaccine (groups 1-5) or VZV-gE-del_574_Y569A (group 6). The sequence AEAADA depicted throughout Figs. 15A and 15B is SEQ ID NO: 58.

Fig. 16A and 16B are graphs showing the results of an ELISA assay, which shows the levels of anti-VZV gE IgG in serum of rhesus monkeys vaccinated with either VZV-gE-del_574_Y569A or ZOSTAVAX[®] after primary exposure with VZV-gE-del_574_Y569A or ZOSTAVAX. Fig. 16C is a graph showing the determination of EC50 and EC10. FIG. 16D, 16E, and 16F are graphs showing the results of the T cell analysis of rhesus monkeys

vaccinated with either VZV-gE-del_574_Y569A or ZOSTAVAX[®] after primary exposure with VZV-gE-del_574_Y569A or ZOSTAVAX[®].

Fig. 17 shows next generation sequencing (NGS) data of VZV mRNA vaccine construct encoding a modified VZV-gE-del_574_Y569A antigen (VZV-gE-del_574_Y569A-v7). Compared to the VZV-gE-del_574_Y569A mRNA vaccine construct, which encodes the gE antigen (e.g., described in Figs. 13-16), VZV-gE-del_574_Y569A-v7 was modified (6 nucleotide changes were introduced) to reduce the occurrence of indel-causing homopolymeric stretches (stretches of 6 As, 5 As, or 4 As). The data shows that VZV-gE-del_574_Y569A-v7 does not contain significant indels.

Fig. 18A shows the average frequency of FITC⁺ MeWo cells six hours post transfection with VZV-gE-del_574_Y569A or VZV-gE-del_574_Y569A-v7, measured by fluorescence-activated cell sorting (FACS). Fig. 18B shows the FITC geometric mean.

Figs. 19A-19B are Western blots showing detection of VZV-gE-del_574_Y569A antigens in the lysates of HeLa cells 16 hours post transfection. Expression of the VZV-gE-del_574_Y569A antigen was detected in HeLa cells transfected with the VZV-gE-del_574_Y569A mRNA construct and in HeLa cells transfected with the VZV-gE-del_574_Y569A-v7 mRNA construct.

Figs. 20A-20B are graphs showing the expression (Fig. 20A) and localization (Fig. 20B) of the VZV-gE-del_574_Y569A antigen expressed in HeLa cells transfected with either the VZV-gE-del_574_Y569A mRNA construct or the VZV-gE-del_574_Y569A-v7 mRNA construct.

Figs. 21A-21C are microscopy images showing the localization in HeLa cells (Fig. 21) or MeWo cells (Figs. 21B and 21C) of full length VZV-gE and VZV-gE-del_574_Y569A antigens encoded by the VZV-gE-del_574_Y569A mRNA construct or the VZV-gE-del_574_Y569A-v7 mRNA construct. The full length VZV gE antigen localized to the golgi, and VZV-gE-del_574_Y569A antigen localized to the cell membrane.

DETAILED DESCRIPTION

Embodiments of the present disclosure provide RNA (e.g., mRNA) vaccines that include at least one RNA (e.g., mRNA) polynucleotide encoding a varicella zoster virus (VZV) antigen. There are at least five clades of varicella zoster virus (VZV). Clades 1 and 3 include European/North American strains; clade 2 includes Asian strains, especially from Japan; and clade 5 appears to be based in India. Clade 4 includes some strains from Europe, but its geographic origins need further clarification. Phylogenetic analysis of VZV genomic sequences resolves wild-type strains into 9 genotypes (E1, E2, J, M1, M2, M3, M4, VIII and

IX). Sequence analysis of 342 clinical varicella and zoster specimens from 18 European countries identified the following distribution of VZV genotypes: E1, 221 (65%); E2, 87 (25%); M1, 20 (6%); M2, 3 (1%); M4, and 11 (3%). No M3 or J strains were observed. Of 165 clinical varicella and zoster isolates from Australia and New Zealand, 67 of 127 eastern Australian isolates were E1, 30 were E2, 16 were J, 10 were M1, and 4 were M2; 25 of 38 New Zealand isolates were E1, 8 were E2, and 5 were M1.

VZV is an alphaherpesvirus that exists as a spherical multilayered structure approximately 200 nm in diameter. The viral genome is surrounded by a protein capsid structure that is covered by an amorphous layer of tegument proteins. These two structures are surrounded by a lipid envelope that is studded with viral glycoproteins, each about 8 nm in length, that are displayed on the exterior of the virion, and encloses the 100 nm nucleocapsid which is comprised of 162 hexameric and pentameric capsomeres arranged in an icosahedral form. The tegument, which is comprised of virally-encoded proteins and enzymes, is located in the space between the nucleocapsid and the viral envelope. The viral envelope is acquired from host cell membranes and contains viral-encoded glycoproteins.

The VZV genome is a single, linear, duplex DNA molecule of 124,884 base pairs having at least 70 open reading frames. The genome has 2 predominant isomers, depending on the orientation of the S segment, P (prototype) and IS (inverted S), which are present with equal frequency for a total frequency of 90-95%. The L segment can also be inverted resulting in a total of four linear isomers (IL and ILS).

VZV is closely related to the herpes simplex viruses (HSV), sharing much genome homology. The VZV genome is the smallest of the human herpesviruses and encodes at least 71 unique proteins (ORF0–ORF68) with three more opening reading frames (ORF69–ORF71) that duplicate earlier open reading frames (ORF64–62, respectively). Only a fraction of the encoded proteins form the structure of the virus particle. Among those proteins are nine glycoproteins: ORF5 (gK), ORF9A (gN), ORF14 (gC), ORF31 (gB), ORF37 (gH), ORF50 (gM), ORF60 (gL), ORF67 (gI), and ORF68 (gE). The known envelope glycoproteins (gB, gC, gE, gH, gI, gK, gL, gN, and gM) correspond with those in HSV; however, there is no equivalent of HSV gD. VZV also fails to produce the LAT (latency-associated transcripts) that play an important role in establishing HSV latency (herpes simplex virus). The encoded glycoproteins gE, gI, gB, gH, gK, gL, gC, gN, and gM function in different steps of the viral replication cycle. The most abundant glycoprotein found in infected cells, as well as in the mature virion, is glycoprotein E (gE, ORF 68), which is a major component of the virion envelope and is essential for viral replication.

Glycoprotein I (gI, ORG 67) forms a complex with gE in infected cells, which facilitates the

endocytosis of both glycoproteins and directs them to the trans-Golgi network (TGN) where the final viral envelope is acquired. Glycoprotein I (gI) is required within the TGN for VZV envelopment and for efficient membrane fusion during VZV replication. VZV gE and gI are found complexed together on the infected host cell surface. Glycoprotein B (ORF 31), which is the second most prevalent glycoprotein and thought to play a role in virus entry, binds to neutralizing antibodies. Glycoprotein H is thought to have a fusion function facilitating cell to cell spread of the virus. Antibodies to gE, gB, and gH are prevalent after natural infection and following vaccination and have been shown to neutralize viral activity in vitro.

Embodiments of the present disclosure provide RNA (*e.g.*, mRNA) vaccines that include at least one polynucleotide encoding at least one VZV antigenic polypeptide. The VZV RNA vaccines provided herein may be used to induce a balanced immune response, comprising both cellular and humoral immunity, without many of the risks associated with DNA vaccines and live attenuated vaccines. The various RNA (*e.g.*, mRNA) vaccines disclosed herein produced an immune response in BALB/C mice, the results of which are discussed in detail in the Examples section. Specifically, RNA (*e.g.*, mRNA) polynucleotide vaccines having an open reading frame encoding one or more of a variety of VZV antigens produced significant immune response, relative to a traditional VZV vaccine (*e.g.* attenuated VZV virus). The VZV RNA (*e.g.*, mRNA) polynucleotide vaccines disclosed herein encoding either VZV gE or variant VZV gE demonstrated significant immune response after two administrations when administered intramuscularly (IM) or intradermally (ID). The VZV glycoproteins and tegument proteins have been shown to be antigenic. VZV glycoproteins, fragments thereof, and epitopes thereof are encompassed within the present disclosure.

The entire contents of International Application No. PCT/US2015/027400, International Publication No. WO2015/164674A, are incorporated herein by reference.

It has been discovered that the mRNA vaccines described herein are superior to current vaccines in several ways. First, the lipid nanoparticle (LNP) delivery is superior to other formulations including a protamine base approach described in the literature and no additional adjuvants are to be necessary. The use of LNPs enables the effective delivery of chemically modified or unmodified mRNA vaccines. Additionally it has been demonstrated herein that both modified and unmodified LNP formulated mRNA vaccines were superior to conventional vaccines by a significant degree. In some embodiments the mRNA vaccines of the invention are superior to conventional vaccines by a factor of at least 10 fold, 20 fold, 40 fold, 50 fold, 100 fold, 500 fold or 1,000 fold.

Although attempts have been made to produce functional RNA vaccines, including mRNA vaccines and self-replicating RNA vaccines, the therapeutic efficacy of these RNA vaccines have not yet been fully established. Quite surprisingly, the inventors have discovered, according to aspects of the invention a class of formulations for delivering mRNA vaccines in vivo that results in significantly enhanced, and in many respects synergistic, immune responses including enhanced antigen generation and functional antibody production with neutralization capability. These results can be achieved even when significantly lower doses of the mRNA are administered in comparison with mRNA doses used in other classes of lipid based formulations. The formulations of the invention have demonstrated significant unexpected in vivo immune responses sufficient to establish the efficacy of functional mRNA vaccines as prophylactic and therapeutic agents. Additionally, self-replicating RNA vaccines rely on viral replication pathways to deliver enough RNA to a cell to produce an immunogenic response. The formulations of the invention do not require viral replication to produce enough protein to result in a strong immune response. Thus, the mRNA of the invention are not self-replicating RNA and do not include components necessary for viral replication.

The invention involves, in some aspects, the surprising finding that lipid nanoparticle (LNP) formulations significantly enhance the effectiveness of mRNA vaccines, including chemically modified and unmodified mRNA vaccines. The efficacy of mRNA vaccines formulated in LNP was examined in vivo using several distinct antigens. The results presented herein demonstrate the unexpected superior efficacy of the mRNA vaccines formulated in LNP over other commercially available vaccines.

In addition to providing an enhanced immune response, the formulations of the invention generate a more rapid immune response with fewer doses of antigen than other vaccines tested. The mRNA-LNP formulations of the invention also produce quantitatively and qualitatively better immune responses than vaccines formulated in a different carriers.

The data described herein demonstrate that the formulations of the invention produced significant unexpected improvements over existing VZV antigen vaccines, including significantly higher levels of IgG production by mRNA chemically modified and unmodified VZV vaccines formulated in LNP compared to VARIVAX and ZOSTAVAX. The onset of IgG production was significantly more rapid for the chemically modified LNP mRNA vaccines than the unmodified or commercially available vaccines tested.

Additionally, the mRNA-LNP formulations of the invention are superior to other vaccines even when the dose of mRNA is lower than other vaccines. The data demonstrate that all gE variants LNP mRNA vaccines induced much stronger immune response than

ZOSTAVAX® after the two 10 µg doses as well as after the two 2 µg doses. When the sera were diluted more than 100 fold, the antibody titer is higher in VZV gE LNP mRNA vaccinated mice sera than in ZOSTAVAX® vaccinated mice sera, suggesting that the VZV gE LNP mRNA vaccines induced much stronger immune response than ZOSTAVAX® in mice.

The results in mice were consistent with the immunogenicity observed in non-human primates. Rhesus monkeys were primed with chemically modified VZV LNP mRNA vaccines or ZOSTAVAX®. The mRNA vaccines provided higher anti-gE titers than ZOSTAVAX® and produced reasonable frequency of CD4 T-cells producing IFN γ , IL-2 or TNF α cells, unlike the ZOSTAVAX® group. The data also demonstrated that a single dose of mRNA vaccination after ZOSTAVAX® exposure was equivalent to two doses of mRNA vaccination in inducing comparable T-cell responses.

Some of the LNP used in the studies described herein has been used previously to deliver siRNA in various animal models as well as in humans. In view of the observations made in association with the siRNA delivery of LNP formulations, the fact that LNP is useful in vaccines is quite surprising. It has been observed that therapeutic delivery of siRNA formulated in LNP causes an undesirable inflammatory response associated with a transient IgM response, typically leading to a reduction in antigen production and a compromised immune response. In contrast to the findings observed with siRNA, the LNP-mRNA formulations of the invention are demonstrated herein to generate enhanced IgG levels, sufficient for prophylactic and therapeutic methods rather than transient IgM responses.

Antigens/Antigenic Polypeptides

In some embodiments, an antigenic polypeptide is a VZV glycoprotein. For example, a VZV glycoprotein may be VZV gE, gI, gB, gH, gK, gL, gC, gN, or gM. In some embodiments, the antigenic polypeptide is a VZV gE polypeptide. In some embodiments, the antigenic polypeptide is a VZV gI polypeptide. In some embodiments, the antigenic polypeptide is a VZV gB polypeptide. In some embodiments, the antigenic polypeptide is a VZV gH polypeptide. In some embodiments, the antigenic polypeptide is a VZV gK polypeptide. In some embodiments, the antigenic polypeptide is a VZV gL polypeptide. In some embodiments, the antigenic polypeptide is a VZV gC polypeptide. In some embodiments, the antigenic polypeptide is a VZV gN polypeptide. In some embodiments, the antigenic polypeptide is a VZV gM polypeptide.

In some embodiments, the antigenic polypeptide comprises two or more glycoproteins. The two or more glycoproteins can be encoded by a single RNA

polynucleotide or can be encoded by two or more RNA polynucleotides, for example, each glycoprotein encoded by a separate RNA polynucleotide. In some embodiments, the two or more glycoproteins can be any combination of VZV gE, gI, gB, gH, gK, gL, gC, gN, and gM polypeptides. In some embodiments, the two or more glycoproteins can be any combination of VZV gE and a glycoprotein selected from gI, gB, gH, gK, gL, gC, gN, and gM polypeptides. In some embodiments, the two or more glycoproteins can be any combination of VZV gI and a glycoprotein selected from gE, gB, gH, gK, gL, gC, gN, and gM polypeptides. In some embodiments, the two or more glycoproteins can be any combination of VZV gE, gI, and a glycoprotein selected from gB, gH, gK, gL, gC, gN, and gM polypeptides. In some embodiments, the two or more VZV glycoproteins are gE and gI. Alternate RNA vaccines comprising RNA polynucleotides encoding other viral protein components of VZV, for example, tegument proteins are encompassed by the present disclosure. Thus, some embodiments of the present disclosure provide VZV vaccines that include at least one ribonucleic acid (RNA) polynucleotide having an open reading frame encoding at least one VZV tegument protein. In some embodiments, the antigenic polypeptide is a VZV tegument protein. In other embodiments, the antigenic fragment(s) of the VZV vaccine may be at least one VZV tegument polypeptide and at least one VZV glycoprotein polypeptide, for example any VZV glycoprotein selected from gE, gI, gB, gH, gK, gL, gC, gN, and gM.

The present disclosure includes variant VZV antigenic polypeptides. In some embodiments, the variant VZV antigenic polypeptide is a variant VZV gE polypeptide. The variant VZV gE polypeptides are designed to avoid ER/golgi retention of polypeptides, leading to increased surface expression of the antigen. In some embodiments, the variant gE polypeptides are truncated to remove the ER retention portion or the cytoplasmic tail portion of the polypeptide. In some embodiments, the variant VZV gE polypeptides are mutated to reduce VZV polypeptide localization to the ER/golgi/TGN. Such modifications inhibit ER trapping and, as such, expedite trafficking to the cell membrane.

Thus, in some embodiments, the VZV glycoprotein is a variant gE polypeptide. VZV gE has targeting sequences for the TGN in its C-terminus and is transported from the ER to the TGN in infected and gE-transfected cells. Most gE in the TGN appears to be retrieved by endocytosis from the plasma membrane and delivered to the TGN by endosomes, which is followed by recycling to the plasma membranes. gE is accumulated in TGN, along with other VZV proteins (*e.g.*, tegument proteins) associated with the production of fully enveloped VZV virions. Thus, mutations to reduce TGN localization and endocytosis aids in the trafficking of gE to the cell membrane.

The variant VZV gE polypeptide can be any truncated polypeptide lacking the anchor domain (ER retention domain). For example, the variant VZV gE polypeptide can be a truncated VZV gE polypeptide comprising at least amino acids 1- 124, including, for example, amino acids 1-124, 1-140, 1-160, 1-200, 1-250, 1-300, 1-350, 1-360, 1-400, 1-450, 1-500, 1-511, 1-550, and 1-561, as well as polypeptide fragments having fragment sizes within the recited size ranges. In one embodiment, the truncated VZV gE polypeptide comprises amino acids 1-561 of SEQ ID NO: 10. In some embodiments, the variant VZV gE polypeptide is a truncated polypeptide lacking the carboxy terminal tail domain. Thus in some embodiments, the truncated VZV gE polypeptide comprises amino acids 1-573 of SEQ ID NO: 10.

In some embodiments, the variant VZV gE polypeptide has at least one mutation in one or more motif(s) associated with ER retention, wherein the mutation(s) in one or more motif(s) results in decreased retention of the VZV gE polypeptide in the ER and/or golgi. In some embodiments, the variant VZV gE polypeptide has at least one mutation in one or more phosphorylated acidic motif(s). For example, the variant VZV gE polypeptide can be a full-length VZV gE polypeptide having a Y582G mutation, a Y569A mutation, or both a Y582G mutation and a Y569A mutation. Alternatively, the variant VZV gE polypeptide can be an antigenic fragment comprising, for example, amino acids 1-573 of VZV gE and having a Y569A mutation. Alternatively, the variant VZV gE polypeptide can be an antigenic fragment having mutation in an acidic phosphorylation motif, such as an SST motif. For example, the variant VZV gE polypeptide can be an antigenic fragment having AEAADA sequence (SEQ ID NO: 58).

In some embodiments, the variant VZV gE polypeptide is a full-length VZV gE polypeptide having additional sequence at the C-terminus which aids in secretion of the polypeptide. For example, the variant VZV gE polypeptide can be a full-length VZV gE polypeptide having an IgKappa sequence at the C-terminus. In some embodiments, the VZV gE polypeptide has additional sequence at the C-terminus that aids in secretion (I., an IgKappa sequence at the C-terminus) and the variant VZV gE polypeptide has at least one mutation in one or more motif(s) associated with ER retention, TGN localization, and/or endocytosis (e.g., a Y582G mutation, a Y569A mutation, or both a Y582G mutation and a Y569A mutation) and/or at least one mutation in one or more phosphorylated acidic motif(s). In some embodiments, the variant VZV gE polypeptide is a truncated polypeptide lacking the anchor domain (ER retention domain) and having an additional sequence at the C-terminus which aids in secretion of the polypeptide, for example, an IgKappa sequence at the C-terminus. In some embodiments, the truncated VZV gE polypeptide comprises amino acids

1-561 of SEQ ID NO: 10 and has an IgKappa sequence at the C-terminus. In some embodiments, the variant VZV gE polypeptide is a truncated polypeptide lacking the carboxy terminal tail domain and having an additional sequence at the C-terminus that aids in secretion of the polypeptide (*e.g.*, having an IgKappa sequence at the C-terminus). In some
5 embodiments, the truncated VZV gE polypeptide comprises amino acids 1-573 of SEQ ID NO: 10 and has an IgKappa sequence at the C-terminus.

In some embodiments, a VZV antigenic polypeptide is longer than 25 amino acids and shorter than 50 amino acids. The term “antigenic polypeptide” and “antigenic protein” includes immunogenic fragments and epitopes thereof. Thus, polypeptides include gene
10 products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing. A polypeptide may be a single molecule or may be a multi-molecular complex such as a dimer, trimer or tetramer. Polypeptides may also comprise single chain or multichain polypeptides such as antibodies or insulin and may be associated or linked. Most commonly, disulfide
15 linkages are found in multichain polypeptides. The term polypeptide may also apply to amino acid polymers in which at least one amino acid residue is an artificial chemical analogue of a corresponding naturally-occurring amino acid.

The term “polypeptide variant” refers to molecules which differ in their amino acid sequence from a native or reference sequence. The amino acid sequence variants may
20 possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence, as compared to a native or reference sequence. Ordinarily, variants possess at least 50% identity to a native or reference sequence. In some embodiments, variants share at least 80%, or at least 90% identity with a native or reference sequence.

In some embodiments “variant mimics” are provided. As used herein, a “variant
25 mimic” contains at least one amino acid that would mimic an activated sequence. For example, glutamate may serve as a mimic for phospho-threonine and/or phospho-serine. Alternatively, variant mimics may result in deactivation or in an inactivated product containing the mimic. For example, phenylalanine may act as an inactivating substitution for tyrosine, or alanine may act as an inactivating substitution for serine.

30 “Orthologs” refers to genes in different species that evolved from a common ancestral gene by speciation. Normally, orthologs retain the same function in the course of evolution. Identification of orthologs is critical for reliable prediction of gene function in newly sequenced genomes.

“Analog” is meant to include polypeptide variants that differ by one or more amino acid alterations, for example, substitutions, additions or deletions of amino acid residues that still maintain one or more of the properties of the parent or starting polypeptide.

“Paralogs” are genes (or proteins) related by duplication within a genome. Orthologs retain the same function in the course of evolution, whereas paralogs evolve new functions, even if these are related to the original one.

The present disclosure provides several types of compositions that are polynucleotide or polypeptide based, including variants and derivatives. These include, for example, substitutional, insertional, deletion and covalent variants and derivatives. The term “derivative” is used synonymously with the term “variant,” but generally refers to a molecule that has been modified and/or changed in any way relative to a reference molecule or starting molecule.

As such, polynucleotides encoding peptides or polypeptides containing substitutions, insertions and/or additions, deletions and covalent modifications with respect to reference sequences, in particular the polypeptide sequences disclosed herein, are included within the scope of this disclosure. For example, sequence tags or amino acids, such as one or more lysines, can be added to peptide sequences (*e.g.*, at the N-terminal or C-terminal ends). Sequence tags can be used for peptide detection, purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy and amino terminal regions of the amino acid sequence of a peptide or protein may optionally be deleted providing for truncated sequences. Certain amino acids (*e.g.*, C-terminal or N-terminal residues) may alternatively be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support. In alternative embodiments, sequences for (or encoding) signal sequences, termination sequences, transmembrane domains, linkers, multimerization domains (such as, *e.g.*, foldon regions) and the like may be substituted with alternative sequences that achieve the same or a similar function. Such sequences are readily identifiable to one of skill in the art. It should also be understood that some of the sequences provided herein contain sequence tags or terminal peptide sequences (*e.g.*, at the N-terminal or C-terminal ends) that may be deleted, for example, prior to use in the preparation of an RNA (*e.g.*, mRNA) vaccine.

“Substitutional variants” when referring to polypeptides are those that have at least one amino acid residue in a native or starting sequence removed and a different amino acid inserted in its place at the same position. Substitutions may be single, where only one amino

acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule.

As used herein the term “conservative amino acid substitution” refers to the substitution of an amino acid that is normally present in the sequence with a different amino acid of similar size, charge, or polarity. Examples of conservative substitutions include the substitution of a non-polar (hydrophobic) residue such as isoleucine, valine and leucine for another non-polar residue. Likewise, examples of conservative substitutions include the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, and between glycine and serine. Additionally, the substitution of a basic residue, such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another acidic residue are additional examples of conservative substitutions. Examples of non-conservative substitutions include the substitution of a non-polar (hydrophobic) amino acid residue such as isoleucine, valine, leucine, alanine, methionine for a polar (hydrophilic) residue such as cysteine, glutamine, glutamic acid or lysine and/or a polar residue for a non-polar residue.

“Features” when referring to polypeptide or polynucleotide are defined as distinct amino acid sequence-based or nucleotide-based components of a molecule respectively. Features of the polypeptides encoded by the polynucleotides include surface manifestations, local conformational shape, folds, loops, half-loops, domains, half-domains, sites, termini or any combination thereof.

As used herein when referring to polypeptides the term “domain” refers to a motif of a polypeptide having one or more identifiable structural or functional characteristics or properties (*e.g.*, binding capacity, serving as a site for protein-protein interactions).

As used herein, when referring to polypeptides the terms “site” as it pertains to amino acid based embodiments, is used synonymously with “amino acid residue” and “amino acid side chain.” As used herein, when referring to polynucleotides the terms “site” as it pertains to nucleotide based embodiments, is used synonymously with “nucleotide.” A site represents a position within a peptide or polypeptide or polynucleotide that may be modified, manipulated, altered, derivatized or varied within the polypeptide or polynucleotide based molecules.

As used herein, the terms “termini” or “terminus,” when referring to polypeptides or polynucleotides, refers to an extremity of a polypeptide or polynucleotide respectively. Such extremity is not limited only to the first or final site of the polypeptide or polynucleotide but may include additional amino acids or nucleotides in the terminal regions. Polypeptide-based molecules may be characterized as having both an N-terminus (terminated by an amino acid

with a free amino group (NH₂)) and a C-terminus (terminated by an amino acid with a free carboxyl group (COOH)). Proteins are in some cases made up of multiple polypeptide chains brought together by disulfide bonds or by non-covalent forces (multimers, oligomers). These proteins have multiple N-termini and C-termini. Alternatively, the termini of the

polypeptides may be modified such that they begin or end, as the case may be, with a non-polypeptide based moiety such as an organic conjugate.

As recognized by those skilled in the art, protein fragments, functional protein domains, and homologous proteins are also considered to be within the scope of polypeptides of interest. For example, provided herein is any protein fragment (meaning a polypeptide sequence at least one amino acid residue shorter than a reference polypeptide sequence but otherwise identical) of a reference protein 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 or greater than 100 amino acids in length. In another example, any protein that includes a stretch of 20, 30, 40, 50, or 100 amino acids that are 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% identical to any of the sequences described herein can be utilized in accordance with the present disclosure. In some embodiments, a polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, or more mutations, as shown in any of the sequences provided or referenced herein. In some embodiments, a protein fragment is longer than 25 amino acids and shorter than 50 amino acids.

Polypeptide or polynucleotide molecules of the present disclosure may share a certain degree of sequence similarity or identity with the reference molecules (*e.g.*, reference polypeptides or reference polynucleotides), for example, with art-described molecules (*e.g.*, engineered or designed molecules or wild-type molecules). The term “identity,” as known in the art, refers to a relationship between the sequences of two or more polypeptides or polynucleotides, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between them as determined by the number of matches between strings of two or more amino acid residues or nucleic acid residues. Identity measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (*e.g.*, “algorithms”). Identity of related peptides can be readily calculated by known methods. “% identity” as it applies to polypeptide or polynucleotide sequences is defined as the percentage of residues (amino acid residues or nucleic acid residues) in the candidate amino acid or nucleic acid sequence that are identical with the residues in the amino acid sequence or nucleic acid sequence of a second sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity. Methods and computer programs for the alignment are well known in the art. It is understood that identity depends

on a calculation of percent identity but may differ in value due to gaps and penalties introduced in the calculation. Generally, variants of a particular polynucleotide or polypeptide have at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% but less than 100% sequence identity to that particular reference polynucleotide or polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art. Such tools for alignment include those of the BLAST suite (Stephen F. Altschul, *et al* (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402). Another popular local alignment technique is based on the Smith-Waterman algorithm (Smith, T.F. & Waterman, M.S. (1981) "Identification of common molecular subsequences." *J. Mol. Biol.* 147:195-197). A general global alignment technique based on dynamic programming is the Needleman-Wunsch algorithm (Needleman, S.B. & Wunsch, C.D. (1970) "A general method applicable to the search for similarities in the amino acid sequences of two proteins." *J. Mol. Biol.* 48:443-453). More recently a Fast Optimal Global Sequence Alignment Algorithm (FOGSAA) has been developed that purportedly produces global alignment of nucleotide and protein sequences faster than other optimal global alignment methods, including the Needleman-Wunsch algorithm. Other tools are described herein, specifically in the definition of "identity" below.

As used herein, the term "homology" refers to the overall relatedness between polymeric molecules, *e.g.* between nucleic acid molecules (*e.g.* DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Polymeric molecules (*e.g.* nucleic acid molecules (*e.g.* DNA molecules and/or RNA molecules) and/or polypeptide molecules) that share a threshold level of similarity or identity determined by alignment of matching residues are termed homologous. Homology is a qualitative term that describes a relationship between molecules and can be based upon the quantitative similarity or identity. Similarity or identity is a quantitative term that defines the degree of sequence match between two compared sequences. In some embodiments, polymeric molecules are considered to be "homologous" to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical or similar. The term "homologous" necessarily refers to a comparison between at least two sequences (polynucleotide or polypeptide sequences). Two polynucleotide sequences are considered homologous if the polypeptides they encode are at least 50%, 60%, 70%, 80%, 90%, 95%, or even 99% for at least one stretch of at least 20 amino acids. In some embodiments, homologous polynucleotide sequences are characterized by the ability to encode a stretch of at least 4–5 uniquely specified amino acids. For polynucleotide sequences less than 60 nucleotides in

length, homology is determined by the ability to encode a stretch of at least 4–5 uniquely specified amino acids. Two protein sequences are considered homologous if the proteins are at least 50%, 60%, 70%, 80%, or 90% identical for at least one stretch of at least 20 amino acids.

5 Homology implies that the compared sequences diverged in evolution from a common origin. The term “homolog” refers to a first amino acid sequence or nucleic acid sequence (*e.g.*, gene (DNA or RNA) or protein sequence) that is related to a second amino acid sequence or nucleic acid sequence by descent from a common ancestral sequence. The term “homolog” may apply to the relationship between genes and/or proteins separated by the
10 event of speciation or to the relationship between genes and/or proteins separated by the event of genetic duplication.

Nucleic Acids/Polynucleotides

Varicella zoster virus (VZV) vaccines, as provided herein, comprise at least one (one
15 or more) ribonucleic acid (RNA, *e.g.*, mRNA) polynucleotide having an open reading frame encoding at least one VZV antigenic polypeptide. The term “nucleic acid,” in its broadest sense, includes any compound and/or substance that comprises a polymer of nucleotides. These polymers are referred to as polynucleotides.

In some embodiments, at least one RNA polynucleotide of a VZV vaccine is encoded
20 by at least one nucleic acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 41.

In some embodiments, at least one RNA (*e.g.*, mRNA) polynucleotide of a VZV vaccine is encoded by at least one fragment of a nucleic acid sequence (*e.g.*, a fragment
25 having an antigenic sequence or at least one epitope) selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 41.

Nucleic acids (also referred to as polynucleotides) may be or may include, for example, ribonucleic acids (RNAs), deoxyribonucleic acids (DNAs), threose nucleic acids
30 (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a β -D-ribo configuration, α -LNA having an α -L-ribo configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino functionalization, and 2'-amino- α -LNA having a 2'-amino functionalization), ethylene nucleic acids (ENA), cyclohexenyl nucleic acids (CeNA) or chimeras or combinations thereof.

In some embodiments, polynucleotides of the present disclosure function as messenger RNA (mRNA). “Messenger RNA” (mRNA) refers to any polynucleotide that encodes a (at least one) polypeptide (a naturally-occurring, non-naturally-occurring, or modified polymer of amino acids) and can be translated to produce the encoded polypeptide *in vitro*, *in vivo*, *in situ* or *ex vivo*. The skilled artisan will appreciate that, except where otherwise noted, polynucleotide sequences set forth in the instant application will recite “T”s in a representative DNA sequence, but where the sequence represents RNA (e.g., mRNA), the “T”s would be substituted for “U”s. Thus, any of the RNA polynucleotides encoded by a DNA identified by a particular sequence identification number may also comprise the corresponding RNA (e.g., mRNA) sequence encoded by the DNA, where each “T” of the DNA sequence is substituted with “U.”

It should be understood that the mRNA polynucleotides of the vaccines as provided herein are synthetic molecules, i.e., they are not naturally-occurring molecules. That is, the mRNA polynucleotides of the present disclosure are isolated mRNA polynucleotides. As is known in the art, “isolated polynucleotides” refer to polynucleotides that are substantially physically separated from other cellular material (e.g., separated from cells and/or systems that produce the polynucleotides) or from other material that hinders their use in the vaccines of the present disclosure. Isolated polynucleotides are substantially pure in that they have been substantially separated from the substances with which they may be associated in living or viral systems. Thus, mRNA polynucleotide vaccines are not associated with living or viral systems, such as cells or viruses. The mRNA polynucleotide vaccines do not include viral components (e.g., viral capsids, viral enzymes, or other viral proteins, for example, those needed for viral-based replication), and the mRNA polynucleotide vaccines are not packaged within, encapsulated within, linked to, or otherwise associated with a virus or viral particle. In some embodiments, the mRNA vaccines comprise a lipid nanoparticle that consists of, or consists essentially of, one or more mRNA polynucleotides (e.g., mRNA polynucleotides encoding one or more VZV antigen(s)).

The basic components of an mRNA molecule typically include at least one coding region, a 5' untranslated region (UTR), a 3' UTR, a 5' cap and a poly-A tail. Polynucleotides of the present disclosure may function as mRNA but can be distinguished from wild-type mRNA in their functional and/or structural design features, which serve to overcome existing problems of effective polypeptide expression using nucleic-acid based therapeutics. In some embodiments, the RNA is a messenger RNA (mRNA) having an open reading frame encoding at least one VZV antigen. In some embodiments, the RNA (e.g., mRNA) further comprises a (at least one) 5' UTR, 3' UTR, a polyA tail and/or a 5' cap.

In some embodiments, a RNA polynucleotide (*e.g.*, mRNA) of a VZV vaccine encodes 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-10, 5-9, 5-8, 5-7, 5-6, 6-10, 6-9, 6-8, 6-7, 7-10, 7-9, 7-8, 8-10, 8-9 or 9-10 antigenic polypeptides. In some embodiments, a RNA polynucleotide (*e.g.*, mRNA) of a VZV RNA (*e.g.*, mRNA) vaccine encodes at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 antigenic polypeptides. In some embodiments, a RNA polynucleotide (*e.g.*, mRNA) of a VZV vaccine encodes at least 100 antigenic polypeptides, or at least 200 antigenic polypeptides. In some embodiments, a RNA polynucleotide (*e.g.*, mRNA) of a VZV vaccine encodes 1-10, 5-15, 10-20, 15-25, 20-30, 25-35, 30-40, 35-45, 40-50, 1-50, 1-100, 2-50 or 2-100 antigenic polypeptides.

Polynucleotides (*e.g.*, mRNAs) of the present disclosure, in some embodiments, are codon optimized. Codon optimization methods are known in the art and may be used as provided herein. For example, any one or more of the sequences SEQ ID NO: 11, 15, 19, 23, 27, 31, 35, 39, 62, 66, 70, 74, 78, 82, 86, 90 or any one or more of the sequences of SEQ ID NO: 92-108 may be codon optimized. Codon optimization, in some embodiments, may be used to match codon frequencies in target and host organisms to ensure proper folding; bias GC content to increase mRNA stability or reduce secondary structures; minimize tandem repeat codons or base runs that may impair gene construction or expression; customize transcriptional and translational control regions; insert or remove protein trafficking sequences; remove/add post translation modification sites in encoded protein (*e.g.*, glycosylation sites); add, remove or shuffle protein domains; insert or delete restriction sites; modify ribosome binding sites and mRNA degradation sites; adjust translational rates to allow the various domains of the protein to fold properly; or reduce or eliminate problem secondary structures within the polynucleotide. Codon optimization tools, algorithms and services are known in the art – non-limiting examples include services from GeneArt (Life Technologies), DNA2.0 (Menlo Park CA) and/or proprietary methods. In some embodiments, the open reading frame (ORF) sequence is optimized using optimization algorithms.

In some embodiments, a codon optimized sequence (*e.g.*, a codon-optimized sequence any one of SEQ ID NO: 92-108) shares less than 95% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)). In some embodiments, a codon optimized sequence shares less than 90% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or

polypeptide)). In some embodiments, a codon optimized sequence shares less than 85% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)). In some embodiments, a codon optimized sequence shares less than 80% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)). In some embodiments, a codon optimized sequence shares less than 75% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)).

In some embodiments, a codon optimized sequence shares between 65% and 85% (*e.g.*, between about 67% and about 85% or between about 67% and about 80%) sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)). In some embodiments, a codon optimized sequence shares between 65% and 75% or about 80% sequence identity to a naturally-occurring or wild-type sequence (*e.g.*, a naturally-occurring or wild-type mRNA sequence encoding a polypeptide or protein of interest (*e.g.*, an antigenic protein or polypeptide)).

In some embodiments, a codon-optimized sequence (*e.g.*, a codon-optimized sequence of any one of SEQ ID NO: 92-108) encodes an antigenic polypeptide that is as immunogenic as, or more immunogenic than (*e.g.*, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 100%, or at least 200% more), than an antigenic polypeptide encoded by a (non-codon-optimized) sequence of any one of SEQ ID NO: 92-108.

In some embodiments, the VZV vaccine includes at least one RNA polynucleotide having an open reading frame encoding at least one VZV antigenic polypeptide having at least one modification, at least one 5' terminal cap, and is formulated within a lipid nanoparticle. 5'-capping of polynucleotides may be completed concomitantly during the *in vitro*-transcription reaction using the following chemical RNA cap analogs to generate the 5'-guanosine cap structure according to manufacturer protocols: 3'-O-Me-m7G(5')ppp(5') G [the ARCA cap]; G(5')ppp(5')A; G(5')ppp(5')G; m7G(5')ppp(5')A; m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). 5'-capping of modified RNA may be completed post-transcriptionally using a Vaccinia Virus Capping Enzyme to generate the "Cap 0" structure: m7G(5')ppp(5')G (New England BioLabs, Ipswich, MA). Cap 1 structure may be generated using both Vaccinia Virus Capping Enzyme and a 2'-O methyl-transferase to generate: m7G(5')ppp(5')G-2'-O-methyl. Cap 2 structure may be generated from the Cap 1 structure

followed by the 2'-O-methylation of the 5'-antepenultimate nucleotide using a 2'-O methyl-transferase. Cap 3 structure may be generated from the Cap 2 structure followed by the 2'-O-methylation of the 5'-preantepenultimate nucleotide using a 2'-O methyl-transferase.

Enzymes may be derived from a recombinant source.

- 5 When transfected into mammalian cells, the modified mRNAs have a stability of between 12-18 hours, or greater than 18 hours, *e.g.*, 24, 36, 48, 60, 72, or greater than 72 hours.

 In some embodiments a codon optimized RNA may be one in which the levels of G/C are enhanced. The G/C-content of nucleic acid molecules (*e.g.*, mRNA) may influence the
10 stability of the RNA. RNA having an increased amount of guanine (G) and/or cytosine (C) residues may be functionally more stable than RNA containing a large amount of adenine (A) and thymine (T) or uracil (U) nucleotides. As an example, WO2002/098443 discloses a pharmaceutical composition containing an mRNA stabilized by sequence modifications in the translated region. Due to the degeneracy of the genetic code, the modifications work by
15 substituting existing codons for those that promote greater RNA stability without changing the resulting amino acid. The approach is limited to coding regions of the RNA.

Signal peptides

 In some embodiments, antigenic polypeptides encoded by VZV polynucleotides
20 comprise a signal peptide. Signal peptides, comprising the N-terminal 15-60 amino acids of proteins, are typically needed for the translocation across the membrane on the secretory pathway and thus universally control the entry of most proteins both in eukaryotes and prokaryotes to the secretory pathway. Signal peptides generally include of three regions: an N-terminal region of differing length, which usually comprises positively charged amino
25 acids; a hydrophobic region; and a short carboxy-terminal peptide region. In eukaryotes, the signal peptide of a nascent precursor protein (pre-protein) directs the ribosome to the rough endoplasmic reticulum (ER) membrane and initiates the transport of the growing peptide chain across it. The signal peptide is not responsible for the final destination of the mature protein, however. Secretory proteins devoid of further address tags in their sequence are by
30 default secreted to the external environment. Signal peptides are cleaved from precursor proteins by an endoplasmic reticulum (ER)-resident signal peptidase or they remain uncleaved and function as a membrane anchor. During recent years, a more advanced view of signal peptides has evolved, showing that the functions and immunodominance of certain signal peptides are much more versatile than previously anticipated.

Signal peptides typically function to facilitate the targeting of newly synthesized protein to the endoplasmic reticulum (ER) for processing. ER processing produces a mature Envelope protein, wherein the signal peptide is cleaved, typically by a signal peptidase of the host cell. A signal peptide may also facilitate the targeting of the protein to the cell membrane. VZV vaccines of the present disclosure may comprise, for example, RNA polynucleotides encoding an artificial signal peptide, wherein the signal peptide coding sequence is operably linked to and is in frame with the coding sequence of the VZV antigenic polypeptide. Thus, VZV vaccines of the present disclosure, in some embodiments, produce an antigenic polypeptide comprising a VZV antigenic polypeptide fused to a signal peptide.

In some embodiments, a signal peptide is fused to the N-terminus of the VZV antigenic polypeptide. In some embodiments, a signal peptide is fused to the C-terminus of the VZV antigenic polypeptide.

In some embodiments, the signal peptide fused to the VZV antigenic polypeptide is an artificial signal peptide. In some embodiments, an artificial signal peptide fused to the VZV antigenic polypeptide encoded by the VZV RNA (*e.g.*, mRNA) vaccine is obtained from an immunoglobulin protein, *e.g.*, an IgE signal peptide or an IgG signal peptide. In some embodiments, a signal peptide fused to the VZV antigenic polypeptide encoded by a VZV RNA (*e.g.*, mRNA) vaccine is an Ig heavy chain epsilon-1 signal peptide (IgE HC SP) having the sequence of: MDWTWILFLVAAATRVHS (SEQ ID NO: 56). In some embodiments, a signal peptide fused to a VZV antigenic polypeptide encoded by the VZV RNA (*e.g.*, mRNA) vaccine is an IgGk chain V-III region HAH signal peptide (IgGk SP) having the sequence of METPAQLLFLLLLWLPDTTG (SEQ ID NO: 57). In some embodiments, the VZV antigenic polypeptide encoded by a VZV RNA (*e.g.*, mRNA) vaccine has an amino acid sequence set forth in one of 10, 14, 18, 22, 26, 30, 34, 38, 42 and 45-55 fused to a signal peptide of any one of SEQ ID NO: 56, 57, 109, 110 and 111. The examples disclosed herein are not meant to be limiting and any signal peptide that is known in the art to facilitate targeting of a protein to ER for processing and/or targeting of a protein to the cell membrane may be used in accordance with the present disclosure.

A signal peptide may have a length of 15-60 amino acids. For example, a signal peptide may have a length of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 amino acids. In some embodiments, a signal peptide may have a length of 20-60, 25-60, 30-60, 35-60, 40-60, 45-60, 50-60, 55-60, 15-55, 20-55, 25-55, 30-55, 35-55, 40-55, 45-55, 50-55, 15-50, 20-50, 25-50, 30-50, 35-50, 40-50, 45-50, 15-45, 20-45, 25-45,

30-45, 35-45, 40-45, 15-40, 20-40, 25-40, 30-40, 35-40, 15-35, 20-35, 25-35, 30-35, 15-30, 20-30, 25-30, 15-25, 20-25, or 15-20 amino acids.

A signal peptide is typically cleaved from the nascent polypeptide at the cleavage junction during ER processing. The mature VZV antigenic polypeptide produced by VZV

5 RNA vaccine of the present disclosure typically does not comprise a signal peptide.

Chemical Modifications

RNA (*e.g.*, mRNA) vaccines of the present disclosure comprise, in some embodiments, at least one ribonucleic acid (RNA) polynucleotide having an open reading
10 frame encoding at least one respiratory syncytial virus (VZV) antigenic polypeptide, wherein said RNA comprises at least one chemical modification.

The terms “chemical modification” and “chemically modified” refer to modification with respect to adenosine (A), guanosine (G), uridine (U), thymidine (T) or cytidine (C) ribonucleosides or deoxyribonucleosides in at least one of their position, pattern, percent or
15 population. Generally, these terms do not refer to the ribonucleotide modifications in naturally occurring 5'-terminal mRNA cap moieties.

Modifications of polynucleotides include, without limitation, those described herein, and include, but are expressly not limited to, those modifications that comprise chemical modifications. Polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides)
20 may comprise modifications that are naturally-occurring, non-naturally-occurring or the polynucleotide may comprise a combination of naturally-occurring and non-naturally-occurring modifications. Polynucleotides may include any useful modification, for example, of a sugar, a nucleobase, or an internucleoside linkage (*e.g.*, to a linking phosphate, to a phosphodiester linkage or to the phosphodiester backbone).

25 With respect to a polypeptide, the term “modification” refers to a modification relative to the canonical set of 20 amino acids. Polypeptides, as provided herein, are also considered “modified” if they contain amino acid substitutions, insertions or a combination of substitutions and insertions.

Polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides), in
30 some embodiments, comprise various (more than one) different modifications. In some embodiments, a particular region of a polynucleotide contains one, two or more (optionally different) nucleoside or nucleotide modifications. In some embodiments, a modified RNA polynucleotide (*e.g.*, a modified mRNA polynucleotide), introduced to a cell or organism, exhibits reduced degradation in the cell or organism, respectively, relative to an unmodified
35 polynucleotide. In some embodiments, a modified RNA polynucleotide (*e.g.*, a modified

mRNA polynucleotide), introduced into a cell or organism, may exhibit reduced immunogenicity in the cell or organism, respectively (*e.g.*, a reduced innate response).

Polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides), in some embodiments, comprise non-natural modified nucleotides that are introduced during synthesis or post-synthesis of the polynucleotides to achieve desired functions or properties. The modifications may be present on internucleotide linkages, purine or pyrimidine bases, or sugars. The modification may be introduced with chemical synthesis or with a polymerase enzyme at the terminal of a chain or anywhere else in the chain. Any of the regions of a polynucleotide may be chemically modified.

The present disclosure provides for modified nucleosides and nucleotides of a polynucleotide (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides). A “nucleoside” refers to a compound containing a sugar molecule (*e.g.*, a pentose or ribose) or a derivative thereof in combination with an organic base (*e.g.*, a purine or pyrimidine) or a derivative thereof (also referred to herein as “nucleobase”). A nucleotide” refers to a nucleoside, including a phosphate group. Modified nucleotides may be synthesized by any useful method, such as, for example, chemically, enzymatically, or recombinantly, to include one or more modified or non-natural nucleosides. Polynucleotides may comprise a region or regions of linked nucleosides. Such regions may have variable backbone linkages. The linkages may be standard phosphodiester linkages, in which case the polynucleotides would comprise regions of nucleotides.

Modified nucleotide base pairing encompasses not only the standard adenosine-thymine, adenosine-uracil, or guanosine-cytosine base pairs, but also base pairs formed between nucleotides and/or modified nucleotides comprising non-standard or modified bases, wherein the arrangement of hydrogen bond donors and hydrogen bond acceptors permits hydrogen bonding between a non-standard base and a standard base or between two complementary non-standard base structures, such as, for example, in those polynucleotides having at least one chemical modification. One example of such non-standard base pairing is the base pairing between the modified nucleotide inosine and adenine, cytosine or uracil. Any combination of base/sugar or linker may be incorporated into polynucleotides of the present disclosure.

Modifications of polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides), including but not limited to chemical modification, that are useful in the compositions, vaccines, methods and synthetic processes of the present disclosure include, but are not limited to the following: 2-methylthio-N6-(*cis*-hydroxyisopentenyl)adenosine; 2-methylthio-N6-methyladenosine; 2-methylthio-N6-threonyl carbamoyladenosine; N6-

glycincylcarbamoyladenosine; N6-isopentenyladenosine; N6-methyladenosine; N6-threonylcarbamoyladenosine; 1,2'-O-dimethyladenosine; 1-methyladenosine; 2'-O-methyladenosine; 2'-O-ribosyladenosine (phosphate); 2-methyladenosine; 2-methylthio-N6 isopentenyladenosine; 2-methylthio-N6-hydroxynorvalyl carbamoyladenosine; 2'-O-methyladenosine; 2'-O-ribosyladenosine (phosphate); Isopentenyladenosine; N6-(cis-hydroxyisopentenyl)adenosine; N6,2'-O-dimethyladenosine; N6,2'-O-dimethyladenosine; N6,N6,2'-O-trimethyladenosine; N6,N6-dimethyladenosine; N6-acetyladenosine; N6-hydroxynorvalylcarbamoyladenosine; N6-methyl-N6-threonylcarbamoyladenosine; 2-methyladenosine; 2-methylthio-N6-isopentenyladenosine; 7-deaza-adenosine; N1-methyladenosine; N6, N6 (dimethyl)adenine; N6-cis-hydroxy-isopentenyl-adenosine; α -thioadenosine; 2 (amino)adenine; 2 (aminopropyl)adenine; 2 (methylthio) N6 (isopentenyl)adenine; 2-(alkyl)adenine; 2-(aminoalkyl)adenine; 2-(aminopropyl)adenine; 2-(halo)adenine; 2-(halo)adenine; 2-(propyl)adenine; 2'-Amino-2'-deoxy-ATP; 2'-Azido-2'-deoxy-ATP; 2'-Deoxy-2'-a-aminoadenosine TP; 2'-Deoxy-2'-a-azidoadenosine TP; 6 (alkyl)adenine; 6 (methyl)adenine; 6-(alkyl)adenine; 6-(methyl)adenine; 7 (deaza)adenine; 8 (alkenyl)adenine; 8 (alkynyl)adenine; 8 (amino)adenine; 8 (thioalkyl)adenine; 8-(alkenyl)adenine; 8-(alkyl)adenine; 8-(alkynyl)adenine; 8-(amino)adenine; 8-(halo)adenine; 8-(hydroxyl)adenine; 8-(thioalkyl)adenine; 8-(thiol)adenine; 8-azido-adenosine; aza adenine; deaza adenine; N6 (methyl)adenine; N6-(isopentyl)adenine; 7-deaza-8-aza-adenosine; 7-methyladenine; 1-Deazaadenosine TP; 2'Fluoro-N6-Bz-deoxyadenosine TP; 2'-OMe-2'-Amino-ATP; 2'O-methyl-N6-Bz-deoxyadenosine TP; 2'-a-Ethynyladenosine TP; 2-aminoadenine; 2-Aminoadenosine TP; 2-Amino-ATP; 2'-a-Trifluoromethyladenosine TP; 2-Azidoadenosine TP; 2'-b-Ethynyladenosine TP; 2-Bromoadenosine TP; 2'-b-Trifluoromethyladenosine TP; 2-Chloroadenosine TP; 2'-Deoxy-2',2'-difluoroadenosine TP; 2'-Deoxy-2'-a-mercaptoadenosine TP; 2'-Deoxy-2'-a-thiomethoxyadenosine TP; 2'-Deoxy-2'-b-aminoadenosine TP; 2'-Deoxy-2'-b-azidoadenosine TP; 2'-Deoxy-2'-b-bromoadenosine TP; 2'-Deoxy-2'-b-chloroadenosine TP; 2'-Deoxy-2'-b-fluoroadenosine TP; 2'-Deoxy-2'-b-iodoadenosine TP; 2'-Deoxy-2'-b-mercaptoadenosine TP; 2'-Deoxy-2'-b-thiomethoxyadenosine TP; 2-Fluoroadenosine TP; 2-Iodoadenosine TP; 2-Mercaptoadenosine TP; 2-methoxy-adenine; 2-methylthio-adenine; 2-Trifluoromethyladenosine TP; 3-Deaza-3-bromoadenosine TP; 3-Deaza-3-chloroadenosine TP; 3-Deaza-3-fluoroadenosine TP; 3-Deaza-3-iodoadenosine TP; 3-Deazaadenosine TP; 4'-Azidoadenosine TP; 4'-Carbocyclic adenosine TP; 4'-Ethynyladenosine TP; 5'-Homo-adenosine TP; 8-Aza-ATP; 8-bromo-adenosine TP; 8-Trifluoromethyladenosine TP; 9-Deazaadenosine TP; 2-aminopurine; 7-deaza-2,6-diaminopurine; 7-deaza-8-aza-2,6-

diaminopurine; 7-deaza-8-aza-2-aminopurine; 2,6-diaminopurine; 7-deaza-8-aza-adenine, 7-deaza-2-aminopurine; 2-thiocytidine; 3-methylcytidine; 5-formylcytidine; 5-hydroxymethylcytidine; 5-methylcytidine; N4-acetylcytidine; 2'-O-methylcytidine; 2'-O-methylcytidine; 5,2'-O-dimethylcytidine; 5-formyl-2'-O-methylcytidine; Lysidine; N4,2'-O-dimethylcytidine; N4-acetyl-2'-O-methylcytidine; N4-methylcytidine; N4,N4-Dimethyl-2'-OMe-Cytidine TP; 4-methylcytidine; 5-aza-cytidine; Pseudo-iso-cytidine; pyrrolo-cytidine; α -thio-cytidine; 2-(thio)cytosine; 2'-Amino-2'-deoxy-CTP; 2'-Azido-2'-deoxy-CTP; 2'-Deoxy-2'-a-aminocytidine TP; 2'-Deoxy-2'-a-azidocytidine TP; 3 (deaza) 5 (aza)cytosine; 3 (methyl)cytosine; 3-(alkyl)cytosine; 3-(deaza) 5 (aza)cytosine; 3-(methyl)cytidine; 4,2'-O-dimethylcytidine; 5 (halo)cytosine; 5 (methyl)cytosine; 5 (propynyl)cytosine; 5 (trifluoromethyl)cytosine; 5-(alkyl)cytosine; 5-(alkynyl)cytosine; 5-(halo)cytosine; 5-(propynyl)cytosine; 5-(trifluoromethyl)cytosine; 5-bromo-cytidine; 5-iodo-cytidine; 5-propynyl cytosine; 6-(azo)cytosine; 6-aza-cytidine; aza cytosine; deaza cytosine; N4 (acetyl)cytosine; 1-methyl-1-deaza-pseudoisocytidine; 1-methyl-pseudoisocytidine; 2-methoxy-5-methyl-cytidine; 2-methoxy-cytidine; 2-thio-5-methyl-cytidine; 4-methoxy-1-methyl-pseudoisocytidine; 4-methoxy-pseudoisocytidine; 4-thio-1-methyl-1-deaza-pseudoisocytidine; 4-thio-1-methyl-pseudoisocytidine; 4-thio-pseudoisocytidine; 5-azazebularine; 5-methyl-zebularine; pyrrolo-pseudoisocytidine; Zebularine; (E)-5-(2-Bromovinyl)cytidine TP; 2,2'-anhydro-cytidine TP hydrochloride; 2'Fluor-N4-Bz-cytidine TP; 2'Fluoro-N4-Acetyl-cytidine TP; 2'-O-Methyl-N4-Acetyl-cytidine TP; 2'O-methyl-N4-Bz-cytidine TP; 2'-a-Ethynylcytidine TP; 2'-a-Trifluoromethylcytidine TP; 2'-b-Ethynylcytidine TP; 2'-b-Trifluoromethylcytidine TP; 2'-Deoxy-2',2'-difluorocytidine TP; 2'-Deoxy-2'-a-mercaptopcytidine TP; 2'-Deoxy-2'-a-thiomethoxycytidine TP; 2'-Deoxy-2'-b-aminocytidine TP; 2'-Deoxy-2'-b-azidocytidine TP; 2'-Deoxy-2'-b-bromocytidine TP; 2'-Deoxy-2'-b-chlorocytidine TP; 2'-Deoxy-2'-b-fluorocytidine TP; 2'-Deoxy-2'-b-iodocytidine TP; 2'-Deoxy-2'-b-mercaptopcytidine TP; 2'-Deoxy-2'-b-thiomethoxycytidine TP; 2'-O-Methyl-5-(1-propynyl)cytidine TP; 3'-Ethynylcytidine TP; 4'-Azidocytidine TP; 4'-Carbocyclic cytidine TP; 4'-Ethynylcytidine TP; 5-(1-Propynyl)ara-cytidine TP; 5-(2-Chloro-phenyl)-2-thiocytidine TP; 5-(4-Amino-phenyl)-2-thiocytidine TP; 5-Aminoallyl-CTP; 5-Cyanocytidine TP; 5-Ethynylara-cytidine TP; 5-Ethynylcytidine TP; 5'-Homo-cytidine TP; 5-Methoxycytidine TP; 5-Trifluoromethyl-Cytidine TP; N4-Amino-cytidine TP; N4-Benzoyl-cytidine TP; Pseudoisocytidine; 7-methylguanosine; N2,2'-O-dimethylguanosine; N2-methylguanosine; Wyosine; 1,2'-O-dimethylguanosine; 1-methylguanosine; 2'-O-methylguanosine; 2'-O-ribosylguanosine (phosphate); 2'-O-methylguanosine; 2'-O-ribosylguanosine (phosphate); 7-aminomethyl-7-deazaguanosine; 7-cyano-7-deazaguanosine;

Archaeosine; Methylwyosine; N2,7-dimethylguanosine; N2,N2,2'-O-trimethylguanosine; N2,N2,7-trimethylguanosine; N2,N2-dimethylguanosine; N2,7,2'-O-trimethylguanosine; 6-thio-guanosine; 7-deaza-guanosine; 8-oxo-guanosine; N1-methyl-guanosine; α -thio-guanosine; 2 (propyl)guanine; 2-(alkyl)guanine; 2'-Amino-2'-deoxy-GTP; 2'-Azido-2'-deoxy-GTP; 2'-Deoxy-2'-a-aminoguanosine TP; 2'-Deoxy-2'-a-azidoguanosine TP; 6 (methyl)guanine; 6-(alkyl)guanine; 6-(methyl)guanine; 6-methyl-guanosine; 7 (alkyl)guanine; 7 (deaza)guanine; 7 (methyl)guanine; 7-(alkyl)guanine; 7-(deaza)guanine; 7-(methyl)guanine; 8 (alkyl)guanine; 8 (alkynyl)guanine; 8 (halo)guanine; 8 (thioalkyl)guanine; 8-(alkenyl)guanine; 8-(alkyl)guanine; 8-(alkynyl)guanine; 8-(amino)guanine; 8- (halo)guanine; 8-(hydroxyl)guanine; 8-(thioalkyl)guanine; 8-(thiol)guanine; aza guanine; deaza guanine; N (methyl)guanine; N-(methyl)guanine; 1-methyl-6-thio-guanosine; 6-methoxy-guanosine; 6-thio-7-deaza-8-aza-guanosine; 6-thio-7-deaza-guanosine; 6-thio-7-methyl-guanosine; 7-deaza-8-aza-guanosine; 7-methyl-8-oxo-guanosine; N2,N2-dimethyl-6-thio-guanosine; N2-methyl-6-thio-guanosine; 1-Me-GTP; 2'Fluoro-N2-isobutyl-guanosine TP; 2'O-methyl-N2-isobutyl-guanosine TP; 2'-a-Ethynylguanosine TP; 2'-a-Trifluoromethylguanosine TP; 2'-b-Ethynylguanosine TP; 2'-b-Trifluoromethylguanosine TP; 2'-Deoxy-2',2'-difluoroguanosine TP; 2'-Deoxy-2'-a-mercaptoguanosine TP; 2'-Deoxy-2'-a-thiomethoxyguanosine TP; 2'-Deoxy-2'-b-aminoguanosine TP; 2'-Deoxy-2'-b-azidoguanosine TP; 2'-Deoxy-2'-b-bromoguanosine TP; 2'-Deoxy-2'-b-chloroguanosine TP; 2'-Deoxy-2'-b-fluoroguanosine TP; 2'-Deoxy-2'-b-iodoguanosine TP; 2'-Deoxy-2'-b-mercaptoguanosine TP; 2'-Deoxy-2'-b-thiomethoxyguanosine TP; 4'-Azidoguanosine TP; 4'-Carbocyclic guanosine TP; 4'-Ethynylguanosine TP; 5'-Homo-guanosine TP; 8-bromo-guanosine TP; 9-Deazaguanosine TP; N2-isobutyl-guanosine TP; 1-methylinosine; Inosine; 1,2'-O-dimethylinosine; 2'-O-methylinosine; 7-methylinosine; 2'-O-methylinosine; Epoxyqueuosine; galactosyl-queuosine; Mannosylqueuosine; Queuosine; allyamino-thymidine; aza thymidine; deaza thymidine; deoxy-thymidine; 2'-O-methyluridine; 2-thiouridine; 3-methyluridine; 5-carboxymethyluridine; 5-hydroxyuridine; 5-methyluridine; 5-taurinomethyl-2-thiouridine; 5-taurinomethyluridine; Dihydrouridine; Pseudouridine; (3-(3-amino-3-carboxypropyl)uridine; 1-methyl-3-(3-amino-5-carboxypropyl)pseudouridine; 1-methylpseudouridine; 1-ethyl-pseudouridine; 2'-O-methyluridine; 2'-O-methylpseudouridine; 2'-O-methyluridine; 2-thio-2'-O-methyluridine; 3-(3-amino-3-carboxypropyl)uridine; 3,2'-O-dimethyluridine; 3-Methyl-pseudo-Uridine TP; 4-thiouridine; 5-(carboxyhydroxymethyl)uridine; 5-(carboxyhydroxymethyl)uridine methyl ester; 5,2'-O-dimethyluridine; 5,6-dihydro-uridine; 5-aminomethyl-2-thiouridine; 5-carbamoylmethyl-2'-O-methyluridine; 5-carbamoylmethyluridine; 5-carboxyhydroxymethyluridine; 5-carboxyhydroxymethyluridine

methyl ester; 5-carboxymethylaminomethyl-2'-O-methyluridine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyluridine; 5-carboxymethylaminomethyluridine; 5-Carbamoylmethyluridine TP; 5-methoxycarbonylmethyl-2'-O-methyluridine; 5-methoxycarbonylmethyl-2-thiouridine; 5-methoxycarbonylmethyluridine; 5-methyluridine, 5-methoxyuridine; 5-methyl-2-thiouridine; 5-methylaminomethyl-2-selenouridine; 5-methylaminomethyl-2-thiouridine; 5-methylaminomethyluridine; 5-Methyldihydrouridine; 5-Oxyacetic acid- Uridine TP; 5-Oxyacetic acid-methyl ester-Uridine TP; N1-methyl-pseudo-uracil; N1-ethyl-pseudo-uracil; uridine 5-oxyacetic acid; uridine 5-oxyacetic acid methyl ester; 3-(3-Amino-3-carboxypropyl)-Uridine TP; 5-(iso-Pentenylaminomethyl)- 2-thiouridine TP; 5-(iso-Pentenylaminomethyl)-2'-O-methyluridine TP; 5-(iso-Pentenylaminomethyl)uridine TP; 5-propynyl uracil; α -thio-uridine; 1 (aminoalkylamino-carbonylethylenyl)-2(thio)-pseudouracil; 1 (aminoalkylaminocarbonylethylenyl)-2,4-(dithio)pseudouracil; 1 (aminoalkylaminocarbonylethylenyl)-4 (thio)pseudouracil; 1 (aminoalkylaminocarbonylethylenyl)-pseudouracil; 1 (aminocarbonylethylenyl)-2(thio)-pseudouracil; 1 (aminocarbonylethylenyl)-2,4-(dithio)pseudouracil; 1 (aminocarbonylethylenyl)-4 (thio)pseudouracil; 1 (aminocarbonylethylenyl)-pseudouracil; 1 substituted 2(thio)-pseudouracil; 1 substituted 2,4-(dithio)pseudouracil; 1 substituted 4 (thio)pseudouracil; 1 substituted pseudouracil; 1-(aminoalkylamino-carbonylethylenyl)-2-(thio)-pseudouracil; 1-Methyl-3-(3-amino-3-carboxypropyl) pseudouridine TP; 1-Methyl-3-(3-amino-3-carboxypropyl)pseudo-UTP; 1-Methyl-pseudo-UTP; 1-Ethyl-pseudo-UTP; 2 (thio)pseudouracil; 2' deoxy uridine; 2' fluorouridine; 2-(thio)uracil; 2,4-(dithio)pseudouracil; 2' methyl, 2' amino, 2' azido, 2' fluoro-guanosine; 2'-Amino-2'-deoxy-UTP; 2'-Azido-2'-deoxy-UTP; 2'-Azido-deoxyuridine TP; 2'-O-methylpseudouridine; 2' deoxy uridine; 2' fluorouridine; 2'-Deoxy-2'-a-aminouridine TP; 2'-Deoxy-2'-a-azidouridine TP; 2-methylpseudouridine; 3 (3 amino-3 carboxypropyl)uracil; 4 (thio)pseudouracil; 4-(thio)pseudouracil; 4-(thio)uracil; 4-thiouracil; 5 (1,3-diazole-1-alkyl)uracil; 5 (2-aminopropyl)uracil; 5 (aminoalkyl)uracil; 5 (dimethylaminoalkyl)uracil; 5 (guanidiniumalkyl)uracil; 5 (methoxycarbonylmethyl)-2-(thio)uracil; 5 (methoxycarbonylmethyl)uracil; 5 (methyl) 2 (thio)uracil; 5 (methyl) 2,4 (dithio)uracil; 5 (methyl) 4 (thio)uracil; 5 (methylaminomethyl)-2 (thio)uracil; 5 (methylaminomethyl)-2,4 (dithio)uracil; 5 (methylaminomethyl)-4 (thio)uracil; 5 (propynyl)uracil; 5 (trifluoromethyl)uracil; 5-(2-aminopropyl)uracil; 5-(alkyl)-2-(thio)pseudouracil; 5-(alkyl)-2,4 (dithio)pseudouracil; 5-(alkyl)-4 (thio)pseudouracil; 5-(alkyl)pseudouracil; 5-(alkyl)uracil; 5-(alkynyl)uracil; 5-(allylamino)uracil; 5-(cyanoalkyl)uracil; 5-(dialkylaminoalkyl)uracil; 5-

(dimethylaminoalkyl)uracil; 5-(guanidiniumalkyl)uracil; 5-(halo)uracil; 5-(1,3-diazole-1-alkyl)uracil; 5-(methoxy)uracil; 5-(methoxycarbonylmethyl)-2-(thio)uracil; 5-(methoxycarbonyl-methyl)uracil; 5-(methyl) 2(thio)uracil; 5-(methyl) 2,4 (dithio)uracil; 5-(methyl) 4 (thio)uracil; 5-(methyl)-2-(thio)pseudouracil; 5-(methyl)-2,4 (dithio)pseudouracil;

5 5-(methyl)-4 (thio)pseudouracil; 5-(methyl)pseudouracil; 5-(methylaminomethyl)-2 (thio)uracil; 5-(methylaminomethyl)-2,4(dithio)uracil; 5-(methylaminomethyl)-4-(thio)uracil; 5-(propynyl)uracil; 5-(trifluoromethyl)uracil; 5-aminoallyl-uridine; 5-bromo-uridine; 5-iodo-uridine; 5-uracil; 6 (azo)uracil; 6-(azo)uracil; 6-aza-uridine; allyamino-uracil; aza uracil; deaza uracil; N3 (methyl)uracil; P pseudo-UTP-1-2-ethanoic acid; Pseudouracil; 4-Thio-

10 pseudo-UTP; 1-carboxymethyl-pseudouridine; 1-methyl-1-deaza-pseudouridine; 1-propynyl-uridine; 1-taurinomethyl-1-methyl-uridine; 1-taurinomethyl-4-thio-uridine; 1-taurinomethyl-pseudouridine; 2-methoxy-4-thio-pseudouridine; 2-thio-1-methyl-1-deaza-pseudouridine; 2-thio-1-methyl-pseudouridine; 2-thio-5-aza-uridine; 2-thio-dihdropseudouridine; 2-thio-dihydrouridine; 2-thio-pseudouridine; 4-methoxy-2-thio-pseudouridine; 4-methoxy-

15 pseudouridine; 4-thio-1-methyl-pseudouridine; 4-thio-pseudouridine; 5-aza-uridine; Dihdropseudouridine; ($\pm$)1-(2-Hydroxypropyl)pseudouridine TP; (2R)-1-(2-Hydroxypropyl)pseudouridine TP; (2S)-1-(2-Hydroxypropyl)pseudouridine TP; (E)-5-(2-Bromo-vinyl)ara-uridine TP; (E)-5-(2-Bromo-vinyl)uridine TP; (Z)-5-(2-Bromo-vinyl)ara-uridine TP; (Z)-5-(2-Bromo-vinyl)uridine TP; 1-(2,2,2-Trifluoroethyl)-pseudo-UTP; 1-

20 (2,2,3,3,3-Pentafluoropropyl)pseudouridine TP; 1-(2,2-Diethoxyethyl)pseudouridine TP; 1-(2,4,6-Trimethylbenzyl)pseudouridine TP; 1-(2,4,6-Trimethyl-benzyl)pseudo-UTP; 1-(2,4,6-Trimethyl-phenyl)pseudo-UTP; 1-(2-Amino-2-carboxyethyl)pseudo-UTP; 1-(2-Amino-ethyl)pseudo-UTP; 1-(2-Hydroxyethyl)pseudouridine TP; 1-(2-Methoxyethyl)pseudouridine TP; 1-(3,4-Bis-trifluoromethoxybenzyl)pseudouridine TP; 1-(3,4-

25 Dimethoxybenzyl)pseudouridine TP; 1-(3-Amino-3-carboxypropyl)pseudo-UTP; 1-(3-Amino-propyl)pseudo-UTP; 1-(3-Cyclopropyl-prop-2-ynyl)pseudouridine TP; 1-(4-Amino-4-carboxybutyl)pseudo-UTP; 1-(4-Amino-benzyl)pseudo-UTP; 1-(4-Amino-butyl)pseudo-UTP; 1-(4-Amino-phenyl)pseudo-UTP; 1-(4-Azidobenzyl)pseudouridine TP; 1-(4-Bromobenzyl)pseudouridine TP; 1-(4-Chlorobenzyl)pseudouridine TP; 1-(4-

30 Fluorobenzyl)pseudouridine TP; 1-(4-Iodobenzyl)pseudouridine TP; 1-(4-Methanesulfonylbenzyl)pseudouridine TP; 1-(4-Methoxybenzyl)pseudouridine TP; 1-(4-Methoxy-benzyl)pseudo-UTP; 1-(4-Methoxy-phenyl)pseudo-UTP; 1-(4-Methylbenzyl)pseudouridine TP; 1-(4-Methyl-benzyl)pseudo-UTP; 1-(4-Nitrobenzyl)pseudouridine TP; 1-(4-Nitro-benzyl)pseudo-UTP; 1(4-Nitro-phenyl)pseudo-

35 UTP; 1-(4-Thiomethoxybenzyl)pseudouridine TP; 1-(4-

Trifluoromethoxybenzyl)pseudouridine TP; 1-(4-Trifluoromethylbenzyl)pseudouridine TP;
 1-(5-Amino-pentyl)pseudo-UTP; 1-(6-Amino-hexyl)pseudo-UTP; 1,6-Dimethyl-pseudo-
 UTP; 1-[3-(2-{2-[2-(2-Aminoethoxy)-ethoxy]-ethoxy}-ethoxy)-propionyl]pseudouridine TP;
 1-{3-[2-(2-Aminoethoxy)-ethoxy]-propionyl } pseudouridine TP; 1-Acetylpsudouridine TP;
 5 1-Alkyl-6-(1-propynyl)-pseudo-UTP; 1-Alkyl-6-(2-propynyl)-pseudo-UTP; 1-Alkyl-6-allyl-
 pseudo-UTP; 1-Alkyl-6-ethynyl-pseudo-UTP; 1-Alkyl-6-homoallyl-pseudo-UTP; 1-Alkyl-6-
 vinyl-pseudo-UTP; 1-Allylpsudouridine TP; 1-Aminomethyl-pseudo-UTP; 1-
 Benzoylpsudouridine TP; 1-Benzyloxymethylpsudouridine TP; 1-Benzyl-pseudo-UTP; 1-
 Biotinyl-PEG2-pseudouridine TP; 1-Biotinylpsudouridine TP; 1-Butyl-pseudo-UTP; 1-
 10 Cyanomethylpsudouridine TP; 1-Cyclobutylmethyl-pseudo-UTP; 1-Cyclobutyl-pseudo-
 UTP; 1-Cycloheptylmethyl-pseudo-UTP; 1-Cycloheptyl-pseudo-UTP; 1-Cyclohexylmethyl-
 pseudo-UTP; 1-Cyclohexyl-pseudo-UTP; 1-Cyclooctylmethyl-pseudo-UTP; 1-Cyclooctyl-
 pseudo-UTP; 1-Cyclopentylmethyl-pseudo-UTP; 1-Cyclopentyl-pseudo-UTP; 1-
 Cyclopropylmethyl-pseudo-UTP; 1-Cyclopropyl-pseudo-UTP; 1-Ethyl-pseudo-UTP; 1-
 15 Hexyl-pseudo-UTP; 1-Homoallylpsudouridine TP; 1-Hydroxymethylpsudouridine TP; 1-
 iso-propyl-pseudo-UTP; 1-Me-2-thio-pseudo-UTP; 1-Me-4-thio-pseudo-UTP; 1-Me-alpha-
 thio-pseudo-UTP; 1-Methanesulfonylmethylpsudouridine TP; 1-
 Methoxymethylpsudouridine TP; 1-Methyl-6-(2,2,2-Trifluoroethyl)pseudo-UTP; 1-Methyl-
 6-(4-morpholino)-pseudo-UTP; 1-Methyl-6-(4-thiomorpholino)-pseudo-UTP; 1-Methyl-6-
 20 (substituted phenyl)pseudo-UTP; 1-Methyl-6-amino-pseudo-UTP; 1-Methyl-6-azido-pseudo-
 UTP; 1-Methyl-6-bromo-pseudo-UTP; 1-Methyl-6-butyl-pseudo-UTP; 1-Methyl-6-chloro-
 pseudo-UTP; 1-Methyl-6-cyano-pseudo-UTP; 1-Methyl-6-dimethylamino-pseudo-UTP; 1-
 Methyl-6-ethoxy-pseudo-UTP; 1-Methyl-6-ethylcarboxylate-pseudo-UTP; 1-Methyl-6-ethyl-
 pseudo-UTP; 1-Methyl-6-fluoro-pseudo-UTP; 1-Methyl-6-formyl-pseudo-UTP; 1-Methyl-6-
 25 hydroxyamino-pseudo-UTP; 1-Methyl-6-hydroxy-pseudo-UTP; 1-Methyl-6-iodo-pseudo-
 UTP; 1-Methyl-6-iso-propyl-pseudo-UTP; 1-Methyl-6-methoxy-pseudo-UTP; 1-Methyl-6-
 methylamino-pseudo-UTP; 1-Methyl-6-phenyl-pseudo-UTP; 1-Methyl-6-propyl-pseudo-
 UTP; 1-Methyl-6-tert-butyl-pseudo-UTP; 1-Methyl-6-trifluoromethoxy-pseudo-UTP; 1-
 Methyl-6-trifluoromethyl-pseudo-UTP; 1-Morpholinomethylpsudouridine TP; 1-Pentyl-
 30 pseudo-UTP; 1-Phenyl-pseudo-UTP; 1-Pivaloylpsudouridine TP; 1-Propargylpsudouridine
 TP; 1-Propyl-pseudo-UTP; 1-propynyl-pseudouridine; 1-p-tolyl-pseudo-UTP; 1-tert-Butyl-
 pseudo-UTP; 1-Thiomethoxymethylpsudouridine TP; 1-
 Thiomorpholinomethylpsudouridine TP; 1-Trifluoroacetylpsudouridine TP; 1-
 Trifluoromethyl-pseudo-UTP; 1-Vinylpsudouridine TP; 2,2'-anhydro-uridine TP; 2'-bromo-
 35 deoxyuridine TP; 2'-F-5-Methyl-2'-deoxy-UTP; 2'-OMe-5-Me-UTP; 2'-OMe-pseudo-UTP;

2'-a-Ethynyluridine TP; 2'-a-Trifluoromethyluridine TP; 2'-b-Ethynyluridine TP; 2'-b-Trifluoromethyluridine TP; 2'-Deoxy-2',2'-difluorouridine TP; 2'-Deoxy-2'-a-mercaptopuridine TP; 2'-Deoxy-2'-a-thiomethoxyuridine TP; 2'-Deoxy-2'-b-aminouridine TP; 2'-Deoxy-2'-b-azidouridine TP; 2'-Deoxy-2'-b-bromouridine TP; 2'-Deoxy-2'-b-chlorouridine TP; 2'-Deoxy-2'-b-fluorouridine TP; 2'-Deoxy-2'-b-iodouridine TP; 2'-Deoxy-2'-b-mercaptopuridine TP; 2'-Deoxy-2'-b-thiomethoxyuridine TP; 2-methoxy-4-thio-uridine; 2-methoxyuridine; 2'-O-Methyl-5-(1-propynyl)uridine TP; 3-Alkyl-pseudo-UTP; 4'-Azidouridine TP; 4'-Carbocyclic uridine TP; 4'-Ethynyluridine TP; 5-(1-Propynyl)ara-uridine TP; 5-(2-Furanyl)uridine TP; 5-Cyanouridine TP; 5-Dimethylaminouridine TP; 5'-Homo-uridine TP; 5-iodo-2'-fluoro-deoxyuridine TP; 5-Phenylethynyluridine TP; 5-Trideuteromethyl-6-deuterouridine TP; 5-Trifluoromethyl-Uridine TP; 5-Vinylarauridine TP; 6-(2,2,2-Trifluoroethyl)-pseudo-UTP; 6-(4-Morpholino)-pseudo-UTP; 6-(4-Thiomorpholino)-pseudo-UTP; 6-(Substituted-Phenyl)-pseudo-UTP; 6-Amino-pseudo-UTP; 6-Azido-pseudo-UTP; 6-Bromo-pseudo-UTP; 6-Butyl-pseudo-UTP; 6-Chloro-pseudo-UTP; 6-Cyano-pseudo-UTP; 6-Dimethylamino-pseudo-UTP; 6-Ethoxy-pseudo-UTP; 6-Ethylcarboxylate-pseudo-UTP; 6-Ethyl-pseudo-UTP; 6-Fluoro-pseudo-UTP; 6-Formyl-pseudo-UTP; 6-Hydroxyamino-pseudo-UTP; 6-Hydroxy-pseudo-UTP; 6-Iodo-pseudo-UTP; 6-iso-Propyl-pseudo-UTP; 6-Methoxy-pseudo-UTP; 6-Methylamino-pseudo-UTP; 6-Methyl-pseudo-UTP; 6-Phenyl-pseudo-UTP; 6-Phenyl-pseudo-UTP; 6-Propyl-pseudo-UTP; 6-tert-Butyl-pseudo-UTP; 6-Trifluoromethoxy-pseudo-UTP; 6-Trifluoromethyl-pseudo-UTP; Alpha-thio-pseudo-UTP; Pseudouridine 1-(4-methylbenzenesulfonic acid) TP; Pseudouridine 1-(4-methylbenzoic acid) TP; Pseudouridine TP 1-[3-(2-ethoxy)]propionic acid; Pseudouridine TP 1-[3-{2-(2-[2-(2-ethoxy)-ethoxy]-ethoxy)-ethoxy}]propionic acid; Pseudouridine TP 1-[3-{2-(2-[2-{2(2-ethoxy)-ethoxy}-ethoxy]-ethoxy)-ethoxy}]propionic acid; Pseudouridine TP 1-[3-{2-(2-[2-ethoxy]-ethoxy)-ethoxy}]propionic acid; Pseudouridine TP 1-[3-{2-(2-ethoxy)-ethoxy}] propionic acid; Pseudouridine TP 1-methylphosphonic acid; Pseudouridine TP 1-methylphosphonic acid diethyl ester; Pseudo-UTP-N1-3-propionic acid; Pseudo-UTP-N1-4-butanoic acid; Pseudo-UTP-N1-5-pentanoic acid; Pseudo-UTP-N1-6-hexanoic acid; Pseudo-UTP-N1-7-heptanoic acid; Pseudo-UTP-N1-methyl-p-benzoic acid; Pseudo-UTP-N1-p-benzoic acid; Wybutosine; Hydroxywybutosine; Isowyosine; Peroxywybutosine; undermodified hydroxywybutosine; 4-demethylwyosine; 2,6-(diamino)purine; 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 1,3-(diaz)-2-(oxo)-phenthiazin-1-yl; 1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 1,3,5-(triaz)-2,6-(diox)-naphthalene; 2 (amino)purine; 2,4,5-(trimethyl)phenyl; 2' methyl, 2' amino, 2' azido, 2' fluoro-cytidine; 2' methyl, 2' amino, 2' azido, 2' fluoro-adenine; 2' methyl, 2' amino, 2' azido, 2' fluoro-uridine; 2'-amino-2'-deoxyribose; 2-amino-6-Chloro-purine; 2-aza-inosinyl; 2'-azido-2'-

deoxyribose; 2'-fluoro-2'-deoxyribose; 2'-fluoro-modified bases; 2'-O-methyl-ribose; 2-oxo-7-aminopyridopyrimidin-3-yl; 2-oxo-pyridopyrimidine-3-yl; 2-pyridinone; 3-nitropyrrole; 3-(methyl)-7-(propynyl)isocarbostyryl; 3-(methyl)isocarbostyryl; 4-(fluoro)-6-(methyl)benzimidazole; 4-(methyl)benzimidazole; 4-(methyl)indolyl; 4,6-(dimethyl)indolyl;

5 5-nitroindole; 5-substituted pyrimidines; 5-(methyl)isocarbostyryl; 5-nitroindole; 6-(aza)pyrimidine; 6-(azo)thymine; 6-(methyl)-7-(aza)indolyl; 6-chloro-purine; 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl; 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 7-(aminoalkylhydroxy)-1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 7-(aminoalkylhydroxy)-1,3-(diaz)-2-(oxo)-phenthiazin-

10 1-yl; 7-(aminoalkylhydroxy)-1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 7-(aza)indolyl; 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl; 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 7-(guanidiniumalkylhydroxy)-1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 7-(guanidiniumalkyl-

15 hydroxy)-1,3-(diaz)-2-(oxo)-phenthiazin-1-yl; 7-(guanidiniumalkylhydroxy)-1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 7-(propynyl)isocarbostyryl; 7-(propynyl)isocarbostyryl, propynyl-7-(aza)indolyl; 7-deaza-inosinyl; 7-substituted 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl; 7-substituted 1,3-(diaz)-2-(oxo)-phenoxazin-1-yl; 9-(methyl)-imidizopyridinyl; Aminoindolyl; Anthracenyl; bis-ortho-(aminoalkylhydroxy)-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; bis-

20 ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; Difluorotolyl; Hypoxanthine; Imidizopyridinyl; Inosinyl; Isocarbostyryl; Isoguanisine; N2-substituted purines; N6-methyl-2-amino-purine; N6-substituted purines; N-alkylated derivative; Napthalenyl; Nitrobenzimidazolyl; Nitroimidazolyl; Nitroindazolyl; Nitropyrazolyl; Nubularine; O6-substituted purines; O-alkylated derivative; ortho-(aminoalkylhydroxy)-6-phenyl-pyrrolo-

25 pyrimidin-2-on-3-yl; ortho-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; Oxoformycin TP; para-(aminoalkylhydroxy)-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; para-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl; Pentacenyl; Phenanthracenyl; Phenyl; propynyl-7-(aza)indolyl; Pyrenyl; pyridopyrimidin-3-yl; pyridopyrimidin-3-yl, 2-oxo-7-amino-pyridopyrimidin-3-yl; pyrrolo-pyrimidin-2-on-3-yl; Pyrrolopyrimidinyl; Pyrrolopyrizinyl;

30 Stilbenzyl; substituted 1,2,4-triazoles; Tetracenyl; Tubercidine; Xanthine; Xanthosine-5'-TP; 2-thio-zebularine; 5-aza-2-thio-zebularine; 7-deaza-2-amino-purine; pyridin-4-one ribonucleoside; 2-Amino-riboside-TP; Formycin A TP; Formycin B TP; Pyrrolosine TP; 2'-OH-ara-adenosine TP; 2'-OH-ara-cytidine TP; 2'-OH-ara-uridine TP; 2'-OH-ara-guanosine TP; 5-(2-carbomethoxyvinyl)uridine TP; and N6-(19-Amino-pentaoxanonadecyl)adenosine

35 TP.

In some embodiments, polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) include a combination of at least two (*e.g.*, 2, 3, 4 or more) of the aforementioned modified nucleobases.

In some embodiments, modified nucleobases in polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) are selected from the group consisting of pseudouridine (ψ), 2-thiouridine (s2U), 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methyluridine, 5-methoxyuridine, 2'-O-methyl uridine, 1-methyl-pseudouridine (m1 ψ), 1-ethyl-pseudouridine (e1 ψ), 5-methoxy-uridine (mo5U), 5-methyl-cytidine (m5C), α -thio-guanosine, α -thio-adenosine, 5-cyano uridine, 4'-thio uridine 7-deaza-adenine, 1-methyl-adenosine (m1A), 2-methyl-adenine (m2A), N6-methyl-adenosine (m6A), and 2,6-Diaminopurine, (I), 1-methyl-inosine (m1I), wyosine (imG), methylwyosine (mimG), 7-deaza-guanosine, 7-cyano-7-deaza-guanosine (preQ0), 7-aminomethyl-7-deaza-guanosine (preQ1), 7-methyl-guanosine (m7G), 1-methyl-guanosine (m1G), 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 2,8-dimethyladenosine, 2-geranylthiouridine, 2-lysidine, 2-selenouridine, 3-(3-amino-3-carboxypropyl)-5,6-dihydrouridine, 3-(3-amino-3-carboxypropyl)pseudouridine, 3-methylpseudouridine, 5-(carboxyhydroxymethyl)-2'-O-methyluridine methyl ester, 5-aminomethyl-2-geranylthiouridine, 5-aminomethyl-2-selenouridine, 5-aminomethyluridine, 5-carbamoylhydroxymethyluridine, 5-carbamoylmethyl-2-thiouridine, 5-carboxymethyl-2-thiouridine, 5-carboxymethylaminomethyl-2-geranylthiouridine, 5-carboxymethylaminomethyl-2-selenouridine, 5-cyanomethyluridine, 5-hydroxycytidine, 5-methylaminomethyl-2-geranylthiouridine, 7-aminocarboxypropyl-demethylwyosine, 7-aminocarboxypropylwyosine, 7-aminocarboxypropylwyosine methyl ester, 8-methyladenosine, N4,N4-dimethylcytidine, N6-formyladenosine, N6-hydroxymethyladenosine, agmatidine, cyclic N6-threonylcarbamoyladenosine, glutamyl-queuosine, methylated undermodified hydroxywybutosine, N4,N4,2'-O-trimethylcytidine, geranylated 5-methylaminomethyl-2-thiouridine, geranylated 5-carboxymethylaminomethyl-2-thiouridine, Qbase, preQ0base, preQ1base, and combinations of two or more thereof. In some embodiments, the at least one chemically modified nucleoside is selected from the group consisting of pseudouridine, 1-methyl-pseudouridine, 1-ethyl-pseudouridine, 5-methylcytosine, 5-methoxyuridine, and a combination thereof. In some embodiments, the polyribonucleotide (*e.g.*, RNA polyribonucleotide, such as mRNA polyribonucleotide)

includes a combination of at least two (*e.g.*, 2, 3, 4 or more) of the aforementioned modified nucleobases. In some embodiments, polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) include a combination of at least two (*e.g.*, 2, 3, 4 or more) of the aforementioned modified nucleobases.

5 In some embodiments, modified nucleobases in polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) are selected from the group consisting of 1-methyl-pseudouridine (m1 ψ), 1-ethyl-pseudouridine (e1 ψ), 5-methoxy-uridine (mo5U), 5-methyl-cytidine (m5C), pseudouridine (ψ), α -thio-guanosine and α -thio-adenosine. In some embodiments, the polyribonucleotide includes a combination of at least two (*e.g.*, 2, 3, 4 or
10 more) of the aforementioned modified nucleobases, including but not limited to chemical modifications.

In some embodiments, polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) comprise pseudouridine (ψ) and 5-methyl-cytidine (m5C). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 1-methyl-pseudouridine (m1 ψ). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as
15 mRNA) comprise 1-ethyl-pseudouridine (e1 ψ). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 1-methyl-pseudouridine (m1 ψ) and 5-methyl-cytidine (m5C). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 1-ethyl-pseudouridine (e1 ψ) and 5-methyl-cytidine (m5C). In
20 some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 2-thiouridine (s2U). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 2-thiouridine and 5-methyl-cytidine (m5C). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise methoxy-uridine (mo5U). In some
25 embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 5-methoxy-uridine (mo5U) and 5-methyl-cytidine (m5C). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 2'-O-methyl uridine. In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise 2'-O-methyl uridine and 5-methyl-cytidine (m5C). In some embodiments, the polyribonucleotides (*e.g.*, RNA, such as mRNA) comprise N6-methyl-adenosine (m6A). In some embodiments, the polyribonucleotides (*e.g.*,
30 RNA, such as mRNA) comprise N6-methyl-adenosine (m6A) and 5-methyl-cytidine (m5C).

In some embodiments, polynucleotides (*e.g.*, RNA polynucleotides, such as mRNA polynucleotides) are uniformly modified (*e.g.*, fully modified, modified throughout the entire sequence) for a particular modification. For example, a polynucleotide can be uniformly modified with 1-methyl-pseudouridine, meaning that all uridine residues in the mRNA
35 sequence are replaced with 1-methyl-pseudouridine. Similarly, a polynucleotide can be

uniformly modified for any type of nucleoside residue present in the sequence by replacement with a modified residue such as those set forth above.

Exemplary nucleobases and nucleosides having a modified cytosine include N4-acetyl-cytidine (ac4C), 5-methyl-cytidine (m5C), 5-halo-cytidine (*e.g.*, 5-iodo-cytidine), 5-hydroxymethyl-cytidine (hm5C), 1-methyl-pseudoisocytidine, 2-thio-cytidine (s2C), and 2-thio-5-methyl-cytidine.

In some embodiments, a modified nucleobase is a modified uridine. Exemplary nucleobases and nucleosides having a modified uridine include 1-methyl-pseudouridine (m1ψ), 1-ethyl-pseudouridine (e1ψ), 5-methoxy uridine, 2-thio uridine, 5-cyano uridine, 2'-O-methyl uridine and 4'-thio uridine.

In some embodiments, a modified nucleobase is a modified adenine. Exemplary nucleobases and nucleosides having a modified adenine include 7-deaza-adenine, 1-methyl-adenosine (m1A), 2-methyl-adenine (m2A), and N6-methyl-adenosine (m6A).

In some embodiments, a modified nucleobase is a modified guanine. Exemplary nucleobases and nucleosides having a modified guanine include inosine (I), 1-methyl-inosine (m1I), wyosine (imG), methylwyosine (mimG), 7-deaza-guanosine, 7-cyano-7-deaza-guanosine (preQ0), 7-aminomethyl-7-deaza-guanosine (preQ1), 7-methyl-guanosine (m7G), 1-methyl-guanosine (m1G), 8-oxo-guanosine, and 7-methyl-8-oxo-guanosine.

The polynucleotides of the present disclosure may be partially or fully modified along the entire length of the molecule. For example, one or more or all or a given type of nucleotide (*e.g.*, purine or pyrimidine, or any one or more or all of A, G, U, C) may be uniformly modified in a polynucleotide of the invention, or in a given predetermined sequence region thereof (*e.g.*, in the mRNA including or excluding the polyA tail). In some embodiments, all nucleotides X in a polynucleotide of the present disclosure (or in a given sequence region thereof) are modified nucleotides, wherein X may be any one of nucleotides A, G, U, C, or any one of the combinations A+G, A+U, A+C, G+U, G+C, U+C, A+G+U, A+G+C, G+U+C or A+G+C.

The polynucleotide may contain from about 1% to about 100% modified nucleotides (either in relation to overall nucleotide content, or in relation to one or more types of nucleotide, *i.e.*, any one or more of A, G, U or C) or any intervening percentage (*e.g.*, from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to

100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100%). It will be understood that any remaining

percentage is accounted for by the presence of unmodified A, G, U, or C.

The polynucleotides may contain at a minimum 1% and at maximum 100% modified nucleotides, or any intervening percentage, such as at least 5% modified nucleotides, at least 10% modified nucleotides, at least 25% modified nucleotides, at least 50% modified nucleotides, at least 80% modified nucleotides, or at least 90% modified nucleotides. For example, the polynucleotides may contain a modified pyrimidine such as a modified uracil or cytosine. In some embodiments, at least 5%, at least 10%, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the uracil in the polynucleotide is replaced with a modified uracil (*e.g.*, a 5-substituted uracil). The modified uracil can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (*e.g.*, 2, 3, 4 or more unique structures). In some embodiments, at least 5%, at least 10%, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the cytosine in the polynucleotide is replaced with a modified cytosine (*e.g.*, a 5-substituted cytosine). The modified cytosine can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (*e.g.*, 2, 3, 4 or more unique structures).

Thus, in some embodiments, the RNA vaccines comprise a 5'UTR element, an optionally codon optimized open reading frame, and a 3'UTR element, a poly(A) sequence and/or a polyadenylation signal wherein the RNA is not chemically modified.

In some embodiments, the modified nucleobase is a modified uracil. Exemplary nucleobases and nucleosides having a modified uracil include pseudouridine (ψ), pyridin-4-one ribonucleoside, 5-aza-uridine, 6-aza-uridine, 2-thio-5-aza-uridine, 2-thio-uridine (s^2U), 4-thio-uridine (s^4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxy-uridine (ho^5U), 5-aminoallyl-uridine, 5-halo-uridine (*e.g.*, 5-iodo-uridine or 5-bromo-uridine), 3-methyl-uridine (m^3U), 5-methoxy-uridine (mo^5U), uridine 5-oxyacetic acid (cmo^5U), uridine 5-oxyacetic acid methyl ester ($mcmo^5U$), 5-carboxymethyl-uridine (cm^5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uridine (chm^5U), 5-carboxyhydroxymethyl-uridine methyl ester ($mchm^5U$), 5-methoxycarbonylmethyl-uridine (mcm^5U), 5-methoxycarbonylmethyl-2-thio-uridine (mcm^5s^2U), 5-aminomethyl-2-thio-uridine (nm^5s^2U), 5-methylaminomethyl-uridine (mnm^5U), 5-methylaminomethyl-2-thio-uridine (mnm^5s^2U), 5-methylaminomethyl-2-seleno-uridine (mnm^5se^2U), 5-carbamoylmethyl-uridine (ncm^5U), 5-

carboxymethylaminomethyl-uridine (cmnm⁵U), 5-carboxymethylaminomethyl-2-thio-uridine (cmnm⁵s²U), 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyl-uridine (τm⁵U), 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine(τm⁵s²U), 1-taurinomethyl-4-thio-pseudouridine, 5-methyl-uridine (m⁵U, i.e., having the nucleobase deoxythymine), 1-methyl-pseudouridine (m¹ψ), 1-ethyl-pseudouridine (e1ψ), 5-methyl-2-thio-uridine (m⁵s²U), 1-methyl-4-thio-pseudouridine (m¹s⁴ψ), 4-thio-1-methyl-pseudouridine, 3-methyl-pseudouridine (m³ψ), 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine (D), dihydropseudouridine, 5,6-dihydrouridine, 5-methyl-dihydrouridine (m⁵D), 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxy-uridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uridine (acp³U), 1-methyl-3-(3-amino-3-carboxypropyl)pseudouridine (acp³ψ), 5-(isopentenylaminomethyl)uridine (inm⁵U), 5-(isopentenylaminomethyl)-2-thio-uridine (inm⁵s²U), α-thio-uridine, 2'-O-methyl-uridine (Um), 5,2'-O-dimethyl-uridine (m⁵Um), 2'-O-methyl-pseudouridine (ψm), 2-thio-2'-O-methyl-uridine (s²Um), 5-methoxycarbonylmethyl-2'-O-methyl-uridine (mcm⁵Um), 5-carbamoylmethyl-2'-O-methyl-uridine (ncm⁵Um), 5-carboxymethylaminomethyl-2'-O-methyl-uridine (cmnm⁵Um), 3,2'-O-dimethyl-uridine (m³Um), and 5-(isopentenylaminomethyl)-2'-O-methyl-uridine (inm⁵Um), 1-thio-uridine, deoxythymidine, 2'-F-ara-uridine, 2'-F-uridine, 2'-OH-ara-uridine, 5-(2-carbomethoxyvinyl)uridine, and 5-[3-(1-E-propenylamino)]uridine.

In some embodiments, the modified nucleobase is a modified cytosine. Exemplary nucleobases and nucleosides having a modified cytosine include 5-aza-cytidine, 6-aza-cytidine, pseudoisocytidine, 3-methyl-cytidine (m³C), N4-acetyl-cytidine (ac⁴C), 5-formyl-cytidine (f⁵C), N4-methyl-cytidine (m⁴C), 5-methyl-cytidine (m⁵C), 5-halo-cytidine (e.g., 5-iodo-cytidine), 5-hydroxymethyl-cytidine (hm⁵C), 1-methyl-pseudoisocytidine, pyrrolo-cytidine, pyrrolo-pseudoisocytidine, 2-thio-cytidine (s²C), 2-thio-5-methyl-cytidine, 4-thio-pseudoisocytidine, 4-thio-1-methyl-pseudoisocytidine, 4-thio-1-methyl-1-deaza-pseudoisocytidine, 1-methyl-1-deaza-pseudoisocytidine, zebularine, 5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine, 2-methoxy-cytidine, 2-methoxy-5-methyl-cytidine, 4-methoxy-pseudoisocytidine, 4-methoxy-1-methyl-pseudoisocytidine, lysidine (k₂C), α-thio-cytidine, 2'-O-methyl-cytidine (Cm), 5,2'-O-dimethyl-cytidine (m⁵Cm), N4-acetyl-2'-O-methyl-cytidine (ac⁴Cm), N4,2'-O-dimethyl-cytidine (m⁴Cm), 5-formyl-2'-O-methyl-cytidine (f⁵Cm), N4,N4,2'-O-trimethyl-cytidine (m⁴₂Cm), 1-thio-cytidine, 2'-F-ara-cytidine, 2'-F-cytidine, and 2'-OH-ara-cytidine.

In some embodiments, the modified nucleobase is a modified adenine. Exemplary nucleobases and nucleosides having a modified adenine include 2-amino-purine, 2, 6-diaminopurine, 2-amino-6-halo-purine (*e.g.*, 2-amino-6-chloro-purine), 6-halo-purine (*e.g.*, 6-chloro-purine), 2-amino-6-methyl-purine, 8-azido-adenosine, 7-deaza-adenine, 7-deaza-8-aza-adenine, 7-deaza-2-amino-purine, 7-deaza-8-aza-2-amino-purine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 1-methyl-adenosine (m^1A), 2-methyl-adenine (m^2A), N6-methyl-adenosine (m^6A), 2-methylthio-N6-methyl-adenosine (ms^2m^6A), N6-isopentenyl-adenosine (i^6A), 2-methylthio-N6-isopentenyl-adenosine (ms^2i^6A), N6-(*cis*-hydroxyisopentenyl)adenosine (io^6A), 2-methylthio-N6-(*cis*-hydroxyisopentenyl)adenosine (ms^2io^6A), N6-glycylcarbamoyl-adenosine (g^6A), N6-threonylcarbamoyl-adenosine (t^6A), N6-methyl-N6-threonylcarbamoyl-adenosine (m^6t^6A), 2-methylthio-N6-threonylcarbamoyl-adenosine (ms^2g^6A), N6,N6-dimethyl-adenosine (m^6_2A), N6-hydroxynorvalylcarbamoyl-adenosine (hn^6A), 2-methylthio-N6-hydroxynorvalylcarbamoyl-adenosine (ms^2hn^6A), N6-acetyl-adenosine (ac^6A), 7-methyl-adenine, 2-methylthio-adenine, 2-methoxy-adenine, α -thio-adenosine, 2'-O-methyl-adenosine (Am), N6,2'-O-dimethyl-adenosine (m^6Am), N6,N6,2'-O-trimethyl-adenosine (m^6_2Am), 1,2'-O-dimethyl-adenosine (m^1Am), 2'-O-ribosyladenosine (phosphate) ($Ar(p)$), 2-amino-N6-methyl-purine, 1-thio-adenosine, 8-azido-adenosine, 2'-F-ara-adenosine, 2'-F-adenosine, 2'-OH-ara-adenosine, and N6-(19-amino-pentaoxanonadecyl)-adenosine.

In some embodiments, the modified nucleobase is a modified guanine. Exemplary nucleobases and nucleosides having a modified guanine include inosine (I), 1-methyl-inosine (m^1I), wyosine (imG), methylwyosine ($mimG$), 4-demethyl-wyosine ($imG-14$), isowyosine ($imG2$), wybutosine (yW), peroxywybutosine (o_2yW), hydroxywybutosine ($OhyW$), undermodified hydroxywybutosine ($OhyW^*$), 7-deaza-guanosine, queuosine (Q), epoxyqueuosine (oQ), galactosyl-queuosine ($galQ$), mannosyl-queuosine ($manQ$), 7-cyano-7-deaza-guanosine ($preQ_0$), 7-aminomethyl-7-deaza-guanosine ($preQ_1$), archaeosine (G^+), 7-deaza-8-aza-guanosine, 6-thio-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-deaza-8-aza-guanosine, 7-methyl-guanosine (m^7G), 6-thio-7-methyl-guanosine, 7-methyl-inosine, 6-methoxy-guanosine, 1-methyl-guanosine (m^1G), N2-methyl-guanosine (m^2G), N2,N2-dimethyl-guanosine (m^2_2G), N2,7-dimethyl-guanosine ($m^{2,7}G$), N2, N2,7-dimethyl-guanosine ($m^{2,2,7}G$), 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 1-methyl-6-thio-guanosine, N2-methyl-6-thio-guanosine, N2,N2-dimethyl-6-thio-guanosine, α -thio-guanosine, 2'-O-methyl-guanosine (Gm), N2-methyl-2'-O-methyl-guanosine (m^2Gm), N2,N2-dimethyl-2'-O-methyl-guanosine (m^2_2Gm), 1-methyl-2'-O-methyl-guanosine (m^1Gm), N2,7-dimethyl-2'-O-methyl-guanosine ($m^{2,7}Gm$), 2'-O-methyl-inosine (Im), 1,2'-O-dimethyl-inosine (m^1Im), 2'-O-

ribosylguanosine (phosphate) (Gr(p)), 1-thio-guanosine, O6-methyl-guanosine, 2'-F-ara-guanosine, and 2'-F-guanosine.

In Vitro Transcription of RNA (e.g., mRNA)

5 VZV vaccines of the present disclosure comprise at least one RNA polynucleotide, such as a mRNA (*e.g.*, modified mRNA). mRNA, for example, is transcribed *in vitro* from template DNA, referred to as an “*in vitro* transcription template.” In some embodiments, the at least one RNA polynucleotide has at least one chemical modification. The at least one chemical modification may include, but is expressly not limited to, any modification
10 described herein.

In vitro transcription of RNA is known in the art and is described in International Publication WO2014/152027, which is incorporated by reference herein in its entirety. For example, in some embodiments, the RNA transcript is generated using a non-amplified, linearized DNA template in an *in vitro* transcription reaction to generate the RNA transcript.

15 In some embodiments the RNA transcript is capped *via* enzymatic capping. In some embodiments the RNA transcript is purified via chromatographic methods, *e.g.*, use of an oligo dT substrate. Some embodiments exclude the use of DNase. In some embodiments the RNA transcript is synthesized from a non-amplified, linear DNA template coding for the gene of interest via an enzymatic *in vitro* transcription reaction utilizing a T7 phage RNA
20 polymerase and nucleotide triphosphates of the desired chemistry. Any number of RNA polymerases or variants may be used in the method of the present invention. The polymerase may be selected from, but is not limited to, a phage RNA polymerase, *e.g.*, a T7 RNA polymerase, a T3 RNA polymerase, a SP6 RNA polymerase, and/or mutant polymerases such as, but not limited to, polymerases able to incorporate modified nucleic acids and/or modified
25 nucleotides, including chemically modified nucleic acids and/or nucleotides.

In some embodiments a non-amplified, linearized plasmid DNA is utilized as the template DNA for *in vitro* transcription. In some embodiments, the template DNA is isolated DNA. In some embodiments, the template DNA is cDNA. In some embodiments, the cDNA is formed by reverse transcription of a RNA polynucleotide, for example, but not limited to
30 VZV RNA, *e.g.* VZV mRNA. In some embodiments, Cells, *e.g.*, bacterial cells, *e.g.*, *E. coli*, *e.g.*, DH-1 cells are transfected with the plasmid DNA template. In some embodiments, the transfected cells are cultured to replicate the plasmid DNA which is then isolated and purified. In some embodiments, the DNA template includes a RNA polymerase promoter, *e.g.*, a T7 promoter located 5' to and operably linked to the gene of interest.

In some embodiments, an *in vitro* transcription template encodes a 5' untranslated (UTR) region, contains an open reading frame, and encodes a 3' UTR and a polyA tail. The particular nucleic acid sequence composition and length of an *in vitro* transcription template will depend on the mRNA encoded by the template.

5 A "5' untranslated region" (UTR) refers to a region of an mRNA that is directly upstream (*i.e.*, 5') from the start codon (*i.e.*, the first codon of an mRNA transcript translated by a ribosome) that does not encode a polypeptide.

A "3' untranslated region" (UTR) refers to a region of an mRNA that is directly downstream (*i.e.*, 3') from the stop codon (*i.e.*, the codon of an mRNA transcript that signals
10 a termination of translation) that does not encode a polypeptide.

An "open reading frame" is a continuous stretch of DNA or RNA beginning with a start codon (*e.g.*, methionine (ATG or AUG)), and ending with a stop codon (*e.g.*, TAA, TAG or TGA, or UAA, UAG or UGA) and typically encodes a polypeptide (*e.g.*, protein). It will be understood that the sequences disclosed herein may further comprise additional
15 elements, *e.g.*, 5' and 3' UTRs, but that those elements, unlike the ORF, need not necessarily be present in a vaccine of the present disclosure.

A "polyA tail" is a region of mRNA that is downstream, *e.g.*, directly downstream (*i.e.*, 3'), from the 3' UTR that contains multiple, consecutive adenosine monophosphates. A polyA tail may contain 10 to 300 adenosine monophosphates. For example, a polyA tail may
20 contain 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290 or 300 adenosine monophosphates. In some embodiments, a polyA tail contains 50 to 250 adenosine monophosphates. In a relevant biological setting (*e.g.*, in cells, *in vivo*) the poly(A) tail functions to protect mRNA from enzymatic degradation, *e.g.*, in the cytoplasm, and aids in transcription termination, and/or
25 export of the mRNA from the nucleus and translation.

In some embodiments, a polynucleotide includes 200 to 3,000 nucleotides. For example, a polynucleotide may include 200 to 500, 200 to 1000, 200 to 1500, 200 to 3000, 500 to 1000, 500 to 1500, 500 to 2000, 500 to 3000, 1000 to 1500, 1000 to 2000, 1000 to 3000, 1500 to 3000, or 2000 to 3000 nucleotides).

30 *Methods of Treatment*

Provided herein are compositions (*e.g.*, pharmaceutical compositions), methods, kits and reagents for prevention and/or treatment of VZV in humans and other mammals. VZV RNA vaccines can be used as therapeutic or prophylactic agents. They may be used in
35 medicine to prevent and/or treat infectious disease. In exemplary aspects, the VZV RNA

vaccines of the invention are used to provide prophylactic protection from varicella and herpes zoster. Varicella is an acute infectious disease caused by VZV. The primary varicella zoster virus infection that results in chickenpox (varicella) may result in complications, including viral or secondary bacterial pneumonia. Even when the clinical symptoms of chickenpox have resolved, VZV remains dormant in the nervous system of the infected person in the trigeminal and dorsal root ganglia and may reactivate later in life, travelling from the sensory ganglia back to the skin where it produces a disease (rash) known as shingles or herpes zoster, and can also cause a number of neurologic conditions ranging from aseptic meningitis to encephalitis. The VZV vaccines of the present disclosure can be used to prevent and/or treat both the primary infection (Chicken pox) and also the re-activated viral infection (shingles or herpes zoster) and may be particularly useful for prevention and/or treatment of immunocompromised and elderly patients to prevent or to reduce the severity and/or duration of herpes zoster.

Prophylactic protection from VZV can be achieved following administration of a VZV RNA vaccine of the present disclosure. Vaccines can be administered once, twice, three times, four times or more but it is likely sufficient to administer the vaccine once (optionally followed by a single booster). It is possible, although less desirable, to administer the vaccine to an infected individual to achieve a therapeutic response. Dosing may need to be adjusted accordingly.

A method of eliciting an immune response in a subject against a VZV is provided in aspects of the present disclosure. The method involves administering to the subject a VZV RNA vaccine comprising at least one RNA (e.g., mRNA) polynucleotide having an open reading frame encoding at least one VZV antigenic polypeptide, thereby inducing in the subject an immune response specific to VZV antigenic polypeptide, wherein anti-antigenic polypeptide antibody titer in the subject is increased following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the VZV. An “anti-antigenic polypeptide antibody” is a serum antibody the binds specifically to the antigenic polypeptide.

A prophylactically effective dose is a therapeutically effective dose that prevents infection with the virus at a clinically acceptable level. In some embodiments, the therapeutically effective dose is a dose listed in a package insert for the vaccine. A traditional vaccine, as used herein, refers to a vaccine other than the mRNA vaccines of the present disclosure. For instance, a traditional vaccine includes, but is not limited, to live microorganism vaccines, killed microorganism vaccines, subunit vaccines, protein antigen vaccines, DNA vaccines, VLP vaccines, *etc.* In exemplary embodiments, a traditional

vaccine is a vaccine that has achieved regulatory approval and/or is registered by a national drug regulatory body, for example the Food and Drug Administration (FDA) in the United States or the European Medicines Agency (EMA).

A method of eliciting an immune response in a subject against a VZV is provided in aspects of the invention. The method involves administering to the subject a VZV RNA vaccine comprising at least one RNA polynucleotide having an open reading frame encoding at least one VZV antigenic polypeptide, thereby inducing in the subject an immune response specific to VZV antigenic polypeptide, wherein anti-antigenic polypeptide antibody titer in the subject is increased following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the VZV. An “anti-antigenic polypeptide antibody” is a serum antibody the binds specifically to the antigenic polypeptide.

A prophylactically effective dose is a therapeutically effective dose that prevents infection with the virus at a clinically acceptable level. In some embodiments the therapeutically effective dose is a dose listed in a package insert for the vaccine. A traditional vaccine, as used herein, refers to a vaccine other than the mRNA vaccines of the invention. For instance, a traditional vaccine includes but is not limited to live microorganism vaccines, killed microorganism vaccines, subunit vaccines, protein antigen vaccines, DNA vaccines, *etc.*

In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 1 log to 10 log following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the VZV.

In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 1 log following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the VZV.

In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 2 log following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the VZV.

In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 3 log following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the VZV.

In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 5 log following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the VZV.

5 In some embodiments the anti-antigenic polypeptide antibody titer in the subject is increased 10 log following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the VZV.

10 A method of eliciting an immune response in a subject against a VZV is provided in other aspects of the invention. The method involves administering to the subject a VZV RNA vaccine comprising at least one RNA polynucleotide having an open reading frame encoding at least one VZV antigenic polypeptide, thereby inducing in the subject an immune response specific to VZV antigenic polypeptide, wherein the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine against
15 the VZV at 2 times to 100 times the dosage level relative to the RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at twice the dosage level relative to the VZV RNA vaccine.

20 In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at three times the dosage level relative to the VZV RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 4 times the dosage level relative to the VZV RNA vaccine.

25 In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 5 times the dosage level relative to the VZV RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 10 times the dosage level
30 relative to the VZV RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 50 times the dosage level relative to the VZV RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 100 times the dosage level relative to the VZV RNA vaccine.

5 In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 10 times to 1000 times the dosage level relative to the VZV RNA vaccine.

In some embodiments the immune response in the subject is equivalent to an immune response in a subject vaccinated with a traditional vaccine at 100 times to 1000 times the dosage level relative to the VZV RNA vaccine.

10 In other embodiments the immune response is assessed by determining [protein] antibody titer in the subject.

In other aspects the invention is a method of eliciting an immune response in a subject against a VZV by administering to the subject a VZV RNA vaccine comprising at least one RNA polynucleotide having an open reading frame encoding at least one VZV antigenic polypeptide, thereby inducing in the subject an immune response specific to VZV antigenic polypeptide, wherein the immune response in the subject is induced 2 days to 10 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the VZV. In some embodiments the immune response in the subject is induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine at 2 times to 100 times the dosage level relative to the RNA vaccine.

20 In some embodiments the immune response in the subject is induced 2 days earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

25 In some embodiments the immune response in the subject is induced 3 days earlier relative to an immune response induced in a subject vaccinated a prophylactically effective dose of a traditional vaccine.

In some embodiments the immune response in the subject is induced 1 week earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

30 In some embodiments the immune response in the subject is induced 2 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

In some embodiments the immune response in the subject is induced 3 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

In some embodiments the immune response in the subject is induced 5 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

In some embodiments the immune response in the subject is induced 10 weeks earlier relative to an immune response induced in a subject vaccinated with a prophylactically effective dose of a traditional vaccine.

A method of v eliciting an immune response in a subject against a VZV by administering to the subject a VZV RNA vaccine having an open reading frame encoding a first antigenic polypeptide, wherein the RNA polynucleotide does not include a stabilization element, and wherein an adjuvant is not co-formulated or co-administered with the vaccine.

Flagellin Adjuvants

Flagellin is an approximately 500 amino acid monomeric protein that polymerizes to form the flagella associated with bacterial motion. Flagellin is expressed by a variety of flagellated bacteria (*Salmonella typhimurium* for example) as well as non-flagellated bacteria (such as *Escherichia coli*). Sensing of flagellin by cells of the innate immune system (dendritic cells, macrophages, *etc.*) is mediated by the Toll-like receptor 5 (TLR5) as well as by Nod-like receptors (NLRs) Ipaf and Naip5. TLRs and NLRs have been identified as playing a role in the activation of innate immune response and adaptive immune response. As such, flagellin provides an adjuvant effect in a vaccine.

The nucleotide and amino acid sequences encoding known flagellin polypeptides are publicly available in the NCBI GenBank database. The flagellin sequences from *S. Typhimurium*, *H. Pylori*, *V. Cholera*, *S. marcesens*, *S. flexneri*, *T. Pallidum*, *L. pneumophila*, *B. burgdorferi*, *C. difficile*, *R. meliloti*, *A. tumefaciens*, *R. lupini*, *B. clarridgeiae*, *P. Mirabilis*, *B. subtilis*, *L. monocytogenes*, *P. aeruginosa*, and *E. coli*, among others are known.

A flagellin polypeptide, as used herein, refers to a full length flagellin protein, immunogenic fragments thereof, and peptides having at least 50% sequence identify to a flagellin protein or immunogenic fragments thereof. Exemplary flagellin proteins include flagellin from *Salmonella typhi* (UniPro Entry number: Q56086), *Salmonella typhimurium* (A0A0C9DG09), *Salmonella enteritidis* (A0A0C9BAB7), and *Salmonella choleraesuis* (Q6V2X8), and SEQ ID NO: 115-117. In some embodiments, the flagellin polypeptide has

at least 60%, 70%, 75%, 80%, 90%, 95%, 97%, 98%, or 99% sequence identify to a flagellin protein or immunogenic fragments thereof.

In some embodiments, the flagellin polypeptide is an immunogenic fragment. An immunogenic fragment is a portion of a flagellin protein that provokes an immune response.

5 In some embodiments, the immune response is a TLR5 immune response. An example of an immunogenic fragment is a flagellin protein in which all or a portion of a hinge region has been deleted or replaced with other amino acids. For example, an antigenic polypeptide may be inserted in the hinge region. Hinge regions are the hypervariable regions of a flagellin. Hinge regions of a flagellin are also referred to as “D3 domain or region,” “propeller domain
10 or region,” “hypervariable domain or region” and “variable domain or region.” “At least a portion of a hinge region,” as used herein, refers to any part of the hinge region of the flagellin, or the entirety of the hinge region. In other embodiments an immunogenic fragment of flagellin is a 20, 25, 30, 35, or 40 amino acid C-terminal fragment of flagellin.

The flagellin monomer is formed by domains D0 through D3. D0 and D1, which form
15 the stem, are composed of tandem long alpha helices and are highly conserved among different bacteria. The D1 domain includes several stretches of amino acids that are useful for TLR5 activation. The entire D1 domain or one or more of the active regions within the domain are immunogenic fragments of flagellin. Examples of immunogenic regions within the D1 domain include residues 88-114 and residues 411-431 (in *Salmonella typhimurium*
20 FliC flagellin. Within the 13 amino acids in the 88-100 region, at least 6 substitutions are permitted between *Salmonella* flagellin and other flagellin proteins that still preserve TLR5 activation. Thus, immunogenic fragments of flagellin include flagellin like sequences that activate TLR5 and contain a 13 amino acid motif that is 53% or more identical to the *Salmonella* sequence in 88-100 of FliC (LQRVRELAVQSAN; SEQ ID NO: 118).

25 In some embodiments, the RNA (*e.g.*, mRNA) vaccine includes an RNA that encodes a fusion protein of flagellin and one or more antigenic polypeptides. A “fusion protein” as used herein, refers to a linking of two components of the construct. In some embodiments, a carboxy-terminus of the antigenic polypeptide is fused or linked to an amino terminus of the flagellin polypeptide. In other embodiments, an amino-terminus of the antigenic polypeptide
30 is fused or linked to a carboxy-terminus of the flagellin polypeptide. The fusion protein may include, for example, one, two, three, four, five, six or more flagellin polypeptides linked to one, two, three, four, five, six or more antigenic polypeptides. When two or more flagellin polypeptides and/or two or more antigenic polypeptides are linked such a construct may be referred to as a “multimer.”

Each of the components of a fusion protein may be directly linked to one another or they may be connected through a linker. For instance, the linker may be an amino acid linker. The amino acid linker encoded for by the RNA (*e.g.*, mRNA) vaccine to link the components of the fusion protein may include, for instance, at least one member selected from the group consisting of a lysine residue, a glutamic acid residue, a serine residue and an arginine residue. In some embodiments the linker is 1-30, 1-25, 1-25, 5-10, 5, 15, or 5-20 amino acids in length.

In other embodiments the RNA (*e.g.*, mRNA) vaccine includes at least two separate RNA polynucleotides, one encoding one or more antigenic polypeptides and the other encoding the flagellin polypeptide. The at least two RNA polynucleotides may be co-formulated in a carrier such as a lipid nanoparticle.

Therapeutic and Prophylactic Compositions

Provided herein are compositions (*e.g.*, pharmaceutical compositions), methods, kits and reagents for prevention, treatment or diagnosis of VZV in humans and other mammals, for example. VZV RNA (*e.g.*, mRNA) vaccines can be used as therapeutic or prophylactic agents. They may be used in medicine to prevent and/or treat infectious disease. In some embodiments, the VZV vaccines of the invention can be envisioned for use in the priming of immune effector cells, for example, to activate peripheral blood mononuclear cells (PBMCs) *ex vivo*, which are then infused (re-infused) into a subject.

In exemplary embodiments, a VZV vaccine containing RNA polynucleotides as described herein can be administered to a subject (*e.g.*, a mammalian subject, such as a human subject), and the RNA polynucleotides are translated *in vivo* to produce an antigenic polypeptide.

The VZV RNA vaccines may be induced for translation of a polypeptide (*e.g.*, antigen or immunogen) in a cell, tissue or organism. In exemplary embodiments, such translation occurs *in vivo*, although there can be envisioned embodiments where such translation occurs *ex vivo*, in culture or *in vitro*. In exemplary embodiments, the cell, tissue or organism is contacted with an effective amount of a composition containing a VZV RNA vaccine that contains a polynucleotide that has at least one a translatable region encoding an antigenic polypeptide.

An “effective amount” of the VZV RNA vaccine is provided based, at least in part, on the target tissue, target cell type, means of administration, physical characteristics of the polynucleotide (*e.g.*, size, and extent of modified nucleosides) and other components of the VZV RNA vaccine, and other determinants. In general, an effective amount of the VZV

RNA vaccine composition provides an induced or boosted immune response as a function of antigen production in the cell. In general, an effective amount of the VZV RNA vaccine containing RNA polynucleotides having at least one chemical modifications are preferably more efficient than a composition containing a corresponding unmodified polynucleotide encoding the same antigen or a peptide antigen. Increased antigen production may be demonstrated by increased cell transfection (the percentage of cells transfected with the RNA vaccine), increased protein translation from the polynucleotide, decreased nucleic acid degradation (as demonstrated, for example, by increased duration of protein translation from a modified polynucleotide), or altered antigen specific immune response of the host cell.

The term "pharmaceutical composition" refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use *in vivo* or *ex vivo*. A "pharmaceutically acceptable carrier," after administered to or upon a subject, does not cause undesirable physiological effects. The carrier in the pharmaceutical composition must be "acceptable" also in the sense that it is compatible with the active ingredient and can be capable of stabilizing it. One or more solubilizing agents can be utilized as pharmaceutical carriers for delivery of an active agent. Examples of a pharmaceutically acceptable carrier include, but are not limited to, biocompatible vehicles, adjuvants, additives, and diluents to achieve a composition usable as a dosage form. Examples of other carriers include colloidal silicon oxide, magnesium stearate, cellulose, and sodium lauryl sulfate. Additional suitable pharmaceutical carriers and diluents, as well as pharmaceutical necessities for their use, are described in Remington's Pharmaceutical Sciences.

In some embodiments, RNA vaccines (including polynucleotides and their encoded polypeptides) in accordance with the present disclosure may be used for treatment or prevention of VZV.

VZV RNA vaccines may be administered prophylactically or therapeutically as part of an active immunization scheme to healthy individuals or early in infection during the incubation phase or during active infection after onset of symptoms. In some embodiments, the amount of RNA vaccines of the present disclosure provided to a cell, a tissue or a subject may be an amount effective for immune prophylaxis.

VZV RNA (*e.g.*, mRNA) vaccines may be administered with other prophylactic or therapeutic compounds. As a non-limiting example, a prophylactic or therapeutic compound may be an adjuvant or a booster. As used herein, when referring to a prophylactic composition, such as a vaccine, the term "booster" refers to an extra administration of the prophylactic (vaccine) composition. A booster (or booster vaccine) may be given after an

earlier administration of the prophylactic composition. The time of administration between the initial administration of the prophylactic composition and the booster may be, but is not limited to, 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes, 10 minutes, 15 minutes, 20 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 36 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 10 days, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 18 months, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years, 17 years, 18 years, 19 years, 20 years, 25 years, 30 years, 35 years, 40 years, 45 years, 50 years, 55 years, 60 years, 65 years, 70 years, 75 years, 80 years, 85 years, 90 years, 95 years or more than 99 years. In exemplary embodiments, the time of administration between the initial administration of the prophylactic composition and the booster may be, but is not limited to, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 6 months or 1 year.

In some embodiments, VZV RNA vaccines may be administered intramuscularly, intranasally or intradermally, similarly to the administration of inactivated vaccines known in the art.

The VZV RNA vaccines may be utilized in various settings depending on the prevalence of the infection or the degree or level of unmet medical need. As a non-limiting example, the RNA vaccines may be utilized to treat and/or prevent a variety of infectious disease. RNA vaccines have superior properties in that they produce much larger antibody titers and produce responses early than commercially available anti-virals.

Provided herein are pharmaceutical compositions including VZV RNA vaccines and RNA vaccine compositions and/or complexes optionally in combination with one or more pharmaceutically acceptable excipients.

VZV RNA (*e.g.*, mRNA) vaccines may be formulated or administered alone or in conjunction with one or more other components. For instance, VZV RNA vaccines (vaccine compositions) may comprise other components including, but not limited to, adjuvants.

In some embodiments, VZV RNA vaccines do not include an adjuvant (they are adjuvant free).

VZV RNA (*e.g.*, mRNA) vaccines may be formulated or administered in combination with one or more pharmaceutically-acceptable excipients. In some embodiments, vaccine compositions comprise at least one additional active substances, such as, for example, a

therapeutically-active substance, a prophylactically-active substance, or a combination of both. Vaccine compositions may be sterile, pyrogen-free or both sterile and pyrogen-free. General considerations in the formulation and/or manufacture of pharmaceutical agents, such as vaccine compositions, may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference in its entirety).

In some embodiments, VZV RNA vaccines are administered to humans, human patients or subjects. For the purposes of the present disclosure, the phrase “active ingredient” generally refers to the RNA vaccines or the polynucleotides contained therein, for example, RNA polynucleotides (*e.g.*, mRNA polynucleotides) encoding antigenic polypeptides.

Formulations of the vaccine compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient (*e.g.*, mRNA polynucleotide) into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the disclosure will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100%, *e.g.*, between 0.5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

VZV RNA vaccines can be formulated using one or more excipients to: (1) increase stability; (2) increase cell transfection; (3) permit the sustained or delayed release (*e.g.*, from a depot formulation); (4) alter the biodistribution (*e.g.*, target to specific tissues or cell types); (5) increase the translation of encoded protein *in vivo*; and/or (6) alter the release profile of encoded protein (antigen) *in vivo*. In addition to traditional excipients such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, excipients can include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with VZV RNA vaccines (*e.g.*, for transplantation into a subject), hyaluronidase, nanoparticle mimics and combinations thereof.

Stabilizing Elements

Naturally-occurring eukaryotic mRNA molecules have been found to contain stabilizing elements, including, but not limited to untranslated regions (UTR) at their 5'-end (5'UTR) and/or at their 3'-end (3'UTR), in addition to other structural features, such as a 5'-cap structure or a 3'-poly(A) tail. Both the 5'UTR and the 3'UTR are typically transcribed from the genomic DNA and are elements of the premature mRNA. Characteristic structural features of mature mRNA, such as the 5'-cap and the 3'-poly(A) tail are usually added to the transcribed (premature) mRNA during mRNA processing. The 3'-poly(A) tail is typically a stretch of adenine nucleotides added to the 3'-end of the transcribed mRNA. It can comprise up to about 400 adenine nucleotides. In some embodiments the length of the 3'-poly(A) tail may be an essential element with respect to the stability of the individual mRNA.

In some embodiments the RNA vaccine may include one or more stabilizing elements. Stabilizing elements may include for instance a histone stem-loop. A stem-loop binding protein (SLBP), a 32 kDa protein has been identified. It is associated with the histone stem-loop at the 3'-end of the histone messages in both the nucleus and the cytoplasm. Its expression level is regulated by the cell cycle; it peaks during the S-phase, when histone mRNA levels are also elevated. The protein has been shown to be essential for efficient 3'-end processing of histone pre-mRNA by the U7 snRNP. SLBP continues to be associated with the stem-loop after processing, and then stimulates the translation of mature histone mRNAs into histone proteins in the cytoplasm. The RNA binding domain of SLBP is conserved through metazoa and protozoa; its binding to the histone stem-loop depends on the structure of the loop. The minimum binding site includes at least three nucleotides 5' and two nucleotides 3' relative to the stem-loop.

In some embodiments, the RNA vaccines include a coding region, at least one histone stem-loop, and optionally, a poly(A) sequence or polyadenylation signal. The poly(A) sequence or polyadenylation signal generally should enhance the expression level of the encoded protein. The encoded protein, in some embodiments, is not a histone protein, a reporter protein (*e.g.* Luciferase, GFP, EGFP, β -Galactosidase, EGFP), or a marker or selection protein (*e.g.* alpha-Globin, Galactokinase and Xanthine:guanine phosphoribosyl transferase (GPT)).

In some embodiments, the combination of a poly(A) sequence or polyadenylation signal and at least one histone stem-loop, even though both represent alternative mechanisms in nature, acts synergistically to increase the protein expression beyond the level observed with either of the individual elements. It has been found that the synergistic effect of the combination of poly(A) and at least one histone stem-loop does not depend on the order of the elements or the length of the poly(A) sequence.

In some embodiments, the RNA vaccine does not comprise a histone downstream element (HDE). "Histone downstream element" (HDE) includes a purine-rich polynucleotide stretch of approximately 15 to 20 nucleotides 3' of naturally occurring stem-loops, representing the binding site for the U7 snRNA, which is involved in processing of histone pre-mRNA into mature histone mRNA. In some embodiments, the nucleic acid does not include an intron.

In some embodiments, the RNA vaccine may or may not contain an enhancer and/or promoter sequence, which may be modified or unmodified or which may be activated or inactivated. In some embodiments, the histone stem-loop is generally derived from histone genes, and includes an intramolecular base pairing of two neighbored partially or entirely reverse complementary sequences separated by a spacer, consisting of a short sequence, which forms the loop of the structure. The unpaired loop region is typically unable to base pair with either of the stem loop elements. It occurs more often in RNA, as is a key component of many RNA secondary structures, but may be present in single-stranded DNA as well. Stability of the stem-loop structure generally depends on the length, number of mismatches or bulges, and base composition of the paired region. In some embodiments, wobble base pairing (non-Watson-Crick base pairing) may result. In some embodiments, the at least one histone stem-loop sequence comprises a length of 15 to 45 nucleotides.

In other embodiments the RNA vaccine may have one or more AU-rich sequences removed. These sequences, sometimes referred to as AURES are destabilizing sequences found in the 3'UTR. The AURES may be removed from the RNA vaccines. Alternatively the AURES may remain in the RNA vaccine.

Nanoparticle Formulations

In some embodiments, VZV RNA (e.g., mRNA) vaccines are formulated in a nanoparticle. In some embodiments, VZV RNA vaccines are formulated in a lipid nanoparticle. In some embodiments, VZV RNA vaccines are formulated in a lipid-polycation complex, referred to as a cationic lipid nanoparticle. The formation of the lipid nanoparticle may be accomplished by methods known in the art and/or as described in U.S. Publication No. 2012/0178702, herein incorporated by reference in its entirety. As a non-limiting example, the polycation may include a cationic peptide or a polypeptide such as, but not limited to, polylysine, polyornithine and/or polyarginine and the cationic peptides described in International Publication No. WO2012/013326 or U.S. Publication No. US2013/0142818; each of which is herein incorporated by reference in its entirety. In some embodiments, VZV

RNA vaccines are formulated in a lipid nanoparticle that includes a non-cationic lipid such as, but not limited to, cholesterol or dioleoyl phosphatidylethanolamine (DOPE).

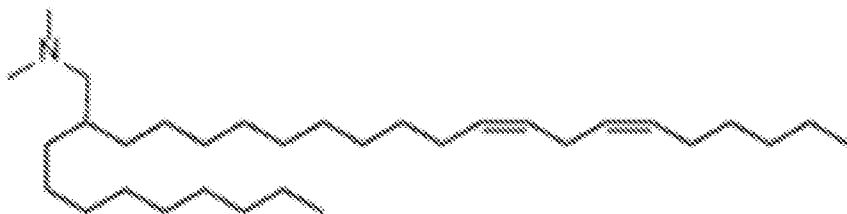
A lipid nanoparticle formulation may be influenced by, but not limited to, the selection of the cationic lipid component, the degree of cationic lipid saturation, the nature of the PEGylation, ratio of all components and biophysical parameters such as size. In one example by Semple *et al.* (*Nature Biotech.* 2010 28:172-176; herein incorporated by reference in its entirety), the lipid nanoparticle formulation is composed of 57.1 % cationic lipid, 7.1% dipalmitoylphosphatidylcholine, 34.3 % cholesterol, and 1.4% PEG-c-DMA. As another example, changing the composition of the cationic lipid was shown to more effectively deliver siRNA to various antigen presenting cells (Basha *et al.* *Mol Ther.* 2011 19:2186-2200; herein incorporated by reference in its entirety).

In some embodiments, lipid nanoparticle formulations may comprise 35 to 45% cationic lipid, 40% to 50% cationic lipid, 50% to 60% cationic lipid and/or 55% to 65% cationic lipid. In some embodiments, the ratio of lipid to RNA (*e.g.*, mRNA) in lipid nanoparticles may be 5:1 to 20:1, 10:1 to 25:1, 15:1 to 30:1 and/or at least 30:1.

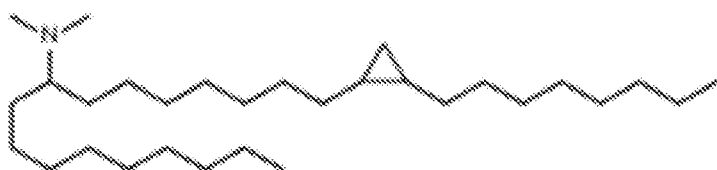
In some embodiments, the ratio of PEG in the lipid nanoparticle formulations may be increased or decreased and/or the carbon chain length of the PEG lipid may be modified from C14 to C18 to alter the pharmacokinetics and/or biodistribution of the lipid nanoparticle formulations. As a non-limiting example, lipid nanoparticle formulations may contain 0.5% to 3.0%, 1.0% to 3.5%, 1.5% to 4.0%, 2.0% to 4.5%, 2.5% to 5.0% and/or 3.0% to 6.0% of the lipid molar ratio of PEG-c-DOMG (R-3-[(ω -methoxy-poly(ethyleneglycol)2000)carbamoyl]-1,2-dimyristyloxypropyl-3-amine) (also referred to herein as PEG-DOMG) as compared to the cationic lipid, DSPC and cholesterol. In some embodiments, the PEG-c-DOMG may be replaced with a PEG lipid such as, but not limited to, PEG-DSG (1,2-Distearoyl-sn-glycerol, methoxypolyethylene glycol), PEG-DMG (1,2-Dimyristoyl-sn-glycerol) and/or PEG-DPG (1,2-Dipalmitoyl-sn-glycerol, methoxypolyethylene glycol). The cationic lipid may be selected from any lipid known in the art such as, but not limited to, DLin-MC3-DMA, DLin-DMA, C12-200 and DLin-KC2-DMA.

In some embodiments, a VZV RNA (*e.g.*, mRNA) vaccine formulation is a nanoparticle that comprises at least one lipid. The lipid may be selected from, but is not limited to, DLin-DMA, DLin-K-DMA, 98N12-5, C12-200, DLin-MC3-DMA, DLin-KC2-DMA, DODMA, PLGA, PEG, PEG-DMG, (12Z,15Z)-N,N-dimethyl-2-nonylhenicos-12,15-dien-1-amine, N,N-dimethyl-1-[(1S,2R)-2-octylcyclopropyl]heptadecan-8-amine, PEGylated lipids and amino alcohol lipids.

In some embodiments, the cationic lipid is



In some embodiments, the cationic lipid is



5 In some embodiments, the lipid may be a cationic lipid such as, but not limited to, DLin-DMA, DLin-D-DMA, DLin-MC3-DMA, DLin-KC2-DMA, DODMA and amino alcohol lipids. The amino alcohol cationic lipid may be the lipids described in and/or made by the methods described in U.S. Publication No. US2013/0150625, herein incorporated by reference in its entirety. As a non-limiting example, the cationic lipid may be 2-amino-3-
 10 [(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-2-{[(9Z,2Z)-octadeca-9,12-dien-1-yloxy]methyl}propan-1-ol (Compound 1 in US2013/0150625); 2-amino-3-[(9Z)-octadec-9-en-1-yloxy]-2-{[(9Z)-octadec-9-en-1-yloxy]methyl}propan-1-ol (Compound 2 in US2013/0150625); 2-amino-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-2-
 15 [(octyloxy)methyl]propan-1-ol (Compound 3 in US2013/0150625); and 2-(dimethylamino)-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]-2-{[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]methyl}propan-1-ol (Compound 4 in US2013/0150625); or any pharmaceutically acceptable salt or stereoisomer thereof.

Lipid nanoparticle formulations typically comprise a lipid, in particular, an ionizable cationic lipid, for example, 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-
 20 DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), or di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, and further comprise a neutral lipid, a sterol and a molecule capable of reducing particle aggregation, for example a PEG or PEG-modified lipid.

In some embodiments, a lipid nanoparticle formulation consists essentially of (i) at
 25 least one lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate; (ii) a neutral lipid selected from DSPC, DPPC, POPC, DOPE and SM; (iii) a sterol, *e.g.*,

cholesterol; and (iv) a PEG-lipid, e.g., PEG-DMG or PEG-cDMA, in a molar ratio of 20-60% cationic lipid: 5-25% neutral lipid (non-cationic lipid): 25-55% sterol; 0.5-15% PEG-lipid.

In some embodiments, a lipid nanoparticle formulation includes 25% to 75% on a molar basis of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-
 5 dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, e.g., 35 to 65%, 45 to 65%, 60%, 57.5%, 50% or 40% on a molar basis.

In some embodiments, a lipid nanoparticle formulation includes 0.5% to 15% on a
 10 molar basis of the neutral lipid, e.g., 3 to 12%, 5 to 10% or 15%, 10%, or 7.5% on a molar basis. Examples of neutral lipids include, without limitation, DSPC, POPC, DPPC, DOPE and SM. In some embodiments, the formulation includes 5% to 50% on a molar basis of the sterol (e.g., 15 to 45%, 20 to 40%, 40%, 38.5%, 35%, or 31% on a molar basis. A non-limiting example of a sterol is cholesterol. In some embodiments, a lipid nanoparticle
 15 formulation includes 0.5% to 20% on a molar basis of the PEG or PEG-modified lipid (e.g., 0.5 to 10%, 0.5 to 5%, 1.5%, 0.5%, 1.5%, 3.5%, or 5% on a molar basis. In some embodiments, a PEG or PEG modified lipid comprises a PEG molecule of an average molecular weight of 2,000 Da. In some embodiments, a PEG or PEG modified lipid comprises a PEG molecule of an average molecular weight of less than 2,000, for example
 20 around 1,500 Da, around 1,000 Da, or around 500 Da. Non-limiting examples of PEG-modified lipids include PEG-distearoyl glycerol (PEG-DMG) (also referred herein as PEG-C14 or C14-PEG), PEG-cDMA (further discussed in Reyes *et al. J. Controlled Release*, 107, 276-287 (2005) the content of which is herein incorporated by reference in its entirety).

In some embodiments, lipid nanoparticle formulations include 25-75% of a cationic
 25 lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, 0.5-15% of the neutral lipid, 5-50% of the sterol, and 0.5-20% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 35-65% of a cationic
 30 lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, 3-12% of the neutral lipid, 15-45% of the sterol, and 0.5-10% of the PEG or PEG-modified
 35 lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 45-65% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, 5-10% of the neutral lipid, 25-40% of the sterol, and 0.5-10% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 60% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, 7.5% of the neutral lipid, 31 % of the sterol, and 1.5% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 50% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, 10% of the neutral lipid, 38.5 % of the sterol, and 1.5% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 50% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, 10% of the neutral lipid, 35 % of the sterol, 4.5% or 5% of the PEG or PEG-modified lipid, and 0.5% of the targeting lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 40% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, 15% of the neutral lipid, 40% of the sterol, and 5% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 57.2% of a cationic lipid selected from the group consisting of 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, 7.1% of the neutral lipid, 34.3% of the sterol, and 1.4% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations include 57.5% of a cationic lipid selected from the PEG lipid is PEG-cDMA (PEG-cDMA is further discussed in Reyes et al. (J. Controlled Release, 107, 276-287 (2005), the content of which is herein incorporated by reference in its entirety), 7.5% of the neutral lipid, 31.5 % of the sterol, and 3.5% of the PEG or PEG-modified lipid on a molar basis.

In some embodiments, lipid nanoparticle formulations consists essentially of a lipid mixture in molar ratios of 20-70% cationic lipid: 5-45% neutral lipid: 20-55% cholesterol: 0.5-15% PEG-modified lipid. In some embodiments, lipid nanoparticle formulations consists essentially of a lipid mixture in a molar ratio of 20-60% cationic lipid: 5-25% neutral lipid (non-cationic lipid): 25-55% cholesterol: 0.5-15% PEG-modified lipid.

In some embodiments, the molar lipid ratio is 50/10/38.5/1.5 (mol% cationic lipid/neutral lipid, e.g., DSPC/Chol/PEG-modified lipid, e.g., PEG-DMG, PEG-DSG or PEG-DPG), 57.2/7.1134.3/1.4 (mol% cationic lipid/ neutral lipid, e.g., DPPC/Chol/ PEG-modified lipid, e.g., PEG-cDMA), 40/15/40/5 (mol% cationic lipid/ neutral lipid, e.g., DSPC/Chol/ PEG-modified lipid, e.g., PEG-DMG), 50/10/35/4.5/0.5 (mol% cationic lipid/ neutral lipid, e.g., DSPC/Chol/ PEG-modified lipid, e.g., PEG-DSG), 50/10/35/5 (cationic lipid/ neutral lipid, e.g., DSPC/Chol/ PEG-modified lipid, e.g., PEG-DMG), 40/10/40/10 (mol% cationic lipid/ neutral lipid, e.g., DSPC/Chol/ PEG-modified lipid, e.g., PEG-DMG or PEG-cDMA), 35/15/40/10 (mol% cationic lipid/ neutral lipid, e.g., DSPC/Chol/ PEG-modified lipid, e.g., PEG-DMG or PEG-cDMA) or 52/13/30/5 (mol% cationic lipid/ neutral lipid, e.g., DSPC/Chol/ PEG-modified lipid, e.g., PEG-DMG or PEG-cDMA).

Non-limiting examples of lipid nanoparticle compositions and methods of making them are described, for example, in Semple *et al.* (2010) *Nat. Biotechnol.* 28:172-176; Jayarama *et al.* (2012), *Angew. Chem. Int. Ed.*, 51: 8529–8533; and Maier *et al.* (2013) *Molecular Therapy* 21, 1570-1578 (the contents of each of which are incorporated herein by reference in their entirety).

In some embodiments, lipid nanoparticle formulations may comprise a cationic lipid, a PEG lipid and a structural lipid and optionally comprise a non-cationic lipid. As a non-limiting example, a lipid nanoparticle may comprise 40-60% of cationic lipid, 5-15% of a non-cationic lipid, 1-2% of a PEG lipid and 30-50% of a structural lipid. As another non-limiting example, the lipid nanoparticle may comprise 50% cationic lipid, 10% non-cationic lipid, 1.5% PEG lipid and 38.5% structural lipid. As yet another non-limiting example, a lipid nanoparticle may comprise 55% cationic lipid, 10% non-cationic lipid, 2.5% PEG lipid and 32.5% structural lipid. In some embodiments, the cationic lipid may be any cationic lipid

described herein such as, but not limited to, DLin-KC2-DMA, DLin-MC3-DMA and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate.

In some embodiments, the lipid nanoparticle formulations described herein may be 4 component lipid nanoparticles. The lipid nanoparticle may comprise a cationic lipid, a non-cationic lipid, a PEG lipid and a structural lipid. As a non-limiting example, the lipid nanoparticle may comprise 40-60% of cationic lipid, 5-15% of a non-cationic lipid, 1-2% of a PEG lipid and 30-50% of a structural lipid. As another non-limiting example, the lipid nanoparticle may comprise 50% cationic lipid, 10% non-cationic lipid, 1.5% PEG lipid and 38.5% structural lipid. As yet another non-limiting example, the lipid nanoparticle may comprise 55% cationic lipid, 10% non-cationic lipid, 2.5% PEG lipid and 32.5% structural lipid. In some embodiments, the cationic lipid may be any cationic lipid described herein such as, but not limited to, DLin-KC2-DMA, DLin-MC3-DMA and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate.

In some embodiments, the lipid nanoparticle formulations described herein may comprise a cationic lipid, a non-cationic lipid, a PEG lipid and a structural lipid. As a non-limiting example, the lipid nanoparticle comprise 50% of the cationic lipid DLin-KC2-DMA, 10% of the non-cationic lipid DSPC, 1.5% of the PEG lipid PEG-DOMG and 38.5% of the structural lipid cholesterol. As a non-limiting example, the lipid nanoparticle comprise 50% of the cationic lipid DLin-MC3-DMA, 10% of the non-cationic lipid DSPC, 1.5% of the PEG lipid PEG-DOMG and 38.5% of the structural lipid cholesterol. As a non-limiting example, the lipid nanoparticle comprise 50% of the cationic lipid DLin-MC3-DMA, 10% of the non-cationic lipid DSPC, 1.5% of the PEG lipid PEG-DMG and 38.5% of the structural lipid cholesterol. As yet another non-limiting example, the lipid nanoparticle comprise 55% of the cationic lipid di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate, 10% of the non-cationic lipid DSPC, 2.5% of the PEG lipid PEG-DMG and 32.5% of the structural lipid cholesterol.

Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a vaccine composition may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered. For example, the composition may comprise between 0.1% and 99% (w/w) of the active ingredient. By way of example, the composition may comprise between 0.1% and 100%, *e.g.*, between 0.5 and 50%, between 1-30%, between 5-80%, at least 80% (w/w) active ingredient.

In some embodiments, the RNA vaccine composition may comprise the polynucleotide described herein, formulated in a lipid nanoparticle comprising MC3,

Cholesterol, DSPC and PEG2000-DMG, the buffer trisodium citrate, sucrose and water for injection. As a non-limiting example, the composition comprises: 2.0 mg/mL of drug substance (*e.g.*, polynucleotides encoding VZV), 21.8 mg/mL of MC3, 10.1 mg/mL of cholesterol, 5.4 mg/mL of DSPC, 2.7 mg/mL of PEG2000-DMG, 5.16 mg/mL of trisodium citrate, 71 mg/mL of sucrose and 1.0 mL of water for injection.

In some embodiments, a nanoparticle (*e.g.*, a lipid nanoparticle) has a mean diameter of 10-500 nm, 20-400 nm, 30-300 nm, 40-200 nm. In some embodiments, a nanoparticle (*e.g.*, a lipid nanoparticle) has a mean diameter of 50-150 nm, 50-200 nm, 80-100 nm or 80-200 nm.

Liposomes, Lipoplexes, and Lipid Nanoparticles

In some embodiments, the RNA vaccine pharmaceutical compositions may be formulated in liposomes such as, but not limited to, DiLa2 liposomes (Marina Biotech, Bothell, WA), SMARTICLES® (Marina Biotech, Bothell, WA), neutral DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) based liposomes (*e.g.*, siRNA delivery for ovarian cancer (Landen et al. Cancer Biology & Therapy 2006 5(12)1708-1713); herein incorporated by reference in its entirety) and hyaluronan-coated liposomes (Quiet Therapeutics, Israel).

In some embodiments, the RNA vaccines may be formulated in a lyophilized gel-phase liposomal composition as described in U.S. Publication No. US2012/0060293, herein incorporated by reference in its entirety.

The nanoparticle formulations may comprise a phosphate conjugate. The phosphate conjugate may increase *in vivo* circulation times and/or increase the targeted delivery of the nanoparticle. Phosphate conjugates for use with the present invention may be made by the methods described in International Publication No. WO2013/033438 or U.S. Publication No. US2013/0196948, the content of each of which is herein incorporated by reference in its entirety. As a non-limiting example, the phosphate conjugates may include a compound of any one of the formulas described in International Publication No. WO2013/033438, herein incorporated by reference in its entirety.

The nanoparticle formulation may comprise a polymer conjugate. The polymer conjugate may be a water soluble conjugate. The polymer conjugate may have a structure as described in U.S. Publication No. 2013/0059360, the content of which is herein incorporated by reference in its entirety. In some aspects, polymer conjugates with the polynucleotides of the present invention may be made using the methods and/or segmented polymeric reagents described in U.S. Publication No. 2013/0072709, herein incorporated by reference in its entirety.

In other aspects, the polymer conjugate may have pendant side groups comprising ring moieties

such as, but not limited to, the polymer conjugates described in U.S. Publication No. US2013/0196948, the contents of which is herein incorporated by reference in its entirety.

The nanoparticle formulations may comprise a conjugate to enhance the delivery of nanoparticles of the present invention in a subject. Further, the conjugate may inhibit
5 phagocytic clearance of the nanoparticles in a subject. In some aspects, the conjugate may be a “self” peptide designed from the human membrane protein CD47 (*e.g.*, the “self” particles described by Rodriguez *et al* (*Science* 2013, 339, 971-975), herein incorporated by reference in its entirety). As shown by Rodriguez *et al.* the self peptides delayed macrophage-mediated clearance of nanoparticles which enhanced delivery of the nanoparticles. In other aspects, the
10 conjugate may be the membrane protein CD47 (*e.g.*, *see* Rodriguez *et al.* *Science* 2013, 339, 971-975, herein incorporated by reference in its entirety). Rodriguez *et al.* showed that, similarly to “self” peptides, CD47 can increase the circulating particle ratio in a subject as compared to scrambled peptides and PEG coated nanoparticles.

In some embodiments, the RNA vaccines of the present invention are formulated in
15 nanoparticles which comprise a conjugate to enhance the delivery of the nanoparticles of the present invention in a subject. The conjugate may be the CD47 membrane or the conjugate may be derived from the CD47 membrane protein, such as the “self” peptide described previously. In other embodiments, the nanoparticle may comprise PEG and a conjugate of CD47 or a derivative thereof. In yet other embodiments, the nanoparticle may comprise both the “self”
20 peptide described above and the membrane protein CD47.

In some embodiments, a “self” peptide and/or CD47 protein may be conjugated to a virus-like particle or pseudovirion, as described herein for delivery of the RNA vaccines of the present invention.

In other embodiments, RNA vaccine pharmaceutical compositions comprising the
25 polynucleotides of the present invention and a conjugate, which may have a degradable linkage. Non-limiting examples of conjugates include an aromatic moiety comprising an ionizable hydrogen atom, a spacer moiety, and a water-soluble polymer. As a non-limiting example, pharmaceutical compositions comprising a conjugate with a degradable linkage and methods for delivering such pharmaceutical compositions are described in U.S. Publication No.
30 US2013/0184443, the content of which is herein incorporated by reference in its entirety.

The nanoparticle formulations may be a carbohydrate nanoparticle comprising a carbohydrate carrier and a RNA vaccine. As a non-limiting example, the carbohydrate carrier may include, but is not limited to, an anhydride-modified phytoglycogen or glycogen-type material, phytoglycogen octenyl succinate, phytoglycogen beta-dextrin, anhydride-modified

phytoglycogen beta-dextrin. (*See e.g.*, International Publication No. WO2012/109121, the content of which is herein incorporated by reference in its entirety).

Nanoparticle formulations of the present invention may be coated with a surfactant or polymer in order to improve the delivery of the particle. In some embodiments, the nanoparticle may be coated with a hydrophilic coating such as, but not limited to, PEG coatings and/or coatings that have a neutral surface charge. The hydrophilic coatings may help to deliver nanoparticles with larger payloads such as, but not limited to, RNA vaccines within the central nervous system. As a non-limiting example nanoparticles comprising a hydrophilic coating and methods of making such nanoparticles are described in U.S. Publication No.

US2013/0183244, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the lipid nanoparticles of the present invention may be hydrophilic polymer particles. Non-limiting examples of hydrophilic polymer particles and methods of making hydrophilic polymer particles are described in U.S. Publication No. US2013/0210991, the content of which is herein incorporated by reference in its entirety.

In other embodiments, the lipid nanoparticles of the present invention may be hydrophobic polymer particles.

Lipid nanoparticle formulations may be improved by replacing the cationic lipid with a biodegradable cationic lipid which is known as a rapidly eliminated lipid nanoparticle (reLNP). Ionizable cationic lipids, such as, but not limited to, DLinDMA, DLin-KC2-DMA, and DLin-MC3-DMA, have been shown to accumulate in plasma and tissues over time and may be a potential source of toxicity. The rapid metabolism of the rapidly eliminated lipids can improve the tolerability and therapeutic index of the lipid nanoparticles by an order of magnitude from a 1 mg/kg dose to a 10 mg/kg dose in rat. Inclusion of an enzymatically degraded ester linkage can improve the degradation and metabolism profile of the cationic component, while still maintaining the activity of the reLNP formulation. The ester linkage can be internally located within the lipid chain or it may be terminally located at the terminal end of the lipid chain. The internal ester linkage may replace any carbon in the lipid chain.

In some embodiments, the internal ester linkage may be located on either side of the saturated carbo.

In some embodiments, an immune response may be elicited by delivering a lipid nanoparticle which may include a nanospecies, a polymer and an immunogen. (U.S. Publication No. 2012/0189700 and International Publication No. WO2012/099805, each of which is herein incorporated by reference in its entirety).

The polymer may encapsulate the nanospecies or partially encapsulate the nanospecies. The immunogen may be a recombinant protein, a modified RNA and/or a

polynucleotide described herein. In some embodiments, the lipid nanoparticle may be formulated for use in a vaccine such as, but not limited to, against a pathogen.

Lipid nanoparticles may be engineered to alter the surface properties of particles so the lipid nanoparticles may penetrate the mucosal barrier. Mucus is located on mucosal tissue such as, but not limited to, oral (*e.g.*, the buccal and esophageal membranes and tonsil tissue), ophthalmic, gastrointestinal (*e.g.*, stomach, small intestine, large intestine, colon, rectum), nasal, respiratory (*e.g.*, nasal, pharyngeal, tracheal and bronchial membranes), genital (*e.g.*, vaginal, cervical and urethral membranes). Nanoparticles larger than 10-200 nm which are preferred for higher drug encapsulation efficiency and the ability to provide the sustained delivery of a wide array of drugs have been thought to be too large to rapidly diffuse through mucosal barriers. Mucus is continuously secreted, shed, discarded or digested and recycled so most of the trapped particles may be removed from the mucosal tissue within seconds or within a few hours. Large polymeric nanoparticles (200 nm to 500 nm in diameter) which have been coated densely with a low molecular weight polyethylene glycol (PEG) diffused through mucus only 4 to 6-fold lower than the same particles diffusing in water (Lai *et al.* *PNAS* 2007 104(5):1482-487; Lai *et al.* *Adv Drug Deliv Rev.* 2009 61(2): 158-171; each of which is herein incorporated by reference in its entirety). The transport of nanoparticles may be determined using rates of permeation and/or fluorescent microscopy techniques including, but not limited to, fluorescence recovery after photobleaching (FRAP) and high resolution multiple particle tracking (MPT). As a non-limiting example, compositions which can penetrate a mucosal barrier may be made as described in U.S. Patent No. 8,241,670 or International Publication No. WO2013/110028, the content of each of which is herein incorporated by reference in its entirety.

The lipid nanoparticle engineered to penetrate mucus may comprise a polymeric material (*e.g.*, a polymeric core) and/or a polymer-vitamin conjugate and/or a tri-block co-polymer. The polymeric material may include, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, poly(styrenes), polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. The polymeric material may be biodegradable and/or biocompatible. Non-limiting examples of biocompatible polymers are described in International Publication No. WO2013/116804, the content of which is herein incorporated by reference in its entirety. The polymeric material may additionally be irradiated. As a non-limiting example, the polymeric material may be gamma irradiated (*see e.g.*, International Publication No. WO2012/082165, herein incorporated by reference in its entirety). Non-limiting examples of specific polymers include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA),

poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacralate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate, polyoxymethylene, poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), PEG-PLGA-PEG and trimethylene carbonate, polyvinylpyrrolidone. The lipid nanoparticle may be coated or associated with a copolymer such as, but not limited to, a block co-polymer (such as a branched polyether-polyamide block copolymer described in International Publication No. WO2013/012476, herein incorporated by reference in its entirety), and (poly(ethylene glycol))-(poly(propylene oxide))-(poly(ethylene glycol)) triblock copolymer (*see e.g.*, U.S. Publication 2012/0121718, U.S. Publication 2010/0003337 and U.S. Patent No. 8,263,665, each of which is herein incorporated by reference in its entirety). The co-polymer may be a polymer that is generally regarded as safe (GRAS) and the formation of the lipid nanoparticle may be in such a way that no new chemical entities are created. For example, the lipid nanoparticle may comprise poloxamers coating PLGA nanoparticles without forming new chemical entities which are still able to rapidly penetrate human mucus (Yang *et al. Angew. Chem. Int. Ed.* 2011 50:25972600, the content of which is herein incorporated by reference in its entirety). A non-limiting scalable method to produce nanoparticles which can penetrate human mucus is described by Xu *et al.* (*see e.g.*, *J Control*

Release 2013, 170(2):279-86, the content of which is herein incorporated by reference in its entirety).

The vitamin of the polymer-vitamin conjugate may be vitamin E. The vitamin portion of the conjugate may be substituted with other suitable components such as, but not limited to, vitamin A, vitamin E, other vitamins, cholesterol, a hydrophobic moiety, or a hydrophobic component of other surfactants (*e.g.*, sterol chains, fatty acids, hydrocarbon chains and alkylene oxide chains).

In some embodiments, the RNA (*e.g.*, mRNA) vaccine pharmaceutical compositions may be formulated in liposomes such as, but not limited to, DiLa2 liposomes (Marina Biotech, Bothell, WA), SMARTICLES® (Marina Biotech, Bothell, WA), neutral DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) based liposomes (*e.g.*, siRNA delivery for ovarian cancer (Landen *et al. Cancer Biology & Therapy* 2006 5(12)1708-1713, herein incorporated by reference in its entirety)) and hyaluronan-coated liposomes (Quiet Therapeutics, Israel).

In some embodiments, the RNA vaccines may be formulated in a lyophilized gel-phase liposomal composition as described in U.S. Publication No. US2012/0060293, herein incorporated by reference in its entirety.

The nanoparticle formulations may comprise a phosphate conjugate. The phosphate conjugate may increase *in vivo* circulation times and/or increase the targeted delivery of the nanoparticle. Phosphate conjugates for use with the present invention may be made by the methods described in International Publication No. WO2013/033438 or U.S. Publication No. 2013/0196948, the content of each of which is herein incorporated by reference in its entirety. As a non-limiting example, the phosphate conjugates may include a compound of any one of the formulas described in International Publication No. WO2013/033438, herein incorporated by reference in its entirety.

The nanoparticle formulation may comprise a polymer conjugate. The polymer conjugate may be a water soluble conjugate. The polymer conjugate may have a structure as described in U.S. Application No. 2013/0059360, the content of which is herein incorporated by reference in its entirety. In some aspects, polymer conjugates with the polynucleotides of the present invention may be made using the methods and/or segmented polymeric reagents described in U.S. Patent Application No. 2013/0072709, herein incorporated by reference in its entirety. In other aspects, the polymer conjugate may have pendant side groups comprising ring moieties such as, but not limited to, the polymer conjugates described in U.S. Publication No. US2013/0196948, the content of which is herein incorporated by reference in its entirety.

The nanoparticle formulations may comprise a conjugate to enhance the delivery of nanoparticles of the present invention in a subject. Further, the conjugate may inhibit

phagocytic clearance of the nanoparticles in a subject. In some aspects, the conjugate may be a “self” peptide designed from the human membrane protein CD47 (*e.g.*, the “self” particles described by Rodriguez *et al.* (*Science* 2013, 339, 971-975), herein incorporated by reference in its entirety). As shown by Rodriguez *et al.* the self peptides delayed macrophage-mediated clearance of nanoparticles which enhanced delivery of the nanoparticles. In other aspects, the conjugate may be the membrane protein CD47 (*e.g.*, see Rodriguez *et al.* *Science* 2013, 339, 971-975, herein incorporated by reference in its entirety). Rodriguez *et al.* showed that, similarly to “self” peptides, CD47 can increase the circulating particle ratio in a subject as compared to scrambled peptides and PEG coated nanoparticles.

In some embodiments, the RNA vaccines of the present invention are formulated in nanoparticles that comprise a conjugate to enhance the delivery of the nanoparticles of the present disclosure in a subject. The conjugate may be the CD47 membrane or the conjugate may be derived from the CD47 membrane protein, such as the “self” peptide described previously. In other aspects the nanoparticle may comprise PEG and a conjugate of CD47 or a derivative thereof. In yet other aspects, the nanoparticle may comprise both the “self” peptide described above and the membrane protein CD47.

In other aspects, a “self” peptide and/or CD47 protein may be conjugated to a virus-like particle or pseudovirion, as described herein for delivery of the RNA vaccines of the present invention.

In other embodiments, RNA vaccine pharmaceutical compositions comprising the polynucleotides of the present invention and a conjugate which may have a degradable linkage. Non-limiting examples of conjugates include an aromatic moiety comprising an ionizable hydrogen atom, a spacer moiety, and a water-soluble polymer. As a non-limiting example, pharmaceutical compositions comprising a conjugate with a degradable linkage and methods for delivering such pharmaceutical compositions are described in U.S. Publication No. US2013/0184443, the content of which is herein incorporated by reference in its entirety.

The nanoparticle formulations may be a carbohydrate nanoparticle comprising a carbohydrate carrier and a RNA (*e.g.*, mRNA) vaccine. As a non-limiting example, the carbohydrate carrier may include, but is not limited to, an anhydride-modified phytoglycogen or glycogen-type material, phytoglycogen octenyl succinate, phytoglycogen beta-dextrin, anhydride-modified phytoglycogen beta-dextrin. (See *e.g.*, International Publication No. WO2012/109121; the content of which is herein incorporated by reference in its entirety).

Nanoparticle formulations of the present invention may be coated with a surfactant or polymer in order to improve the delivery of the particle. In some embodiments, the nanoparticle may be coated with a hydrophilic coating such as, but not limited to, PEG coatings

and/or coatings that have a neutral surface charge. The hydrophilic coatings may help to deliver nanoparticles with larger payloads such as, but not limited to, RNA vaccines within the central nervous system. As a non-limiting example nanoparticles comprising a hydrophilic coating and methods of making such nanoparticles are described in U.S. Publication No.

5 US2013/0183244, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the lipid nanoparticles of the present invention may be hydrophilic polymer particles. Non-limiting examples of hydrophilic polymer particles and methods of making hydrophilic polymer particles are described in U.S. Publication No. US2013/0210991, the content of which is herein incorporated by reference in its entirety.

10 In other embodiments, the lipid nanoparticles of the present invention may be hydrophobic polymer particles.

Lipid nanoparticle formulations may be improved by replacing the cationic lipid with a biodegradable cationic lipid which is known as a rapidly eliminated lipid nanoparticle (reLNP). Ionizable cationic lipids, such as, but not limited to, DLinDMA, DLin-KC2-DMA, and DLin-MC3-DMA, have been shown to accumulate in plasma and tissues over time and may be a potential source of toxicity. The rapid metabolism of the rapidly eliminated lipids can improve the tolerability and therapeutic index of the lipid nanoparticles by an order of magnitude from a 1 mg/kg dose to a 10 mg/kg dose in rat. Inclusion of an enzymatically degraded ester linkage can improve the degradation and metabolism profile of the cationic component, while still maintaining the activity of the reLNP formulation. The ester linkage can be internally located within the lipid chain or it may be terminally located at the terminal end of the lipid chain. The internal ester linkage may replace any carbon in the lipid chain.

20 In some embodiments, the internal ester linkage may be located on either side of the saturated carbo.

25 In some embodiments, an immune response may be elicited by delivering a lipid nanoparticle which may include a nanospecies, a polymer and an immunogen (U.S. Publication No. 2012/0189700 and International Publication No. WO2012/099805, each of which is herein incorporated by reference in its entirety).

The polymer may encapsulate the nanospecies or partially encapsulate the nanospecies. The immunogen may be a recombinant protein, a modified RNA and/or a polynucleotide described herein. In some embodiments, the lipid nanoparticle may be formulated for use in a vaccine such as, but not limited to, against a pathogen.

Lipid nanoparticles may be engineered to alter the surface properties of particles so the lipid nanoparticles may penetrate the mucosal barrier. Mucus is located on mucosal tissue such as, but not limited to, oral (*e.g.*, the buccal and esophageal membranes and tonsil tissue),

ophthalmic, gastrointestinal (*e.g.*, stomach, small intestine, large intestine, colon, rectum), nasal, respiratory (*e.g.*, nasal, pharyngeal, tracheal and bronchial membranes), genital (*e.g.*, vaginal, cervical and urethral membranes). Nanoparticles larger than 10-200 nm which are preferred for higher drug encapsulation efficiency and the ability to provide the sustained delivery of a wide array of drugs have been thought to be too large to rapidly diffuse through mucosal barriers.

Mucus is continuously secreted, shed, discarded or digested and recycled so most of the trapped particles may be removed from the mucosal tissue within seconds or within a few hours. Large polymeric nanoparticles (200nm -500nm in diameter) which have been coated densely with a low molecular weight polyethylene glycol (PEG) diffused through mucus only 4 to 6-fold lower than the same particles diffusing in water (Lai *et al. PNAS* 2007 104(5):1482-487; Lai *et al. Adv Drug Deliv Rev.* 2009 61(2): 158-171; each of which is herein incorporated by reference in its entirety). The transport of nanoparticles may be determined using rates of permeation and/or fluorescent microscopy techniques including, but not limited to, fluorescence recovery after photobleaching (FRAP) and high resolution multiple particle tracking (MPT). As a non-limiting example, compositions which can penetrate a mucosal barrier may be made as described in U.S. Patent No. 8,241,670 or International Publication No. WO2013/110028, the content of each of which is herein incorporated by reference in its entirety.

The lipid nanoparticle engineered to penetrate mucus may comprise a polymeric material (*i.e.* a polymeric core) and/or a polymer-vitamin conjugate and/or a tri-block co-polymer. The polymeric material may include, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, poly(styrenes), polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. The polymeric material may be biodegradable and/or biocompatible. Non-limiting examples of biocompatible polymers are described in International Publication No. WO2013/116804, the content of which is herein incorporated by reference in its entirety. The polymeric material may additionally be irradiated. As a non-limiting example, the polymeric material may be gamma irradiated (*see e.g.*, International Publication No. WO2012/082165, herein incorporated by reference in its entirety). Non-limiting examples of specific polymers include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacralate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol,

poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl

5 alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone, polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate)

10 (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate, polyoxymethylene,

15 poloxamers, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), PEG-PLGA-PEG and trimethylene carbonate, polyvinylpyrrolidone. The lipid nanoparticle may be coated or associated with a copolymer such as, but not limited to, a block co-polymer (such as a branched polyether-polyamide block copolymer described in International Publication No. WO2013/012476, herein incorporated by reference in its

20 entirety), and (poly(ethylene glycol))-(poly(propylene oxide))-(poly(ethylene glycol)) triblock copolymer (see *e.g.*, U.S. Publication 2012/0121718 and U.S. Publication 2010/0003337 and U.S. Pat. No. 8,263,665; each of which is herein incorporated by reference in its entirety). The co-polymer may be a polymer that is generally regarded as safe (GRAS) and the formation of the lipid nanoparticle may be in such a way that no new chemical entities are created. For

25 example, the lipid nanoparticle may comprise poloxamers coating PLGA nanoparticles without forming new chemical entities which are still able to rapidly penetrate human mucus (Yang *et al. Angew. Chem. Int. Ed.* 2011 50:25972600; the content of which is herein incorporated by reference in its entirety). A non-limiting scalable method to produce nanoparticles which can penetrate human mucus is described by Xu *et al. (see e.g., J Control*

30 *Release* 2013, 170(2):279-86, the content of which is herein incorporated by reference in its entirety).

The vitamin of the polymer-vitamin conjugate may be vitamin E. The vitamin portion of the conjugate may be substituted with other suitable components such as, but not limited to, vitamin A, vitamin E, other vitamins, cholesterol, a hydrophobic moiety, or a hydrophobic

component of other surfactants (*e.g.*, sterol chains, fatty acids, hydrocarbon chains and alkylene oxide chains).

The lipid nanoparticle engineered to penetrate mucus may include surface altering agents such as, but not limited to, polynucleotides, anionic proteins (*e.g.*, bovine serum albumin), surfactants (*e.g.*, cationic surfactants such as for example dimethyldioctadecyl-ammonium bromide), sugars or sugar derivatives (*e.g.*, cyclodextrin), nucleic acids, polymers (*e.g.*, heparin, polyethylene glycol and poloxamer), mucolytic agents (*e.g.*, N-acetylcysteine, mugwort, bromelain, papain, clerodendrum, acetylcysteine, bromhexine, carbocysteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin β 4 dornase alfa, nelteneine, erdosteine) and various DNases including rhDNase. The surface altering agent may be embedded or enmeshed in the particle's surface or disposed (*e.g.*, by coating, adsorption, covalent linkage, or other process) on the surface of the lipid nanoparticle (see *e.g.*, U.S. Publication 2010/0215580 and U.S. Publication 2008/0166414 and US2013/0164343 the content of each of which is herein incorporated by reference in its entirety).

In some embodiments, the mucus penetrating lipid nanoparticles may comprise at least one polynucleotide described herein. The polynucleotide may be encapsulated in the lipid nanoparticle and/or disposed on the surface of the particle. The polynucleotide may be covalently coupled to the lipid nanoparticle. Formulations of mucus penetrating lipid nanoparticles may comprise a plurality of nanoparticles. Further, the formulations may contain particles which may interact with the mucus and alter the structural and/or adhesive properties of the surrounding mucus to decrease mucoadhesion which may increase the delivery of the mucus penetrating lipid nanoparticles to the mucosal tissue.

In other embodiments, the mucus penetrating lipid nanoparticles may be a hypotonic formulation comprising a mucosal penetration enhancing coating. The formulation may be hypotonic for the epithelium to which it is being delivered.

Non-limiting examples of hypotonic formulations may be found in International Publication No. WO2013/110028, the content of which is herein incorporated by reference in its entirety.

In some embodiments, in order to enhance the delivery through the mucosal barrier the RNA vaccine formulation may comprise or be a hypotonic solution. Hypotonic solutions were found to increase the rate at which mucoinert particles such as, but not limited to, mucus-penetrating particles, were able to reach the vaginal epithelial surface (*see e.g.*, Ensign *et al. Biomaterials* 2013, 34(28):6922-9, the content of which is herein incorporated by reference in its entirety).

In some embodiments, the RNA vaccine is formulated as a lipoplex, such as, without limitation, the ATUPLEXTM system, the DACC system, the DBTC system and other siRNA-lipoplex technology from Silence Therapeutics (London, United Kingdom), STEMFECTTM from STEMGENT® (Cambridge, MA), and polyethylenimine (PEI) or protamine-based targeted and non-targeted delivery of nucleic acids (Aleku *et al.* *Cancer Res.* 2008 68:9788-9798; Strumberg *et al.* *Int J Clin Pharmacol Ther* 2012 50:76-78; Santel *et al.*, *Gene Ther* 2006 13:1222-1234; Santel *et al.*, *Gene Ther* 2006 13:1360-1370; Gutbier *et al.*, *Pulm Pharmacol. Ther.* 2010 23:334-344; Kaufmann *et al.* *Microvasc Res* 2010 80:286-293; Weide *et al.* *J Immunother.* 2009 32:498-507; Weide *et al.* *J Immunother.* 2008 31:180-188; Pascolo, *Expert Opin. Biol. Ther.* 4:1285-1294; Fotin-Mleczek *et al.*, 2011 *J. Immunother.* 34:1-15; Song *et al.*, *Nature Biotechnol.* 2005, 23:709-717; Peer *et al.*, *Proc Natl Acad Sci U S A.* 2007 6;104:4095-4100; deFougerolles *Hum Gene Ther.* 2008 19:125-132; each of which is incorporated herein by reference in its entirety).

In some embodiments, such formulations may also be constructed or compositions altered such that they passively or actively are directed to different cell types *in vivo*, including but not limited to hepatocytes, immune cells, tumor cells, endothelial cells, antigen presenting cells, and leukocytes (Akinc *et al.* *Mol Ther.* 2010 18:1357-1364; Song *et al.*, *Nat Biotechnol.* 2005 23:709-717; Judge *et al.*, *J Clin Invest.* 2009 119:661-673; Kaufmann *et al.*, *Microvasc Res* 2010 80:286-293; Santel *et al.*, *Gene Ther* 2006 13:1222-1234; Santel *et al.*, *Gene Ther* 2006 13:1360-1370; Gutbier *et al.*, *Pulm Pharmacol. Ther.* 2010 23:334-344; Basha *et al.*, *Mol. Ther.* 2011 19:2186-2200; Fenske and Cullis, *Expert Opin Drug Deliv.* 2008 5:25-44; Peer *et al.*, *Science.* 2008 319:627-630; Peer and Lieberman, *Gene Ther.* 2011 18:1127-1133; each of which is incorporated herein by reference in its entirety). One example of passive targeting of formulations to liver cells includes the DLin-DMA, DLin-KC2-DMA and DLin-MC3-DMA-based lipid nanoparticle formulations which have been shown to bind to apolipoprotein E and promote binding and uptake of these formulations into hepatocytes *in vivo* (Akinc *et al.* *Mol Ther.* 2010 18:1357-1364; herein incorporated by reference in its entirety). Formulations can also be selectively targeted through expression of different ligands on their surface as exemplified by, but not limited by, folate, transferrin, N-acetylgalactosamine (GalNAc), and antibody targeted approaches (Kolhatkar *et al.*, *Curr Drug Discov Technol.* 2011 8:197-206; Musacchio and Torchilin, *Front Biosci.* 2011 16:1388-1412; Yu *et al.*, *Mol Membr Biol.* 2010 27:286-298; Patil *et al.*, *Crit Rev Ther Drug Carrier Syst.* 2008 25:1-61; Benoit *et al.*, *Biomacromolecules.* 2011 12:2708-2714; Zhao *et al.*, *Expert Opin Drug Deliv.* 2008 5:309-319; Akinc *et al.*, *Mol Ther.* 2010 18:1357-1364; Srinivasan *et al.*, *Methods Mol Biol.* 2012 820:105-116; Ben-Arie *et al.*, *Methods Mol Biol.* 2012 757:497-507; Peer 2010 *J Control*

Release. 20:63-68; Peer *et al.*, *Proc Natl Acad Sci U S A.* 2007 104:4095-4100; Kim *et al.*, *Methods Mol Biol.* 2011 721:339-353; Subramanya *et al.*, *Mol Ther.* 2010 18:2028-2037; Song *et al.*, *Nat Biotechnol.* 2005 23:709-717; Peer *et al.*, *Science.* 2008 319:627-630; Peer and Lieberman, *Gene Ther.* 2011 18:1127-1133; each of which is incorporated herein by
5 reference in its entirety).

In some embodiments, the RNA (*e.g.*, mRNA) vaccine is formulated as a solid lipid nanoparticle. A solid lipid nanoparticle (SLN) may be spherical with an average diameter between 10 to 1000 nm. SLN possess a solid lipid core matrix that can solubilize lipophilic molecules and may be stabilized with surfactants and/or emulsifiers. In other embodiments, the
10 lipid nanoparticle may be a self-assembly lipid-polymer nanoparticle (*see* Zhang *et al.*, *ACS Nano*, 2008, 2 (8), pp 1696–1702; the content of which is herein incorporated by reference in its entirety). As a non-limiting example, the SLN may be the SLN described in International Publication No. WO2013/105101, the content of which is herein incorporated by reference in its entirety. As another non-limiting example, the SLN may be made by the methods or processes
15 described in International Publication No. WO2013/105101, the content of which is herein incorporated by reference in its entirety).

Liposomes, lipoplexes, or lipid nanoparticles may be used to improve the efficacy of polynucleotides directed protein production as these formulations may be able to increase cell transfection by the RNA vaccine; and/or increase the translation of encoded protein. One such
20 example involves the use of lipid encapsulation to enable the effective systemic delivery of polyplex plasmid DNA (Heyes *et al.*, *Mol Ther.* 2007 15:713-720; herein incorporated by reference in its entirety). The liposomes, lipoplexes, or lipid nanoparticles may also be used to increase the stability of the polynucleotide.

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present invention can be
25 formulated for controlled release and/or targeted delivery. As used herein, “controlled release” refers to a pharmaceutical composition or compound release profile that conforms to a particular pattern of release to effect a therapeutic outcome. In some embodiments, the RNA vaccines may be encapsulated into a delivery agent described herein and/or known in the art for controlled release and/or targeted delivery. As used herein, the term “encapsulate” means to
30 enclose, surround or encase. As it relates to the formulation of the compounds of the invention, encapsulation may be substantial, complete or partial. The term “substantially encapsulated” means that at least greater than 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.9 or greater than 99.999% of the pharmaceutical composition or compound of the invention may be enclosed, surrounded or encased within the delivery agent. “Partially
35 encapsulation” means that less than 10, 10, 20, 30, 40 50 or less of the pharmaceutical

composition or compound of the invention may be enclosed, surrounded or encased within the delivery agent. Advantageously, encapsulation may be determined by measuring the escape or the activity of the pharmaceutical composition or compound of the invention using fluorescence and/or electron micrograph. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the pharmaceutical composition or compound of the present disclosure are encapsulated in the delivery agent.

In some embodiments, the controlled release formulation may include, but is not limited to, tri-block co-polymers. As a non-limiting example, the formulation may include two different types of tri-block co-polymers (International Pub. No. WO2012/131104 and WO2012/131106; the contents of each of which is herein incorporated by reference in its entirety).

In other embodiments, the RNA vaccines may be encapsulated into a lipid nanoparticle or a rapidly eliminated lipid nanoparticle and the lipid nanoparticles or a rapidly eliminated lipid nanoparticle may then be encapsulated into a polymer, hydrogel and/or surgical sealant described herein and/or known in the art. As a non-limiting example, the polymer, hydrogel or surgical sealant may be PLGA, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, FL), HYLENEX® (Halozyme Therapeutics, San Diego CA), surgical sealants such as fibrinogen polymers (Ethicon Inc. Cornelia, GA), TISSELL® (Baxter International, Inc. Deerfield, IL), PEG-based sealants, and COSEAL® (Baxter International, Inc. Deerfield, IL).

In other embodiments, the lipid nanoparticle may be encapsulated into any polymer known in the art which may form a gel when injected into a subject. As another non-limiting example, the lipid nanoparticle may be encapsulated into a polymer matrix which may be biodegradable.

In some embodiments, the RNA vaccine formulation for controlled release and/or targeted delivery may also include at least one controlled release coating. Controlled release coatings include, but are not limited to, OPADRY®, polyvinylpyrrolidone/vinyl acetate copolymer, polyvinylpyrrolidone, hydroxypropyl methylcellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, EUDRAGIT RL®, EUDRAGIT RS® and cellulose derivatives such as ethylcellulose aqueous dispersions (AQUACOAT® and SURELEASE®).

In some embodiments, the RNA (*e.g.*, mRNA) vaccine controlled release and/or targeted delivery formulation may comprise at least one degradable polyester which may contain polycationic side chains. Degradable polyesters include, but are not limited to, poly(*serine ester*), poly(*L-lactide-co-L-lysine*), poly(*4-hydroxy-L-proline ester*), and combinations thereof. In other embodiments, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

In some embodiments, the RNA vaccine controlled release and/or targeted delivery formulation comprising at least one polynucleotide may comprise at least one PEG and/or PEG related polymer derivatives as described in U.S. Patent No. 8,404,222, herein incorporated by reference in its entirety.

5 In other embodiments, the RNA vaccine controlled release delivery formulation comprising at least one polynucleotide may be the controlled release polymer system described in U.S. Publication No. 2013/0130348, herein incorporated by reference in its entirety.

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present invention may be encapsulated in a therapeutic nanoparticle, referred to herein as “therapeutic nanoparticle RNA
10 vaccines.” Therapeutic nanoparticles may be formulated by methods described herein and known in the art such as, but not limited to, International Publication Nos. WO2010/005740, WO2010/030763, WO2010/005721, WO2010/005723, WO2012/054923, U.S. Publication Nos. US2011/0262491, US2010/0104645, US2010/0087337, US2010/0068285, US2011/0274759, US2010/0068286, US2012/0288541, US2013/0123351 and
15 US2013/0230567 and US Patent Nos. 8,206,747, 8,293,276, 8,318,208 and 8,318,211, the content of each of which is herein incorporated by reference in its entirety. In other embodiments, therapeutic polymer nanoparticles may be identified by the methods described in U.S. Publication No. US2012/0140790, the content of which is herein incorporated by reference in its entirety.

20 In some embodiments, the therapeutic nanoparticle RNA vaccine may be formulated for sustained release. As used herein, “sustained release” refers to a pharmaceutical composition or compound that conforms to a release rate over a specific period of time. The period of time may include, but is not limited to, hours, days, weeks, months and years. As a non-limiting example, the sustained release nanoparticle may comprise a polymer and a therapeutic agent
25 such as, but not limited to, the polynucleotides of the present invention (*see* International Publication No. 2010/075072 and U.S. Publication Nos. US2010/0216804, US2011/0217377 and US2012/0201859, each of which is herein incorporated by reference in its entirety). In another non-limiting example, the sustained release formulation may comprise agents which permit persistent bioavailability such as, but not limited to, crystals, macromolecular gels
30 and/or particulate suspensions (*see* U.S. Publication No. US2013/0150295, the content of which is herein incorporated by reference in its entirety).

In some embodiments, the therapeutic nanoparticle RNA vaccines may be formulated to be target specific. As a non-limiting example, the therapeutic nanoparticles may include a corticosteroid (*see* International Publication No. WO2011/084518, herein incorporated by
35 reference in its entirety). As a non-limiting example, the therapeutic nanoparticles may be

formulated in nanoparticles described in International Publication Nos. WO2008/121949, WO2010/005726, WO2010/005725, WO2011/084521 and U.S. Publication Nos. US2010/0069426, US2012/0004293 and US2010/0104655, each of which is herein incorporated by reference in its entirety.

5 In some embodiments, the nanoparticles of the present invention may comprise a polymeric matrix. As a non-limiting example, the nanoparticle may comprise two or more polymers such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, 10 polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester) or combinations thereof.

In some embodiments, the therapeutic nanoparticle comprises a diblock copolymer. In some embodiments, the diblock copolymer may include PEG in combination with a polymer 15 such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines, polylysine, poly(ethylene imine), poly(serine ester), poly(L-lactide-co-L-lysine), poly(4- 20 hydroxy-L-proline ester) or combinations thereof. In yet other embodiments, the diblock copolymer may be a high-X diblock copolymer such as those described in International Publication No. WO2013/120052, the content of which is herein incorporated by reference in its entirety.

As a non-limiting example, the therapeutic nanoparticle comprises a PLGA-PEG 25 block copolymer (*see* U.S. Publication No. US2012/0004293 and U.S. Patent No. 8,236,330, each of which is herein incorporated by reference in its entirety). In another non-limiting example, the therapeutic nanoparticle is a stealth nanoparticle comprising a diblock copolymer of PEG and PLA or PEG and PLGA (*see* U.S. Patent No. 8,246,968 and International Publication No. WO2012/166923, the content of each of which is herein incorporated by 30 reference in its entirety). In yet another non-limiting example, the therapeutic nanoparticle is a stealth nanoparticle or a target-specific stealth nanoparticle as described in U.S. Publication No. 2013/0172406, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the therapeutic nanoparticle may comprise a multiblock copolymer (*see e.g.*, U.S. Patent Nos. 8,263,665 and 8,287,910 and U.S. Publication No.

2013/0195987, the content of each of which is herein incorporated by reference in its entirety).

In yet another non-limiting example, the lipid nanoparticle comprises the block copolymer PEG-PLGA-PEG (*see e.g.*, the thermosensitive hydrogel (PEG-PLGA-PEG) used as a TGF-beta1 gene delivery vehicle in Lee *et al.* "Thermosensitive Hydrogel as a TGF-β1 Gene Delivery Vehicle Enhances Diabetic Wound Healing." *Pharmaceutical Research*, 2003 20(12): 1995-2000; and used as a controlled gene delivery system in Li *et al.* "Controlled Gene Delivery System Based on Thermosensitive Biodegradable Hydrogel" *Pharmaceutical Research* 2003 20(6):884- 888; and Chang *et al.*, "Non-ionic amphiphilic biodegradable PEG-PLGA-PEG copolymer enhances gene delivery efficiency in rat skeletal muscle." *J Controlled Release*. 2007 118:245-253; each of which is herein incorporated by reference in its entirety). The RNA (*e.g.*, mRNA) vaccines of the present disclosure may be formulated in lipid nanoparticles comprising the PEG-PLGA-PEG block copolymer.

In some embodiments, the block copolymers described herein may be included in a polyion complex comprising a non-polymeric micelle and the block copolymer. (*see e.g.*, U.S. Publication No. 2012/0076836, herein incorporated by reference in its entirety).

In some embodiments, the therapeutic nanoparticle may comprise at least one acrylic polymer. Acrylic polymers include but are not limited to, acrylic acid, methacrylic acid, acrylic acid and methacrylic acid copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, amino alkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), polycyanoacrylates and combinations thereof.

In some embodiments, the therapeutic nanoparticles may comprise at least one poly(vinyl ester) polymer. The poly(vinyl ester) polymer may be a copolymer such as a random copolymer. As a non-limiting example, the random copolymer may have a structure such as those described in International Publication No. WO2013/032829 or U.S. Publication No. 2013/0121954, the content of which is herein incorporated by reference in its entirety. In some aspects, the poly(vinyl ester) polymers may be conjugated to the polynucleotides described herein.

In some embodiments, the therapeutic nanoparticle may comprise at least one diblock copolymer. The diblock copolymer may be, but it not limited to, a poly(lactic) acid-poly(ethylene)glycol copolymer (*see e.g.*, International Publication No. WO2013/044219; herein incorporated by reference in its entirety). As a non-limiting example, the therapeutic nanoparticle may be used to treat cancer (*see* International publication No. WO2013/044219, herein incorporated by reference in its entirety).

In some embodiments, the therapeutic nanoparticles may comprise at least one cationic polymer described herein and/or known in the art.

In some embodiments, the therapeutic nanoparticles may comprise at least one amine-containing polymer such as, but not limited to polylysine, polyethyleneimine, poly(amidoamine) dendrimers, poly(beta-amino esters) (*see e.g.*, U.S. Patent No. 8,287,849, herein incorporated by reference in its entirety) and combinations thereof. In other embodiments, the nanoparticles described herein may comprise an amine cationic lipid such as those described in International Publication No. WO2013/059496, the content of which is herein incorporated by reference in its entirety. In some aspects the cationic lipids may have an amino-amine or an amino-amide moiety.

In some embodiments, the therapeutic nanoparticles may comprise at least one degradable polyester, which may contain polycationic side chains. Degradable polyesters include, but are not limited to, poly(serine ester), poly(L-lactide-co-L-lysine), poly(4-hydroxy-L-proline ester), and combinations thereof. In other embodiments, the degradable polyesters may include a PEG conjugation to form a PEGylated polymer.

In other embodiments, the therapeutic nanoparticle may include a conjugation of at least one targeting ligand. The targeting ligand may be any ligand known in the art such as, but not limited to, a monoclonal antibody (Kirpotin *et al*, *Cancer Res.* 2006 66:6732-6740, herein incorporated by reference in its entirety).

In some embodiments, the therapeutic nanoparticle may be formulated in an aqueous solution, which may be used to target cancer (*see* International Publication No. WO2011/084513 and U.S. Publication No. 2011/0294717, each of which is herein incorporated by reference in its entirety).

In some embodiments, the therapeutic nanoparticle RNA vaccines, *e.g.*, therapeutic nanoparticles comprising at least one RNA vaccine may be formulated using the methods described by Podobinski *et al* in U.S. Patent No. 8,404,799, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the RNA (*e.g.*, mRNA) vaccines may be encapsulated in, linked to and/or associated with synthetic nanocarriers. Synthetic nanocarriers include, but are not limited to, those described in International Publication Nos. WO2010/005740, WO2012/149454 and WO2013/019669, and U.S. Publication Nos. US2011/0262491, US2010/0104645, US2010/0087337 and US2012/0244222, each of which is herein incorporated by reference in its entirety. The synthetic nanocarriers may be formulated using methods known in the art and/or described herein. As a non-limiting example, the synthetic nanocarriers may be formulated by the methods described in International Publication Nos.

WO2010/005740, WO2010/030763 and WO2012/13501, and U.S. Publication Nos. US2011/0262491, US2010/0104645, US2010/0087337 and US2012/024422, each of which is herein incorporated by reference in its entirety. In other embodiments, the synthetic nanocarrier formulations may be lyophilized by methods described in International Publication No.

5 WO2011/072218 and U.S. Patent No. 8,211,473, the content of each of which is herein incorporated by reference in its entirety. In yet other embodiments, formulations of the present invention, including, but not limited to, synthetic nanocarriers, may be lyophilized or reconstituted by the methods described in U.S. Publication No. 2013/0230568, the content of which is herein incorporated by reference in its entirety.

10 In some embodiments, the synthetic nanocarriers may contain reactive groups to release the polynucleotides described herein (*see* International Publication No. WO2012/092552 and U.S. Publication No. US2012/0171229, each of which is herein incorporated by reference in its entirety).

In some embodiments, the synthetic nanocarriers may contain an immunostimulatory agent to enhance the immune response from delivery of the synthetic nanocarrier. As a non-limiting example, the synthetic nanocarrier may comprise a Th1 immunostimulatory agent which may enhance a Th1-based response of the immune system (*see* International Publication No. WO2010/123569 and U.S. Publication No. 2011/0223201, each of which is herein incorporated by reference in its entirety).

20 In some embodiments, the synthetic nanocarriers may be formulated for targeted release. In some embodiments, the synthetic nanocarrier is formulated to release the polynucleotides at a specified pH and/or after a desired time interval. As a non-limiting example, the synthetic nanoparticle may be formulated to release the RNA vaccines after 24 hours and/or at a pH of 4.5 (*see* International Publication Nos. WO2010/138193 and
25 WO2010/138194 and U.S. Publication Nos. US2011/0020388 and US2011/0027217, each of which is herein incorporated by reference in their entireties).

In some embodiments, the synthetic nanocarriers may be formulated for controlled and/or sustained release of the polynucleotides described herein. As a non-limiting example, the synthetic nanocarriers for sustained release may be formulated by methods known in the art,
30 described herein and/or as described in International Publication No. WO2010/138192 and U.S. Publication No. 2010/0303850, each of which is herein incorporated by reference in its entirety.

In some embodiments, the RNA vaccine may be formulated for controlled and/or sustained release wherein the formulation comprises at least one polymer that is a crystalline

side chain (CYSC) polymer. CYSC polymers are described in U.S. Patent No. 8,399,007, herein incorporated by reference in its entirety.

In some embodiments, the synthetic nanocarrier may be formulated for use as a vaccine. In some embodiments, the synthetic nanocarrier may encapsulate at least one polynucleotide which encode at least one antigen. As a non-limiting example, the synthetic nanocarrier may include at least one antigen and an excipient for a vaccine dosage form (*see* International Publication No. WO2011/150264 and U.S. Publication No. 2011/0293723, each of which is herein incorporated by reference in its entirety). As another non-limiting example, a vaccine dosage form may include at least two synthetic nanocarriers with the same or different antigens and an excipient (*see* International Publication No. WO2011/150249 and U.S. Publication No. 2011/0293701, each of which is herein incorporated by reference in its entirety). The vaccine dosage form may be selected by methods described herein, known in the art and/or described in International Publication No. WO2011/150258 and U.S. Publication No. US2012/0027806, each of which is herein incorporated by reference in its entirety).

In some embodiments, the synthetic nanocarrier may comprise at least one polynucleotide which encodes at least one adjuvant. As non-limiting example, the adjuvant may comprise dimethyldioctadecylammonium-bromide, dimethyldioctadecylammonium-chloride, dimethyldioctadecylammonium-phosphate or dimethyldioctadecylammonium-acetate (DDA) and an apolar fraction or part of said apolar fraction of a total lipid extract of a mycobacterium (*see e.g.*, U.S. Patent No. 8,241,610; herein incorporated by reference in its entirety). In other embodiments, the synthetic nanocarrier may comprise at least one polynucleotide and an adjuvant. As a non-limiting example, the synthetic nanocarrier comprising and adjuvant may be formulated by the methods described in International Publication No. WO2011/150240 and U.S. Publication No. US2011/0293700, each of which is herein incorporated by reference in its entirety).

In some embodiments, the synthetic nanocarrier may encapsulate at least one polynucleotide which encodes a peptide, fragment or region from a virus. As a non-limiting example, the synthetic nanocarrier may include, but is not limited to, the nanocarriers described in International Publication Nos. WO2012/024621, WO2012/02629, WO2012/024632 and U.S. Publication No. US2012/0064110, US2012/0058153 and US2012/0058154, each of which is herein incorporated by reference in its entirety).

In some embodiments, the synthetic nanocarrier may be coupled to a polynucleotide which may be able to trigger a humoral and/or cytotoxic T lymphocyte (CTL) response (*See e.g.*, International Publication No. WO2013/019669, herein incorporated by reference in its entirety).

In some embodiments, the RNA vaccine may be encapsulated in, linked to and/or associated with zwitterionic lipids. Non-limiting examples of zwitterionic lipids and methods of using zwitterionic lipids are described in U.S. Publication No. 2013/0216607, the content of which is herein incorporated by reference in its entirety. In some aspects, the zwitterionic lipids may be used in the liposomes and lipid nanoparticles described herein.

In some embodiments, the RNA vaccine may be formulated in colloid nanocarriers as described in U.S. Publication No. 2013/0197100, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the nanoparticle may be optimized for oral administration. The nanoparticle may comprise at least one cationic biopolymer such as, but not limited to, chitosan or a derivative thereof. As a non-limiting example, the nanoparticle may be formulated by the methods described in U.S. Publication No. 2012/0282343; herein incorporated by reference in its entirety.

In some embodiments, LNPs comprise the lipid KL52 (an amino-lipid disclosed in U.S. Application Publication No. 2012/0295832 expressly incorporated herein by reference in its entirety). Activity and/or safety (as measured by examining one or more of ALT/AST, white blood cell count and cytokine induction) of LNP administration may be improved by incorporation of such lipids. LNPs comprising KL52 may be administered intravenously and/or in one or more doses. In some embodiments, administration of LNPs comprising KL52 results in equal or improved mRNA and/or protein expression as compared to LNPs comprising MC3.

In some embodiments, RNA vaccine may be delivered using smaller LNPs. Such particles may comprise a diameter from below 0.1 μm up to 100 nm such as, but not limited to, less than 0.1 μm , less than 1.0 μm , less than 5 μm , less than 10 μm , less than 15 μm , less than 20 μm , less than 25 μm , less than 30 μm , less than 35 μm , less than 40 μm , less than 50 μm , less than 55 μm , less than 60 μm , less than 65 μm , less than 70 μm , less than 75 μm , less than 80 μm , less than 85 μm , less than 90 μm , less than 95 μm , less than 100 μm , less than 125 μm , less than 150 μm , less than 175 μm , less than 200 μm , less than 225 μm , less than 250 μm , less than 275 μm , less than 300 μm , less than 325 μm , less than 350 μm , less than 375 μm , less than 400 μm , less than 425 μm , less than 450 μm , less than 475 μm , less than 500 μm , less than 525 μm , less than 550 μm , less than 575 μm , less than 600 μm , less than 625 μm , less than 650 μm , less than 675 μm , less than 700 μm , less than 725 μm , less than 750 μm , less than 775 μm , less than 800 μm , less than 825 μm , less than 850 μm , less than 875 μm , less than 900 μm , less than 925 μm , less than 950 μm , or less than 975 μm .

In other embodiments, RNA (*e.g.*, mRNA) vaccines may be delivered using smaller LNPs which may comprise a diameter from about 1 nm to about 100 nm, from about 1 nm to about 10 nm, about 1 nm to about 20 nm, from about 1 nm to about 30 nm, from about 1 nm to about 40 nm, from about 1 nm to about 50 nm, from about 1 nm to about 60 nm, from about 1 nm to about 70 nm, from about 1 nm to about 80 nm, from about 1 nm to about 90 nm, from about 5 nm to about 100 nm, from about 5 nm to about 10 nm, about 5 nm to about 20 nm, from about 5 nm to about 30 nm, from about 5 nm to about 40 nm, from about 5 nm to about 50 nm, from about 5 nm to about 60 nm, from about 5 nm to about 70 nm, from about 5 nm to about 80 nm, from about 5 nm to about 90 nm, about 10 to about 50 nm, from about 20 to about 50 nm, from about 30 to about 50 nm, from about 40 to about 50 nm, from about 20 to about 60 nm, from about 30 to about 60 nm, from about 40 to about 60 nm, from about 20 to about 70 nm, from about 30 to about 70 nm, from about 40 to about 70 nm, from about 50 to about 70 nm, from about 60 to about 70 nm, from about 20 to about 80 nm, from about 30 to about 80 nm, from about 40 to about 80 nm, from about 50 to about 80 nm, from about 60 to about 80 nm, from about 20 to about 90 nm, from about 30 to about 90 nm, from about 40 to about 90 nm, from about 50 to about 90 nm, from about 60 to about 90 nm and/or from about 70 to about 90 nm.

In some embodiments, such LNPs are synthesized using methods comprising microfluidic mixers. Exemplary microfluidic mixers may include, but are not limited to a slit interdigital micromixer including, but not limited to those manufactured by Microinnova (Allerheiligen bei Wildon, Austria) and/or a staggered herringbone micromixer (SHM) (Zhigaltsev, I.V. *et al.*, Bottom-up design and synthesis of limit size lipid nanoparticle systems with aqueous and triglyceride cores using millisecond microfluidic mixing have been published (Langmuir. 2012. 28:3633-40; Belliveau, N.M. *et al.*, Microfluidic synthesis of highly potent limit-size lipid nanoparticles for *in vivo* delivery of siRNA. *Molecular Therapy-Nucleic Acids*. 2012. 1:e37; Chen, D. *et al.*, Rapid discovery of potent siRNA-containing lipid nanoparticles enabled by controlled microfluidic formulation. *J Am Chem Soc*. 2012. 134(16):6948-51; each of which is herein incorporated by reference in its entirety).

In some embodiments, methods of LNP generation comprising SHM, further comprise the mixing of at least two input streams wherein mixing occurs by microstructure-induced chaotic advection (MICA). According to this method, fluid streams flow through channels present in a herringbone pattern causing rotational flow and folding the fluids around each other. This method may also comprise a surface for fluid mixing wherein the surface changes orientations during fluid cycling. Methods of generating LNPs using SHM include those disclosed in U.S.

Application Publication Nos. 2004/0262223 and 2012/0276209, each of which is expressly incorporated herein by reference in their entirety.

In some embodiments, the RNA vaccine of the present invention may be formulated in lipid nanoparticles created using a micromixer such as, but not limited to, a Slit Interdigital
5 Microstructured Mixer (SIMM-V2) or a Standard Slit Interdigital Micro Mixer (SSIMM) or Caterpillar (CPMM) or Impinging-jet (IJMM) from the Institut für Mikrotechnik Mainz GmbH, Mainz Germany).

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present disclosure may be formulated in lipid nanoparticles created using microfluidic technology (*see* Whitesides, George M. The Origins and the Future of Microfluidics. *Nature*, 2006 442: 368-373; and
10 Abraham *et al.* Chaotic Mixer for Microchannels. *Science*, 2002 295: 647-651; each of which is herein incorporated by reference in its entirety). As a non-limiting example, controlled microfluidic formulation includes a passive method for mixing streams of steady pressure-driven flows in micro channels at a low Reynolds number (*see e.g.*, Abraham *et al.* Chaotic
15 Mixer for Microchannels. *Science*, 2002 295: 647651; which is herein incorporated by reference in its entirety).

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present invention may be formulated in lipid nanoparticles created using a micromixer chip such as, but not limited to, those from Harvard Apparatus (Holliston, MA) or Dolomite Microfluidics (Royston, UK).
20 A micromixer chip can be used for rapid mixing of two or more fluid streams with a split and recombine mechanism.

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the invention may be formulated for delivery using the drug encapsulating microspheres described in International Publication No. WO2013/063468 or U.S. Patent No. 8,440,614, each of which is herein
25 incorporated by reference in its entirety. The microspheres may comprise a compound of the formula (I), (II), (III), (IV), (V) or (VI) as described in International Publication No. WO2013/063468, the content of which is herein incorporated by reference in its entirety. In other aspects, the amino acid, peptide, polypeptide, lipids (APPL) are useful in delivering the RNA vaccines of the invention to cells (*see* International Publication No. WO2013/063468,
30 the contents of which is herein incorporated by reference in its entirety).

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present disclosure may be formulated in lipid nanoparticles having a diameter from about 10 to about 100 nm such as, but not limited to, about 10 to about 20 nm, about 10 to about 30 nm, about 10 to about 40 nm, about 10 to about 50 nm, about 10 to about 60 nm, about 10 to about 70 nm, about 10 to
35 about 80 nm, about 10 to about 90 nm, about 20 to about 30 nm, about 20 to about 40 nm, about

20 to about 50 nm, about 20 to about 60 nm, about 20 to about 70 nm, about 20 to about 80 nm, about 20 to about 90 nm, about 20 to about 100 nm, about 30 to about 40 nm, about 30 to about 50 nm, about 30 to about 60 nm, about 30 to about 70 nm, about 30 to about 80 nm, about 30 to about 90 nm, about 30 to about 100 nm, about 40 to about 50 nm, about 40 to about 60 nm, about 40 to about 70 nm, about 40 to about 80 nm, about 40 to about 90 nm, about 40 to about 100 nm, about 50 to about 60 nm, about 50 to about 70 nm, about 50 to about 80 nm, about 50 to about 90 nm, about 50 to about 100 nm, about 60 to about 70 nm, about 60 to about 80 nm, about 60 to about 90 nm, about 60 to about 100 nm, about 70 to about 80 nm, about 70 to about 90 nm, about 70 to about 100 nm, about 80 to about 90 nm, about 80 to about 100 nm and/or about 90 to about 100 nm.

In some embodiments, the lipid nanoparticles may have a diameter from about 10 to 500 nm.

In some embodiments, the lipid nanoparticle may have a diameter greater than 100 nm, greater than 150 nm, greater than 200 nm, greater than 250 nm, greater than 300 nm, greater than 350 nm, greater than 400 nm, greater than 450 nm, greater than 500 nm, greater than 550 nm, greater than 600 nm, greater than 650 nm, greater than 700 nm, greater than 750 nm, greater than 800 nm, greater than 850 nm, greater than 900 nm, greater than 950 nm or greater than 1000 nm.

In some aspects, the lipid nanoparticle may be a limit size lipid nanoparticle described in International Publication No. WO2013/059922, the content of which is herein incorporated by reference in its entirety. The limit size lipid nanoparticle may comprise a lipid bilayer surrounding an aqueous core or a hydrophobic core; where the lipid bilayer may comprise a phospholipid such as, but not limited to, diacylphosphatidylcholine, a diacylphosphatidylethanolamine, a ceramide, a sphingomyelin, a dihydrosphingomyelin, a cephalin, a cerebroside, a C8-C20 fatty acid diacylphosphatidylcholine, and 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC). In other aspects the limit size lipid nanoparticle may comprise a polyethylene glycol-lipid such as, but not limited to, DLPE-PEG, DMPE-PEG, DPPC-PEG and DSPE-PEG.

In some embodiments, the RNA vaccines may be delivered, localized and/or concentrated in a specific location using the delivery methods described in International Publication No. WO2013063530, the content of which is herein incorporated by reference in its entirety. As a non-limiting example, a subject may be administered an empty polymeric particle prior to, simultaneously with or after delivering the RNA vaccines to the subject. The empty polymeric particle undergoes a change in volume once in contact with the subject and becomes lodged, embedded, immobilized or entrapped at a specific location in the subject.

In some embodiments, the RNA vaccines may be formulated in an active substance release system (*see e.g.*, U.S. Publication No. US2013/0102545, the contents of which is herein incorporated by reference in its entirety). The active substance release system may comprise 1) at least one nanoparticle bonded to an oligonucleotide inhibitor strand which is hybridized with a catalytically active nucleic acid and 2) a compound bonded to at least one substrate molecule bonded to a therapeutically active substance (*e.g.*, polynucleotides described herein), where the therapeutically active substance is released by the cleavage of the substrate molecule by the catalytically active nucleic acid.

In some embodiments, the RNA (*e.g.*, mRNA) vaccines may be formulated in a nanoparticle comprising an inner core comprising a non-cellular material and an outer surface comprising a cellular membrane. The cellular membrane may be derived from a cell or a membrane derived from a virus. As a non-limiting example, the nanoparticle may be made by the methods described in International Publication No. WO2013/052167, herein incorporated by reference in its entirety. As another non-limiting example, the nanoparticle described in International Publication No. WO2013/052167, herein incorporated by reference in its entirety, may be used to deliver the RNA vaccines described herein.

In some embodiments, the RNA vaccines may be formulated in porous nanoparticle-supported lipid bilayers (protocells). Protocells are described in International Publication No. WO2013/056132, the content of which is herein incorporated by reference in its entirety.

In some embodiments, the RNA vaccines described herein may be formulated in polymeric nanoparticles as described in or made by the methods described in US Patent Nos. 8,420,123 and 8,518,963 and European Patent No. EP2073848B1, the contents of each of which are herein incorporated by reference in their entirety. As a non-limiting example, the polymeric nanoparticle may have a high glass transition temperature such as the nanoparticles described in or nanoparticles made by the methods described in US Patent No. 8,518,963, the content of which is herein incorporated by reference in its entirety. As another non-limiting example, the polymer nanoparticle for oral and parenteral formulations may be made by the methods described in European Patent No. EP2073848B1, the content of which is herein incorporated by reference in its entirety.

In other embodiments, the RNA (*e.g.*, mRNA) vaccines described herein may be formulated in nanoparticles used in imaging. The nanoparticles may be liposome nanoparticles such as those described in U.S. Publication No. 2013/0129636, herein incorporated by reference in its entirety. As a non-limiting example, the liposome may comprise gadolinium(III)-2-{4,7-bis-carboxymethyl-10-[(N,N-distearylamidomethyl-N'-amido-methyl)-1,4,7,10-tetra-azacyclododec-1-yl]}-acetic acid and a neutral, fully saturated phospholipid

component (*see e.g.*, U.S. Publication No US2013/0129636, the contents of which is herein incorporated by reference in its entirety).

In some embodiments, the nanoparticles which may be used in the present invention are formed by the methods described in U.S. Patent Application No. 2013/0130348, the contents of which is herein incorporated by reference in its entirety.

The nanoparticles of the present invention may further include nutrients such as, but not limited to, those which deficiencies can lead to health hazards from anemia to neural tube defects (*see e.g.*, the nanoparticles described in International Patent Publication No. WO2013/072929, the contents of which is herein incorporated by reference in its entirety). As a non-limiting example, the nutrient may be iron in the form of ferrous, ferric salts or elemental iron, iodine, folic acid, vitamins or micronutrients.

In some embodiments, the RNA (*e.g.*, mRNA) vaccines of the present invention may be formulated in a swellable nanoparticle. The swellable nanoparticle may be, but is not limited to, those described in U.S. Patent No. 8,440,231, the contents of which is herein incorporated by reference in its entirety. As a non-limiting embodiment, the swellable nanoparticle may be used for delivery of the RNA (*e.g.*, mRNA) vaccines of the present invention to the pulmonary system (*see e.g.*, U.S. Patent No. 8,440,231, the contents of which is herein incorporated by reference in its entirety).

The RNA (*e.g.*, mRNA) vaccines of the present invention may be formulated in polyanhydride nanoparticles such as, but not limited to, those described in U.S. Patent No. 8,449,916, the contents of which is herein incorporated by reference in its entirety. The nanoparticles and microparticles of the present invention may be geometrically engineered to modulate macrophage and/or the immune response. In some aspects, the geometrically engineered particles may have varied shapes, sizes and/or surface charges in order to incorporated the polynucleotides of the present invention for targeted delivery such as, but not limited to, pulmonary delivery (*see e.g.*, International Publication No. WO2013/082111, the content of which is herein incorporated by reference in its entirety). Other physical features the geometrically engineering particles may have include, but are not limited to, fenestrations, angled arms, asymmetry and surface roughness, charge which can alter the interactions with cells and tissues. As a non-limiting example, nanoparticles of the present invention may be made by the methods described in International Publication No. WO2013/082111, the contents of which is herein incorporated by reference in its entirety.

In some embodiments, the nanoparticles of the present invention may be water soluble nanoparticles such as, but not limited to, those described in International Publication No. WO2013/090601, the content of which is herein incorporated by reference in its entirety.

The nanoparticles may be inorganic nanoparticles which have a compact and zwitterionic ligand in order to exhibit good water solubility. The nanoparticles may also have small hydrodynamic diameters (HD), stability with respect to time, pH, and salinity and a low level of non-specific protein binding.

5 In some embodiments the nanoparticles of the present invention may be developed by the methods described in U.S. Publication No. US2013/0172406, the content of which is herein incorporated by reference in its entirety.

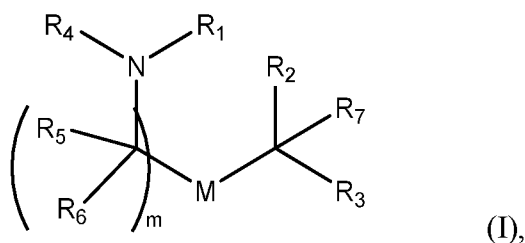
In some embodiments, the nanoparticles of the present invention are stealth nanoparticles or target-specific stealth nanoparticles such as, but not limited to, those
10 described in U.S. Publication No. 2013/0172406, the content of which is herein incorporated by reference in its entirety. The nanoparticles of the present invention may be made by the methods described in U.S. Publication No. 2013/0172406, the content of which is herein incorporated by reference in its entirety.

In other embodiments, the stealth or target-specific stealth nanoparticles may comprise a
15 polymeric matrix. The polymeric matrix may comprise two or more polymers such as, but not limited to, polyethylenes, polycarbonates, polyanhydrides, polyhydroxyacids, polypropylfumerates, polycaprolactones, polyamides, polyacetals, polyethers, polyesters, poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polycyanoacrylates, polyureas, polystyrenes, polyamines,
20 polyesters, polyanhydrides, polyethers, polyurethanes, polymethacrylates, polyacrylates, polycyanoacrylates or combinations thereof.

In some embodiments, the nanoparticle may be a nanoparticle-nucleic acid hybrid structure having a high density nucleic acid layer. As a non-limiting example, the nanoparticle-nucleic acid hybrid structure may made by the methods described in U.S. Publication No.
25 2013/0171646, the content of which is herein incorporated by reference in its entirety. The nanoparticle may comprise a nucleic acid such as, but not limited to, polynucleotides described herein and/or known in the art.

At least one of the nanoparticles of the present invention may be embedded in in the core a nanostructure or coated with a low density porous 3-D structure or coating which is
30 capable of carrying or associating with at least one payload within or on the surface of the nanostructure. Non-limiting examples of the nanostructures comprising at least one nanoparticle are described in International Publication No. WO2013/123523, the content of which is herein incorporated by reference in its entirety.

In some embodiments, a nanoparticle comprises compounds of Formula (I):



or a salt or isomer thereof, wherein:

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, $-R^*YR''$, $-YR''$, and $-R''M'R'$;

5 R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, $-R^*YR''$, $-YR''$, and $-R^*OR''$, or R_2 and R_3 , together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, $-CHQR$, $-CQ(R)_2$, and unsubstituted C_{1-6} alkyl, where Q is selected from a
 10 carbocycle, heterocycle, $-OR$, $-O(CH_2)_nN(R)_2$, $-C(O)OR$, $-OC(O)R$, $-CX_3$, $-CX_2H$, $-CXH_2$, $-CN$, $-N(R)_2$, $-C(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)C(O)N(R)_2$, $-N(R)C(S)N(R)_2$, $-N(R)R_8$, $-O(CH_2)_nOR$, $-N(R)C(=NR_9)N(R)_2$, $-N(R)C(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, $-N(OR)C(O)R$, $-N(OR)S(O)_2R$, $-N(OR)C(O)OR$, $-N(OR)C(O)N(R)_2$, $-N(OR)C(S)N(R)_2$, $-N(OR)C(=NR_9)N(R)_2$, $-N(OR)C(=CHR_9)N(R)_2$, $-C(=NR_9)N(R)_2$,
 15 $-C(=NR_9)R$, $-C(O)N(R)OR$, and $-C(R)N(R)_2C(O)OR$, and each n is independently selected from 1, 2, 3, 4, and 5;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl,
 20 and H;

M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')-$, $-N(R')C(O)-$, $-C(O)-$, $-C(S)-$, $-C(S)S-$, $-SC(S)-$, $-CH(OH)-$, $-P(O)(OR')O-$, $-S(O)_2-$, $-S-S-$, an aryl group, and a heteroaryl group;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

25 R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;

R_9 is selected from the group consisting of H, CN, NO_2 , C_{1-6} alkyl, $-OR$, $-S(O)_2R$, $-S(O)_2N(R)_2$, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

30 each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, $-R^*YR''$, $-YR''$, and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

5 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

In some embodiments, a subset of compounds of Formula (I) includes those in which when R₄ is -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, or -CQ(R)₂, then (i) Q is not -N(R)₂ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

In some embodiments, another subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

15 R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, mono- or di-alkylamino, and C₁₋₃ alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;

30 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

5 R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

10 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

15 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

20 In some embodiments, another subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

25 R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

30 R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂,

-C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R₄ is -(CH₂)_nQ in which n is 1 or 2, or (ii) R₄ is -(CH₂)_nCHQR in which n is 1, or (iii) R₄ is -CHQR, and -CQ(R)₂, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered

5 heterocycloalkyl;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

10 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

15 R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈

20 alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

25 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, another subset of compounds of Formula (I) includes those in

30 which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are

35 attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

In some embodiments, another subset of compounds of Formula (I) includes those in

which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₂₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is -(CH₂)_nQ or -(CH₂)_nCHQR, where Q is -N(R)₂, and n is selected from 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13, or salts or isomers thereof.

In some embodiments, another subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, $-CHQR$, and $-CQ(R)_2$, where Q is $-N(R)_2$, and n is selected from 1, 2, 3, 4, and 5;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

5 each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')$, $-N(R')C(O)-$, $-C(O)-$, $-C(S)-$, $-C(S)S-$, $-SC(S)-$, $-CH(OH)-$, $-P(O)(OR')O-$, $-S(O)_2-$, $-S-S-$, an aryl group, and a heteroaryl group;

10 R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, $-R^*YR''$, $-YR''$, and H;

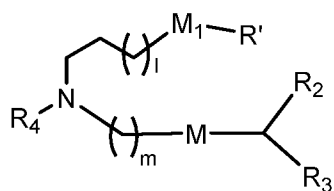
15 each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

each R^* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;

each Y is independently a C_{3-6} carbocycle;

20 each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13, or salts or isomers thereof.

In some embodiments, a subset of compounds of Formula (I) includes those of Formula (IA):

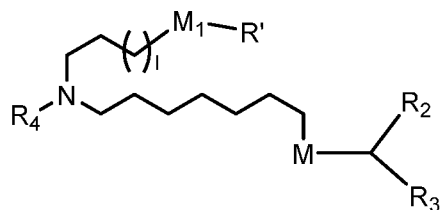


25 (IA),

or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M_1 is a bond or M' ; R_4 is unsubstituted C_{1-3} alkyl, or $-(CH_2)_nQ$, in which Q is OH, $-NHC(S)N(R)_2$, $-NHC(O)N(R)_2$, $-N(R)C(O)R$, $-N(R)S(O)_2R$, $-N(R)R_8$, $-NHC(=NR_9)N(R)_2$, $-NHC(=CHR_9)N(R)_2$, $-OC(O)N(R)_2$, $-N(R)C(O)OR$, heteroaryl or

30 heterocycloalkyl; M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')$, $-P(O)(OR')O-$, $-S-S-$, an aryl group, and a heteroaryl group; and R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, and C_{2-14} alkenyl.

In some embodiments, a subset of compounds of Formula (I) includes those of Formula (II):



(II) or a salt or isomer thereof, wherein l

is selected from 1, 2, 3, 4, and 5; M₁ is a bond or M'; R₄ is unsubstituted C₁₋₃ alkyl,

5 or -(CH₂)_nQ, in which n is 2, 3, or 4, and Q is OH, -NHC(S)N(R)₂, -NHC(O)N(R)₂,

-N(R)C(O)R, -N(R)S(O)₂R, -N(R)R₈, -NHC(=NR₉)N(R)₂, -NHC(=CHR₉)N(R)₂,

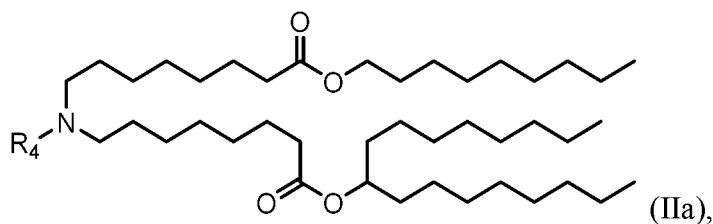
-OC(O)N(R)₂, -N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently

selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a

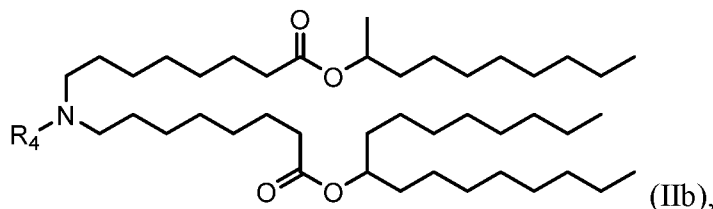
heteroaryl group; and R₂ and R₃ are independently selected from the group consisting of H,

10 C₁₋₁₄ alkyl, and C₂₋₁₄ alkenyl.

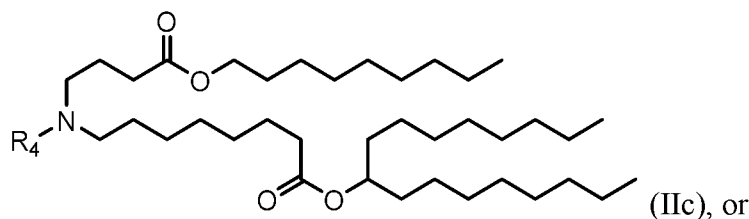
In some embodiments, a subset of compounds of Formula (I) includes those of Formula (IIa), (IIb), (IIc), or (IIe):



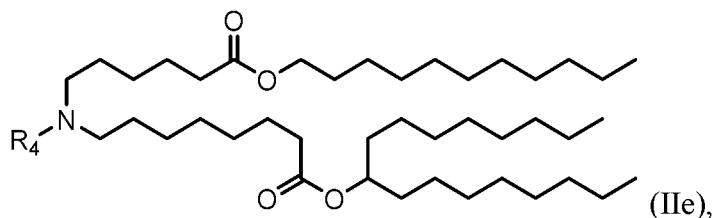
(IIa),



(IIb),



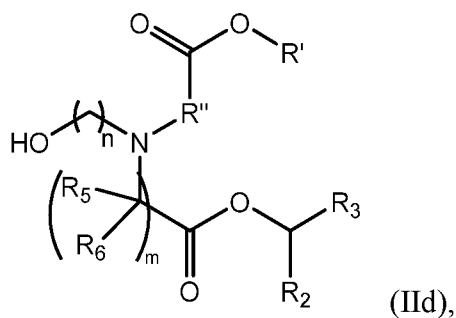
(IIc), or



(Ile),

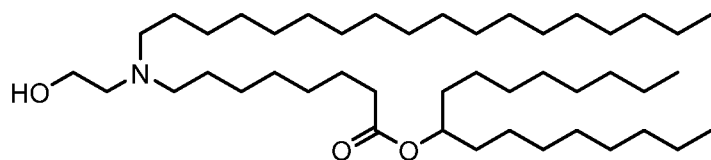
or a salt or isomer thereof, wherein R₄ is as described herein.

In some embodiments, a subset of compounds of Formula (I) includes those of Formula (II_d):

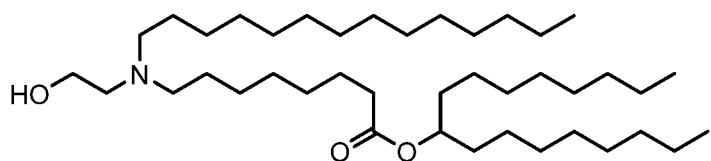


or a salt or isomer thereof, wherein *n* is 2, 3, or 4; and *m*, *R'*, *R''*, and *R*₂ through *R*₆ are as described herein. For example, each of *R*₂ and *R*₃ may be independently selected from the group consisting of C₅₋₁₄ alkyl and C₅₋₁₄ alkenyl.

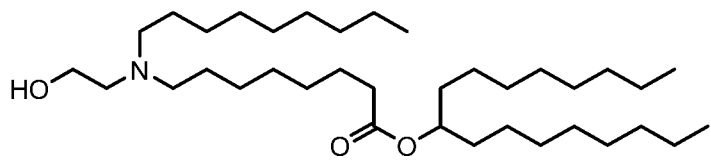
In some embodiments, the compound of Formula (I) is selected from the group consisting of:



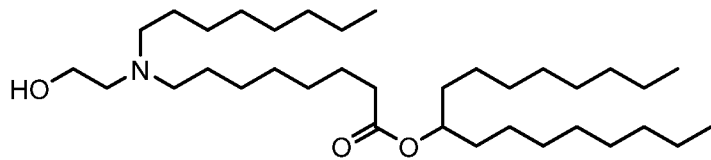
(Compound 1),



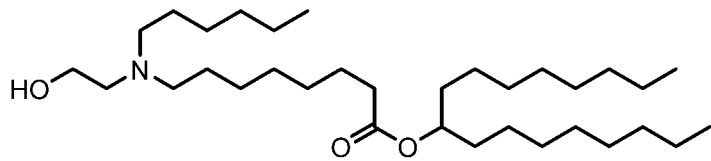
(Compound 2),



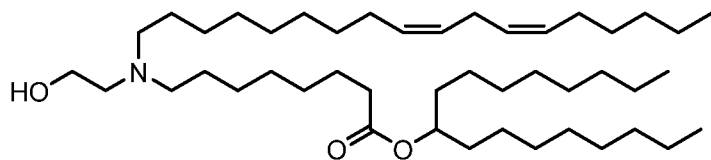
(Compound 3),



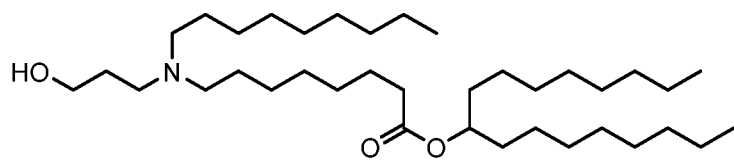
(Compound 4),



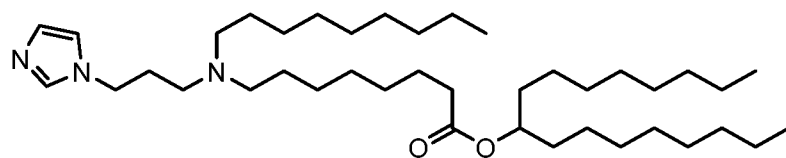
(Compound 5),



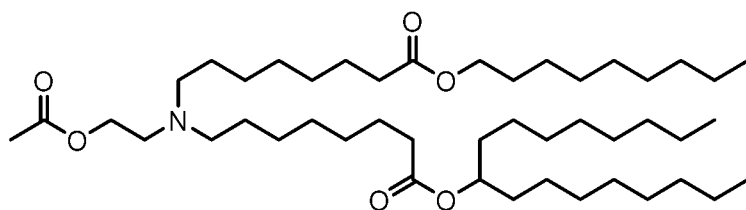
(Compound 6),



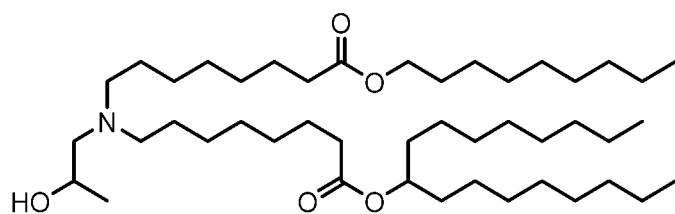
(Compound 7),



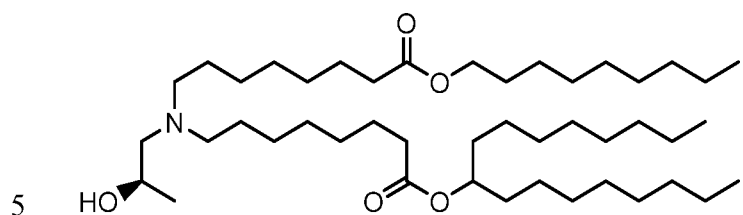
(Compound 8),



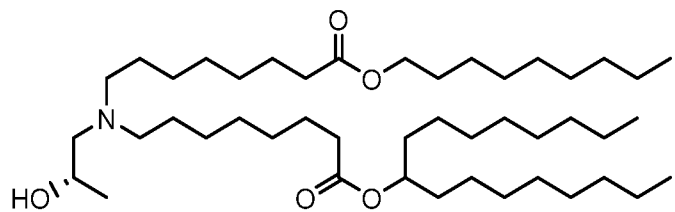
(Compound 9),



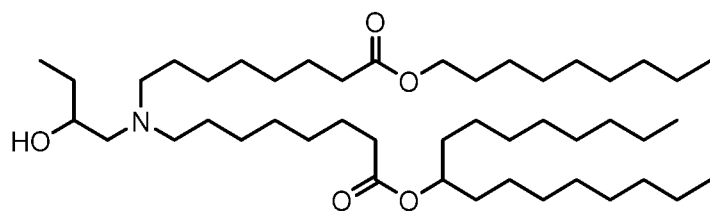
(Compound 10),



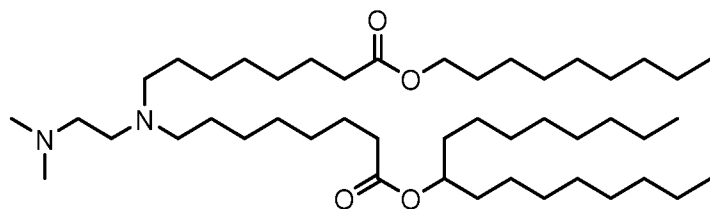
(Compound 11),



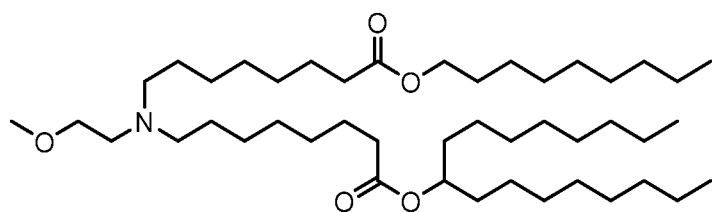
(Compound 12),



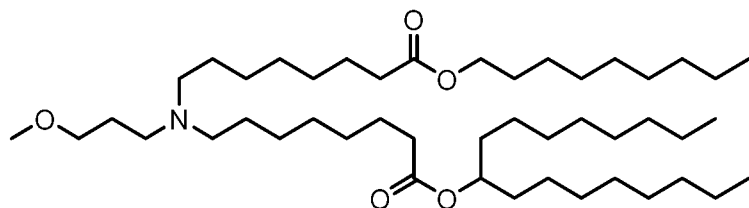
(Compound 13),



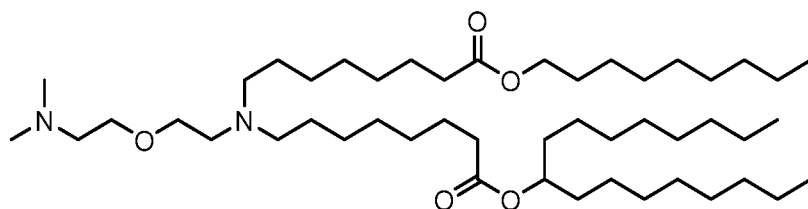
(Compound 14).



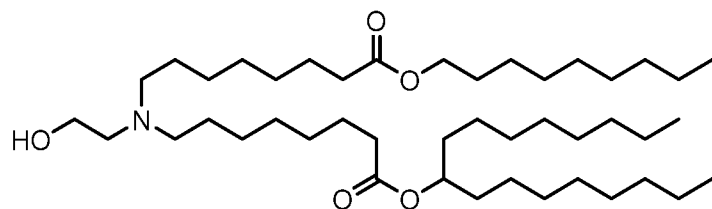
(Compound 15),



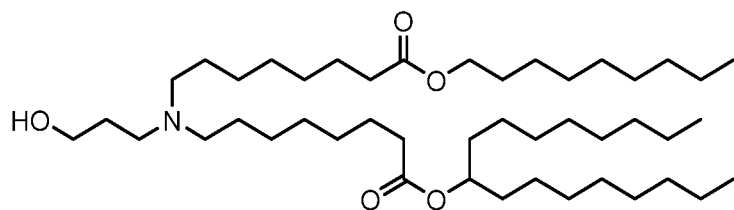
(Compound 16),



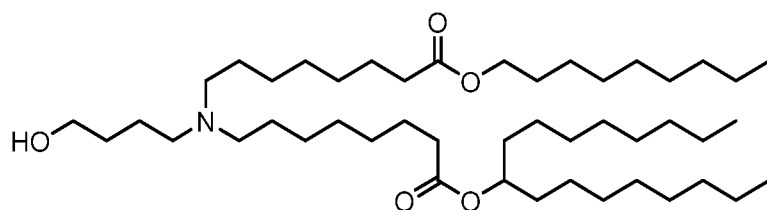
(Compound 17),



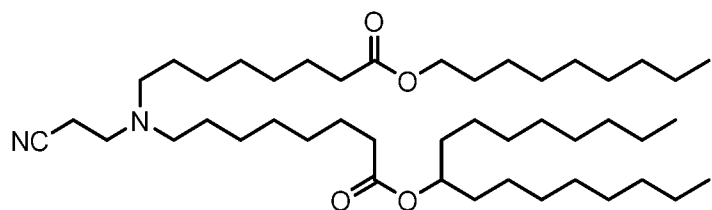
(Compound 18),



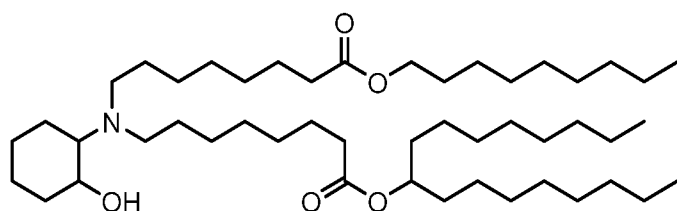
(Compound 19),



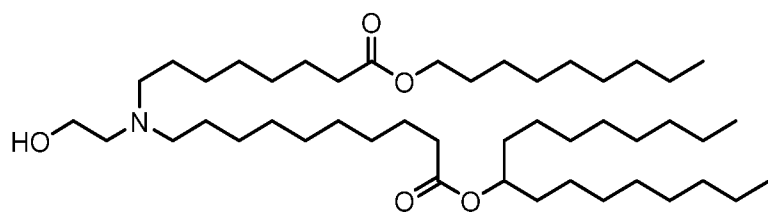
(Compound 20),



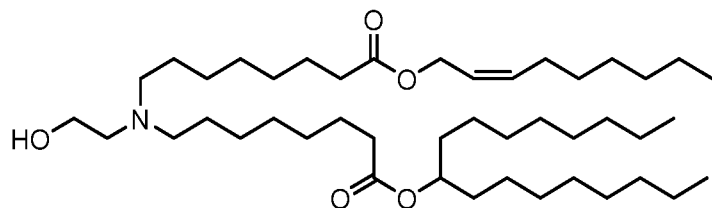
(Compound 21),



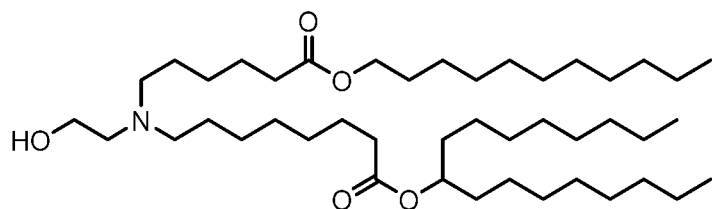
(Compound 22),



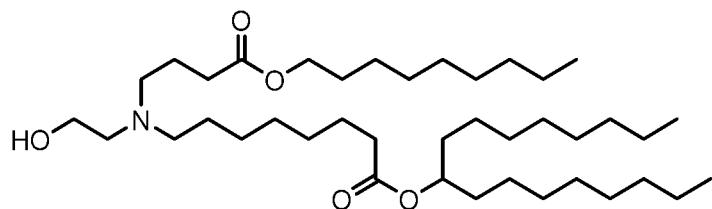
(Compound 23),



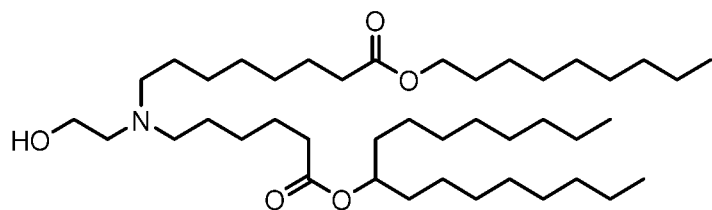
(Compound 24),



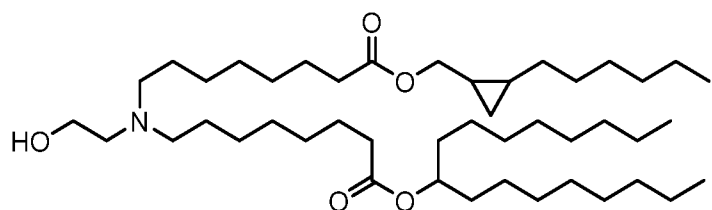
(Compound 25),



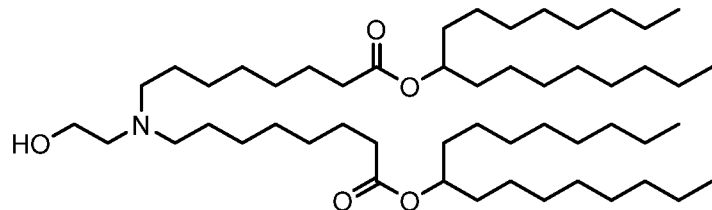
(Compound 26),



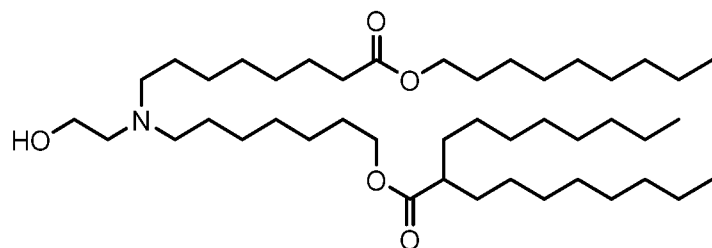
(Compound 27),



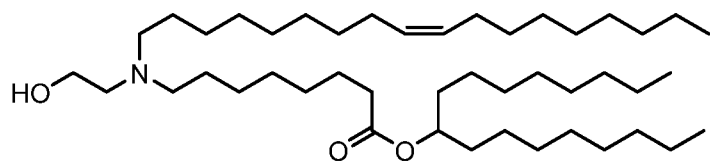
(Compound 28),



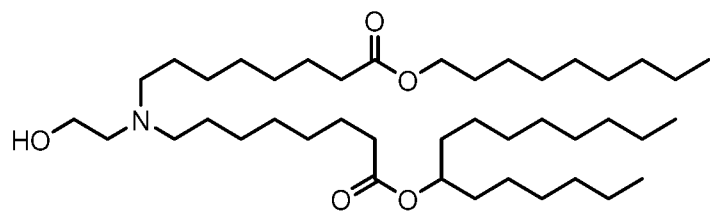
(Compound 29),



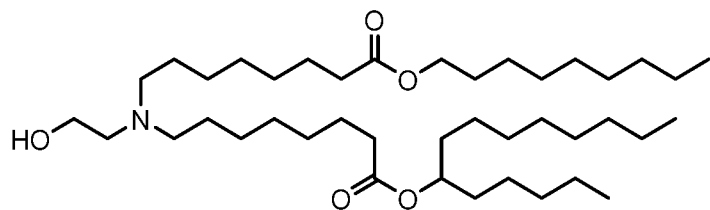
(Compound 30),



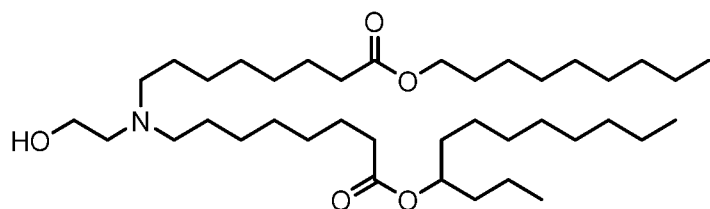
(Compound 31),



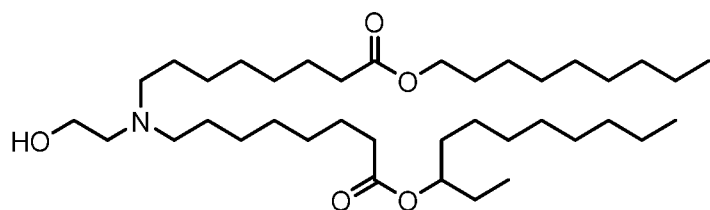
(Compound 32),



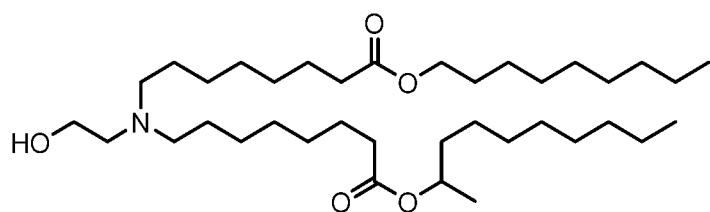
(Compound 33),



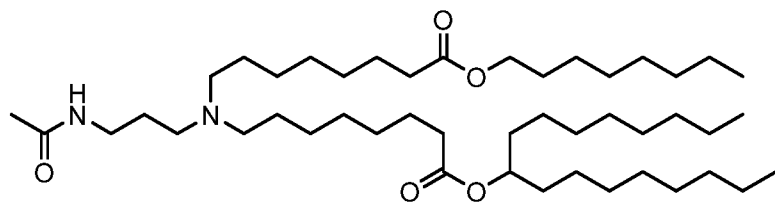
(Compound 34),



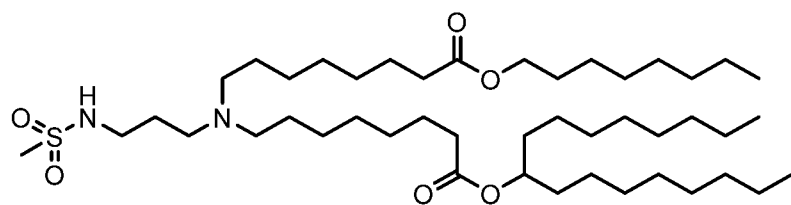
(Compound 35),



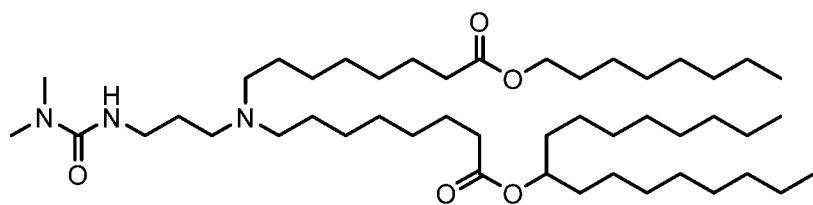
(Compound 36),



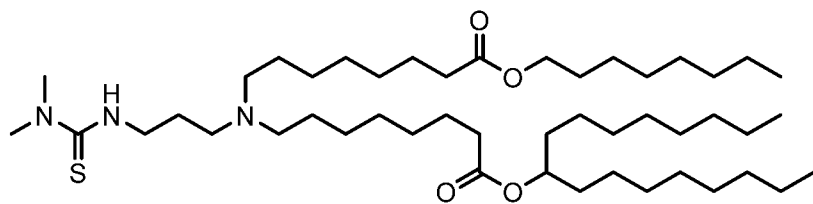
(Compound 37),



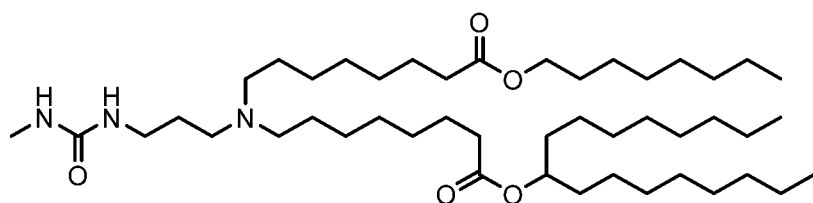
(Compound 38),



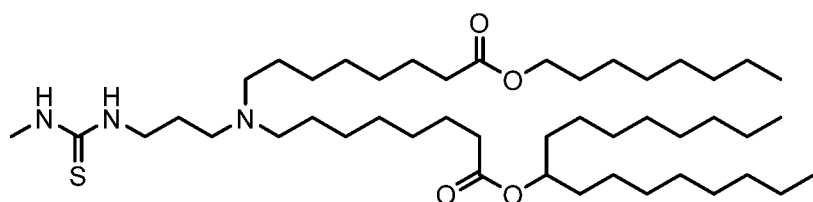
(Compound 39),



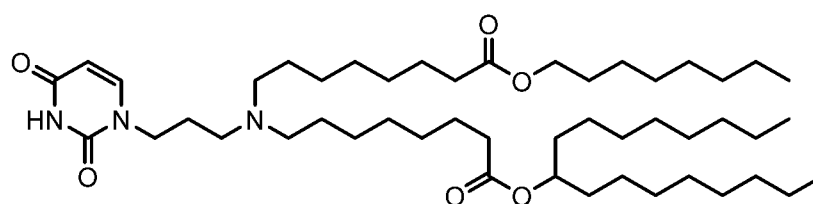
(Compound 40),



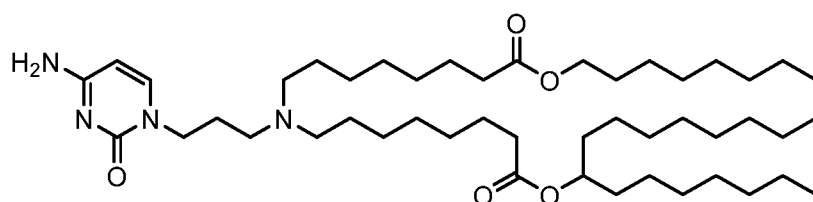
(Compound 41),



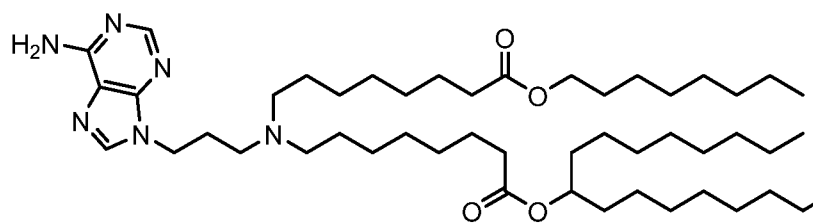
(Compound 42),



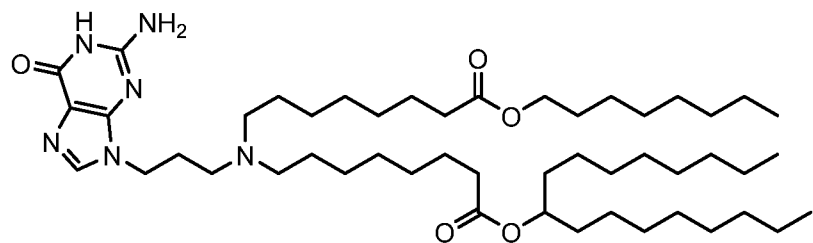
(Compound 43),



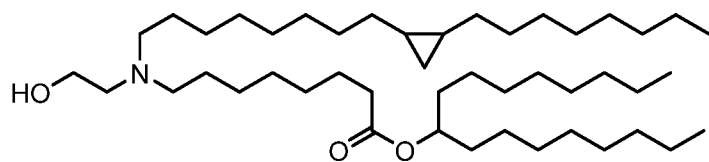
(Compound 44),



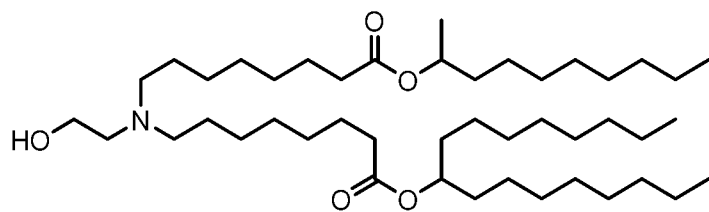
(Compound 45),



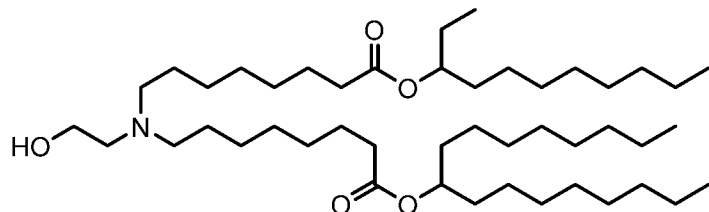
(Compound 46),



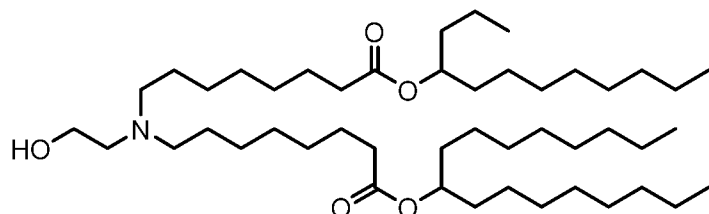
(Compound 47),



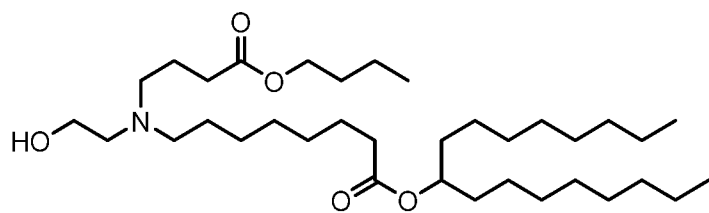
(Compound 48),



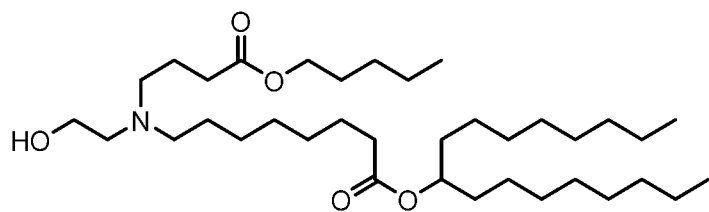
(Compound 49),



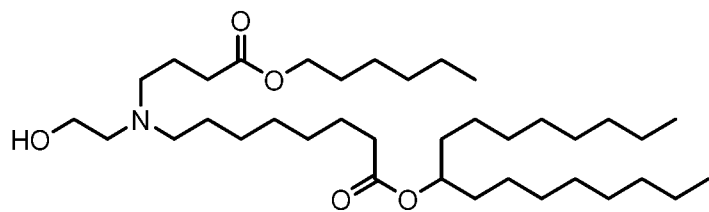
(Compound 50),



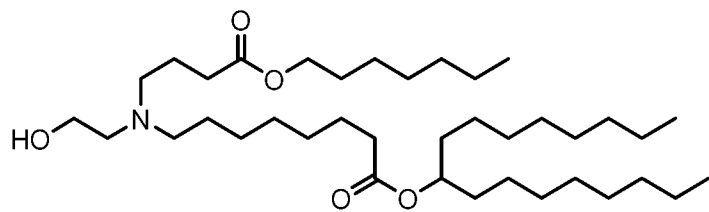
(Compound 51),



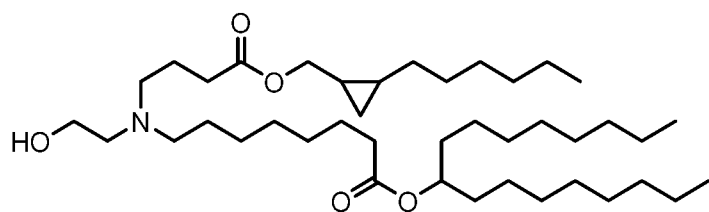
(Compound 52),



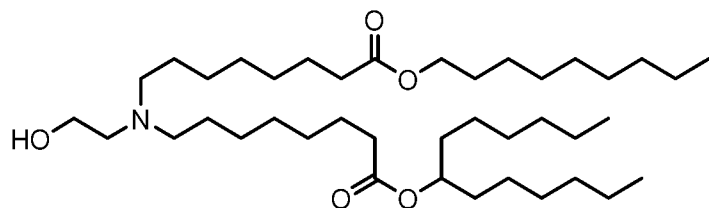
(Compound 53),



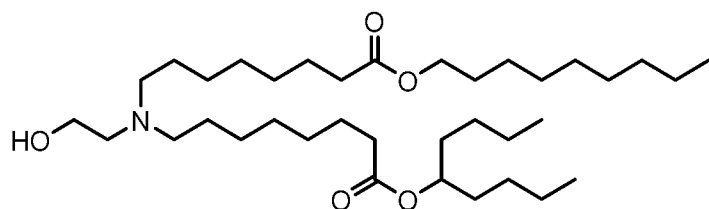
(Compound 54),



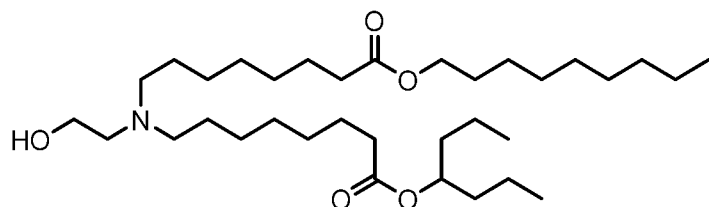
(Compound 55),



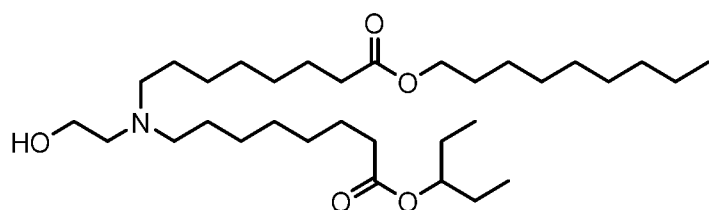
(Compound 56),



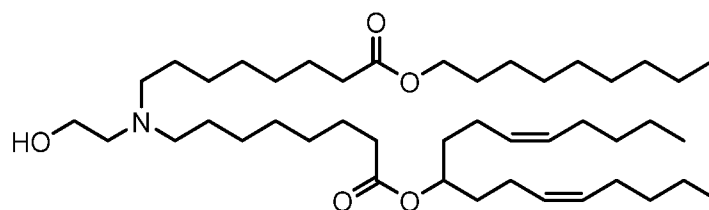
(Compound 57),



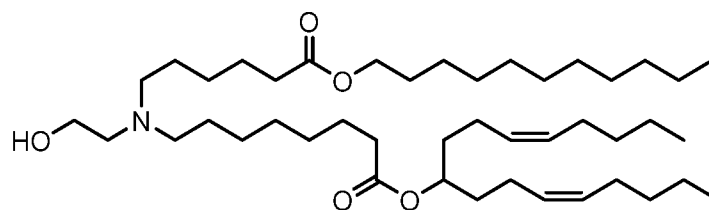
(Compound 58),



(Compound 59),

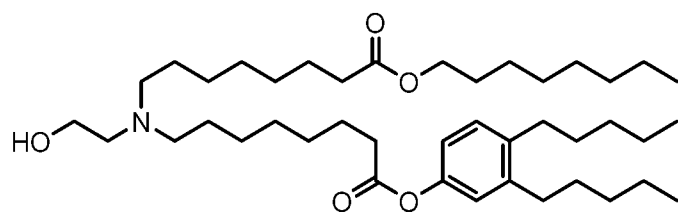


(Compound 60), and

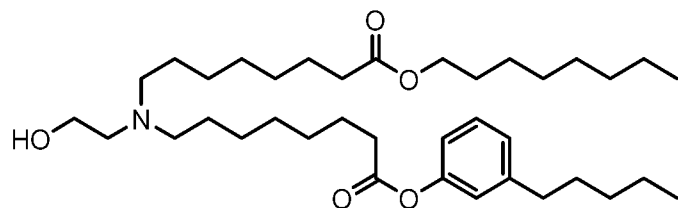


(Compound 61).

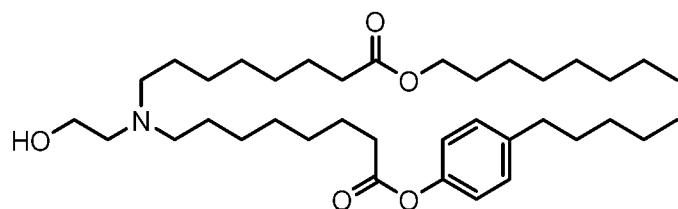
In further embodiments, the compound of Formula (I) is selected from the group consisting of:



(Compound 62),

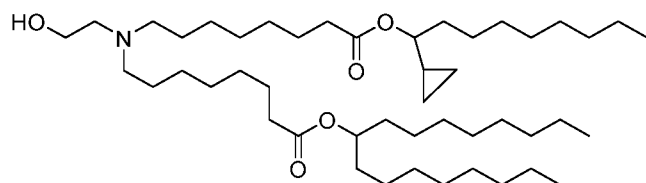


(Compound 63), and

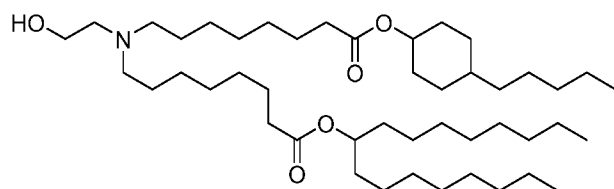


(Compound 64).

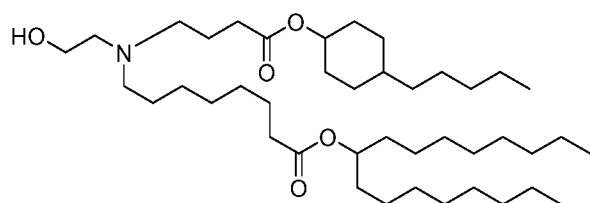
- 5 In some embodiments, the compound of Formula (I) is selected from the group consisting of:



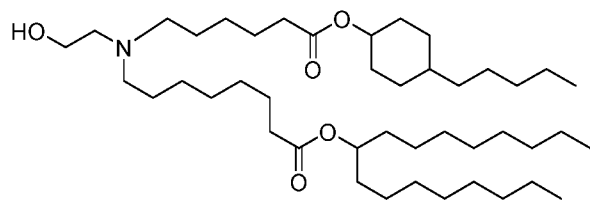
(Compound 65),



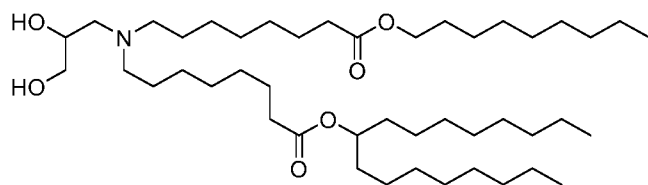
(Compound 66),



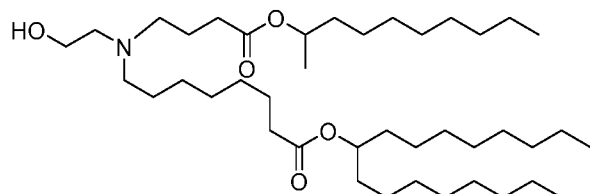
(Compound 67),



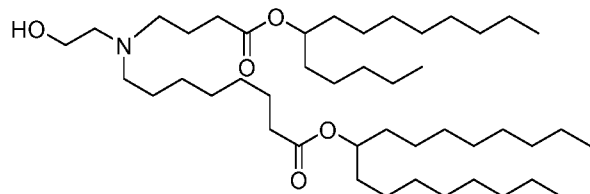
(Compound 68),



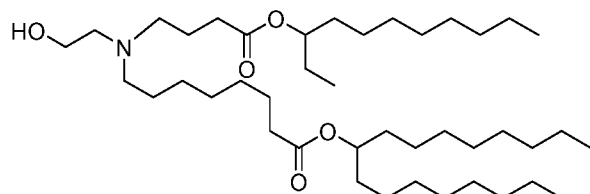
(Compound 69),



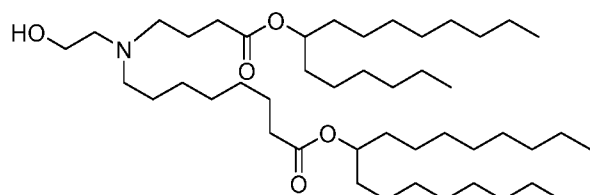
(Compound 70),



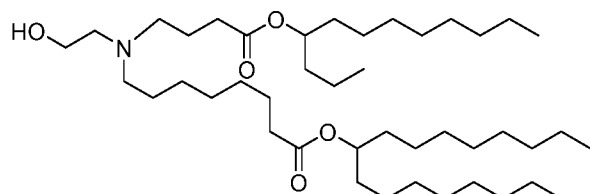
(Compound 71),



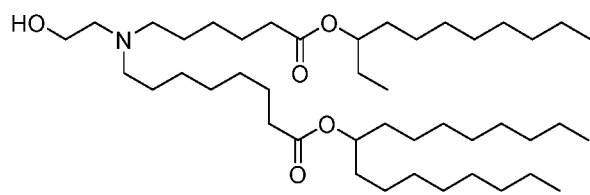
(Compound 72),



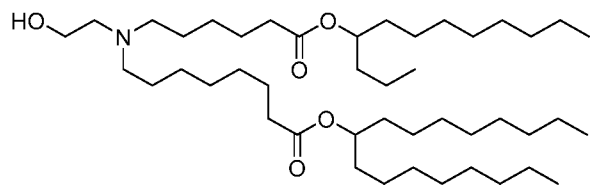
(Compound 73),



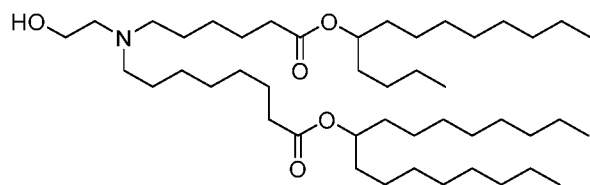
(Compound 74),



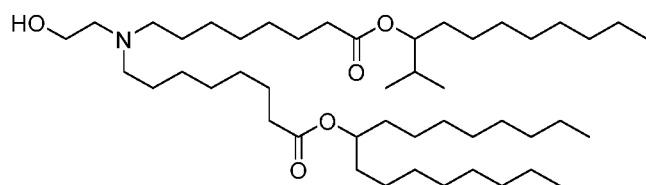
(Compound 75),



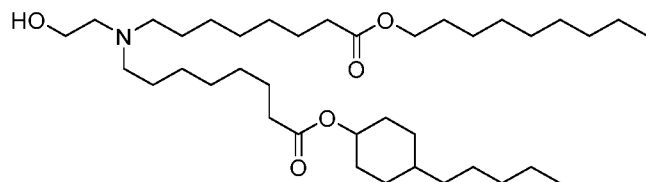
(Compound 76),



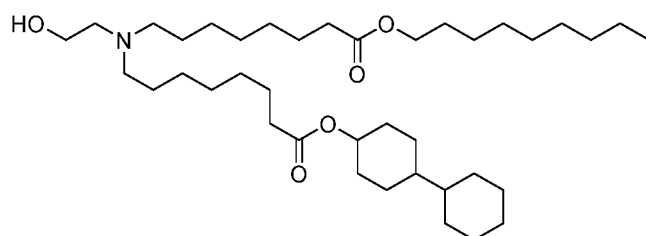
(Compound 77),



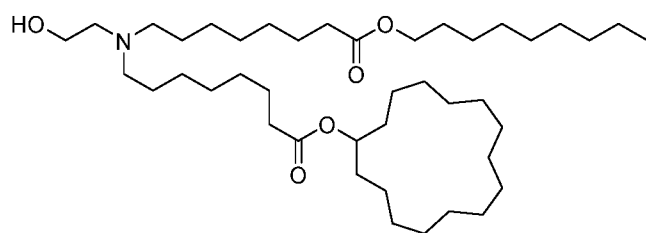
(Compound 78),



(Compound 79),

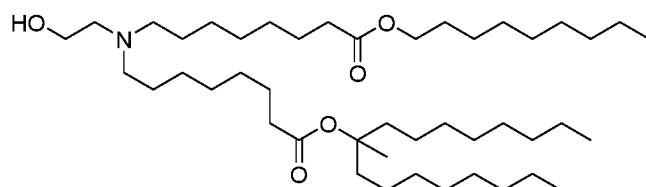


(Compound 80),

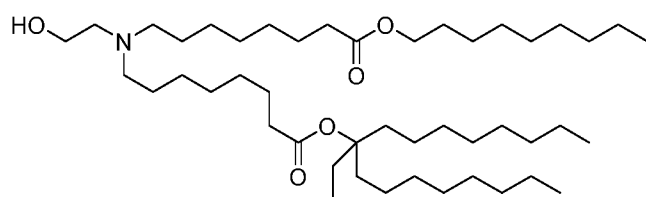


(Compound 81),

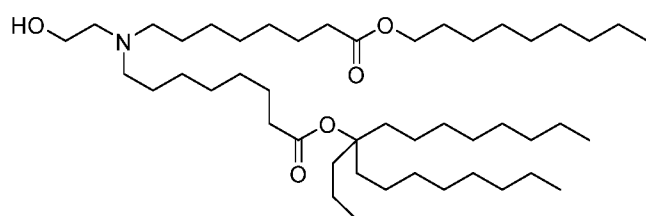
5



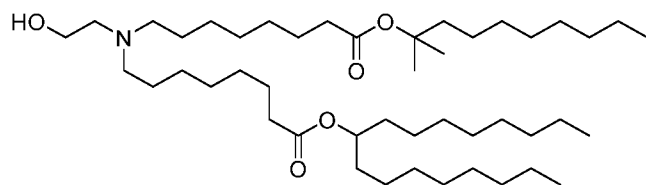
(Compound 82),



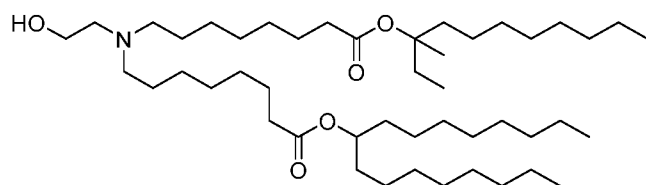
(Compound 83),



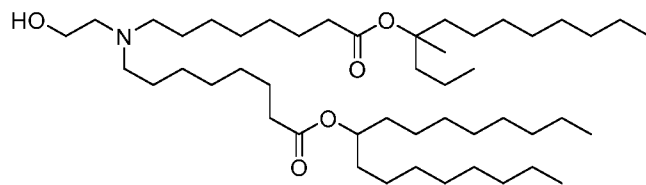
(Compound 84),



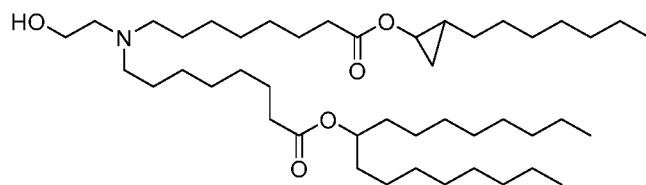
(Compound 85),



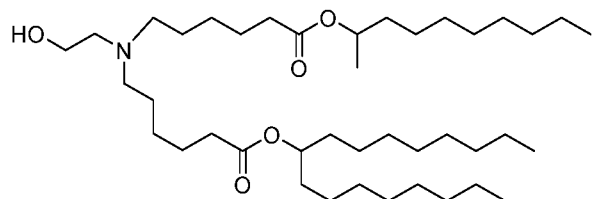
(Compound 86),



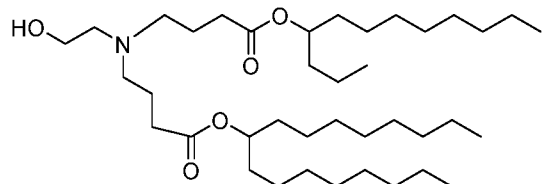
(Compound 87),



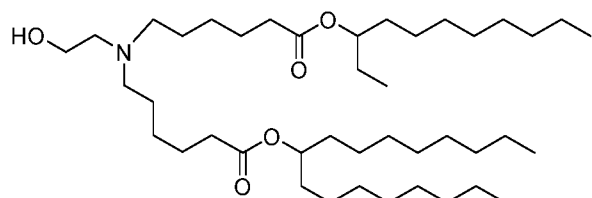
(Compound 88),



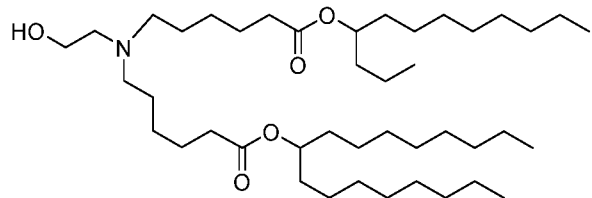
(Compound 89),



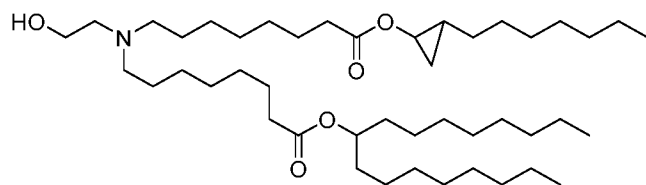
(Compound 90),



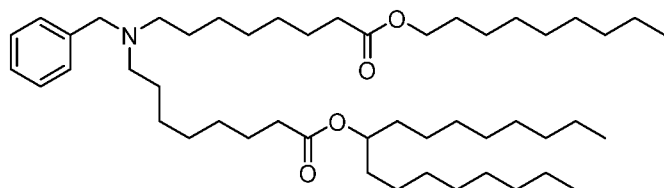
(Compound 91),



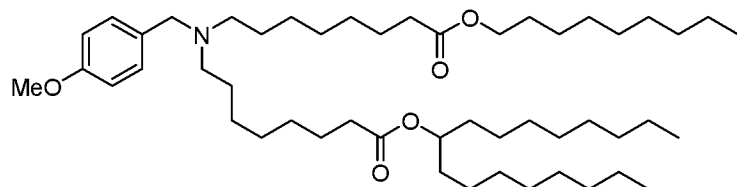
(Compound 92),



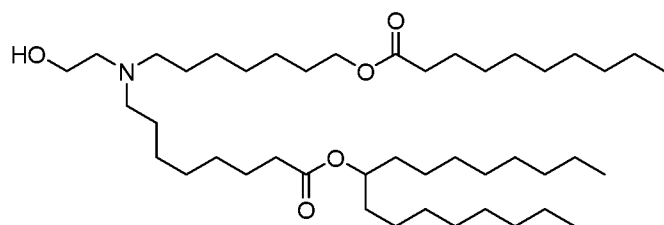
(Compound 93),



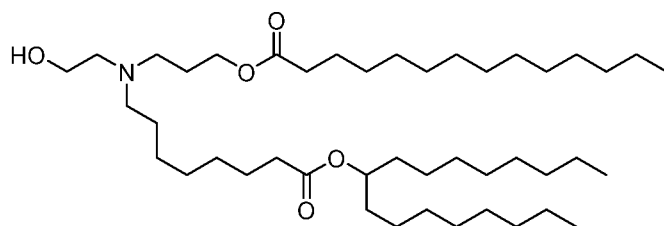
(Compound 94),



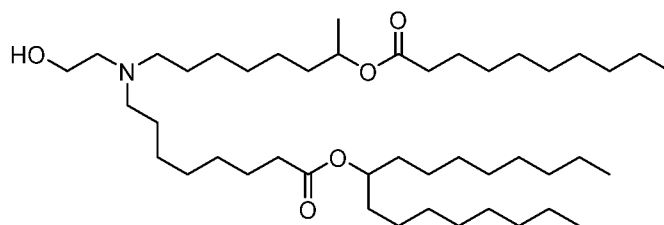
(Compound 95),



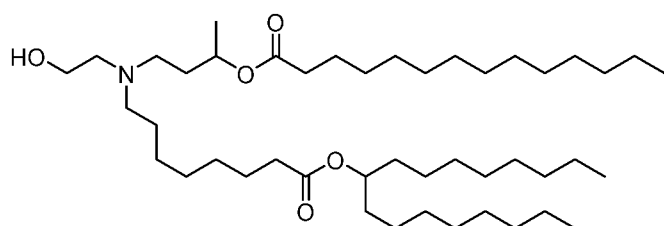
(Compound 96),



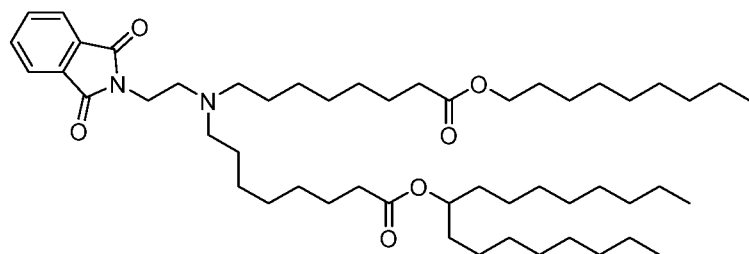
(Compound 97),



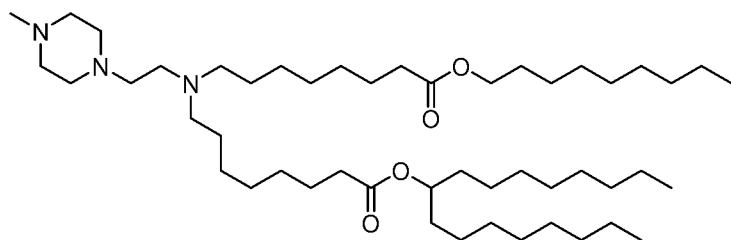
(Compound 98),



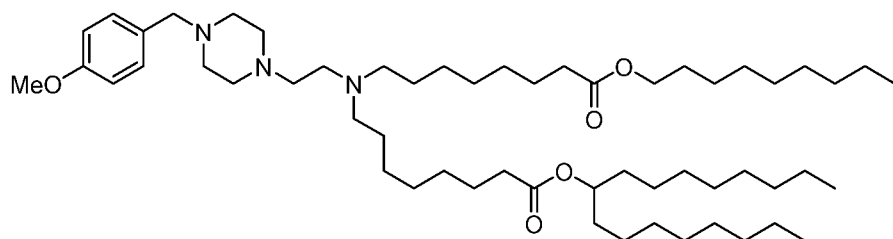
(Compound 99),



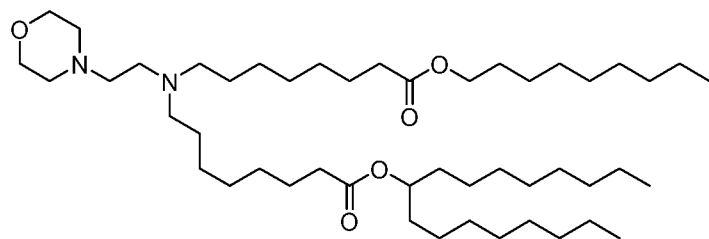
(Compound 100),



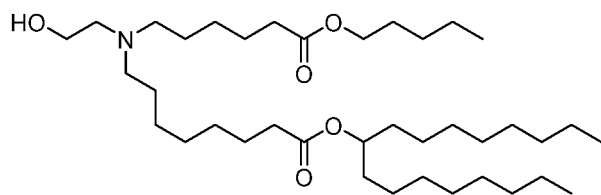
(Compound 101),



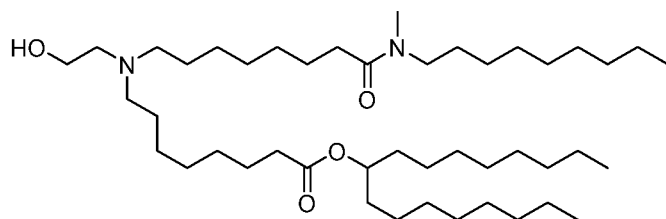
(Compound 102),



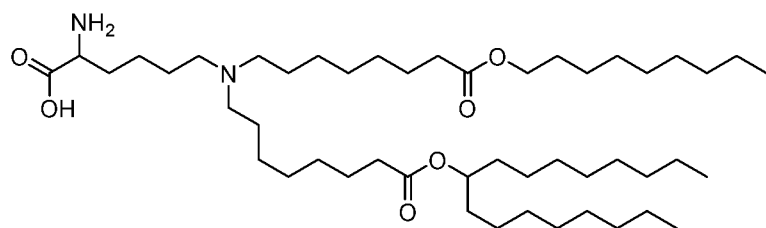
(Compound 103),



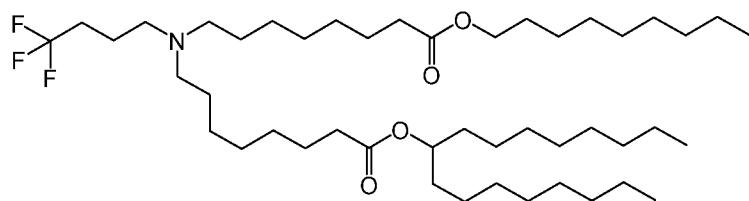
(Compound 104),



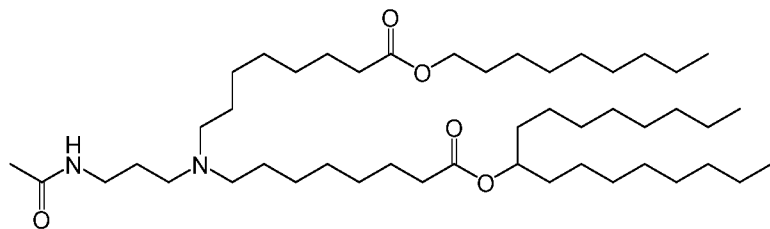
(Compound 105),



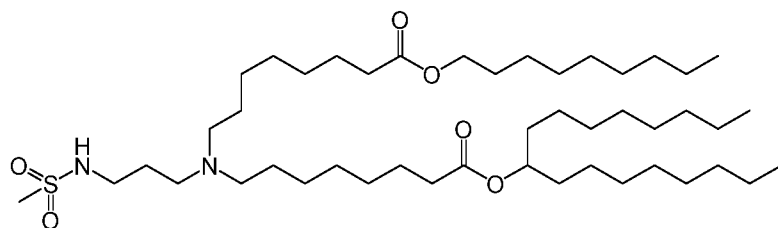
(Compound 106),



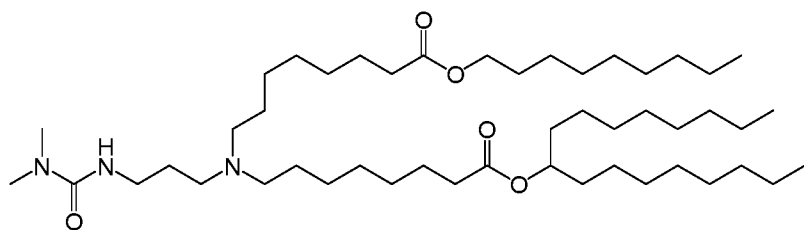
(Compound 107),



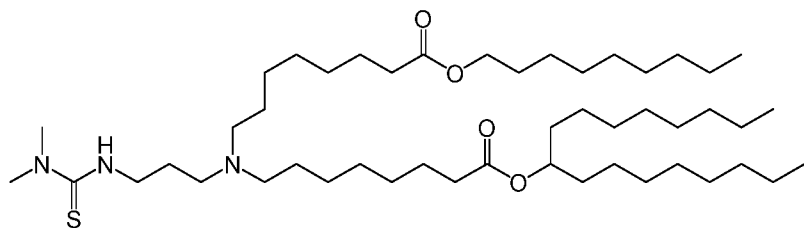
(Compound 108),



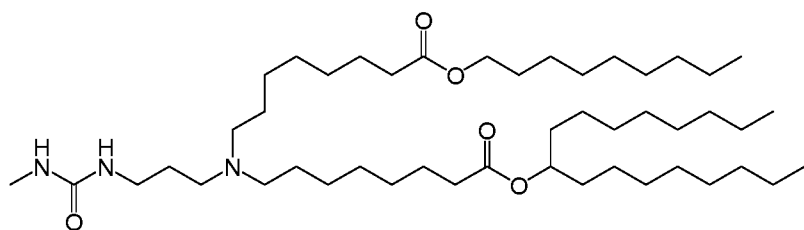
(Compound 109),



(Compound 110),

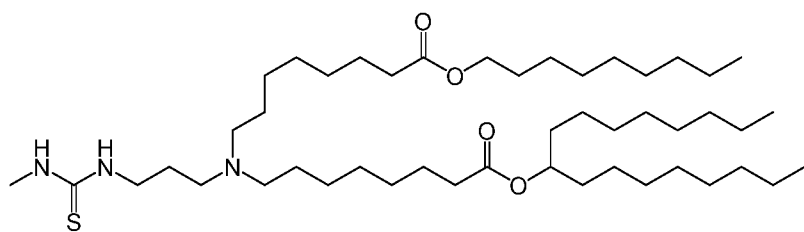


(Compound 111),

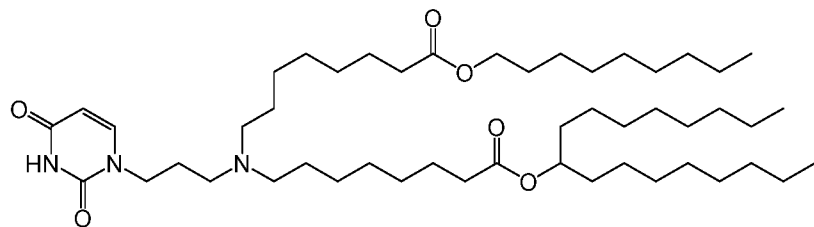


5

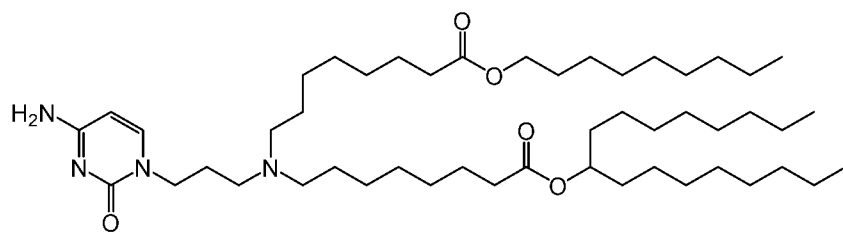
(Compound 112),



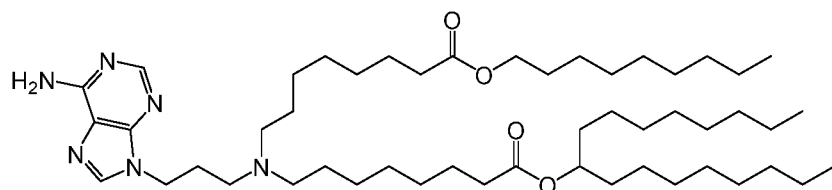
(Compound 113),



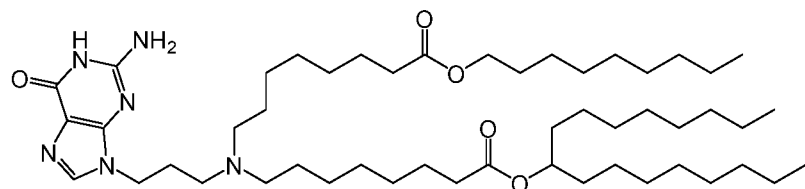
(Compound 114),



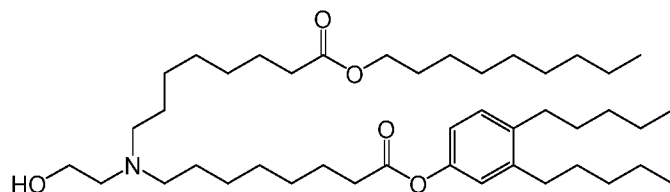
(Compound 115),



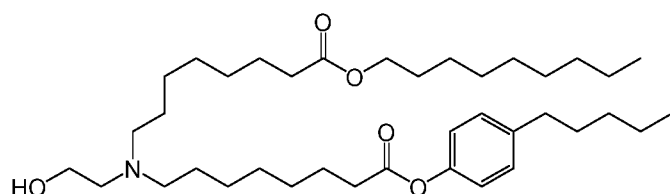
(Compound 116),



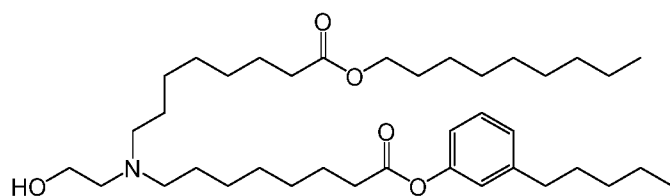
(Compound 117),



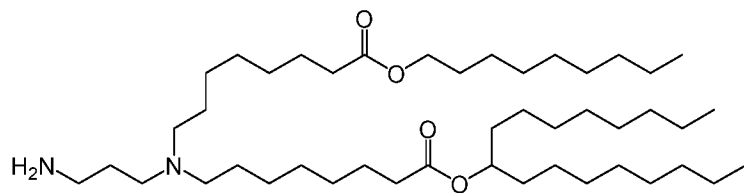
(Compound 118),



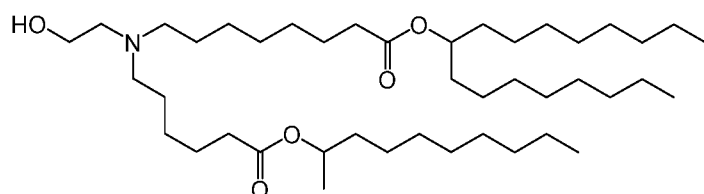
(Compound 119),



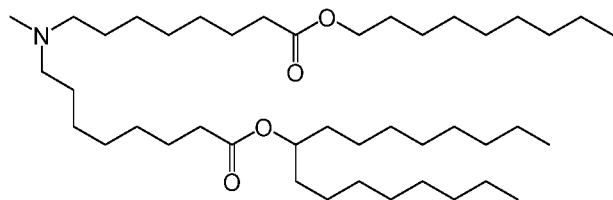
(Compound 120),



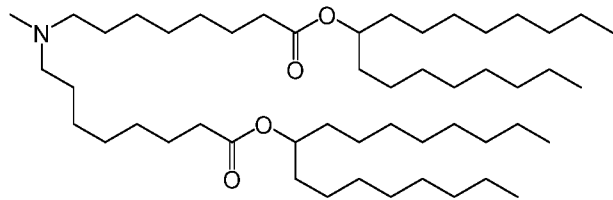
(Compound 121),



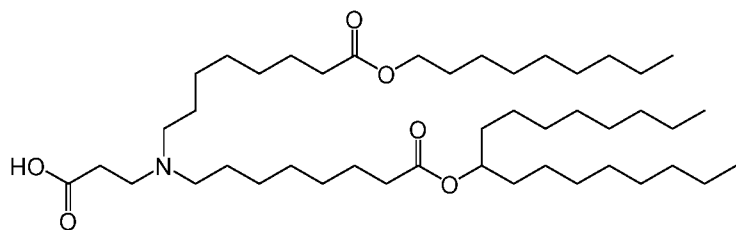
(Compound 122),



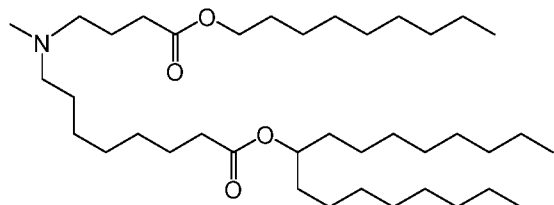
(Compound 123),



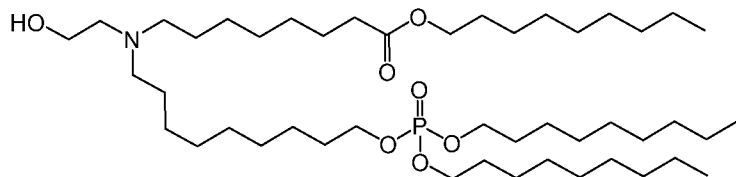
(Compound 124),



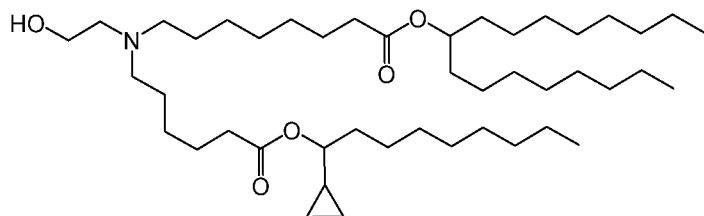
(Compound 125),



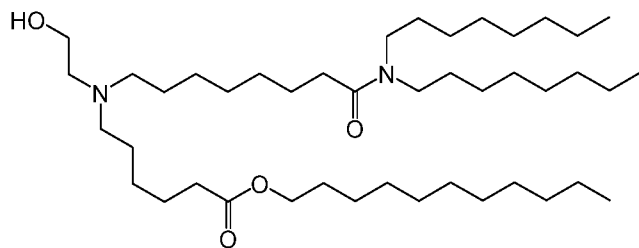
(Compound 126),



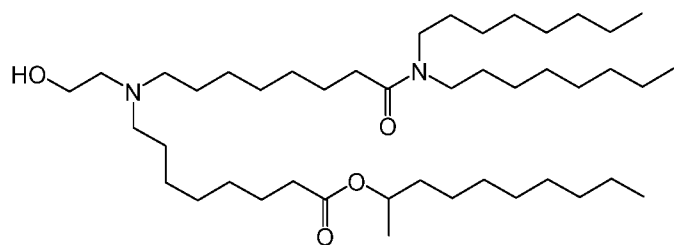
(Compound 127),



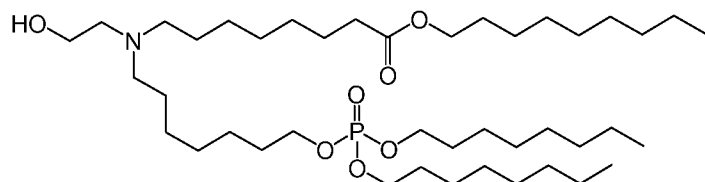
(Compound 128),



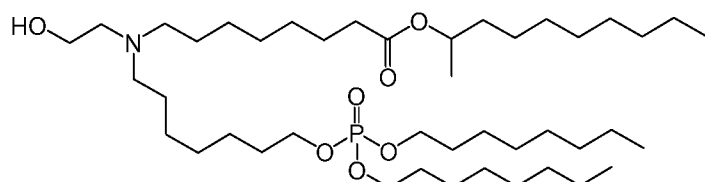
(Compound 129),



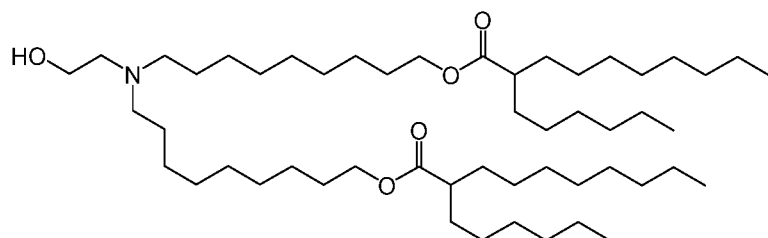
(Compound 130),



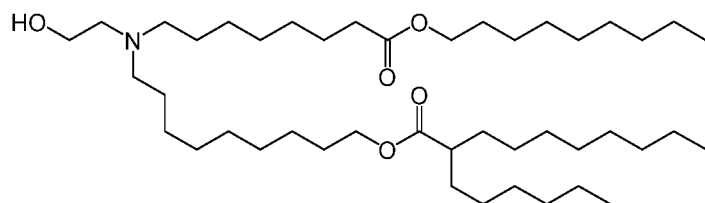
(Compound 131),



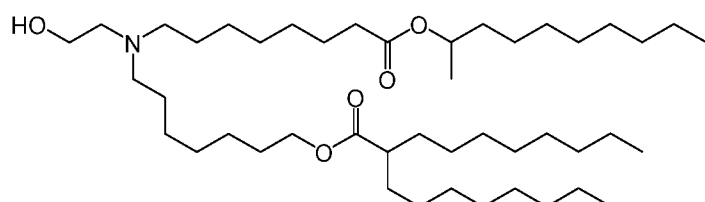
(Compound 132),



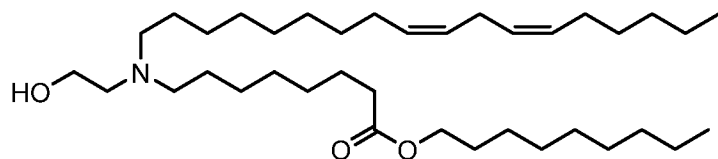
(Compound 133),



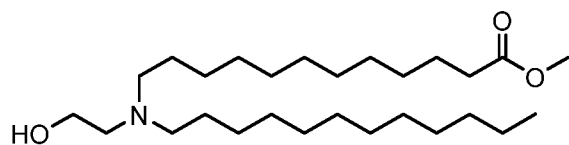
(Compound 134),



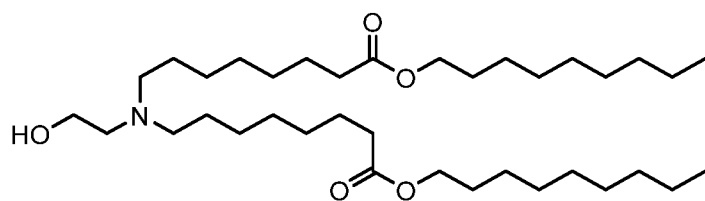
(Compound 135),



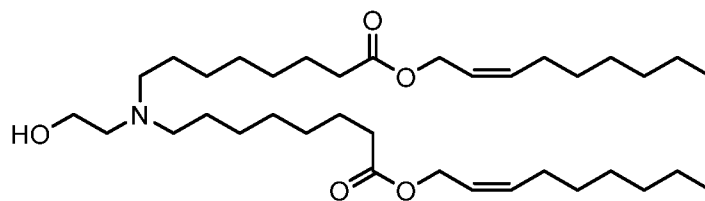
(Compound 136),



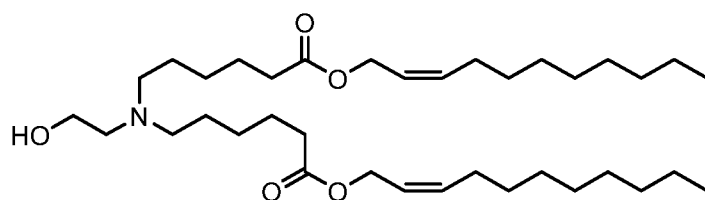
(Compound 137),



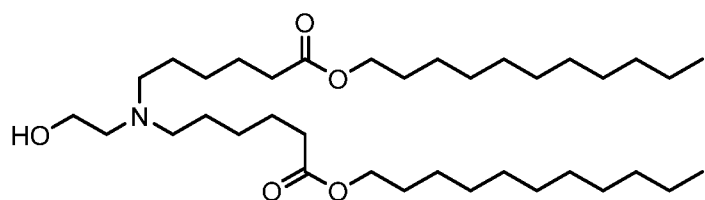
(Compound 138),



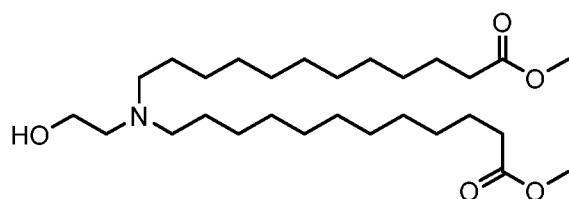
(Compound 139),



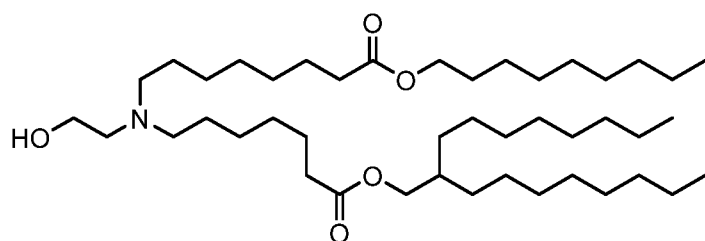
(Compound 140),



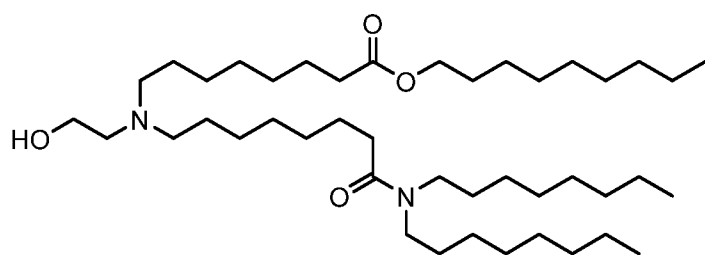
(Compound 141),



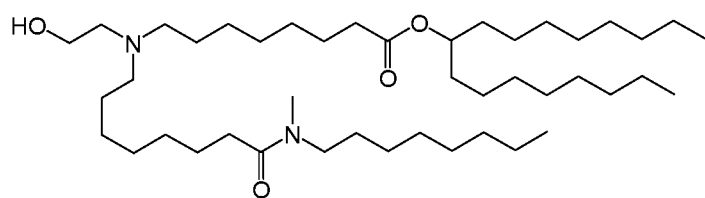
(Compound 142),



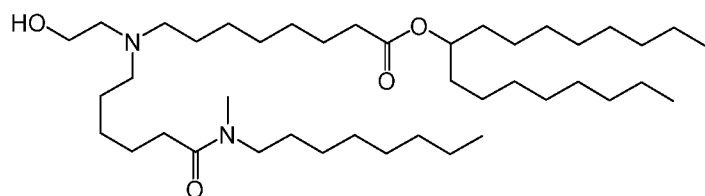
(Compound 143),



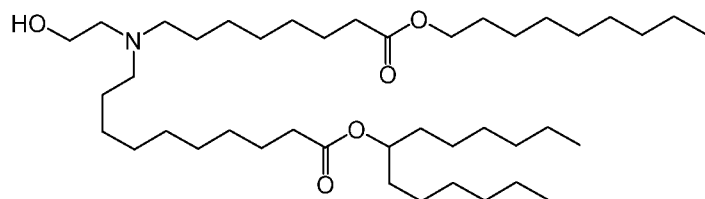
(Compound 144),



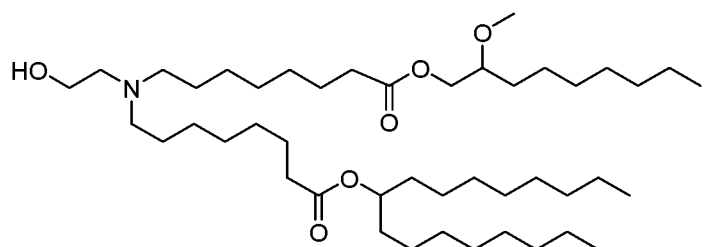
(Compound 145),



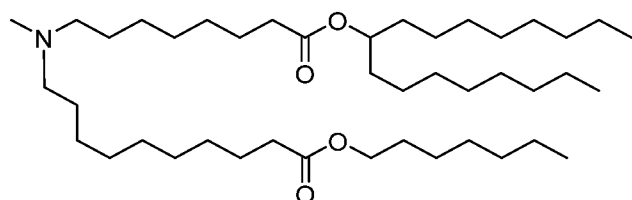
(Compound 146),



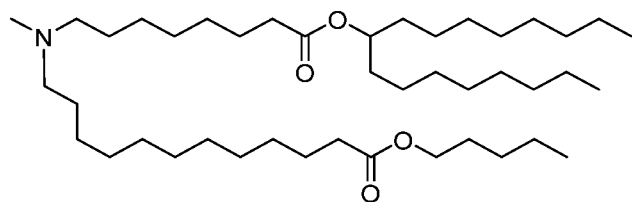
(Compound 147),



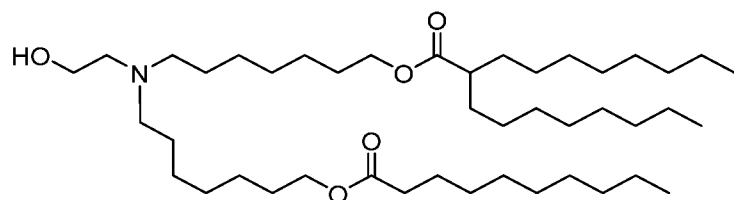
(Compound 148),



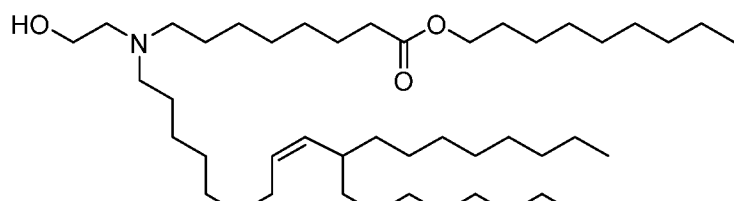
(Compound 149),



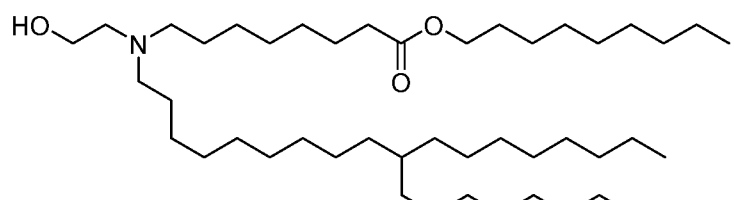
(Compound 150),



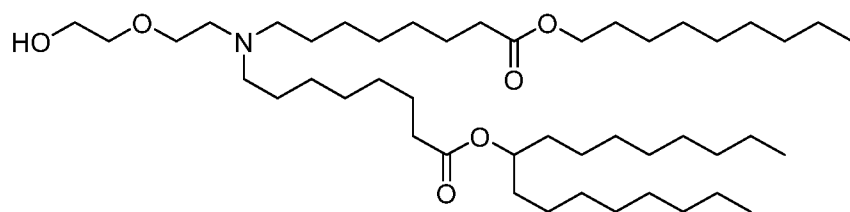
(Compound 151),



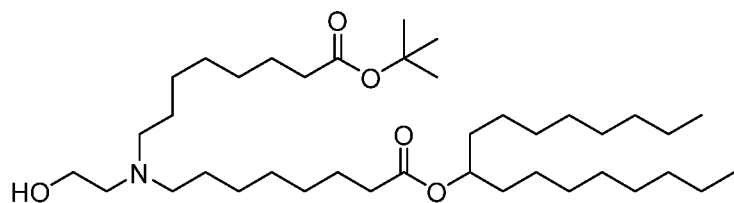
(Compound 152),



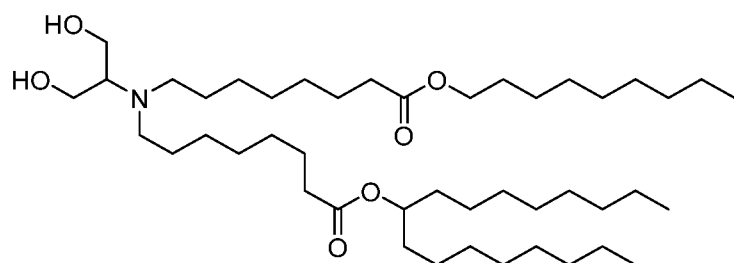
(Compound 153),



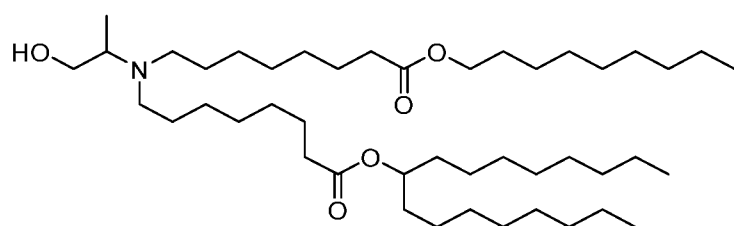
(Compound 154),



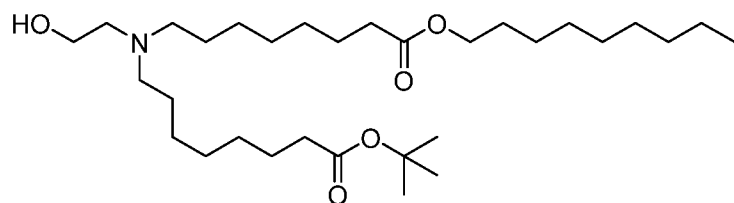
(Compound 155),



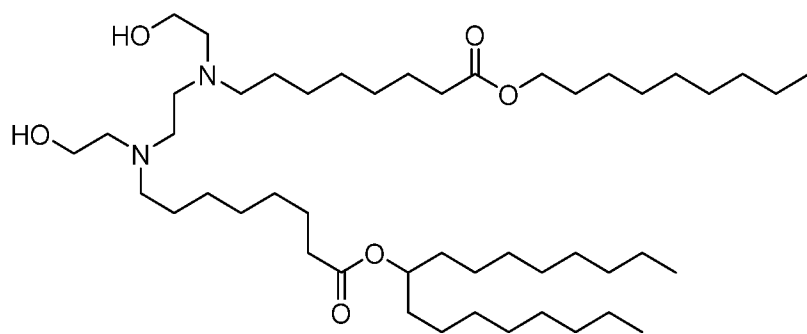
(Compound 156),



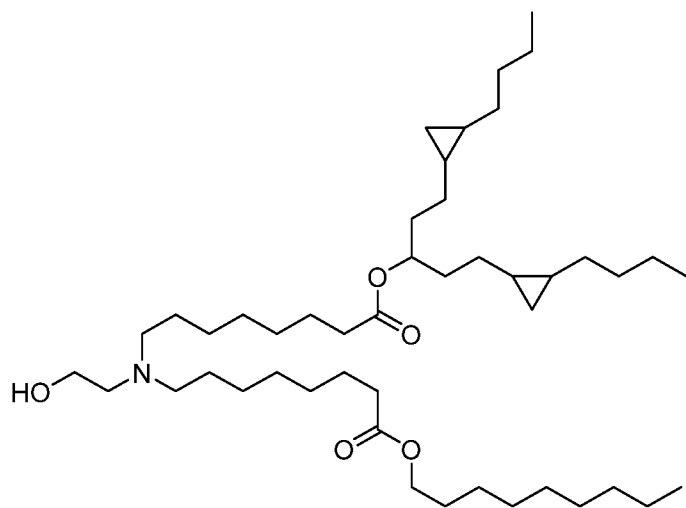
(Compound 157),



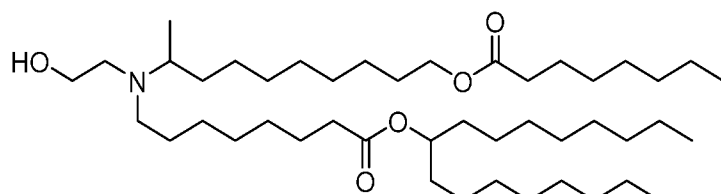
(Compound 158),



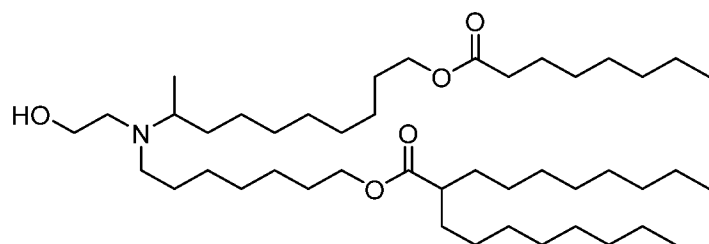
(Compound 159),



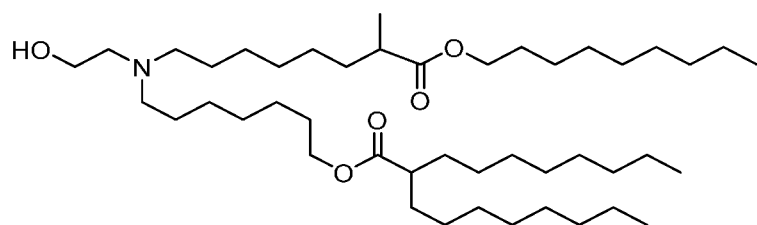
(Compound 160),



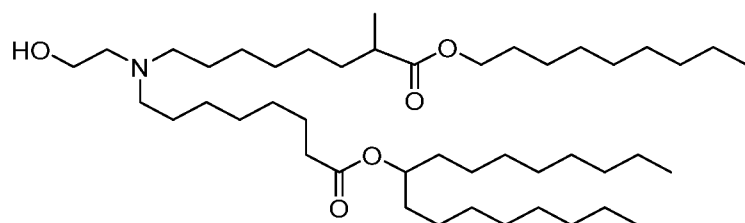
(Compound 161),



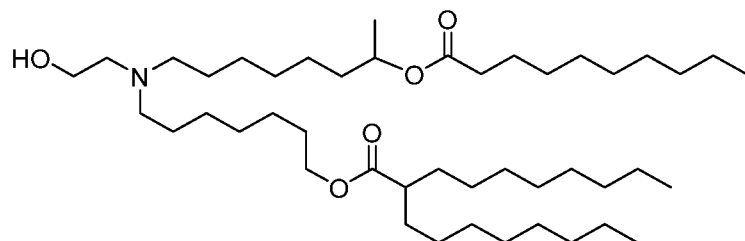
(Compound 162),



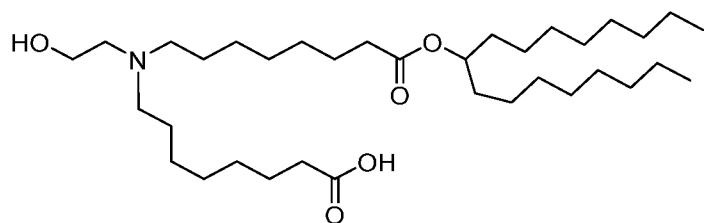
(Compound 163),



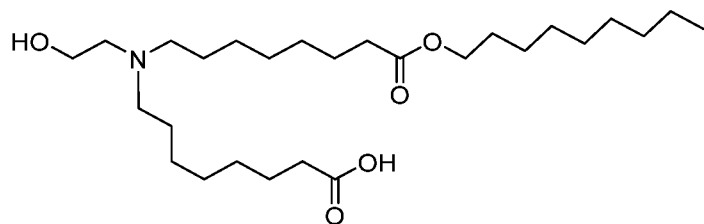
(Compound 164),



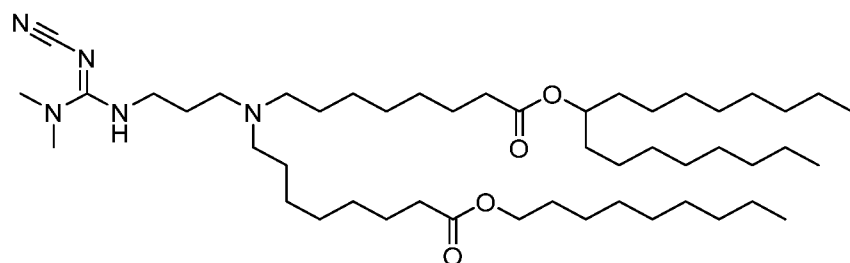
(Compound 165),



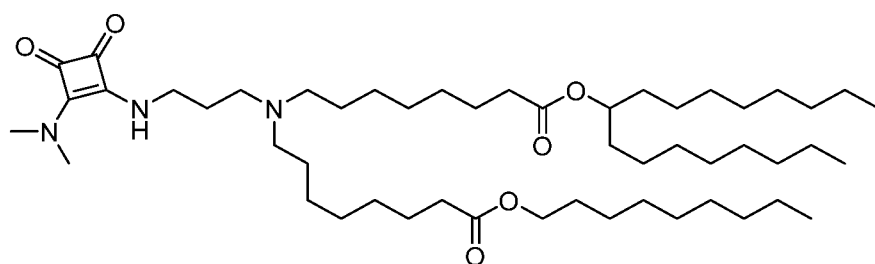
(Compound 166),



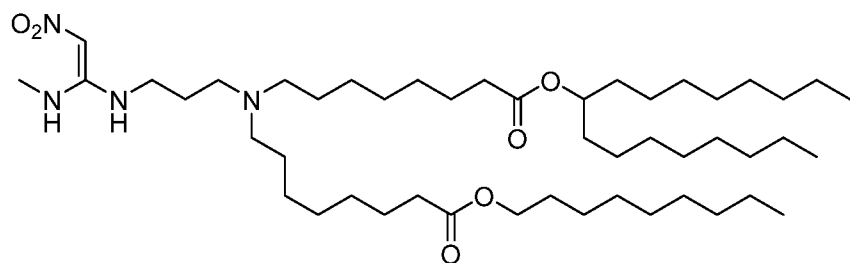
(Compound 167),



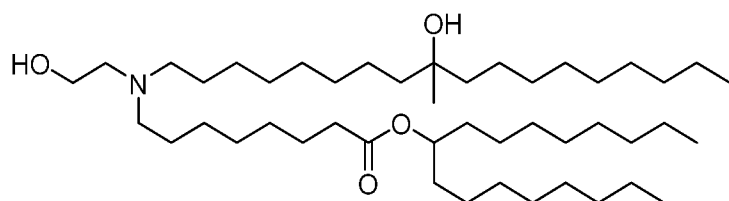
(Compound 168),



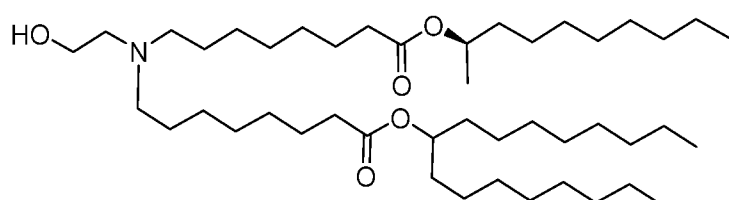
(Compound 169),



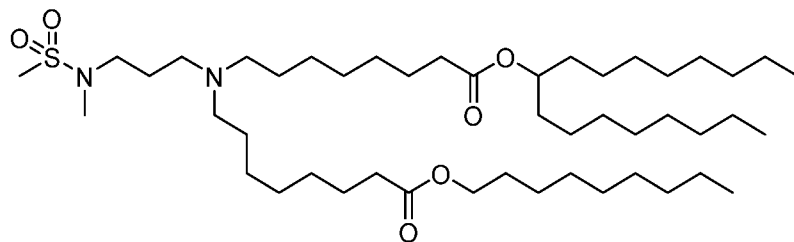
(Compound 170),



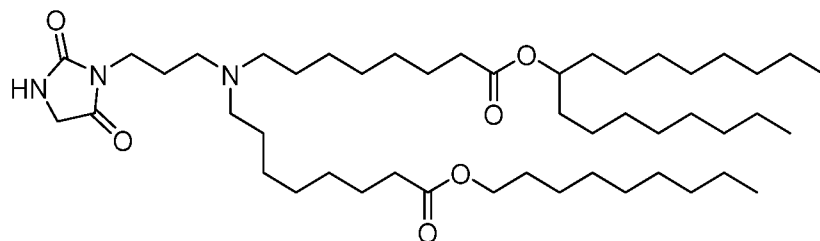
(Compound 171),



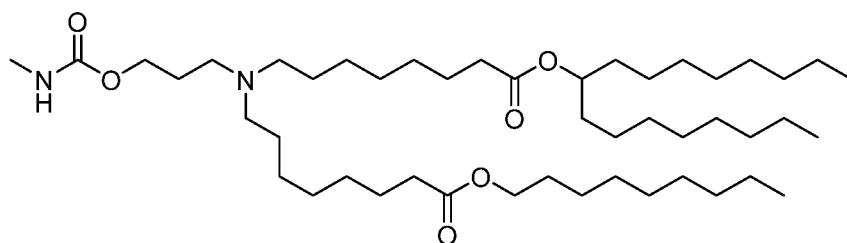
(Compound 172),



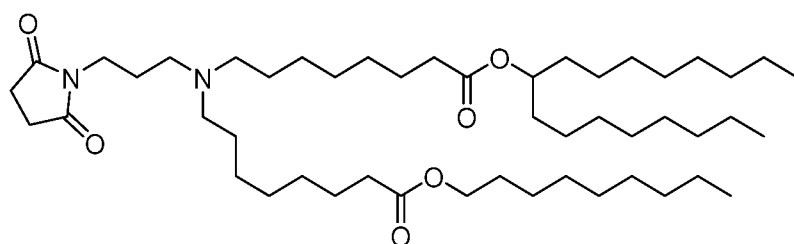
(Compound 173),



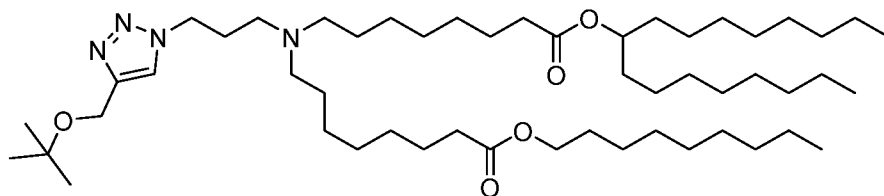
(Compound 174),



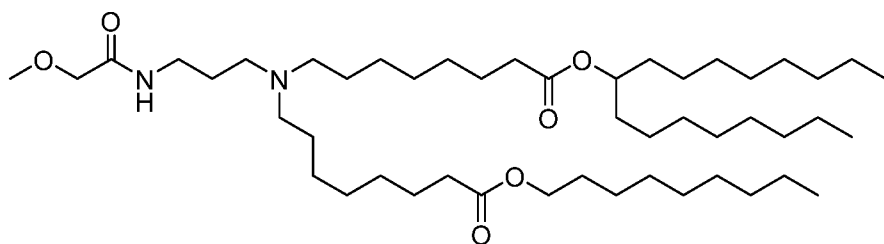
(Compound 175),



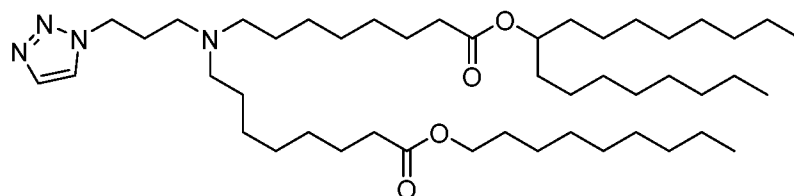
(Compound 176),



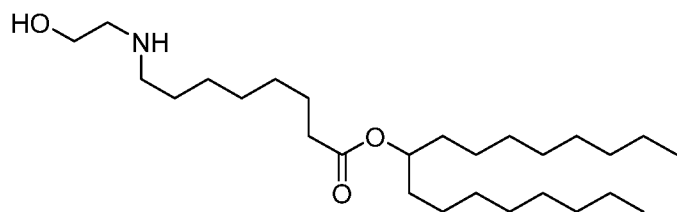
(Compound 177),



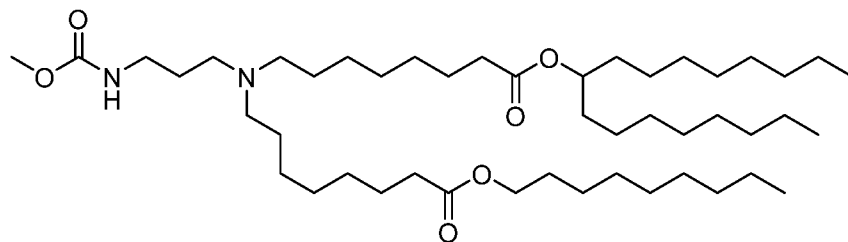
(Compound 178),



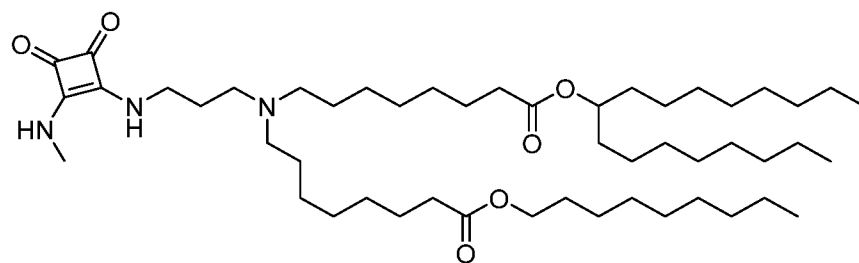
(Compound 179),



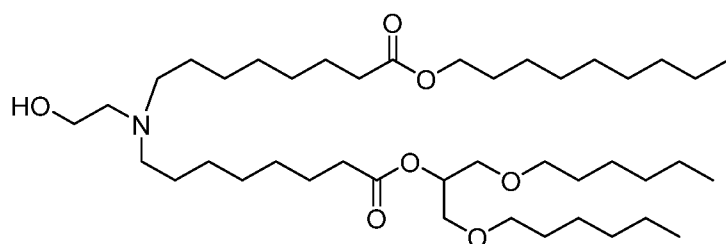
(Compound 180),



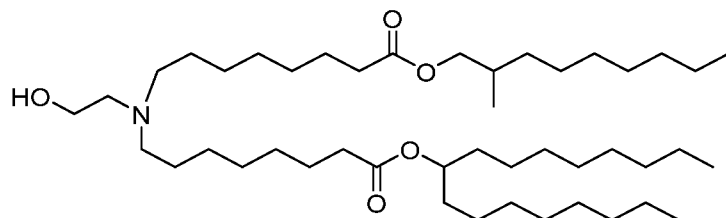
(Compound 181),



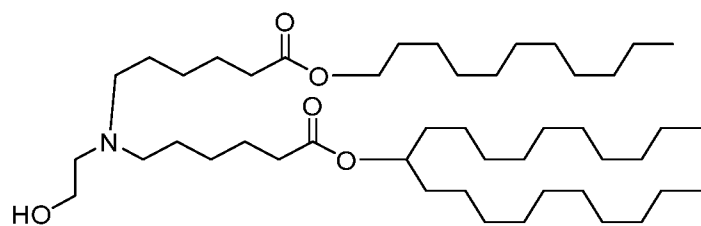
(Compound 182),



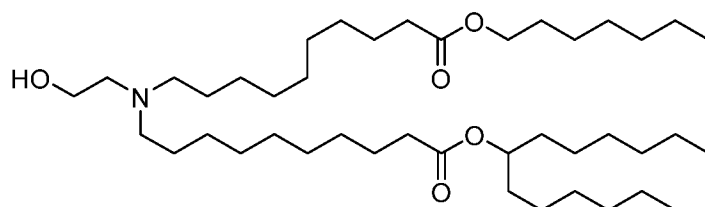
(Compound 183),



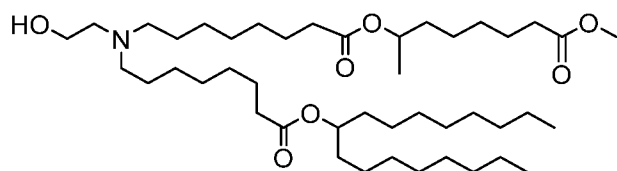
(Compound 184),



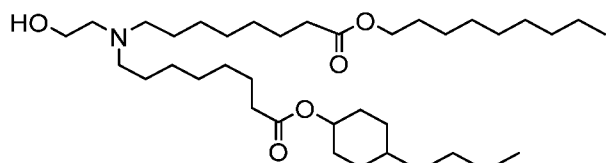
(Compound 185),



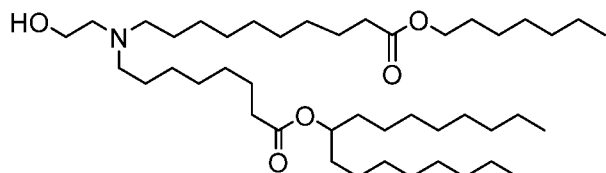
(Compound 186),



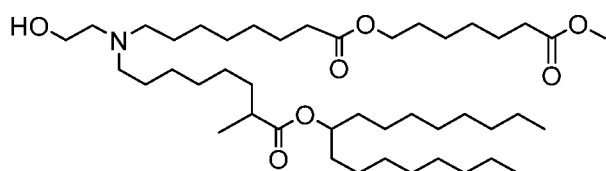
(Compound 187),



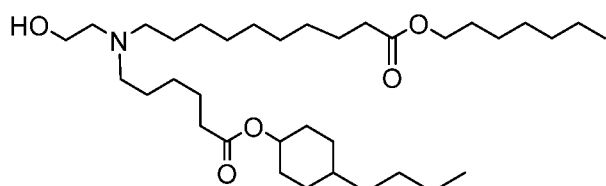
(Compound 188),



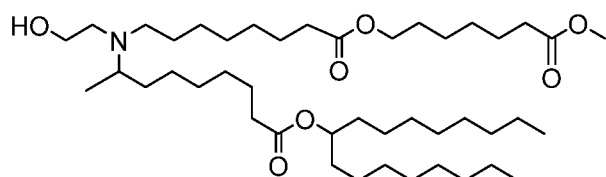
(Compound 189),



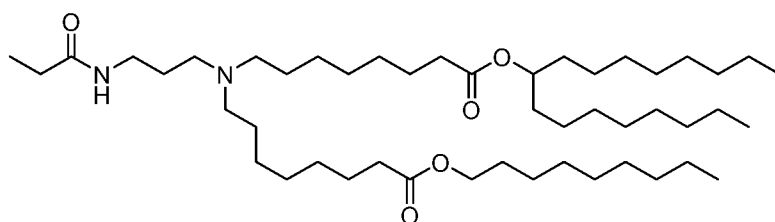
(Compound 190),



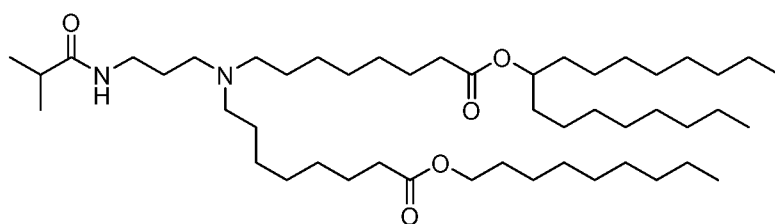
(Compound 191),



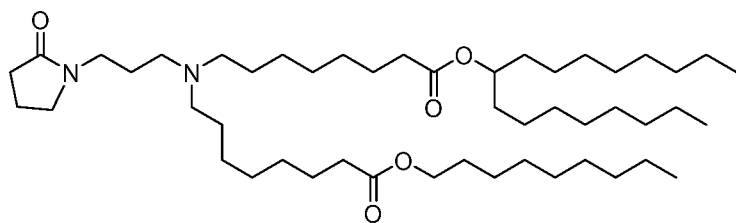
(Compound 192),



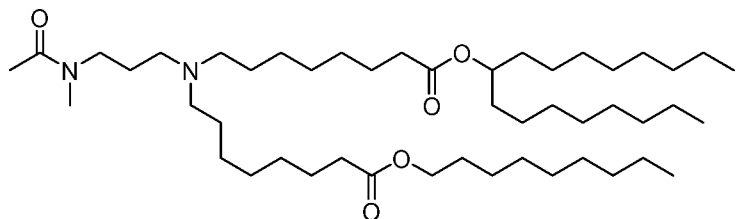
(Compound 193),



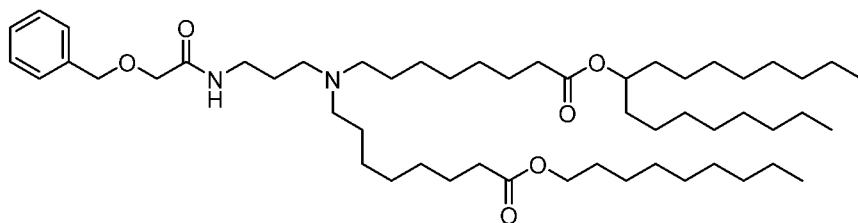
(Compound 194),



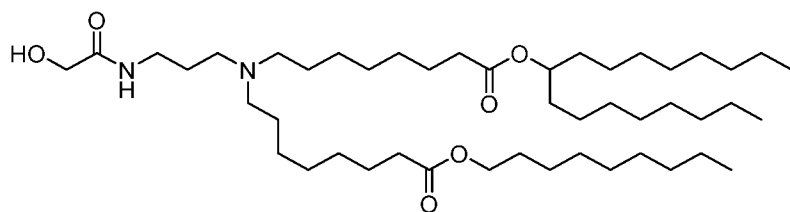
(Compound 195),



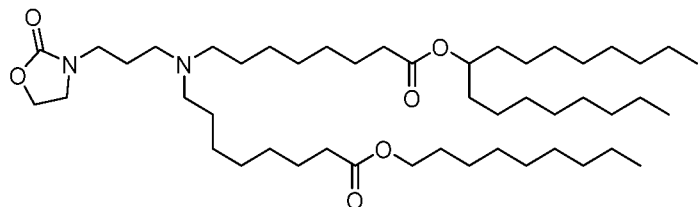
(Compound 196),



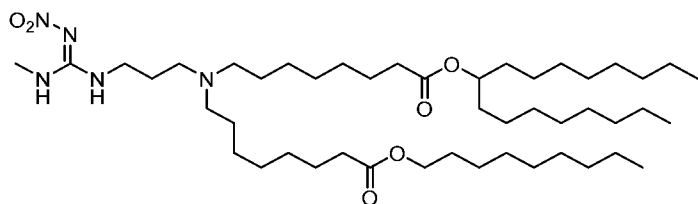
(Compound 197),



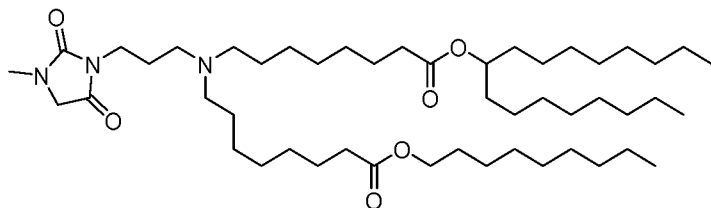
(Compound 198),



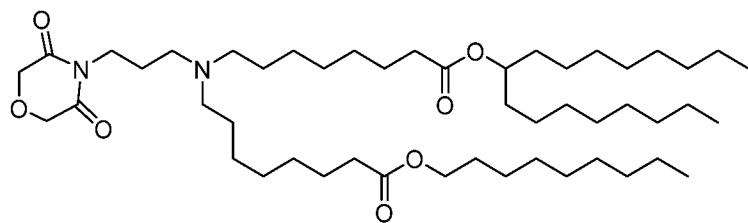
(Compound 199),



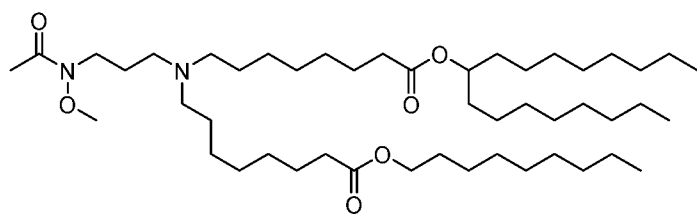
(Compound 200),



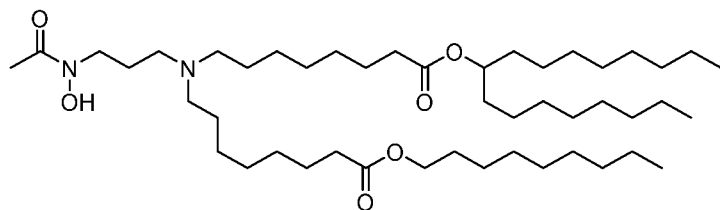
(Compound 201),



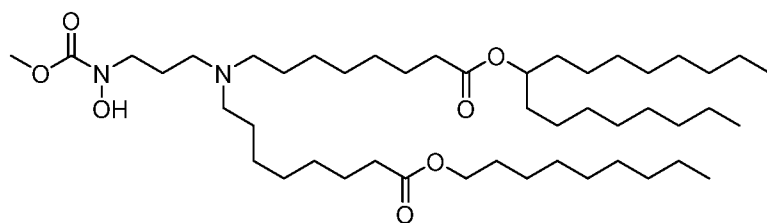
(Compound 202),



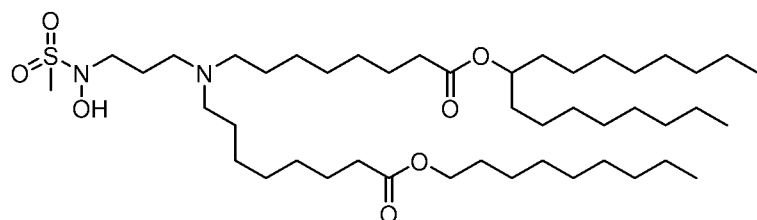
(Compound 203),



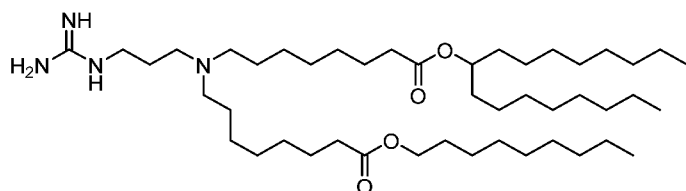
(Compound 204),



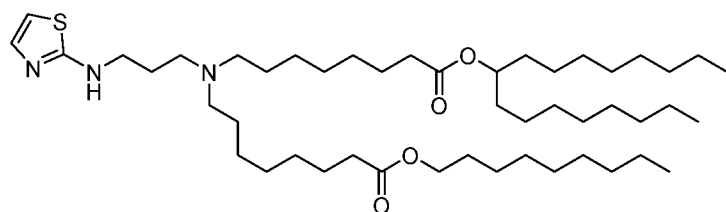
(Compound 205),



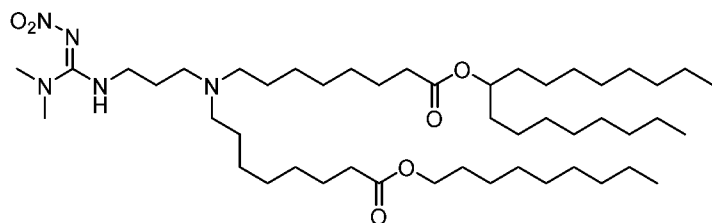
(Compound 206),



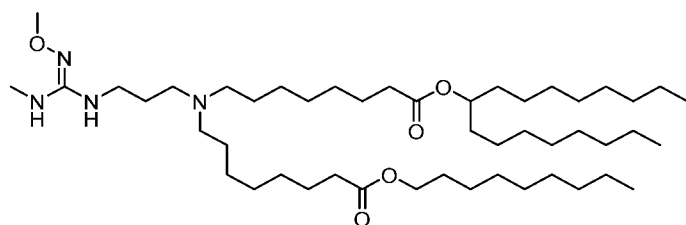
(Compound 207),



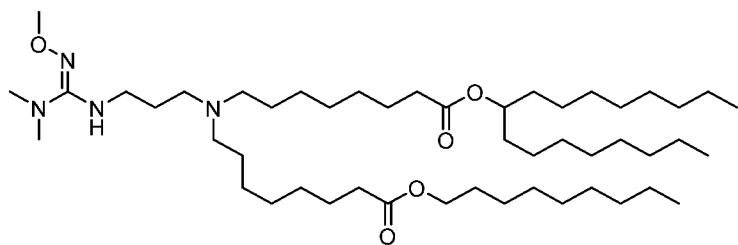
(Compound 208),



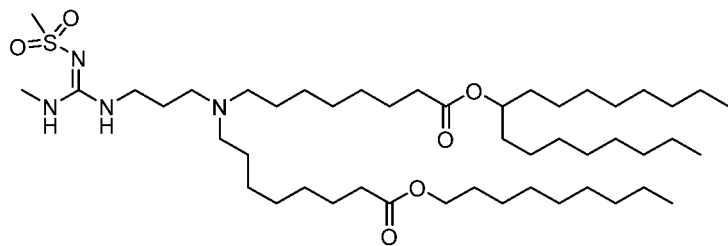
(Compound 209),



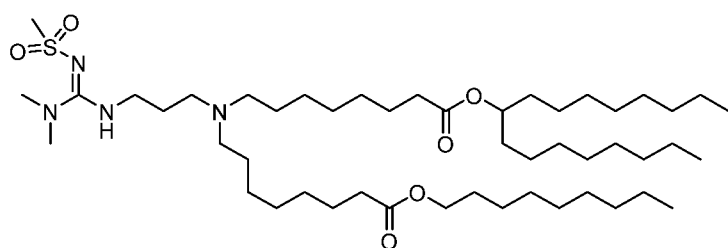
(Compound 210),



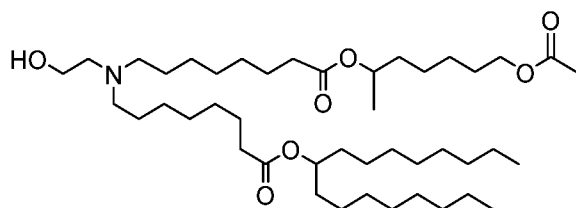
(Compound 211),



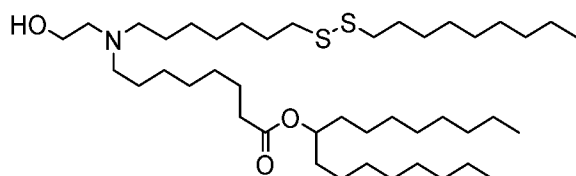
(Compound 212),



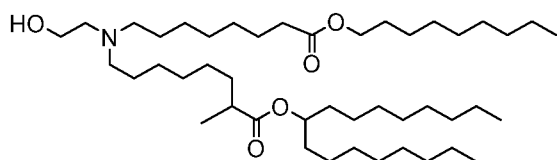
(Compound 213),



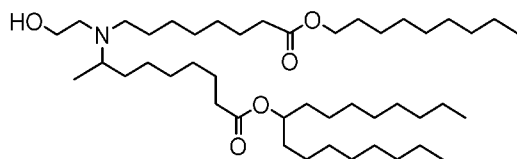
(Compound 214),



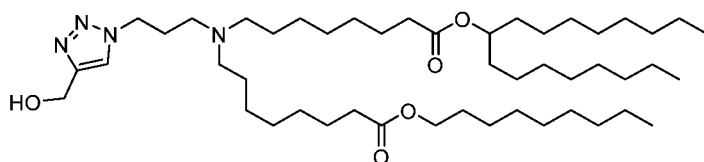
(Compound 215),



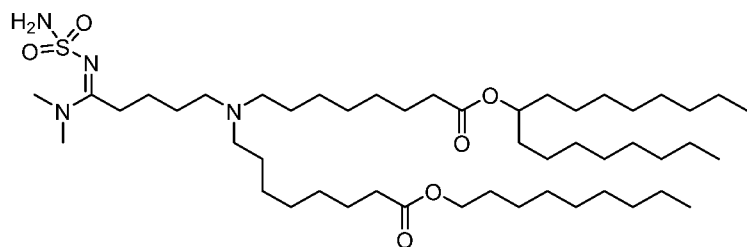
(Compound 216),



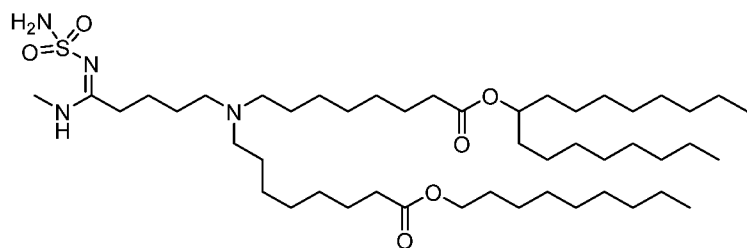
(Compound 217),



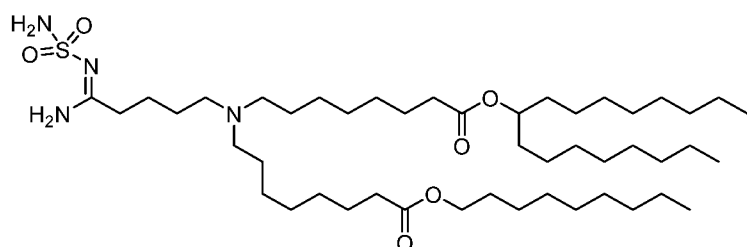
(Compound 218),



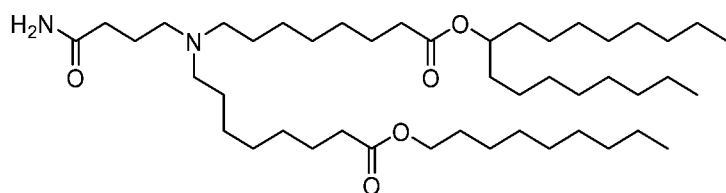
(Compound 219),



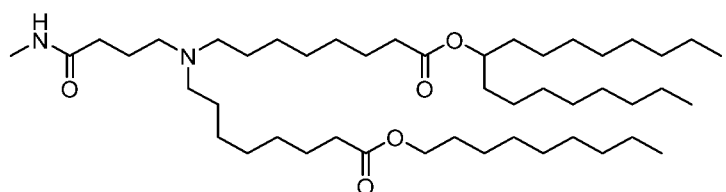
(Compound 220),



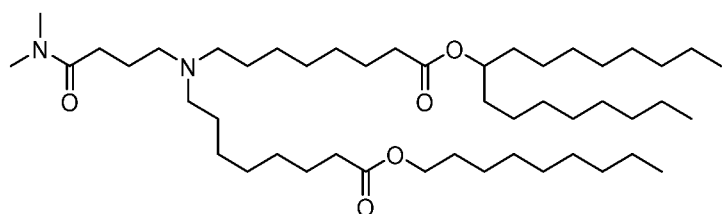
(Compound 221),



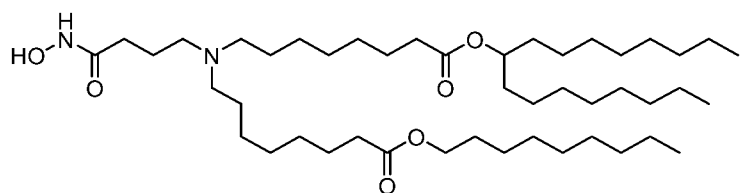
(Compound 222),



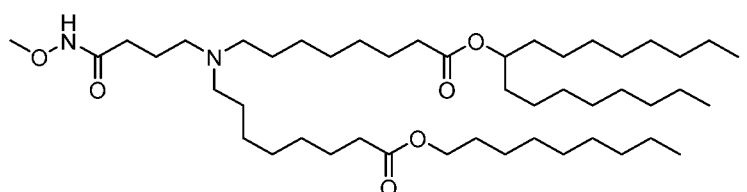
(Compound 223),



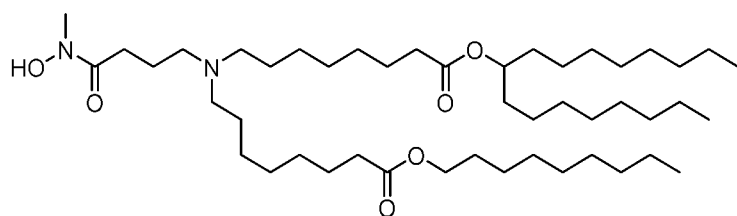
(Compound 224),



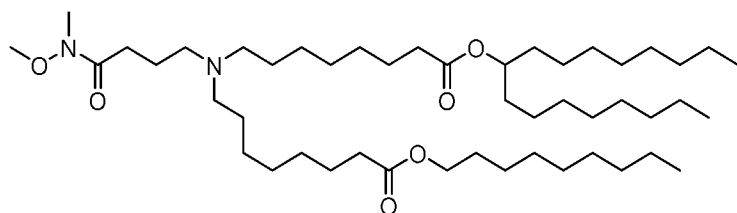
(Compound 225),



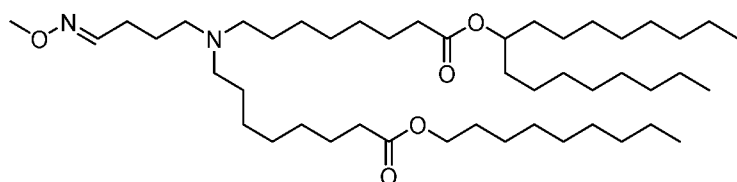
(Compound 226),



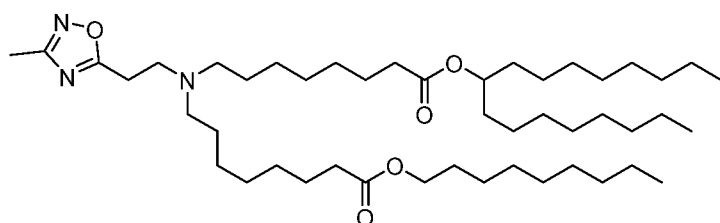
(Compound 227),



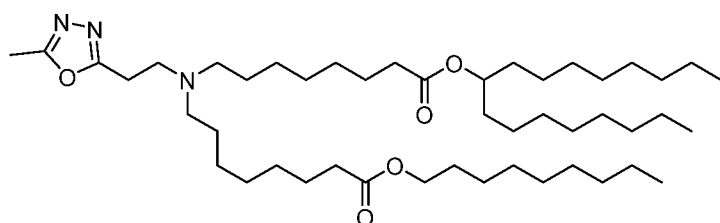
(Compound 228),



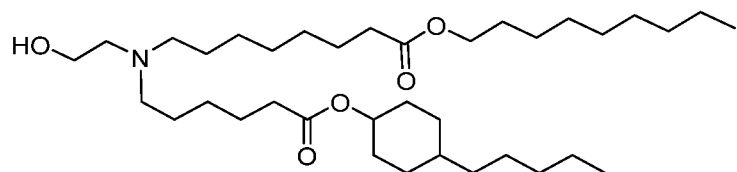
(Compound 229),



(Compound 230),



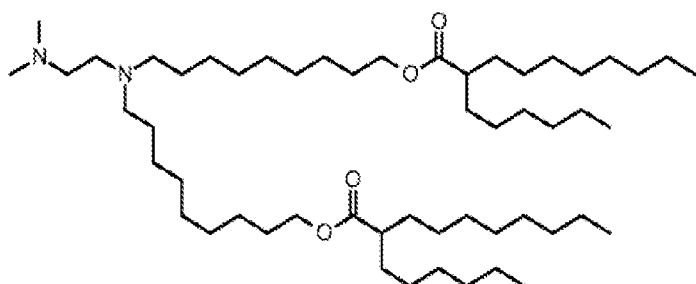
(Compound 231),



(Compound 232), and

salts and isomers thereof.

In some embodiments, a nanoparticle comprises the following compound:



(Compound 233) or salts

and isomers thereof.

In some embodiments, the disclosure features a nanoparticle composition including a lipid component comprising a compound as described herein (*e.g.*, a compound according to Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe)).

5 In some embodiments, the disclosure features a pharmaceutical composition comprising a nanoparticle composition according to the preceding embodiments and a pharmaceutically acceptable carrier. For example, the pharmaceutical composition is refrigerated or frozen for storage and/or shipment (*e.g.*, being stored at a temperature of 4 °C or lower, such as a temperature between about -150 °C and about 0 °C or between about -80 °C and about -20 °C (*e.g.*, about -5 °C, -10 °C, -15 °C, -20 °C, -25 °C, -30 °C, -40 °C, -50 °C, 10 -60 °C, -70 °C, -80 °C, -90 °C, -130 °C or -150 °C). For example, the pharmaceutical composition is a solution that is refrigerated for storage and/or shipment at, for example, about -20° C, -30 °C, -40 °C, -50 °C, -60 °C, -70 °C, or -80 °C.

In some embodiments, the disclosure provides a method of delivering a therapeutic and/or prophylactic (*e.g.*, RNA, such as mRNA) to a cell (*e.g.*, a mammalian cell). This 15 method includes the step of administering to a subject (*e.g.*, a mammal, such as a human) a nanoparticle composition including (i) a lipid component including a phospholipid (such as a polyunsaturated lipid), a PEG lipid, a structural lipid, and a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe) and (ii) a therapeutic and/or prophylactic, in which administering involves contacting the cell with the nanoparticle composition, whereby the 20 therapeutic and/or prophylactic is delivered to the cell.

In some embodiments, the disclosure provides a method of producing a polypeptide of interest in a cell (*e.g.*, a mammalian cell). The method includes the step of contacting the cell with a nanoparticle composition including (i) a lipid component including a phospholipid (such as a polyunsaturated lipid), a PEG lipid, a structural lipid, and a compound of Formula 25 (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe) and (ii) an mRNA encoding the polypeptide of interest, whereby the mRNA is capable of being translated in the cell to produce the polypeptide.

In some embodiments, the disclosure provides a method of treating a disease or disorder in a mammal (*e.g.*, a human) in need thereof. The method includes the step of 30 administering to the mammal a therapeutically effective amount of a nanoparticle composition including (i) a lipid component including a phospholipid (such as a polyunsaturated lipid), a PEG lipid, a structural lipid, and a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe) and (ii) a therapeutic and/or prophylactic (*e.g.*, an mRNA). In some embodiments, the disease or disorder is characterized by dysfunctional or aberrant

protein or polypeptide activity. For example, the disease or disorder is selected from the group consisting of rare diseases, infectious diseases, cancer and proliferative diseases, genetic diseases (*e.g.*, cystic fibrosis), autoimmune diseases, diabetes, neurodegenerative diseases, cardio- and reno-vascular diseases, and metabolic diseases.

5 In some embodiments, the disclosure provides a method of delivering (*e.g.*, specifically delivering) a therapeutic and/or prophylactic to a mammalian organ (*e.g.*, a liver, spleen, lung, or femur). This method includes the step of administering to a subject (*e.g.*, a mammal) a nanoparticle composition including (i) a lipid component including a phospholipid, a PEG lipid, a structural lipid, and a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe) and (ii) a therapeutic and/or prophylactic (*e.g.*, an mRNA), in which
10 administering involves contacting the cell with the nanoparticle composition, whereby the therapeutic and/or prophylactic is delivered to the target organ (*e.g.*, a liver, spleen, lung, or femur).

In some embodiments, the disclosure features a method for the enhanced delivery of a
15 therapeutic and/or prophylactic (*e.g.*, an mRNA) to a target tissue (*e.g.*, a liver, spleen, lung, or femur). This method includes administering to a subject (*e.g.*, a mammal) a nanoparticle composition, the composition including (i) a lipid component including a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe), a phospholipid, a structural lipid, and a PEG lipid; and (ii) a therapeutic and/or prophylactic, the administering including contacting
20 the target tissue with the nanoparticle composition, whereby the therapeutic and/or prophylactic is delivered to the target tissue.

In some embodiments, the disclosure features a method of lowering immunogenicity comprising introducing the nanoparticle composition of the disclosure into cells, wherein the nanoparticle composition reduces the induction of the cellular immune response of the cells
25 to the nanoparticle composition, as compared to the induction of the cellular immune response in cells induced by a reference composition which comprises a reference lipid instead of a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe). For example, the cellular immune response is an innate immune response, an adaptive immune response, or both.

30 The disclosure also includes methods of synthesizing a compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe) and methods of making a nanoparticle composition including a lipid component comprising the compound of Formula (I), (IA), (II), (IIa), (IIb), (IIc), (IId) or (IIe).

Modes of Vaccine Administration

VZV RNA (*e.g.*, mRNA) vaccines may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited, to intradermal, intramuscular, intranasal, and/or subcutaneous administration. The present disclosure provides methods comprising administering RNA vaccines to a subject in need thereof. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. VZV RNA (*e.g.*, mRNA) vaccines compositions are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of VZV RNA (*e.g.*, mRNA) vaccines compositions may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

In some embodiments, VZV RNA (*e.g.*, mRNA) vaccines compositions may be administered at dosage levels sufficient to deliver 0.0001 mg/kg to 100 mg/kg, 0.001 mg/kg to 0.05 mg/kg, 0.005 mg/kg to 0.05 mg/kg, 0.001 mg/kg to 0.005 mg/kg, 0.05 mg/kg to 0.5 mg/kg, 0.01 mg/kg to 50 mg/kg, 0.1 mg/kg to 40 mg/kg, 0.5 mg/kg to 30 mg/kg, 0.01 mg/kg to 10 mg/kg, 0.1 mg/kg to 10 mg/kg, or 1 mg/kg to 25 mg/kg, of subject body weight per day, one or more times a day, per week, per month, etc. to obtain the desired therapeutic, diagnostic, prophylactic, or imaging effect (*see e.g.*, the range of unit doses described in International Publication No. WO2013/078199, herein incorporated by reference in its entirety). The desired dosage may be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, every four weeks, every 2 months, every three months, every 6 months, *etc.* In certain embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). When multiple administrations are employed, split dosing regimens such as those described herein may be used. In exemplary embodiments, VZV RNA (*e.g.*, mRNA) vaccines compositions may be administered at dosage levels sufficient to deliver 0.0005

mg/kg to 0.01 mg/kg, *e.g.*, about 0.0005 mg/kg to about 0.0075 mg/kg, *e.g.*, about 0.0005 mg/kg, about 0.001 mg/kg, about 0.002 mg/kg, about 0.003 mg/kg, about 0.004 mg/kg or about 0.005 mg/kg.

In some embodiments, VZV RNA (*e.g.*, mRNA) vaccine compositions may be administered once or twice (or more) at dosage levels sufficient to deliver 0.025 mg/kg to 0.250 mg/kg, 0.025 mg/kg to 0.500 mg/kg, 0.025 mg/kg to 0.750 mg/kg, or 0.025 mg/kg to 1.0 mg/kg.

In some embodiments, VZV RNA (*e.g.*, mRNA) vaccine compositions may be administered twice (*e.g.*, Day 0 and Day 7, Day 0 and Day 14, Day 0 and Day 21, Day 0 and Day 28, Day 0 and Day 60, Day 0 and Day 90, Day 0 and Day 120, Day 0 and Day 150, Day 0 and Day 180, Day 0 and 3 months later, Day 0 and 6 months later, Day 0 and 9 months later, Day 0 and 12 months later, Day 0 and 18 months later, Day 0 and 2 years later, Day 0 and 5 years later, or Day 0 and 10 years later) at a total dose of or at dosage levels sufficient to deliver a total dose of 0.0100 mg, 0.025 mg, 0.050 mg, 0.075 mg, 0.100 mg, 0.125 mg, 0.150 mg, 0.175 mg, 0.200 mg, 0.225 mg, 0.250 mg, 0.275 mg, 0.300 mg, 0.325 mg, 0.350 mg, 0.375 mg, 0.400 mg, 0.425 mg, 0.450 mg, 0.475 mg, 0.500 mg, 0.525 mg, 0.550 mg, 0.575 mg, 0.600 mg, 0.625 mg, 0.650 mg, 0.675 mg, 0.700 mg, 0.725 mg, 0.750 mg, 0.775 mg, 0.800 mg, 0.825 mg, 0.850 mg, 0.875 mg, 0.900 mg, 0.925 mg, 0.950 mg, 0.975 mg, or 1.0 mg. Higher and lower dosages and frequency of administration are encompassed by the present disclosure. For example, a VZV RNA (*e.g.*, mRNA) vaccine composition may be administered three or four times.

In some embodiments, VZV RNA (*e.g.*, mRNA) vaccine compositions may be administered twice (*e.g.*, Day 0 and Day 7, Day 0 and Day 14, Day 0 and Day 21, Day 0 and Day 28, Day 0 and Day 60, Day 0 and Day 90, Day 0 and Day 120, Day 0 and Day 150, Day 0 and Day 180, Day 0 and 3 months later, Day 0 and 6 months later, Day 0 and 9 months later, Day 0 and 12 months later, Day 0 and 18 months later, Day 0 and 2 years later, Day 0 and 5 years later, or Day 0 and 10 years later) at a total dose of or at dosage levels sufficient to deliver a total dose of 0.010 mg, 0.025 mg, 0.100 mg or 0.400 mg.

In some embodiments the VZV RNA (*e.g.*, mRNA) vaccine for use in a method of vaccinating a subject is administered the subject a single dosage of between 10 µg/kg and 400 µg/kg of the nucleic acid vaccine in an effective amount to vaccinate the subject. In some embodiments the RNA vaccine for use in a method of vaccinating a subject is administered the subject a single dosage of between 10 µg and 400 µg of the nucleic acid vaccine in an effective amount to vaccinate the subject. In some embodiments, a VZV RNA (*e.g.*, mRNA) vaccine for use in a method of vaccinating a subject is administered to the subject as a single

dosage of 25-1000 µg (*e.g.*, a single dosage of mRNA encoding an VZV antigen). In some embodiments, a VZV RNA vaccine is administered to the subject as a single dosage of 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 µg. For example, a VZV RNA vaccine may be administered to a subject as a single
5 dose of 25-100, 25-500, 50-100, 50-500, 50-1000, 100-500, 100-1000, 250-500, 250-1000, or 500-1000 µg. In some embodiments, a VZV RNA (*e.g.*, mRNA) vaccine for use in a method of vaccinating a subject is administered to the subject as two dosages, the combination of which equals 25-1000 µg of the VZV RNA (*e.g.*, mRNA) vaccine.

A VZV RNA (*e.g.*, mRNA) vaccine pharmaceutical composition described herein can
10 be formulated into a dosage form described herein, such as an intranasal, intratracheal, or injectable (*e.g.*, intravenous, intraocular, intravitreal, intramuscular, intradermal, intracardiac, intraperitoneal, and subcutaneous).

VZV RNA vaccine formulations and methods of use

Some aspects of the present disclosure provide formulations of the VZV RNA (*e.g.*, mRNA) vaccine, wherein the VZV RNA vaccine is formulated in an effective amount to produce an antigen specific immune response in a subject (*e.g.*, production of antibodies specific to an anti-VZV antigenic polypeptide). “An effective amount” is a dose of an VZV RNA (*e.g.*, mRNA) vaccine effective to produce an antigen-specific immune response. Also
20 provided herein are methods of inducing an antigen-specific immune response in a subject.

In some embodiments, the antigen-specific immune response is characterized by measuring an anti-VZV antigenic polypeptide antibody titer produced in a subject administered a VZV RNA (*e.g.*, mRNA) vaccine as provided herein. An antibody titer is a measurement of the amount of antibodies within a subject, for example, antibodies that are
25 specific to a particular antigen (*e.g.*, an anti-VZV antigenic polypeptide) or epitope of an antigen. Antibody titer is typically expressed as the inverse of the greatest dilution that provides a positive result. Enzyme-linked immunosorbent assay (ELISA) is a common assay for determining antibody titers, for example.

In some embodiments, an antibody titer is used to assess whether a subject has had an
30 infection or to determine whether immunizations are required. In some embodiments, an antibody titer is used to determine the strength of an autoimmune response, to determine whether a booster immunization is needed, to determine whether a previous vaccine was effective, and to identify any recent or prior infections. In accordance with the present disclosure, an antibody titer may be used to determine the strength of an immune response
35 induced in a subject by the VZV RNA (*e.g.*, mRNA) vaccine.

In some embodiments, an anti-VZV antigenic polypeptide antibody titer produced in a subject is increased by at least 1 log relative to a control. For example, anti-VZV antigenic polypeptide antibody titer produced in a subject may be increased by at least 1.5, at least 2, at least 2.5, or at least 3 log relative to a control. In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in the subject is increased by 1, 1.5, 2, 2.5 or 3 log relative to a control. In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a control. For example, the anti-VZV antigenic polypeptide antibody titer produced in a subject may be increased by 1-1.5, 1-2, 1-2.5, 1-3, 1.5-2, 1.5-2.5, 1.5-3, 2-2.5, 2-3, or 2.5-3 log relative to a control.

In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in a subject is increased at least 2 times relative to a control. For example, the anti-VZV antigenic polypeptide antibody titer produced in a subject may be increased at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, or at least 10 times relative to a control. In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in the subject is increased 2, 3, 4, 5, 6, 7, 8, 9, or 10 times relative to a control. In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in a subject is increased 2-10 times relative to a control. For example, the anti-VZV antigenic polypeptide antibody titer produced in a subject may be increased 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-10, 5-9, 5-8, 5-7, 5-6, 6-10, 6-9, 6-8, 6-7, 7-10, 7-9, 7-8, 8-10, 8-9, or 9-10 times relative to a control.

A control, in some embodiments, is the anti-VZV antigenic polypeptide antibody titer produced in a subject who has not been administered a VZV RNA (*e.g.*, mRNA) vaccine. In some embodiments, a control is an anti-VZV antigenic polypeptide antibody titer produced in a subject who has been administered a live attenuated VZV vaccine. An attenuated vaccine is a vaccine produced by reducing the virulence of a viable (live). An attenuated virus is altered in a manner that renders it harmless or less virulent relative to live, unmodified virus. In some embodiments, a control is an anti-VZV antigenic polypeptide antibody titer produced in a subject administered inactivated VZV vaccine. In some embodiments, a control is an anti-VZV antigenic polypeptide antibody titer produced in a subject administered a recombinant or purified VZV protein vaccine. Recombinant protein vaccines typically include protein antigens that either have been produced in a heterologous expression system (*e.g.*, bacteria or yeast) or purified from large amounts of the pathogenic organism. In some embodiments, a control is an anti-VZV antigenic polypeptide antibody titer produced in a subject who has been administered a VZV virus-like particle (VLP) vaccine (*e.g.*, particles that contain viral

capsid protein but lack a viral genome and, therefore, cannot replicate/produce progeny virus). In some embodiments, the control is a VLP VZV vaccine that comprises prefusion or postfusion F proteins, or that comprises a combination of the two.

In some embodiments, an effective amount of a VZV RNA (*e.g.*, mRNA) vaccine is a
5 dose that is reduced compared to the standard of care dose of a recombinant VZV protein vaccine. A “standard of care,” as provided herein, refers to a medical or psychological treatment guideline and can be general or specific. “Standard of care” specifies appropriate treatment based on scientific evidence and collaboration between medical professionals involved in the treatment of a given condition. It is the diagnostic and treatment process that
10 a physician/ clinician should follow for a certain type of patient, illness or clinical circumstance. A “standard of care dose,” as provided herein, refers to the dose of a recombinant or purified VZV protein vaccine, or a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine, that a physician/clinician or other medical professional would administer to a subject to treat or prevent VZV, or a VZV-related condition, while
15 following the standard of care guideline for treating or preventing VZV, or a VZV-related condition.

In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in a subject administered an effective amount of a VZV RNA vaccine is equivalent to an anti-VZV antigenic polypeptide antibody titer produced in a control subject administered a
20 standard of care dose of a recombinant or purified VZV protein vaccine, or a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine.

In some embodiments, an effective amount of a VZV RNA (*e.g.*, mRNA) vaccine is a dose equivalent to an at least 2-fold reduction in a standard of care dose of a recombinant or purified VZV protein vaccine. For example, an effective amount of a VZV RNA vaccine
25 may be a dose equivalent to an at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold reduction in a standard of care dose of a recombinant or purified VZV protein vaccine. In some embodiments, an effective amount of a VZV RNA vaccine is a dose equivalent to an at least 100-fold, at least 500-fold, or at least 1000-fold reduction in a standard of care dose of a recombinant or purified VZV
30 protein vaccine. In some embodiments, an effective amount of a VZV RNA vaccine is a dose equivalent to a 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 20-, 50-, 100-, 250-, 500-, or 1000-fold reduction in a standard of care dose of a recombinant or purified VZV protein vaccine. In some embodiments, the anti-VZV antigenic polypeptide antibody titer produced in a subject administered an effective amount of a VZV RNA vaccine is equivalent to an anti-VZV
35 antigenic polypeptide antibody titer produced in a control subject administered the standard

of care dose of a recombinant or protein VZV protein vaccine, or a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine. In some embodiments, an effective amount of a VZV RNA (*e.g.*, mRNA) vaccine is a dose equivalent to a 2-fold to 1000-fold (*e.g.*, 2-fold to 100-fold, 10-fold to 1000-fold) reduction in the standard of care dose of a recombinant or purified VZV protein vaccine, wherein the anti-VZV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-VZV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified VZV protein vaccine, or a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine.

In some embodiments, the effective amount of a VZV RNA (*e.g.*, mRNA) vaccine is a dose equivalent to a 2 to 1000-, 2 to 900-, 2 to 800-, 2 to 700-, 2 to 600-, 2 to 500-, 2 to 400-, 2 to 300-, 2 to 200-, 2 to 100-, 2 to 90-, 2 to 80-, 2 to 70-, 2 to 60-, 2 to 50-, 2 to 40-, 2 to 30-, 2 to 20-, 2 to 10-, 2 to 9-, 2 to 8-, 2 to 7-, 2 to 6-, 2 to 5-, 2 to 4-, 2 to 3-, 3 to 1000-, 3 to 900-, 3 to 800-, 3 to 700-, 3 to 600-, 3 to 500-, 3 to 400-, 3 to 300-, 3 to 200-, 3 to 100-, 3 to 90-, 3 to 80-, 3 to 70-, 3 to 60-, 3 to 50-, 3 to 40-, 3 to 30-, 3 to 20-, 3 to 10-, 3 to 9-, 3 to 8-, 3 to 7-, 3 to 6-, 3 to 5-, 3 to 4-, 4 to 1000-, 4 to 900-, 4 to 800-, 4 to 700-, 4 to 600-, 4 to 500-, 4 to 400-, 4 to 300-, 4 to 200-, 4 to 100-, 4 to 90-, 4 to 80-, 4 to 70-, 4 to 60-, 4 to 50-, 4 to 40-, 4 to 30-, 4 to 20-, 4 to 10-, 4 to 9-, 4 to 8-, 4 to 7-, 4 to 6-, 4 to 5-, 4 to 4-, 5 to 1000-, 5 to 900-, 5 to 800-, 5 to 700-, 5 to 600-, 5 to 500-, 5 to 400-, 5 to 300-, 5 to 200-, 5 to 100-, 5 to 90-, 5 to 80-, 5 to 70-, 5 to 60-, 5 to 50-, 5 to 40-, 5 to 30-, 5 to 20-, 5 to 10-, 5 to 9-, 5 to 8-, 5 to 7-, 5 to 6-, 6 to 1000-, 6 to 900-, 6 to 800-, 6 to 700-, 6 to 600-, 6 to 500-, 6 to 400-, 6 to 300-, 6 to 200-, 6 to 100-, 6 to 90-, 6 to 80-, 6 to 70-, 6 to 60-, 6 to 50-, 6 to 40-, 6 to 30-, 6 to 20-, 6 to 10-, 6 to 9-, 6 to 8-, 6 to 7-, 7 to 1000-, 7 to 900-, 7 to 800-, 7 to 700-, 7 to 600-, 7 to 500-, 7 to 400-, 7 to 300-, 7 to 200-, 7 to 100-, 7 to 90-, 7 to 80-, 7 to 70-, 7 to 60-, 7 to 50-, 7 to 40-, 7 to 30-, 7 to 20-, 7 to 10-, 7 to 9-, 7 to 8-, 8 to 1000-, 8 to 900-, 8 to 800-, 8 to 700-, 8 to 600-, 8 to 500-, 8 to 400-, 8 to 300-, 8 to 200-, 8 to 100-, 8 to 90-, 8 to 80-, 8 to 70-, 8 to 60-, 8 to 50-, 8 to 40-, 8 to 30-, 8 to 20-, 8 to 10-, 8 to 9-, 9 to 1000-, 9 to 900-, 9 to 800-, 9 to 700-, 9 to 600-, 9 to 500-, 9 to 400-, 9 to 300-, 9 to 200-, 9 to 100-, 9 to 90-, 9 to 80-, 9 to 70-, 9 to 60-, 9 to 50-, 9 to 40-, 9 to 30-, 9 to 20-, 9 to 10-, 10 to 1000-, 10 to 900-, 10 to 800-, 10 to 700-, 10 to 600-, 10 to 500-, 10 to 400-, 10 to 300-, 10 to 200-, 10 to 100-, 10 to 90-, 10 to 80-, 10 to 70-, 10 to 60-, 10 to 50-, 10 to 40-, 10 to 30-, 10 to 20-, 20 to 1000-, 20 to 900-, 20 to 800-, 20 to 700-, 20 to 600-, 20 to 500-, 20 to 400-, 20 to 300-, 20 to 200-, 20 to 100-, 20 to 90-, 20 to 80-, 20 to 70-, 20 to 60-, 20 to 50-, 20 to 40-, 20 to 30-, 30 to 1000-, 30 to 900-, 30 to 800-, 30 to 700-, 30 to 600-, 30 to 500-, 30 to 400-, 30 to 300-, 30 to 200-, 30 to 100-, 30 to 90-, 30 to 80-, 30 to 70-, 30 to 60-, 30 to 50-, 30 to 40-, 40 to 1000-, 40 to 900-, 40 to

800-, 40 to 700-, 40 to 600-, 40 to 500-, 40 to 400-, 40 to 300-, 40 to 200-, 40 to 100-, 40 to 90-, 40 to 80-, 40 to 70-, 40 to 60-, 40 to 50-, 50 to 1000-, 50 to 900-, 50 to 800-, 50 to 700-, 50 to 600-, 50 to 500-, 50 to 400-, 50 to 300-, 50 to 200-, 50 to 100-, 50 to 90-, 50 to 80-, 50 to 70-, 50 to 60-, 60 to 1000-, 60 to 900-, 60 to 800-, 60 to 700-, 60 to 600-, 60 to 500-, 60 to 400-, 60 to 300-, 60 to 200-, 60 to 100-, 60 to 90-, 60 to 80-, 60 to 70-, 70 to 1000-, 70 to 900-, 70 to 800-, 70 to 700-, 70 to 600-, 70 to 500-, 70 to 400-, 70 to 300-, 70 to 200-, 70 to 100-, 70 to 90-, 70 to 80-, 80 to 1000-, 80 to 900-, 80 to 800-, 80 to 700-, 80 to 600-, 80 to 500-, 80 to 400-, 80 to 300-, 80 to 200-, 80 to 100-, 80 to 90-, 90 to 1000-, 90 to 900-, 90 to 800-, 90 to 700-, 90 to 600-, 90 to 500-, 90 to 400-, 90 to 300-, 90 to 200-, 90 to 100-, 100 to 1000-, 100 to 900-, 100 to 800-, 100 to 700-, 100 to 600-, 100 to 500-, 100 to 400-, 100 to 300-, 100 to 200-, 200 to 1000-, 200 to 900-, 200 to 800-, 200 to 700-, 200 to 600-, 200 to 500-, 200 to 400-, 200 to 300-, 300 to 1000-, 300 to 900-, 300 to 800-, 300 to 700-, 300 to 600-, 300 to 500-, 300 to 400-, 400 to 1000-, 400 to 900-, 400 to 800-, 400 to 700-, 400 to 600-, 400 to 500-, 500 to 1000-, 500 to 900-, 500 to 800-, 500 to 700-, 500 to 600-, 600 to 1000-, 600 to 900-, 600 to 800-, 600 to 700-, 700 to 1000-, 700 to 900-, 700 to 800-, 800 to 1000-, 800 to 900-, or 900 to 1000-fold reduction in the standard of care dose of a recombinant VZV protein vaccine. In some embodiments, such as the foregoing, the anti-VZV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-VZV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified VZV protein vaccine, or a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine. In some embodiments, the effective amount is a dose equivalent to (or equivalent to an at least) 2-, 3 -,4 -,5 -,6-, 7-, 8-, 9-, 10-, 20-, 30-, 40-, 50-, 60-, 70-, 80-, 90-, 100-, 110-, 120-, 130-, 140-, 150-, 160-, 170-, 1280-, 190-, 200-, 210-, 220-, 230-, 240-, 250-, 260-, 270-, 280-, 290-, 300-, 310-, 320-, 330-, 340-, 350-, 360-, 370-, 380-, 390-, 400-, 410-, 420-, 430-, 440-, 450-, 4360-, 470-, 480-, 490-, 500-, 510-, 520-, 530-, 540-, 550-, 560-, 5760-, 580-, 590-, 600-, 610-, 620-, 630-, 640-, 650-, 660-, 670-, 680-, 690-, 700-, 710-, 720-, 730-, 740-, 750-, 760-, 770-, 780-, 790-, 800-, 810-, 820--, 830-, 840-, 850-, 860-, 870-, 880-, 890-, 900-, 910-, 920-, 930-, 940-, 950-, 960-, 970-, 980-, 990-, or 1000-fold reduction in the standard of care dose of a recombinant VZV protein vaccine. In some embodiments, such as the foregoing, an anti-VZV antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-VZV antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a recombinant or purified VZV protein vaccine, or a live attenuated or inactivated VZV vaccine, or a VZV VLP vaccine.

In some embodiments, the effective amount of a VZV RNA (*e.g.*, mRNA) vaccine is a total dose of 50-1000 μ g. In some embodiments, the effective amount of a VZV RNA (*e.g.*, mRNA) vaccine is a total dose of 50-1000, 50- 900, 50-800, 50-700, 50-600, 50-500, 50-400, 50-300, 50-200, 50-100, 50-90, 50-80, 50-70, 50-60, 60-1000, 60- 900, 60-800, 60-700, 60-600, 60-500, 60-400, 60-300, 60-200, 60-100, 60-90, 60-80, 60-70, 70-1000, 70- 900, 70-800, 70-700, 70-600, 70-500, 70-400, 70-300, 70-200, 70-100, 70-90, 70-80, 80-1000, 80-900, 80-800, 80-700, 80-600, 80-500, 80-400, 80-300, 80-200, 80-100, 80-90, 90-1000, 90-900, 90-800, 90-700, 90-600, 90-500, 90-400, 90-300, 90-200, 90-100, 100-1000, 100- 900, 100-800, 100-700, 100-600, 100-500, 100-400, 100-300, 100-200, 200-1000, 200-900, 200-800, 200-700, 200-600, 200-500, 200-400, 200-300, 300-1000, 300-900, 300-800, 300-700, 300-600, 300-500, 300-400, 400-1000, 400-900, 400-800, 400-700, 400-600, 400-500, 500-1000, 500-900, 500-800, 500-700, 500-600, 600-1000, 600-900, 600-800, 600-700, 700-1000, 700-900, 700-800, 800-1000, 800-900, or 900-1000 μ g. In some embodiments, the effective amount of a VZV RNA (*e.g.*, mRNA) vaccine is a total dose of 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 μ g. In some embodiments, the effective amount is a dose of 25-500 μ g administered to the subject a total of two times. In some embodiments, the effective amount of a VZV RNA (*e.g.*, mRNA) vaccine is a dose of 25-500, 25-400, 25-300, 25-200, 25-100, 25-50, 50-500, 50-400, 50-300, 50-200, 50-100, 100-500, 100-400, 100-300, 100-200, 150-500, 150-400, 150-300, 150-200, 200-500, 200-400, 200-300, 250-500, 250-400, 250-300, 300-500, 300-400, 350-500, 350-400, 400-500 or 450-500 μ g administered to the subject a total of two times. In some embodiments, the effective amount of a VZV RNA (*e.g.*, mRNA) vaccine is a total dose of 25, 50, 100, 150, 200, 250, 300, 350, 400, 450, or 500 μ g administered to the subject a total of two times.

Additional Embodiments

1. A varicella zoster virus (VZV) vaccine, comprising:
at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap, an open reading frame encoding at least one VZV antigenic polypeptide, and a 3' polyA tail.
2. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 11.
3. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 92.

4. The vaccine of paragraph 1, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 10.
5. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 15.
- 5 6. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 93.
7. The vaccine of paragraph 1, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 14.
8. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is
10 encoded by a sequence identified by SEQ ID NO: 19.
9. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 94.
- 10 The vaccine of paragraph 1, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 18.
- 15 11. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 23.
12. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 95.
13. The vaccine of paragraph 1, wherein the at least one antigenic polypeptide comprises
20 a sequence identified by SEQ ID NO: 22.
14. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 27.
15. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 96.
- 25 16. The vaccine of paragraph 1, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 26.
17. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 31.
18. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises
30 a sequence identified by SEQ ID NO: 97.
19. The vaccine of paragraph 1, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 30.
20. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 35.

21. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 98.
22. The vaccine of paragraph 1, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 34.
- 5 23. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 39.
24. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 99.
25. The vaccine of paragraph 1, wherein the at least one antigenic polypeptide comprises
10 a sequence identified by SEQ ID NO: 38.
26. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 62.
27. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 101.
- 15 28. The vaccine of paragraph 26 or 27, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 38.
29. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 66.
30. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises
20 a sequence identified by SEQ ID NO: 102.
31. The vaccine of paragraph 30 or 31, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 38.
32. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 70.
- 25 33. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 103.
34. The vaccine of paragraph 32 or 33, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 38.
35. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is
30 encoded by a sequence identified by SEQ ID NO: 74.
36. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 104.
37. The vaccine of paragraph 35 or 36, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 38.

38. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 78.
39. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 105.
- 5 40. The vaccine of paragraph 38 or 39, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 38.
41. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 82.
42. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises
10 a sequence identified by SEQ ID NO: 106.
43. The vaccine of paragraph 41 or 42, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 38.
44. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is encoded by a sequence identified by SEQ ID NO: 86.
- 15 45. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 107, 134, or 148.
46. The vaccine of paragraph 44 or 45, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 38.
47. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide is
20 encoded by a sequence identified by SEQ ID NO: 90.
48. The vaccine of paragraph 1, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 108.
49. The vaccine of paragraph 47 or 48, wherein the at least one antigenic polypeptide comprises a sequence identified by SEQ ID NO: 38.
- 25 50. The vaccine of any one of paragraphs 1-49, wherein the 5' terminal cap is or comprises 7mG(5')ppp(5')NlmpNp.
51. The vaccine of any one of paragraphs 1-50, wherein 100% of the uracil in the open reading frame is modified to include N1-methyl pseudouridine at the 5-position of the uracil.
52. The vaccine of any one of paragraphs 1-51, wherein the vaccine is formulated in a
30 lipid nanoparticle comprising: DLin-MC3-DMA; cholesterol; 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC); and polyethylene glycol (PEG)2000-DMG.
53. The vaccine of paragraph 52, wherein the lipid nanoparticle further comprises trisodium citrate buffer, sucrose and water.
54. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 92 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 92 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 5 55. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 93 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 93 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 10 56. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 94 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 94 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 15 57. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 95 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 95 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 20 58. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 96 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 96 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 25 59. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 97 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 97 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 30 60. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 98 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 98 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 35 61. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 99 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 99 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 5 62. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 101 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 101 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 10 63. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 102 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 102 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 15 64. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 103 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 103 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 20 65. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 104 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 104 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 25 66. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 105 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 105 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 30 67. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 106 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 106 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

- 35 68. A varicella zoster virus (VZV) vaccine, comprising:

at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 107, 134, or 148 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 107 or 134 are modified to include N1-methyl pseudouridine at the 5-position of the uracil

5 nucleotide.

69. A varicella zoster virus (VZV) vaccine, comprising:

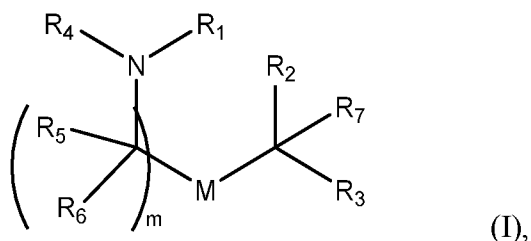
at least one messenger ribonucleic acid (mRNA) polynucleotide having a 5' terminal cap 7mG(5')ppp(5')NlmpNp, a sequence identified by SEQ ID NO: 108 and a 3' polyA tail, wherein the uracil nucleotides of the sequence identified by SEQ ID NO: 108 are modified to include N1-methyl pseudouridine at the 5-position of the uracil nucleotide.

10

70. The vaccine of any one of claims 54-69, wherein the vaccine is formulated in a lipid nanoparticle comprising DLin-MC3-DMA, cholesterol, 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC), and polyethylene glycol (PEG)2000-DMG.

71. The vaccine of any one of paragraphs 1-70 formulated in a lipid nanoparticle comprising at least one cationic lipid selected from compounds of Formula (I):

15



or a salt or isomer thereof, wherein:

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

20

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a carbocycle, heterocycle, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -N(R)₂, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R,

30

-C(O)N(R)OR, and -C(R)N(R)₂C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

5 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

10 R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

15 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

20 72. The vaccine of paragraph 71, wherein a subset of compounds of Formula (I) includes those in which when R₄ is -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, or -CQ(R)₂, then (i) Q is not -N(R)₂ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

73. The vaccine of paragraph 71, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂,

35 -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈,

-O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms

selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, mono- or di-alkylamino, and C₁₋₃ alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R,

-S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

74. The vaccine of paragraph 71, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a

5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S,

- OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂,
 -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈,
 -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR,
 -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂,
 5 -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and
 -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a
 5- to 14-membered heterocycle and (i) R₄ is -(CH₂)_nQ in which n is 1 or 2, or (ii) R₄
 is -(CH₂)_nCHQR in which n is 1, or (iii) R₄ is -CHQR, and -CQ(R)₂, then Q is either a 5- to
 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;
- 10 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,
 -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,
 and a heteroaryl group;
- 15 R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;
 R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R,
 -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;
 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- 20 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,
 -R*YR'', -YR'', and H;
 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;
 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;
 each Y is independently a C₃₋₆ carbocycle;
- 25 each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
 or salts or isomers thereof.
75. The vaccine of paragraph 71, wherein a subset of compounds of Formula (I) includes
 those in which
- 30 R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and
 -R''M'R';
 R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄
 alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
 attached, form a heterocycle or carbocycle;

- R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂,
 5 -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5;
- 10 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;
- 15 R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle; R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle; each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- 20 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H; each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl; each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl; each Y is independently a C₃₋₆ carbocycle;
- 25 each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13, or salts or isomers thereof.
76. The vaccine of paragraph 71, wherein a subset of compounds of Formula (I) includes those in which
- 30 R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R'; R₂ and R₃ are independently selected from the group consisting of H, C₂₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;
- 35 R₄ is -(CH₂)_nQ or -(CH₂)_nCHQR, where Q is -N(R)₂, and n is selected from 3, 4, and 5;

each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,
 -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,
 5 and a heteroaryl group;

R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl,
 -R*YR'', -YR'', and H;

10 each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;

each Y is independently a C_{3-6} carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

15 or salts or isomers thereof.

77. The vaccine of paragraph 71, wherein a subset of compounds of Formula (I) includes those in which

R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR'', -YR'', and
 -R''M'R';

20 R_2 and R_3 are independently selected from the group consisting of C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR'', -YR'', and -R*OR'', or R_2 and R_3 , together with the atom to which they are attached, form a heterocycle or carbocycle;

R_4 is selected from the group consisting of $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, and -CQ(R)₂,
 where Q is -N(R)₂, and n is selected from 1, 2, 3, 4, and 5;

25 each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,
 -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,
 and a heteroaryl group;

30 R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl,
 -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;

35 each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;

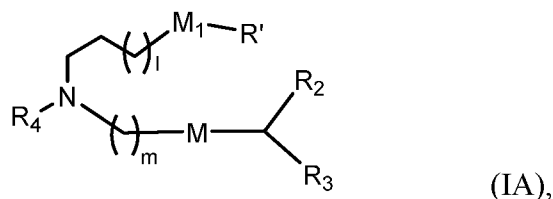
each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

- 5 78. The vaccine of paragraph 71, wherein a subset of compounds of Formula (I) includes those of Formula (IA):



or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6,

7, 8, and 9; M₁ is a bond or M'; R₄ is unsubstituted C₁₋₃ alkyl, or -(CH₂)_nQ, in which Q is OH,

- 10 -NHC(S)N(R)₂, -NHC(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)R₈,

-NHC(=NR₉)N(R)₂, -NHC(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, heteroaryl or

heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-,

-C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, and C₂₋₁₄ alkenyl.

- 15 This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including,"
- 20 "comprising," or "having," "containing," "involving," and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

Broad spectrum VZV vaccines

- It is envisioned that there may be situations where persons are at risk for infection
- 25 with more than one strain of VZV. RNA (*e.g.*, mRNA) therapeutic vaccines are particularly amenable to combination vaccination approaches due to a number of factors including, but not limited to, speed of manufacture, ability to rapidly tailor vaccines to accommodate perceived geographical threat, and the like. Moreover, because the vaccines utilize the human body to produce the antigenic protein, the vaccines are amenable to the production of
- 30 larger, more complex antigenic proteins, allowing for proper folding, surface expression, antigen presentation, *etc.* in the human subject. To protect against more than one strain of VZV, a combination vaccine can be administered that includes RNA encoding at least one

antigenic polypeptide protein (or antigenic portion thereof) of a first VZV and further includes RNA encoding at least one antigenic polypeptide protein (or antigenic portion thereof) of a second VZV. RNAs (mRNAs) can be co-formulated, for example, in a single lipid nanoparticle (LNP) or can be formulated in separate LNPs destined for co-administration.

Multiprotein and Multicomponent Vaccines

The present disclosure encompasses VZV vaccines comprising multiple RNA (*e.g.*, mRNA) polynucleotides, each encoding a single antigenic polypeptide, as well as VZV vaccines comprising a single RNA polynucleotide encoding more than one antigenic polypeptide (*e.g.*, as a fusion polypeptide). Thus, it should be understood that a vaccine composition comprising a RNA (*e.g.*, mRNA) polynucleotide having an open reading frame encoding a first VZV antigenic polypeptide and a RNA polynucleotide (*e.g.*, mRNA) having an open reading frame encoding a second VZV antigenic polypeptide encompasses (a) vaccines that comprise a first RNA polynucleotide encoding a first VZV antigenic polypeptide and a second RNA polynucleotide encoding a second VZV antigenic polypeptide, and (b) vaccines that comprise a single RNA polynucleotide encoding a first and second VZV antigenic polypeptide (*e.g.*, as a fusion polypeptide). VZV RNA vaccines of the present disclosure, in some embodiments, comprise 2-10 (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9 or 10), or more, RNA polynucleotides having an open reading frame, each of which encodes a different VZV antigenic polypeptide (or a single RNA polynucleotide encoding 2-10, or more, different VZV antigenic polypeptides). In some embodiments, a VZV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a VZV gE protein, a RNA polynucleotide having an open reading frame encoding a VZV gI protein, a RNA polynucleotide having an open reading frame encoding a VZV gB protein, a RNA polynucleotide having an open reading frame encoding a VZV gH protein, a RNA polynucleotide having an open reading frame encoding a VZV gK protein, a RNA polynucleotide having an open reading frame encoding a VZV gL protein, a RNA polynucleotide having an open reading frame encoding a VZV gC protein, a RNA polynucleotide having an open reading frame encoding a VZV gN protein, and a RNA polynucleotide having an open reading frame encoding a VZV gM protein. In some embodiments, a VZV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a VZV gE and a RNA polynucleotide having an open reading frame encoding a VZV gI protein. In some embodiments, a VZV RNA vaccine comprises a RNA polynucleotide having an open reading frame encoding a VZV gE protein or a gE variant.

In some embodiments, a RNA polynucleotide encodes a VZV antigenic polypeptide fused to a signal peptide (*e.g.*, SEQ ID NO: 56, 57, 109, 110, or 111). The signal peptide may be fused at the N-terminus or the C-terminus of the antigenic polypeptide.

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EXAMPLES

Example 1: Manufacture of Polynucleotides

According to the present disclosure, the manufacture of polynucleotides and/or parts or regions thereof may be accomplished utilizing the methods taught in International Publication WO2014/152027, entitled “Manufacturing Methods for Production of RNA
10 Transcripts,” the contents of which is incorporated herein by reference in its entirety.

Purification methods may include those taught in International Publication WO2014/152030 and International Publication WO2014/152031, each of which is incorporated herein by reference in its entirety.

Detection and characterization methods of the polynucleotides may be performed as
15 taught in International Publication WO2014/144039, which is incorporated herein by reference in its entirety.

Characterization of the polynucleotides of the disclosure may be accomplished using polynucleotide mapping, reverse transcriptase sequencing, charge distribution analysis, detection of RNA impurities, or any combination of two or more of the foregoing.
20 “Characterizing” comprises determining the RNA transcript sequence, determining the purity of the RNA transcript, or determining the charge heterogeneity of the RNA transcript, for example. Such methods are taught in, for example, International Publication WO2014/144711 and International Publication WO2014/144767, the content of each of which is incorporated herein by reference in its entirety.

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Example 2: Chimeric polynucleotide synthesis

According to the present disclosure, two regions or parts of a chimeric polynucleotide may be joined or ligated using triphosphate chemistry. A first region or part of 100 nucleotides or less is chemically synthesized with a 5' monophosphate and terminal 3'desOH
30 or blocked OH, for example. If the region is longer than 80 nucleotides, it may be synthesized as two strands for ligation.

If the first region or part is synthesized as a non-positionally modified region or part using *in vitro* transcription (IVT), conversion the 5' monophosphate with subsequent capping of the 3' terminus may follow.

Monophosphate protecting groups may be selected from any of those known in the art.

The second region or part of the chimeric polynucleotide may be synthesized using either chemical synthesis or IVT methods. IVT methods may include an RNA polymerase that can utilize a primer with a modified cap. Alternatively, a cap of up to 130 nucleotides may be chemically synthesized and coupled to the IVT region or part.

For ligation methods, ligation with DNA T4 ligase, followed by treatment with DNase should readily avoid concatenation.

The entire chimeric polynucleotide need not be manufactured with a phosphate-sugar backbone. If one of the regions or parts encodes a polypeptide, then such region or part may comprise a phosphate-sugar backbone.

Ligation is then performed using any known click chemistry, orthoclick chemistry, solulink, or other bioconjugate chemistries known to those in the art.

Synthetic route

The chimeric polynucleotide may be made using a series of starting segments. Such segments include:

(a) a capped and protected 5' segment comprising a normal 3'OH (SEG. 1)

(b) a 5' triphosphate segment, which may include the coding region of a polypeptide and a normal 3'OH (SEG. 2)

(c) a 5' monophosphate segment for the 3' end of the chimeric polynucleotide (*e.g.*, the tail) comprising cordycepin or no 3'OH (SEG. 3)

After synthesis (chemical or IVT), segment 3 (SEG. 3) may be treated with cordycepin and then with pyrophosphatase to create the 5' monophosphate.

Segment 2 (SEG. 2) may then be ligated to SEG. 3 using RNA ligase. The ligated polynucleotide is then purified and treated with pyrophosphatase to cleave the diphosphate. The treated SEG.2-SEG. 3 construct may then be purified and SEG. 1 is ligated to the 5' terminus. A further purification step of the chimeric polynucleotide may be performed.

Where the chimeric polynucleotide encodes a polypeptide, the ligated or joined segments may be represented as: 5'UTR (SEG. 1), open reading frame or ORF (SEG. 2) and 3'UTR+PolyA (SEG. 3).

The yields of each step may be as much as 90-95%.

Example 3: PCR for cDNA Production

PCR procedures for the preparation of cDNA may be performed using 2x KAPA HIFI™ HotStart ReadyMix by Kapa Biosystems (Woburn, MA). This system includes 2x KAPA ReadyMix 12.5 µl; Forward Primer (10 µM) 0.75 µl; Reverse Primer (10 µM) 0.75 µl; Template cDNA 100 ng; and dH₂O diluted to 25.0 µl. The reaction conditions may be at 95 °C for 5 min. The reaction may be performed for 25 cycles of 98 °C for 20 sec, then 58 °C for 15 sec, then 72 °C for 45 sec, then 72 °C for 5 min, then 4 °C to termination.

The reaction may be cleaned up using Invitrogen's PURELINK™ PCR Micro Kit (Carlsbad, CA) per manufacturer's instructions (up to 5 µg). Larger reactions may require a cleanup using a product with a larger capacity. Following the cleanup, the cDNA may be quantified using the NANODROP™ and analyzed by agarose gel electrophoresis to confirm that the cDNA is the expected size. The cDNA may then be submitted for sequencing analysis before proceeding to the *in vitro* transcription reaction.

Example 4: In vitro Transcription (IVT)

The *in vitro* transcription reaction generates RNA polynucleotides. Such polynucleotides may comprise a region or part of the polynucleotides of the disclosure, including chemically modified RNA (*e.g.*, mRNA) polynucleotides. The chemically modified RNA polynucleotides can be uniformly modified polynucleotides. The *in vitro* transcription reaction utilizes a custom mix of nucleotide triphosphates (NTPs). The NTPs may comprise chemically modified NTPs, or a mix of natural and chemically modified NTPs, or natural NTPs.

A typical *in vitro* transcription reaction includes the following:

- | | | |
|----|--|--------------------|
| 1) | Template cDNA | 1.0 µg |
| 2) | 10x transcription buffer | 2.0 µl |
| | (400 mM Tris-HCl pH 8.0, 190 mM MgCl ₂ , 50 mM DTT, 10 mM Spermidine) | |
| 3) | Custom NTPs (25mM each) | 0.2 µl |
| 4) | RNase Inhibitor | 20 U |
| 5) | T7 RNA polymerase | 3000 U |
| 6) | dH ₂ O | up to 20.0 µl. and |
| 7) | Incubation at 37 °C for 3 hr-5 hrs. | |

The crude IVT mix may be stored at 4 °C overnight for cleanup the next day. 1 U of RNase-free DNase may then be used to digest the original template. After 15 minutes of incubation at 37 °C, the mRNA may be purified using Ambion's MEGACLEAR™ Kit

(Austin, TX) following the manufacturer's instructions. This kit can purify up to 500 µg of RNA. Following the cleanup, the RNA polynucleotide may be quantified using the NANODROP™ and analyzed by agarose gel electrophoresis to confirm the RNA polynucleotide is the proper size and that no degradation of the RNA has occurred.

5

Example 5: Enzymatic Capping

Capping of a RNA polynucleotide is performed as follows where the mixture includes: IVT RNA 60 µg-180µg and dH₂O up to 72 µl. The mixture is incubated at 65 °C for 5 minutes to denature RNA, and then is transferred immediately to ice.

10

The protocol then involves the mixing of 10x Capping Buffer (0.5 M Tris-HCl (pH 8.0), 60 mM KCl, 12.5 mM MgCl₂) (10.0 µl); 20 mM GTP (5.0 µl); 20 mM S-Adenosyl Methionine (2.5 µl); RNase Inhibitor (100 U); 2'-O-Methyltransferase (400U); Vaccinia capping enzyme (Guanylyl transferase) (40 U); dH₂O (Up to 28 µl); and incubation at 37 °C for 30 minutes for 60 µg RNA or up to 2 hours for 180 µg of RNA.

15

The RNA polynucleotide may then be purified using Ambion's MEGACLEAR™ Kit (Austin, TX) following the manufacturer's instructions. Following the cleanup, the RNA may be quantified using the NANODROP™ (ThermoFisher, Waltham, MA) and analyzed by agarose gel electrophoresis to confirm the RNA polynucleotide is the proper size and that no degradation of the RNA has occurred. The RNA polynucleotide product may also be

20

sequenced by running a reverse-transcription-PCR to generate the cDNA for sequencing.

Example 6: PolyA Tailing Reaction

Without a poly-T in the cDNA, a poly-A tailing reaction must be performed before cleaning the final product. This is done by mixing capped IVT RNA (100 µl); RNase Inhibitor (20 U); 10x Tailing Buffer (0.5 M Tris-HCl (pH 8.0), 2.5 M NaCl, 100 mM MgCl₂)(12.0 µl); 20 mM ATP (6.0 µl); Poly-A Polymerase (20 U); dH₂O up to 123.5 µl and incubation at 37 °C for 30 min. If the poly-A tail is already in the transcript, then the tailing reaction may be skipped and proceed directly to cleanup with Ambion's MEGACLEAR™ kit (Austin, TX) (up to 500 µg). Poly-A Polymerase may be a recombinant enzyme expressed in yeast.

30

It should be understood that the processivity or integrity of the polyA tailing reaction may not always result in an exact size polyA tail. Hence, polyA tails of approximately between 40-200 nucleotides, *e.g.*, about 40, 50, 60, 70, 80, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 150-165, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164 or 165 are within the scope of the present disclosure.

35

Example 7: Capping Assays

Protein Expression Assay

Polynucleotides (e.g., mRNA) encoding a polypeptide, containing any of the caps taught herein, can be transfected into cells at equal concentrations. The amount of protein secreted into the culture medium can be assayed by ELISA at 6, 12, 24 and/or 36 hours post-transfection. Synthetic polynucleotides that secrete higher levels of protein into the medium correspond to a synthetic polynucleotide with a higher translationally-competent cap structure.

Purity Analysis Synthesis

RNA (e.g., mRNA) polynucleotides encoding a polypeptide, containing any of the caps taught herein can be compared for purity using denaturing Agarose-Urea gel electrophoresis or HPLC analysis. RNA polynucleotides with a single, consolidated band by electrophoresis correspond to the higher purity product compared to polynucleotides with multiple bands or streaking bands. Chemically modified RNA polynucleotides with a single HPLC peak also correspond to a higher purity product. The capping reaction with a higher efficiency provides a more pure polynucleotide population.

Cytokine Analysis

RNA (e.g., mRNA) polynucleotides encoding a polypeptide, containing any of the caps taught herein can be transfected into cells at multiple concentrations. The amount of pro-inflammatory cytokines, such as TNF-alpha and IFN-beta, secreted into the culture medium can be assayed by ELISA at 6, 12, 24 and/or 36 hours post-transfection. RNA polynucleotides resulting in the secretion of higher levels of pro-inflammatory cytokines into the medium correspond to a polynucleotides containing an immune-activating cap structure.

Capping Reaction Efficiency

RNA (e.g., mRNA) polynucleotides encoding a polypeptide, containing any of the caps taught herein can be analyzed for capping reaction efficiency by LC-MS after nuclease treatment. Nuclease treatment of capped polynucleotides yield a mixture of free nucleotides and the capped 5'-5-triphosphate cap structure detectable by LC-MS. The amount of capped product on the LC-MS spectra can be expressed as a percent of total polynucleotide from the reaction and correspond to capping reaction efficiency. The cap structure with a higher capping reaction efficiency has a higher amount of capped product by LC-MS.

Example 8: Agarose Gel Electrophoresis of Modified RNA or RT PCR Products

Individual RNA polynucleotides (200-400 ng in a 20 µl volume) or reverse transcribed PCR products (200-400 ng) may be loaded into a well on a non-denaturing 1.2% Agarose E-Gel (Invitrogen, Carlsbad, CA) and run for 12-15 minutes, according to the manufacturer protocol.

Example 9: NANODROP™ Modified RNA Quantification and UV Spectral Data

Chemically modified RNA polynucleotides in TE buffer (1 µl) are used for NANODROP™ UV absorbance readings to quantitate the yield of each polynucleotide from an chemical synthesis or *in vitro* transcription reaction.

Example 10: Formulation of Modified mRNA Using Lipidoids

RNA (*e.g.*, mRNA) polynucleotides may be formulated for *in vitro* experiments by mixing the polynucleotides with the lipidoid at a set ratio prior to addition to cells. *In vivo* formulation may require the addition of extra ingredients to facilitate circulation throughout the body. To test the ability of these lipidoids to form particles suitable for *in vivo* work, a standard formulation process used for siRNA-lipidoid formulations may be used as a starting point. After formation of the particle, polynucleotide is added and allowed to integrate with the complex. The encapsulation efficiency is determined using a standard dye exclusion assays.

Example 11: Exemplary Nucleic Acid encoding gE RNA polynucleotide for use in a VZV vaccine

The following sequence is an exemplary sequence that can be used to encode a VZV RNA polynucleotide gE for use in a VZV vaccine. A VZV vaccine may comprise, for example, at least one RNA polynucleotide encoded by at least one of the following sequence or by at least one fragment of the following sequence. In some embodiments, the mRNA further comprises a 5' cap, for example, any of the caps disclosed herein, *e.g.*, a cap having sequence m7G(5')ppp(5')G-2'-O-methyl. In some embodiments, the mRNA does not have a cap sequence. In some embodiments, the mRNA has at least one chemical modification, for example, any of the chemical modifications disclosed herein, *e.g.*, N1-methylpseudouridine modification or N1-ethylpseudouridine modification. In other embodiments, the mRNA does not have chemical modification.

Each of the sequences described herein encompasses a chemically modified sequence or an unmodified sequence which includes no modified nucleotides.

VZV gE –full-length Oka strain:

5 TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGA
AGAGTAAGAAGAAATATAAGAGCCACCATGGGGACAGTGAATAAGCCGGTTGTGGGCGTGCTTAT
GGGCTTTGGGATTATTACCGGTACATTACGAATTACCAATCCAGTGCGCGCCAGTGTGCTGCGTTA
10 CGACGACTTTCACATTGACGAGGATAAGCTGGATACTAACAGCGTGTACGAACCTTATTACCACTC
AGATCATGCCGAATCAAGCTGGGTTAATAGAGGAGAAAGCAGCCGAAAAGCCTACGACCACAACCT
CACCTTATATTTGGCCAGAAACGATTATGACGGTTTCTGGAACACGCACATGAACACCATGGAG
TCTACAACCAAGGCAGGGGAATCGACAGTGGCGAGCGTCTTATGCAGCCAACACAGATGTCGGCA
CAGGAGGATCTCGGTGATGACACCGGCATACACGTGATTCCCACATTAAACGGCGACGACAGACA
15 TAAGATCGTCAATGTGGATCAGCGTCAGTATGGGGATGTCTTTAAAGGCGATTTGAATCCAAAGCC
CCAAGGACAGAGACTGATCGAGGTCTCTGTAGAAGAAAATCACCCCTTCACTTTGCGCGCTCCAAT
CCAGAGGATTTACGGGGTTCGTTATACCGAACTTGGAGTTTCTTGCCGTCAGTACGTGTACGGG
GGATGCCGCCCCCGCAATCCAGCACATCTGTCTGAAACACACCACATGCTTTCAGGACGTGGTTGT
GGATGTGGATTGCGCGGAAAAACAAAAAGAACCAACTCGCCGAAATCAGCTATCGTTTTACAGG
20 GTAAAAAAGAGGCCGACCAACCGTGGATTGTTGTGAATACGAGCACGCTCTTCGATGAGCTTGAA
CTCGATCCCCCGAAATCGAGCCTGGGGTTCTAAAAGTGTTGAGGACCGAGAAGCAGTACCTCGG
GGTTTATATCTGGAATATGAGAGGCTCCGATGGCACCTCTACCTACGCAACGTTTCTGGTTACCTG
GAAGGGAGACGAGAAGACACGGAATCCAACGCCCGCTGTGACCCCTCAGCCTAGGGGAGCCGAA
TTCCACATGTGGAATATCACTCCCATTGTATTAGTGTGGGTGACACTTTCAGCCTGGCCATGCAC
CTGCACTATAAGATTACGAGGACACCTTCGACCTCTGCTGGAGTGGTTGTACGTACCTATTGAT
25 CCCACTTGTGAGCCCATCGCGCTGTACTCCACTTGCTTGTACCACCCCAATGCACCAAGTGTCTAT
CACACATGAACTCCGGGTGTACCTTTACTTCACCCCATCTTGCCAGCGGGTCCGACGACAGTGT
ATCAGAACTGTGAGCATGCTGACAACTATACTGCTTATTGCCTCGGAATATCCCATATGGAGCCAA
GCTTCGGGCTCATACTGCACGATGGTGGTACGACACTCAAGTTTCGTGGACACCCCCGAAAGCCTTT
CTGGCTTGTACGTGTTCTGTGGTCTACTTCAATGGACATGTGGAGGCAGTGGCTTACACAGTGGTTT
30 CGACAGTTGATCACTTTGTAAATGCCATTGAGGAACGCGGCTTCCCGCCTACAGCGGGCCAGCCCC
CTGCGACAACAAAACCAAAAGAGATTACGCCGTTAATCCTGGGACTAGTCCATTGCTGAGGTAT
GCCGCCTGGACTGGCGGTCTGGCGGCCGTGGTACTTCTGTGTTTAGTCATATTTCTGATCTGTACCG
CTAAACGTATGCGGGTCAAGGCTTACCGTGTGACAAGTCTCCTTACAATCAGTCAATGTACTATG
CAGGACTCCCTGTTGACGATTTTGAAGACTCAGAGAGTACAGACACAGAAGAAGAATTCGGAAAC
35 GCTATAGGTGGCTCTCACGGAGGTAGCTCGTATACAGTGTACATCGATAAAACCAGATGATAATA
GGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCAGCCCCTCCTCCCCTTCTG
CACCCGTACCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC (SEQ ID NO: 1)

VZV gE –full-length Oka strain (mRNA):

40 UCAAGCUUUUGGACCCUCGUACAGAAGCUAAUACGACUCACUUAUAGGGAAAUAAGAGAGAAAA
GAAGAGUAAGAAGAAAUUAAGAGCCACCAUGGGGACAGUGAAUAAGCCGGUUGUGGGCGUGC
UUAUGGGCUUUUGGAUUAUUACCGGUACAUAACGAAUUAACCAUCCAGUGCGCGCCAGUGUGC
UGCGUUAACGACGACUUAUACAUUGACGAGGAUAAGCUGGAUACUAAACAGCGUGUACGAACCUU
45 AUUACCACUCAGAUCAUGCCGAAUUAAGCUGGGUUAUAAGAGGAGAAAGCAGCCGAAAAGCCU
ACGACCACAACUCACCUUAUAUUUGGCCAGAAACGAUUAUGACGGUUUCCUGGAAAACGCAC
AUGAACACCAUGGAGUCUACAACCAAGGCAGGGGAUUCGACAGUGGCGAGCGUCUUAUGCAGC
CAACACAGAUGUCGGCACAGGAGGAUCUCGGUGAUGACACCGGCAUACACGUGAUUCCCAU
50 UAAACGGCGACGACAGACAUAAAGAUUGUCAAUGUGGAUCAGCGUCAGUAUGGGGAUGUCUUUA
AAGGCGAUUUGAAUCCAAAGCCCCAAGGACAGAGACUGAUCGAGGUCUCUGUAGAAGAAAAC
ACCCCUUCACUUUGCGCGCUCCAUAUCCAGAGGAUUUACGGGGUGCGUUUAUACCGAAACUUGGA
GUUUUCUUGCCGUCACUGACGUGUACGGGGGAUGCCGCCCGCAAUCCAGCACAUUCUGUCUGAA
ACACACCACAUUCUUUCAGGACGUGGUUGUGGAUGUGGAUUGCGCGGAAAACAAAAAGAGA
CCAACUCGCCGAAAUCAGCUAUCGUUUUCAGGGUAAAAAAGAGGCCGACCAACCGUGGAUUGU
55 UGUGAAUACGAGCAGCUCUUCGAUGAGCUUGAACUUGAUCUCCCCGAAAUCGAGCCUGGGGU
UCUAAAAGUGUAGAGACCGAGAGAGCAUACCUUGGGUUUAUUCUGGAUUAUGAGAGGCUC
CGAUGGCACCUUAGCUACGCAACGUUUUCUGGUUACCUUGGAAGGAGACGAGAAGACACGGAA
UCCAACGCCCGCUGUGACCCUCAGCCUAGGGGAGCCGAAUUCACAUGUGGAACUUAUCACUCC
CAUGUAUUCAGUGUGGGUGACACUUUCAGCCUGGCCAUGCACCUGCAGUAUAAGAUUCACGAG
60 GCACCCUUCGACCUCUGUGGAGUGGUUGUACGUACCUAUUGAUCCCAUUGUCAGCCCAUGC

GCCUGUACUCCACUUGCUUGUACCACCCCAAUGCACCACAGUGUCUAUCACACAUGAACUCCGG
 GUGUACCUUUACUUCACCCCAUCUUGCCCAGCGGGUCGCCAGCACAGUGUAUCAGAACUGUGAG
 CAUGCUGACAACUUAUCUGCUUAUUGCCUCGGAAUAUCCCAUAUGGAGCCAAGCUUCGGGCUC
 AUACUGCACGAUGGUGGUACGACACUCAAGUUCGUGGACACCCCCGAAAGCCUUUCUGGCUUG
 5 UACGUGUUCGUGGUCUACUCAAUGGACAUGUGGAGGCAGUGGCUUACACAGUGGUUUCGACA
 GUUGAUCACUUUGUAAAUGCCAUGAGGAACGCGGCUUCCCGCCUACAGCGGGCCAGCCCCUG
 CGACAACAAAACCAAAAGAGAUUACGCCCGUUAUCCUGGGACUAGUCCAUUGCUGAGGUAUG
 CCGCCUGGACUGGCGGUCUGGCGGCCGUGGUACUUCUGUGUUUAGUCAUAUUUCUGAUCUGUA
 10 CCGCUAAACGU AUGCGGGUCAAGGCUUACCGUGUUGACAAGUCUCCUUAACAUCAGUCA AUGU
 ACUAUGCAGGACUCCUGUUGACGAUUUCGAAGACUCAGAGAGUACAGACACAGAAGAAGAAU
 UCGGAAACGCUAUAGGUGGCUCUCACGGAGGUAGCUCGUUAUACAGUGUACAUCGAUAAAACCA
 GAUGAUAAUAGGCUGGAGCCUCGUGGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCCU
 CCUCCCCUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC (SEQ ID
 NO: 123)

Example 12: Exemplary Nucleic Acid encoding gI RNA polynucleotide for use in a VZV vaccine

The following sequence is an exemplary sequence that can be used to encode a VZV
 RNA polynucleotide gI for use in a VZV RNA (*e.g.*, mRNA) vaccine. The gI polypeptide
 20 forms a complex with gE in infected cells which facilitates the endocytosis of both
 glycoproteins and directs them to the trans-Golgi network (TGN) where the final viral
 envelope is acquired. A VZV vaccine may comprise, for example, at least one RNA (*e.g.*,
 mRNA) polynucleotide encoded by at least one of the following sequence or by at least one
 fragment of the following sequence. In some embodiments, the mRNA further comprises a
 25 5' cap, for example, any of the caps disclosed herein, *e.g.*, a cap having sequence
 m7G(5')ppp(5')G-2'-O-methyl. In other embodiments, the mRNA does not have a cap
 sequence. In some embodiments, the mRNA has at least one chemical modification, for
 example, any of the chemical modifications disclosed herein, *e.g.*, N1-methylpseudouridine
 modification or N1-ethylpseudouridine modification. In other embodiments, the mRNA does
 30 not have chemical modification.

VZV-GI-full length (Oka strain):

TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGA
 35 AGAGTAAGAAGAAATATAAGAGCCACCATGTTTTAATCCAATGTTTGATATCGGCCGTTATATTT
 TACATACAAGTGACCAACGCTTTGATCTTCAAGGGCGACCACGTGAGCTTGCAAGTTAACAGCAGT
 CTCACGTCTATCCTTATTTCCCATGCAAAATGATAATTATACAGAGATAAAAGGACAGCTTGTCTTT
 ATTGGAGAGCAACTACCTACCGGGACAACTATAGCGGAACACTGGAAGTGTATACGCGGATAC
 40 GGTGGCGTTTTGTTTTCCGGTCAGTACAAAGTAATAAGATACGACGGATGTCCCCGGATTAGAACGAG
 CGCTTTTATTTCTGTAGGTACAAACATTCTGTGGCATTATGGTAACTCAACGGATCGGATATCAAC
 AGAGCCGGATGCTGGTGTAAATGTTGAAAATTACCAACCGGGAATAAATGATGCTGGTGTGTATG
 TACTTCTTGTTCTGGTTAGACCATAGCAGATCCACCGATGGTTTCATTCTTGGTGTAAATGTATATAC
 AGCGGGCTCGCATCACAAACATTCACGGGGTTATCTACACTTCTCCATCTCTACAGAATGGATATTC
 45 TACAAGAGCCCTTTTTCAACAAGCTCGTTTGTGTGATTTACCCGCGACACCCAAAGGGTCCGGTAC
 CTCCTGTTTCAACATATGCTTGATCTTCTGTCGGGTAATCGTTAGAGGATAACCCCTTGTTTACAT
 GAGGACGTTGTTACGACAGAACTAAGTCCGTTGTTAAGGAGGGGATAGAAAATCACGTATATCC
 AACGGATATGTCCACGTTACCCGAAAGTCCCTTAATGATCCTCCAGAAAATCTACTTATAATTAT
 TCCTATAGTAGCGTCTGTATGATCCTACCGCCATGGTTATTGTTATTGTAATAAGCGTTAAGCGA
 50 CGTAGAATTAATAAACATCCAATTTATCGCCCAAATACAAAAACAAGAAGGGGCATACAAAATGC
 GACACCAGAATCCGATGTGATGTTGGAGGCCGCCATTGCACAACTAGCAACGATTCGCGAAGAAT

CCCCCCCACATTCCGTTGTAAACCCGTTTGTAAATAGTGATAATAGGCTGGAGCCTCGGTGGCCA
TGCTTCTTGCCCTTGCGGCTCCCCCAGCCCCCTCTCCCCTTCTGCACCCGTACCCCCGTGGTCTT
TGAATAAAGTCTGAGTGGGCGGC (SEQ ID NO: 2)

5 VZV-GI-full length (Oka strain) (mRNA):

UCAAGCUUUUGGACCCUCGUACAGAAGCUAAUACGACUCACUUAUAGGGAAAUAAGAGAGAAAA
GAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGUUUUUAUCCAAUGUUUGAUUAUCGGCCGUUA
UAUUUUACAUACAAGUGACCAACGCUUUGAUUCUUAAGGGCGACCACGUGAGCUUGCAAGUUA
10 ACAGCAGUCUCACGUCUAUCCUUAUUCCAUGCAAAAUGAUAAUUAUACAGAGAUAAAAGGAC
AGCUUGUCUUUAUUGGAGAGCAACUACCUACCGGGACAAACUUAAGCGGAACACUGGAACUGU
UAUACGCGGAUACGGUGGCGUUUUGUUUCCGGUCAGUACAAGUAAUAAGAUACGACGGAUGUC
CCCGGAUUAGAACGAGCGCUUUUAUUUCGUGUAGGUACAAACAUUCGUGGCAUUAUGGUAACU
15 CAACGGAUCGGAUAUCAACAGAGCCGGAUGCUGGUGUAAUGUUGAAAAUUACCAAACCGGGAA
UAAAUGAUGCUGGUGUGUAUGUACUUCUUGUUCGGUUAAGACCAUAGCAGAUCCACCGAUGGUU
UCAUUCUUGGUGUAAAUGUAUAUACAGCGGGCUCGCAUCACAACAUUCACGGGGUUAUCUACA
CUUCUCCAUCUCUACAGAAUGGAUUAUCUACAAGAGCCCUUUUUAACAAGCUCGUUUGUGUG
AUUUACCCGCGACACCCAAAGGGUCCGGUACCUCUUGUUUAACAUAUGCUUGAUCUUCGUGC
CGGUAAAUCGUUAGAGGAUAACCCUUGGUUACAUGAGGACGUUGUUAACGACAGAAACUAAGUC
20 CGUUGUUAAGGAGGGGAUAGAAAAUCACGUUAUCCAACGGAUAUGUCCACGUUACCCGAAAA
GUCCCUUAAUGAUCCUCCAGAAAAUCUACUUAUAAUUAUUCUUAUAGUAGCGUCUGUCAUGAU
CCUCACCGCCAUGGUUAUUGUUAUUGUAAUAAGCGUUAAGCGACGUAGAAUUAUAAAAACAUC
AAUUUAUCGCCCAAUAUAAAAACAAGAAGGGGCAUACAAAUGCGACACCAGAAUCCGAUGU
GAUGUUGGAGGCCGCCAUUGCACAACUAGCAACGAUUCGCGAAGAAUCCCCCCCACAUUCCGUU
25 GUAAACCCGUUUGUUAUUAUAGUGAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCU
UGGGCCUCCCCCAGCCCCUCCUCCCUUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGU
CUGAGUGGGCGGC (SEQ ID NO: 124)

Example 13: mRNAs encoding variant gE antigens having different C-terminal sequence

30 *for use in a VZV vaccine*

VZV is enveloped in the trans-golgi network. Glycoprotein I(gI) forms a complex
with gE in infected cells which facilitates the endocytosis of both glycoproteins and directs
them to the trans-Golgi network (TGN) where the final viral envelope is acquired. mRNAs
encoding gE antigens having different C-terminal variant sequence were designed to avoid
35 gE being trapped in the ER/golgi/TGN, leading to an increase in the localization of gE
antigen to the plasma membrane and improved immune-stimulating capabilities. A
schematic of the gE antigen is shown in Fig. 4.

Several different gE variant mRNA sequences (Oka strain) were engineered:

(1) gE variant mRNA encoding a truncated polypeptide having the terminal 62 amino acids
40 of the C terminal region deleted (SEQ ID NO: 17-20). The resultant polypeptide has reduced
localization to the trans-golgi network and reduced endocytosis.

(2) gE variant mRNA encoding a truncated polypeptide having the terminal 62 amino acids
of the C terminal region deleted and also having the signal peptide replaced with IgKappa,
which results in a secreted form of the truncated gE polypeptide (SEQ ID NO: 21-24). The
45 resultant polypeptide has reduced localization to the trans-golgi network and reduced
endocytosis.

(3) gE variant mRNA encoding a truncated polypeptide having the terminal 50 amino acids of the C terminal region deleted (SEQ ID NO: 33-36). The resultant polypeptide has reduced localization to the trans-golgi network and reduced endocytosis.

(4) gE variant mRNA encoding a truncated polypeptide having the terminal 50 amino acids of the C terminal region deleted and also having the point mutation Y569A (SEQ ID NO: 37-40). The “AYRV” motif (SEQ ID NO: 119) is a trafficking motif which targets the gE polypeptide to the trans-golgi network. Thus, mutating the AARV sequence SEQ ID NO: 120 to AYRV SEQ ID NO: 119 results in reduced localization of the gE polypeptide to the trans-golgi network.

(5) gE variant mRNA encoding full-length gE polypeptide with an AEAADA (SEQ ID NO: 58) sequence (SEQ ID NO: 25-28). The A-E-A-A-D-A (SEQ ID NO: 58) sequence replaces SESTD (SEQ ID NO: 59). This is a replacement of the Ser/Thr –rich “SSTT” (SEQ ID NO: 122) acidic cluster with an Ala-rich sequence. This reduces CKII phosphorylation, which in turn results in reduced localization of the gE polypeptide to the trans-golgi network.

(6) gE variant mRNA encoding full-length gE polypeptide with an AEAADA (SEQ ID NO: 58) sequence and also having the point mutation Y582G (SEQ ID NO: 29-32). The “YAGL” (SEQ ID NO: 121) motif is an endocytosis motif which enhances localization of the gE polypeptide to the trans-golgi network. Thus, mutating the GAGL sequence (SEQ ID NO: 132) to YAGL (SEQ ID NO: 121) results in reduced endocytosis of the resultant polypeptide.

Each of these variants have modifications that reduce localization of the encoded gE protein to the trans-golgi network and enhance trafficking to the plasma membrane. Table 1 summarizes mRNAs encoding the variant gE antigens having different C-terminal sequence. In some embodiments, the variant mRNA further comprises a 5' cap, for example, any of the caps disclosed herein, *e.g.*, a cap having sequence m7G(5')ppp(5')G-2'-O-methyl. In some embodiments, the variant mRNA does not have a 5' cap. In some embodiments, the variant mRNA has at least one chemical modification, for example, any of the chemical modifications disclosed herein, *e.g.*, N1-methylpseudouridine modification or N1-ethylpseudouridine modification. In some embodiments, the mRNA does not have chemical modification. The sequences encoding the mRNA variants are provided beneath the table.

Table 1: mRNA Constructs

SEQ ID NO:	Name of mRNA construct	Description	Function
3 (DNA) and 125 (mRNA)	VZV-GE-delete-562	Truncated VZV gE sequence - deletion from aa 562 (62 aa deletion from C terminal domain)	The C-terminal sequence targets gE to the trans-Golgi network (TGN); truncation assists in reducing gE localization to TGN
4 (DNA) and 126 (mRNA)	VZV-GE-delete-562-IgKappa	Secreted form of truncated VZV gE sequence - deletion from aa 562 (62 aa deletion from C terminal domain) and signal peptide replaced with IgKappa	The C-terminal sequence targets gE to the trans-Golgi networks (TGN); truncation assists in reducing gE localization to TGN
5 (DNA) and 127 (mRNA)	VZV-GE-delete-574	Truncated VZV gE sequence - deletion from aa 574 (50 aa deletion from C terminal domain)	The C-terminal sequence targets gE to the trans-Golgi network (TGN); truncation assists in reducing gE localization to TGN
6 (DNA) and 128 (mRNA)	VZV-GE-delete-574-Y569A	Truncated VZV gE sequence - deletion from aa 574 (50 aa deletion from C terminal domain) and Y569A point mutation	The C-terminal sequence targets gE to the trans-Golgi network (TGN); the AYRV (SEQ ID NO: 119) sequence is required for targeting gE to the TGN; truncation/mutation reduces localization to TGN)
7 (DNA) and 129 (mRNA)	VZV-GE-full-length-AEAADA (SEQ ID NO: 58)	VZV gE full length sequence with AEAADA (SEQ ID NO: 58) sequence	AEAADA (SEQ ID NO: 58) replaces SSTT (SEQ ID NO: 122) (acid cluster) comprising a phosphorylation motif, which phosphorylation assists in localizing gE to the TGN; mutation reduces localization of gE to TGN

SEQ ID NO:	Name of mRNA construct	Description	Function
8 (DNA) and 130 (mRNA)	VZV-GE-full-length-AEAADA (SEQ ID NO: 58) - Y582G	VZV gE-full length sequence with AEAADA sequence (SEQ ID NO: 58) and Y582G point mutation	Mutations assist in reducing endocytosis and localization of gE to the TGN

VZV-GE-delete-562

5 TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGA
AGAGTAAGAAGAAATATAAGAGCCACCATGGGGACAGTTAATAAACCTGTGGTGGGGGTATTGAT
GGGGTTTCGGAATTATCACGGGAACGTTGCGTATAACGAATCCGGTCAGAGCATCCGTCTTGCGATA
CGATGATTTTCACATCGATGAAGACAACTGGATACAACTCCGTATATGAGCCTTACTACCATTTC
10 AGATCATGCGGAGTCTTCATGGGTAAATCGGGGAGAGTCTTCGCGAAAAGCGTACGATCATAACT
CACCTTATATATGGCCACGTAATGATTATGATGGATTTTTAGAGAACGCACACGAACACCATGGGG
TGTATAATCAGGGCCGTGGTATCGATAGCGGGGAACGGTTAATGCAACCCACACAAATGCTCTGCA
CAGGAGGATCTTGGGGACGATACGGGCATCCACGTTATCCCTACGTTAAACGGCGATGACAGACA
TAAAATTGTAAATGTGGACCAACGTCAATACGGTGACGTGTTTAAAGGAGATCTTAATCCAAAAC
15 CCCAAGGCCAAAGACTCATTGAGGTGTCAGTGGAAAGAAAATCACCCGTTTACTTTACGCGCACCG
ATTGAGCGGATTTATGGAGTCCGGTACACCGAGACTTGGAGCTTTTTGCCGTCATTAACTGTACG
GGAGACGCAGCGCCCGCCATCCAGCATATATGTTTAAACATACAAACATGCTTTCAAGACGTGGT
GGTGGATGTGGATTGCGCGGAAAATACTAAAGAGGATCAGTTGGCCGAAATCAGTTACCGTTTTTC
AAGGTAAGAAGGAAGCGGACCAACCGTGGATTGTTGTAAACACGAGCACACTGTTTGATGAACCTC
20 GAATTAGACCCCCCGAGATTGAACCGGGTGTCTTGAAAGTACTTCGGACAGAAAAACAATACTT
GGGTGTGTACATTTGGAACATGCGCGGCTCCGATGGTACGTCTACCTACGCCACGTTTTTGGTCAC
CTGGAAAGGGGATGAAAAACAAGAAACCCTACGCCCGCAGTAACTCCTCAACCAAGAGGGGCT
GAGTTTCATATGTGGAATTACCACTCGCATGTATTTTCAGTTGGTGATACGTTTAGCTTGGCAATGC
ATCTTCAGTATAAGACATACATGAAGCGCCATTTGATTTGCTGTAGAGTGGTTGATGTCCCCATGC
25 ATCCTACATGTCAACCAATGCGGTTATATTCTACGTGTTTGTATCATCCCAACGCACCCCAATGCCT
CTCTCATATGAATTCCGGTTGTACATTTACCTCGCCACATTTAGCCCAGCGTGTTGCAAGCACAGTG
TATCAAAATTGTGAACATGCAGATAACTACACCGCATATTGTCTGGGAATATCTCATATGGAGCCT
AGCTTTGGTCTAATCTTACACGACGGGGGCACCACGTTAAAGTTTGTAGATACACCCGAGAGTTTG
TCGGGATTATACGTTTTTGTGGTGTATTTTAAACGGGCATGTTGAAGCCGTAGCATACTGTTGTAT
CCACAGTAGATCATTTTGTAAACGCAATTGAAGAGCGTGGATTTCGCCAACGGCCGGTCAGCCAC
30 CGGCGACTACTAAACCAAGGAAATTACCCCCGTAAACCCCGGAACGTCACCACTTCTACGATAT
GCCGCATGGACCGGAGGGCTTGCAGCAGTAGTACTTTTATGTCTCGTAATATTTTAACTGTACG
GCTTGATGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCTTGGGCCTCCCCCAGCCC
CTCCTCCCCTTCTGACCCCGTACCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC (SEQ ID
NO: 3)

35

VZV-GE-delete-562 (mRNA)

UCAAGCUUUUGGACCCUCGUACAGAAGCUAAUACGACUCACUUAUAGGGAAAUAAAGAGAGAAAA
GAAGAGUAAGAAGAAAUUAAGAGCCACCAUGGGGACAGUUAUAAACCUUGUGUGGGGUUAU
40 UGAUGGGGUUCGGAUUUAUCACGGGAACGUUGCGUAUAACGAUCCGGUCAGAGCAUCCGUCU
UGCGAUACGAUGAUUUUACAUCGAUGAAGACAAACUGGAUACAAACUCCGUUAUAGAGCCUU
ACUACCAUUCAGAUCAUGCGGAGUCUUAUGGGUAAAUCGGGGAGAGUCUUCGCGAAAAGCGU
ACGAUCAUAACUCACCUUAUAUAGGCCACGUAAUGAUUAUGAUGGAUUUUUAGAGAACGCAC
ACGAACACCAUGGGGUGUAUAAUCAGGGCCGUGGUUAUCGAUAGCGGGGAACGGUUAUUGCAAC
45 CCACACAAAUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUCCACGUUAUCCCUACGU
UAAACGGCGAUGACAGACAUAAAAUUGUAAAUGUGGACCAACGUCAAUACGGUGACGUGUUUA
AAGGAGAUUUAAUCCAAAACCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUGGAAGAAAAUC
ACCCGUUUACUUUACGCGCACCGAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGA
GCUUUUUGCCGUCAUUAACUGUACGGGAGACGCAGCGCCCGCAUCCAGCAUUAUGUUUAA
50 AACAUACAACAUGCUUUCAAGACGUGGUGGUGGAUGUGGAUUGCGCGGAAAAUACUAAAGAGG
AUCAGUUGGCCGAAAUACAGUUAACGUUUUCAAAGGUAAGAAGGAAGCGGACCAACCGUGGAUUG
UUGUAAACACGAGCACACUGUUUGAUGAACUCGAAUUAAGACCCCCCGAGAUUGAACCGGGUG

UCUUGAAAGUACUUCGGACAGAAAAACAAUACUUGGGUGUGUACAUUUGGAACAUGC CGCGGCU
 CCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCACCUGGAAAGGGGAUGAAAAACAAGAA
 ACCCUACGCCCCGAGUAAACUCCUCAACCAAGAGGGGGCUGAGUUUCAUAUGUGGAAUUAACACUC
 GCAUGUAUUUUCAGUUGGUGAUACGUUUAGCUUGGCAAUGCAUCUUCAGUAUAAGAUACAUGA
 5 AGCGCCAUUUGAUUUGCUGUUAGAGUGGUUGUAUGUCCCCAUCGAUCCUACAUGUCAACCAAU
 GCGGUUAUAUUCUACGUGUUUGUAUCAUCCCAACGCACCCCAUUGCCUCUCUCAUAUGAAUUC
 GGUUGUACAUUUACCUCGCCACAUUUAGCCCAGCGUGUUGCAAGCACAGUGUAUCAAUUAUUGU
 GAACAUGCAGAUAAACUACACCGCAUAUUGUCUGGGAAUAUCUCAUAUGGAGCCUAGCUUUGGU
 10 CUAUUCUACACGACGGGGGCACCACGUUAAAGUUUGUAGAUACACCCGAGAGUUUGUCGGGA
 UUAUACGUUUUUUGUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUGUUGUAUCC
 ACAGUAGAUAUUUUGUAAACGCAAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUCAGCCA
 CCGGCGACUACUAAACCCAAGGAAUUAACCCCGUAAACCCCGGAACGUCACCACUUCUACGAU
 AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUUUUUGUCUGUAAUUAUUUUUAAUCU
 15 GUACGGCUUGAUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCC
 CCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCG
 GC (SEQ ID NO: 125)

VZV-GE-delete-562-IgKappa

20 TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAAGA
 AGAGTAAGAAGAAATATAAGAGCCACCATGGAAACCCCGGCGCAGCTGCTGTTTCTGCTGCTGCT
 GTGGCTGCCGATACCACCGGCTCCGTCTTGCGATACGATGATTTTCACATCGATGAAGACAACT
 GGATACAACTCCGTATATGAGCCTTACTACCATTTCAGATCATGCGGAGTCTTCATGGGTAAATCG
 25 GGGAGAGTCTTCGCGAAAAGCGTACGATCATAACTCACCTTATATATGGCCACGTAATGATTATGA
 TGGATTTTATAGAAACGCACACGAACACCATGGGGTGTATAATCAGGGCCGTGGTATCGATAGCG
 GGGAACGGTTAATGCAACCCACACAAATGTCTGCACAGGAGGATCTTGGGGACGATACGGGCATC
 CACGTTATCCCTACGTTAAACGGCGATGACAGACATAAAATTGTAAATGTGGACCAACGTCAATA
 CGGTGACGTGTTTAAAGGAGATCTTAATCCAAAACCCCAAGGCCAAAGACTCATTGAGGTGTCAG
 30 TGGAAGAAAATCACCCGTTTACTTTACGCGCACCGATTTCAGCGGATTTATGGAGTCCGGTACACCG
 AGACTTGAGCTTTTGGCGTCATTAACCTGTACGGGAGACGCAGCGCCCGCCATCCAGCATATAT
 GTTTAAACATACAACATGCTTTCAAGACGTGGTGGTGGATGTGGATTGCGCGGAAAATACTAAA
 GAGGATCAGTTGGCCGAAATCAGTTACCGTTTCAAGGTAAGAAGGAAGCGGACCAACCGTGGAT
 TGTGTAAACACGAGCACACTGTTTGATGAACCTCGAATTAGACCCCCCGAGATTGAACCGGGTGT
 35 CTTGAAAGTACTTCGGACAGAAAAACAATACTTGGGTGTGTACATTTGGAACATGCGCGGCTCCG
 ATGGTACGTCTACCTACGCCACGTTTGGTACCTGGAAAGGGGATGAAAAAACAAGAAACCCCT
 ACGCCCGCAGTAACCTCTCAACCAAGAGGGGCTGAGTTTCATATGTGGAATTACCACTCGCATGTA
 TTTTCAGTTGGTGATACGTTTAGCTTGGCAATGCATCTTCAGTATAAGATACATGAAGCGCCATTTG
 ATTTGCTGTTAGAGTGGTTGTATGTCCCCATCGATCCTACATGTCAACCAATGCGGTTATATTCTAC
 GTGTTTGTATCATCCCAACGCACCCCAATGCCTCTCTCATATGAATTCCGGTTGTACATTTACCTCG
 40 CCACATTTAGCCCAGCGTGTTGCAAGCACAGTGTATCAAAATTGTGAACATGCAGATAACTACACC
 GCATATTGTCTGGGAATATCTCATATGGAGCCTAGCTTTGGTCTAATCTTACACGACGGGGGCACC
 ACGTTAAAGTTTGTAGATACACCCGAGAGTTTGTGCGGATTATACGTTTTTGTGGTGTATTTTAACG
 GGCATGTTGAAGCCGTAGCATACTGTTGTATCCACAGTAGATCATTTTGTAAACGCAATTGAAG
 AGCGTGGATTTCCGCCAACGGCCGGTCAGCCACCGGCGACTACTAAACCAAGGAAATTACCCCC
 45 GTAAACCCCGGAACGTCACCACTTCTACGATATGCCGCATGGACCGGAGGGCTTGCAGCAGTAGT
 ACTTTTATGTCTCGTAATATTTTAATCTGTACGGCTTGATGATAATAGGCTGGAGCCTCGGTGGCC
 ATGCTTCTTGCCCCTTGGGCCTCCCCCAGCCCCTCCTCCCCTTCTGCACCCGTACCCCGTGGTC
 TTTGAATAAAGTCTGAGTGGGCGGC (SEQ ID NO: 4)

50 VZV-GE-delete-562-IgKappa (mRNA)

UCAAGCUUUUGGACCCUCGUACAGAAGCUAAUACGACUCACUAUAGGGGAAUUAAGAGAGAAAA
 GAAGAGUAAGAAGAAAUUAAGAGCCACCAUGGAAACCCCGGCGCAGCUGCUGUUUCUGCUGC
 UGCUGUGGCUGCCGGAUACCACCGGCUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUGAAG
 55 ACAAACUGGAUACAAACUCCGUUAUAGAGCCUUAUACCAUUCAGAUCAUGCGGAGUCUUCAU
 GGGUAAAUCGGGGAGAGUCUUCGCGAAAAGCGUACGAUCAUAACUACCUUAUAUAUGGCCAC
 GUAAUGAUUAUGAUGGAUUUUUAGAGAACGCACACGAACACCAUGGGGUGUAUAAUCAGGGCC
 GUGGUUAUCGAUAGCGGGGAACGGUUAUUGCAACCCACACAAAUGUCUGCACAGGAGGAUCUUG
 GGGACGAUACGGGCAUCCACGUUAUCCCUACGUUAAACGGCGAUGACAGACAUAAAAUUGUAA
 60 AUGUGGACCAACGUCAAUACGGUGACGUGUUUAAAGGAGAUUUAAUCCAAAACCCCAAGGCC
 AAAGACUCAUUGAGGUGUCAGUGGAAGAAAUCACCCGUUUACUUUACGCGCACCGAUUCAGC

5 GGAUUUAUGGAGUCCGGUACACCGAGACUUGGAGCUUUUUGCCGUCAUUAACCUGUACGGGAG
 ACGCAGCGCCCGCAUCCAGCAUAUAUGUUUAAAACAUACAACAUGCUUUAAGACGUGGUGG
 UGGAUGUGGAUUGCGCGGAAAAUACUAAAGAGGAUCAGUUGGCCGAAAUACAGUUACCGUUUUU
 AAGGUAAGAAGGAAGCGGACCAACCGUGGAUUGUUGUAAACACGAGCACACUGUUUGAUGAAC
 10 UCGAAUUAGACCCCCCGAGAUUGAACCGGGUGUCUUGAAAGUACUUCGGACAGAAAAACAAU
 ACUUGGGUGUGUACAUUUGGAACAUGCGCGGCUCGGAUGGUACGUCUACCUACGCCACGUUUU
 UGGUACACCUGGAAAGGGGAUGAAAAAAACAAGAAACCCUACGCCCGCAGUAAACUCCUCAACCAA
 GAGGGGCUGAGUUUCAUAUGUGGAAUUACCACUCGCAUGUAUUUUCAGUUGGUGAUACGUUUU
 15 GCUUGGCAAUGCAUCUUCAGUAUAAGAUACAUGAAGCGCCAUUUGAUUUGCUGUUAGAGUGGU
 UGUAUGUCCCCAUCGAUCCUACAUGUCAACCAAUGCGGUUAUAUUCUACGUGUUUGUAUCAUC
 CCAACGCACCCCAUGCCUCUCUCAUAUGAAUUCGGUUGUACAUUUACCUCGCCACAUUUAGC
 CCAGCGUGUUGCAAGCACAGUGUAUCAAAAUUGUGAACAUGCAGAUAAACUACACCGCAUAUUG
 UCUGGGAAUAUCUCAUAUGGAGCCUAGCUUUGGUCUAAUCUUAACACGACGGGGGCACCACGUU
 AAAGUUUGUAGAUACACCCGAGAGUUUGUCGGGAUUUAACGUUUUUGUGGUGUAUUUUAACGG
 20 GCAUGUUGAAGCCGUAGCAUACACUGUUGUAUCCACAGUAGAUCAUUUUGUAAACGCAAUUGA
 AGAGCGUGGAUUUCCGCCAACGGCCGGUCAGCCACCGGCGACUACUAAACCCAAGGAAAUUACC
 CCGUAAACCCCGGAACGUCACCACUUCUACGAUAUGCCGCAUGGACCGGAGGGCUUGCAGCAG
 UAGUACUUUUUUGUCUCGUAUAUUAUUUUAUCUGUACGGCUUGAUGAUAAUAGGCUGGAGCCU
 CGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCCUCCUCCCCUCCUGCACCCGUAC
 25 CCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC (SEQ ID NO: 126)

VZV-GE- delete-574

25 TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAAGA
 AGAGTAAGAAGAAATATAAGAGCCACCATGGGGACAGTTAATAAACCTGTGGTGGGGGTATTGAT
 GGGGTTCGGAATTATCACGGGAACGTTGCGTATAACGAATCCGGTCAGAGCATCCGTCTTGCGATA
 CGATGATTTTACATCGATGAAGACAACTGGATACAACTCCGTATATGAGCCTTACTACCATTTC
 AGATCATGCGGAGTCTTCATGGGTAAATCGGGGAGAGTCTTCGCGAAAAGCGTACGATCATAACT
 30 CACCTTATATATGGCCACGTAATGATTATGATGGATTTTATAGAGAACGCACACGAACACCATGGGG
 TGTATAATCAGGGCCGTGGTATCGATAGCGGGGAACGGTTAATGCAACCCACACAAATGTCTGCA
 CAGGAGGATCTTGGGGACGATACGGGCATCCACGTTATCCCTACGTTAAACGGCGATGACAGACA
 TAAAATTGTAAATGTGGACCAACGTCAATACGGTGACGTGTTTAAAGGAGATCTTAATCCAAAAC
 CCAAAGGCCAAAGACTCATTGAGGTGTCAGTGGAAGAAAATCACCCGTTTACTTTACGCGCACCG
 35 ATTCAGCGGATTTATGGAGTCCGGTACACCGAGACTTGAGGCTTTTTGCCGTCATTAACCTGTACG
 GGAGACGCAGCGCCCGCCATCCAGCATATATGTTTAAACATACAACATGCTTTCAAGACGTGGT
 GGTGGATGTGATTGCGCGGAAATACTAAAGAGGATCAGTTGGCCGAAATCAGTTACCGTTTTTC
 AAGGTAAGAAGGAAGCGGACCAACCGTGGATTGTTGTAAACACGAGCACACTGTTTGATGAACCTC
 GAATTAGACCCCCCGAGATTGAACCGGGTGTCTTGAAAGTACTTCGGACAGAAAAACAATACTT
 40 GGGTGTGTACATTTGGAACATGCGCGGCTCCGATGGTACGTCTACCTACGCCACGTTTTTGGTCAC
 CTGGAAAGGGGATGAAAAAACAAGAAACCCTACGCCCGCAGTAACTCCTCAACCAAGAGGGGCT
 GAGTTTCATATGTGGAATTACCACTCGCATGTATTTTCAGTTGGTGATACGTTTAGCTTGGCAATGC
 ATCTTCAGTATAAGATACATGAAGCGCCATTTGATTTGCTGTTAGAGTGGTTGTATGTCCCCATCG
 ATCCTACATGTCAACCAATGCGGTTATATTCTACGTGTTTGTATCATCCCAACGCACCCCAATGCCT
 45 CTCTCATATGAATTCGGTTGTACATTTACCTCGCCACATTTAGCCCAGCGTGTTGCAAGCACAGTG
 TATCAAATTTGTGAACATGCAGATAACTACACCGCATATTGTCTGGGAATATCTCATATGGAGCCT
 AGCTTTGGTCTAATCTTACACGACGGGGGCACCACGTTAAAGTTTGTAGATACACCCGAGAGTTTG
 TCGGGATTATACGTTTTTTGTGGTGTATTTTAACGGGCATGTTGAAGCCGTAGCATACACTGTTGTAT
 CCACAGTAGATCATTTTTGTAAACGCAATTGAAGAGCGTGATTTCGCCAACGGCCGGTCAGCCAC
 50 CGGCGACTACTAAACCCAAGGAAATTACCCCCGTAAACCCCGGAACGTCACCACTTCTACGATAT
 GCCGCATGGACCGGAGGGCTTGACAGCAGTAGTACTTTTATGTCTCGTAATATTTTAAATCTGTACG
 GCTAAACGAATGAGGGTTAAAGCCTATAGGGTAGACAAGTGATGATAATAGGCTGGAGCCTCGGT
 GGCCATGCTTCTTGCCCTTGGGCCTCCCCCAGCCCCCTCCTCCCCCTCCTGCACCCGTACCCCGT
 GGTCTTTGAATAAAGTCTGAGTGGGCGGC (SEQ ID NO: 5)

55 VZV-GE- delete-574 (mRNA)

60 UCAAGCUUUUGGACCCUCGUACAGAAGCUAAUACGACUCACUAUAGGGAAAUAAGAGAGAAAA
 GAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGGGGACAGUUAUAAACCUUGUGGUGGGGGUUAU
 UGAUGGGGUUCGGAUUUAUCACGGGAACGUUGCGUAUAACGAUCCGGUCAGAGCAUCCGUCU
 UGCGAUACGAUGAUUUUACAUCGAUGAAGACAAACUGGAUACAAACUCCGUUAUAGAGCCUU
 ACUACCAUUCAGAUCAUGCGGAGUCUUAUGGGUAAAUCGGGGAGAGUCUUCGCGAAAAGCGU

ACGAUCAUAAACUCACCUUAAUAAUAGGCCACGUAAUGAUUUAUGAUGGAUUUUUAGAGAACGCAC
 ACGAACACCAUGGGGUGUAUAAUCAGGGCCGUGUAUCGAUAGCGGGGAACGGUUAAUGCAAC
 CCACACAAAUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUCCACGUUAUCCCUACGU
 5 UAAACGGCGAUGACAGACAUAAAAUUGUAAAUGUGGACCAACGUCAAUACGGUGACGUGUUUA
 AAGGAGAUUUAAUCCAAAACCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUGGAAGAAAAUC
 ACCCGUUUACUUUACGCGCACCGAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGA
 GCUUUUUGCCGUCAUUAACCGUACGCGGAGACGCAGCGCCCGCAUCCAGCAUUAUGUUUAA
 AACAUACAACAUUCUUUCAAGACGUGGUGGUGGAUGUGGAUUGCGCGGAAAAUACUAAAGAGG
 10 AUCAGUUGGCCGAAAUCAGUUACCGUUUUAAGGUAAGAAGGAAGCGGACCAACCGUGGAUUG
 UUGUAAACACGAGCACACUGUUUGAUGAACUCGAAUUAAGACCCCCCGAGAUUGAACCGGGUG
 UCUUGAAAGUACUUCGGACAGAAAAACAAUACUUGGGUGUGUACAUUUGGAACAUGCGCGGCU
 CCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCACCUGGAAAGGGGAUGAAAAACAAGAA
 ACCCUACGCCCCGAGUAAACUCCUCAACCAAGAGGGGCGUGAGUUUCAUUGUGGAAUUACCACUC
 15 GCAUGUAUUUUCAGUUGGUGAUACGUUUAGCUUGGCAUUGCAUCUUCAGUAUAAGAUACAUGA
 AGCGCCAUUUGAUUUGCUUUAGAGUGGUUGUAUGUCCCCAUCGAUCCUACAUGUCAACCAAU
 GCGGUUAUAUUCUACGUGUUUGUAUCAUCCCAACGCACCCCCAUGCCUCUCUCAUUGAAUUC
 GGUUGUACAUUUACCUCGCCACAUUUAGCCCAGCGUGUUGCAAGCACAGUGUAUCAAUUGU
 GAACAUGCAGAUAAUACACCGCAUUAUGUCUGGGAUUAUCUCAUUGGAGCCUAGCUUUGGU
 20 CUAAUCUUACACGACGGGGGACCACGUUAAAGUUUGUAGAUACACCCGAGAGUUUGUCGGGA
 UUAUACGUUUUUGUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUGUUGUAUCC
 ACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGUCAGCCA
 CCGGCGACUACUAAACCCAAGGAAUUAACCCCGUAAACCCCGGAACGUCACCACUUCUACGAU
 AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUUUAUGUCUCGUAUAUUAUUUAAUCU
 25 GUACGGCUAAACGAAUGAGGGUUAAGCCUUAAGGGUAGACAAGUGAUGAUAAUAGGCUGGAG
 CCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCCUCCUCCCUUCCUGCACCCG
 UACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC (SEQ ID NO: 127)

VZV-GE- delete-574-Y569A

30 TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGA
 AGAGTAAGAAGAAATATAAGAGCCACCATGGGGACAGTTAATAAACCTGTGGTGGGGGTATTGAT
 GGGGTTTCGGAATTATCACGGGAACGTTGCGTATAACGAATCCGGTCAGAGCATCCGTCTTGCGATA
 CGATGATTTTCACATCGATGAAGACAACTGGATACAACTCCGTATATGAGCCTTACTACCATT
 35 AGATGATGCGGAGTCTTCATGGGTAAATCGGGGAGAGTCTTCGCGAAAAGCGTACGATCATA
 CACCTTATATATGGCCACGTAATGATTATGATGGATTTTATAGAGAACGCACACGAACACCATGGG
 TGTATAATCAGGGCCGTGGTATCGATAGCGGGGAACGGTTAATGCAACCCACACAAATGTCTGCA
 CAGGAGGATCTTGGGGACGATACGGGCATCCACGTTATCCCTACGTTAAACGGCGATGACAGACA
 TAAAATTGTAAATGTGGACCAACGTCAATACGGTGACGTGTTTAAAGGAGATCTTAATCCAAAAC
 40 CCAAGGCCAAAGACTCATTGAGGTGTGAGTGGAAAGAAAATCACCCGTTTACTTTACGCGCACCG
 ATTCAGCGGATTTATGGAGTCCGGTACACCGAGACTTGGAGCTTTTGGCGTCATTAACTGTACG
 GGAGACGCAGCGCCCGCCATCCAGCATATATGTTTAAACATACAAACATGCTTTCAAGACGTGGT
 GGTGGATGTGGATTGCGCGGAAAATACTAAAGAGGATCAGTTGGCCGAAATCAGTTACCGTTTTTC
 AAGGTAAGAAGGAAGCGGACCAACCGTGGATTGTTGTAAACACGAGCACACTGTTTGATGAATC
 45 GAATTAGACCCCCCGAGATTGAACCGGGTGTCTTGAAAGTACTTCGGACAGAAAAACAATACTT
 GGGTGTGTACATTTGGAACATGCGCGGCTCCGATGGTACGTCTACCTACGCCACGTTTTTGGTCAC
 CTGGAAAGGGGATGAAAAACAAGAAACCCTACGCCCGCAGTAACCTCTCAACCAAGAGGGGCT
 GAGTTTCATATGTGGAATTACCACTCGCATGTATTTTCAGTTGGTGATACGTTTAGCTTGGCAATGC
 ATCTTCAGTATAAGATACATGAAGCGCCATTTGATTTGCTGTTAGAGTGGTTGTATGTCCCATCG
 50 ATCTTACATGTCAACCAATGCGGTTATATTCTACGTGTTTGTATCATCCCAACGCACCCCAATGCCT
 CTCTCATATGAATTCGGTTGTACATTTACCTCGCCACATTTAGCCCAGCGTGTTGCAAGCACAGTG
 TATCAAAATTGTGAACATGCAGATAACTACACCGCATATTGTCTGGGAATATCTCATATGGAGCCT
 AGCTTTGGTCTAATCTTACACGACGGGGGCACCACGTTAAAGTTTGTAGATACACCCGAGAGTTTG
 TCGGGATTATACGTTTTTGTGGTGTATTTAACGGGCATGTTGAAGCCGTAGCATACACTGTTGTAT
 55 CCACAGTAGATCATTTTGTAAACGCAATTGAAGAGCGTGGATTTCCGCCAACGGCCGGTCAGCCAC
 CGGCGACTACTAAACCCAAGGAAATTACCCCGTAAACCCCGGAACGTCACCACTTCTACGATAT
 GCCGCATGGACCGGAGGGCTTGCAGCAGTAGTACTTTTATGTCTCGTAATATTTTAAATCTGTACG
 GCTAAACGAATGAGGGTTAAAGCCGCCAGGGTAGACAAGTGATGATAATAGGCTGGAGCCTCGGT
 GGCCATGCTTCTTGGCCCTTGGGCCTCCCCCAGCCCCCTCCTCCCTTCTGCAACCCGTACCCCGT
 60 GGTCTTTGAATAAAGTCTGAGTGGGCGGC (SEQ ID NO: 6)

VZV-GE- delete-574-Y569A (mRNA)

UCAAGCUUUUGGACCCUCGUACAGAAGCUAAUACGACUCACUUAUAGGGAAAUAAGAGAGAAAA
 GAAGAGUAGAAGAAAUAUAAAGAGCCACCAUGGGGACAGUAAUAAACCGUGUGGGGGUUAU
 5 UGAUGGGGUUCGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCCGGUCAGAGCAUCCGUCU
 UGCGAUACGAUGAUUUUCACAUCGAUGAAGACAAACUGGAUACAAACUCCGUUAUAGAGCCUU
 ACUACCAUUCAGAUCAUGCGGAGUCUUAUGGGUAAAUCGGGGAGAGUCUUCGCGAAAAGCGU
 ACGAUCAUAACUCACCUUAUAU AUGGCCACGUAAUGAUUAUGAUGGAUUUUUAGAGAAACGCAC
 ACGAACACCAUGGGGUGUAUAAUCAGGGCCGUGGUUAUCGAUAGCGGGGAACGGUUAAUGCAAC
 10 CCACACAAAUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUCCACGUUAUCCCUACGU
 UAAACGGCGAUGACAGACAUAAAAUUGUAAAUGUGGACCAACGUCAAUACGGUGACGUGUUUA
 AAGGAGAUUUAAUCCAAAACCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUGGAAGAAAUC
 ACCCGUUUACUUUACGCGCACCGAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGA
 GCUUUUUGCCGUAUUAACCGUACGCGGAGACGCAGCGCCCGCAUCCAGCAUUAUGUUUAA
 15 AACAUACAACAUUCUUUAAGACGUGGUGGUGGAUGUGGAUUGCGCGGAAAUAACUAAAGAGG
 AUCAGUUGGCCGAAAUCAGUUACCGUUUUAAGGUAGAAGGAAGCGGACCAACCGUGGAUUG
 UUGUAAACACGAGCACACUGUUUGAUGAACUCGAAUUAAGACCCCCCGAGAUUGAACCGGGUG
 UCUUGAAAGUACUUCGGACAGAAAAACAAUACUUGGGUGUGUACAUAUUGGAACAUGCGCGGCU
 CCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCACCUGGAAAGGGGAUGAAAAACAAGAA
 20 ACCCUACGCCCCGAGUAACUCCUCAACCAAGAGGGGCUGAGUUUCAUAUGUGGAUUUACCACUC
 GCAUGUAUUUUCAGUUGGUGAUACGUUUAAGCUUGGCAUUGCAUCUUCAGUAUAAGAUACAUGA
 AGCGCCAUUUGAUUUGCUGUUAGAGUGGUUGUAUGUCCCCAUCGAUCCUACAUGUCAACCAU
 GCGGUUAUAUUCUACGUGUUUGUAUCAUCCCAACGCACCCCAUGCCUCUCUCAUAUGAAUUC
 GGUUGUACAUUUACCUCGCCACAUUUAGCCCAGCGUGUUGCAAGCACAGUGUAUCAAUUUGU
 25 GAACAUGCAGAUAAUACACCGCAUAUUGUCUGGGAUAUCUCAUAUGGAGCCUAGCUUUGGU
 CUAAUCUUACACGACGGGGGCACCACGUUAAAGUUUGUAGAUACACCCGAGAGUUUGUCGGGA
 UUAUACGUUUUUGUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUGUUGUAUCC
 ACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUCAGCCA
 CCGGCGACUACUAAACCCAAGGAAUUAACCCCGUAAACCCCGGAACGUCACCACUUCUACGAU
 30 AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUUUUAUGUCUCGUAAUAUUUUUAAUCU
 GUACGGCUAAACGAUAGGGGUUAAAGCCGCGGAGGUAAGACAAGUGAUGAAUAAUAGGCUGGAG
 CCUCGGUGCCAUAGCUUCUGCCCCUUGGGCCUCCCCCAGCCCCUCCUCCUCCUCCUCCUCCG
 UACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC (SEQ ID NO: 128)

35 VZV-gE-full length-AEAADA (SEQ ID NO: 58)

TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGA
 AGAGTAAGAAGAAATATAAGAGCCACCATGGGGACAGTTAATAAACCTGTGGTGGGGGTATTGAT
 40 GGGGTTCGGAATTATCACGGGAACGTTGCGTATAACGAATCCGGTCAGAGCATCCGTCTTGCGATA
 CGATGATTTTCACATCGATGAAGACAACTGGATACAACTCCGTATATGAGCCTTACTACCATT
 AGATCATGCGGAGTCTTCATGGGTAAATCGGGGAGAGTCTTCGCGAAAAGCGTACGATCATAACT
 CACCTTATATATGGCCACGTAATGATTATGATGGATTTTATGAGAACGCACACGAACACCATGGGG
 TGTATAATCAGGGCCGTGTTATCGATAGCGGGGAACGGTTAATGCAACCCACACAAATGTCTGCA
 CAGGAGGATCTTGGGACGATACGGGCATCCACGTTATCCCTACGTTAAACGGCGATGACAGACA
 45 TAAAATTGTAAATGTGGACCAACGTCAATACGGTACGCTGTTTAAAGGAGATCTTAATCCAAAAC
 CCAAGGCCAAAGACTCATTGAGGTGTGAGTGAAGAAAATCACCCGTTTACTTTACGCGCACCG
 ATTCAGCGGATTTATGGAGTCCGGTACACCGAGACTTGGAGCTTTTTGCGGTCATTAACTGTACG
 GGAGACGCAGCGCCCGCCATCCAGCATATATGTTTAAACATACAACATGCTTTCAAGACGTGGT
 GGTGGATGTGGATTGCGCGGAAAATACTAAAGAGGATCAGTTGGCCGAAATCAGTTACCGTTTTTC
 50 AAGGTAAGAAGGAAGCGGACCAACCGTGGATTGTTGTAAACACGAGCACACTGTTTGATGAAC
 GAATTAGACCCCCCGAGATTGAACCGGGTGTCTTGAAAGTACTTCGGACAGAAAAACAATACTT
 GGGTGTGTACATTTGGAACATGCGCGGCTCCGATGGTACGTCTACCTACGCCACGTTTTTGGTCAC
 CTGGAAAGGGGATGAAAAACAAGAAACCCTACGCCCGCAGTAACTCCTCAACCAAGAGGGGCT
 GAGTTTCATATGTGGAATTACCACTCGCATGTATTTTCAGTTGGTGATACGTTTAGCTTGGCAATGC
 55 ATCTTCAGTATAAGATACATGAAGCGCCATTTGATTTGCTGTTAGAGTGGTTGTATGTCCCCATCG
 ATCCTACATGTCAACCAATGCGGTTATATTCTACGTGTTTGTATCATCCCAACGCACCCCAATGCCT
 CTCTCATATGAATTCGGTTGTACATTTACCTCGCCACATTTAGCCCAGCGTGTGCAAGCACAGTG
 TATCAAAATTGTGAACATGCAGATAACTACACCGCATATTGTCTGGGAATATCTCATATGGAGCCT
 AGCTTTGGTCTAATCTTACACGACGGGGGCACCACGTTAAAGTTTGTAGATACACCCGAGAGTTTG
 60 TCGGGATTATACGTTTTTGTGGTGTATTTTAACGGGCATGTTGAAGCCGTAGCATACACTGTTGTAT
 CCACAGTAGATCATTTTGTAAACGCAATTGAAGAGCGTGGATTTCCGCCAACGGCCGGTCAGCCAC

CGGCGACTACTAAACCCAAGGAAATTACCCCCGTAAACCCCGGAACGTCAACACTTCTACGATAT
 GCCGCATGGACCGGAGGGCTTGCAGCAGTAGTACTTTTATGTCTCGTAATATTTTAAATCTGTACG
 GCTAAACGAATGAGGGTTAAAGCCTATAGGGTAGACAAGTCCCCGTATAACCAAAGCATGTATTA
 CGCTGGCCTTCCAGTGGACGATTTTCGAGGACGCCGAAGCCCGCATGCCGAAGAAGAGTTTGGTA
 5 ACGCGATTGGAGGGAGTACGGGGGTTTCGAGTTACACGGTGTATATAGATAAGACCCGGTGATGA
 TAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCCTTGGGCCTCCCCCAGCCCCCTCCTCCCCT
 TCCTGCACCCGTACCCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC (SEQ ID NO: 7)

VZV-gE-full length-AEAADA (SEQ ID NO: 58) (mRNA)

UCAAGCUUUUGGACCCUCGUACAGAAGCUAAUACGACUCACUAAUAGGGAAAUAAGAGAGAAAA
 GAAGAGUAAGAAGAAAUAUAAAGAGCCACCAUGGGGACAGUUAUAAACCUGUGGUGGGGGUAAU
 UGAUGGGGUUCGGAUUUAUCACGGGAACGUUGCGUAUAACGAUCCGGUCAGAGCAUCCGUCU
 UGCGAUACGAUGAUUUUCACAUUGAUGAAGACAAACUGGAUACAAACUCCGUAAUUGAGCCUU
 15 ACUACCAUUCAGAUCAUGCGGAGUCUUAUGGGUAAAUCGGGGAGAGUCUUCGCGAAAAGCGU
 ACGAUCAUAACUCACCUUAUUAUGGCCACGUAAUGAUUAUGAUGGAUUUUUAGAGAACGCAC
 ACGAACACCAUGGGGUGUAUAAUCAGGGCCGUGGUUAUCGAUAGCGGGGAACGGUUAUGCAAC
 CCACACAAAUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUCCACGUUAUCCCUACGU
 UAAACGGCGAUGACAGACAUAAAAUUGUAAAUGUGGACCAACGUCAAUACGGUGACGUGUUUA
 20 AAGGAGAUCUUAAUCCAAAACCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUGGAAGAAAUC
 ACCCGUUUACUUUACGCGCACCCGAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGA
 GCUUUUUGCCGUCAUUAACCGUACGCGGAGACGCAGCGCCCGCAUCCAGCAUUAUGUUUAA
 AACAUACAACAUGCUUUCAAGACGUGGUGGUGGAUGUGGAUUUGCGCGGAAAUAACUAAAGAGG
 AUCAGUUGGCCGAAAUCAGUUAACCGUUUUAAGGUAAGAAGGAAGCGGACCAACCGUGGAUUG
 25 UUGUAAAACACGAGCACACUGUUUGAUGAACUCGAAUUAAGACCCCCCGAGAUUGAACCGGGUG
 UCUUGAAAGUACUUCGGACAGAAAAACAAUACUUGGGUGUGUACAUUUGGAACAUGCGCGGCU
 CCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCACCUGGAAAGGGGAUGAAAAACAAGAA
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 GCAUGUAUUUUCAGUUGGUGAUACGUUUAGCUUGGCAUUGCAUCUUCAGUAUAAGAUACAUGA
 30 AGCGCCAUUUGAUUUUGUGUUAGAGUGGUUGUAUGUCCCAUCGAUCCUACAUGUACAACCAU
 GCGGUUAUAUUUACGUGUUUGUAUCAUCCCAACGACCCCAUAGCCUUCUCAUAUGAAUUAUCC
 GGUUGUACAUUUACCUCGCCACAUUUAGCCACGCGUGUUGCAAGCACAGUGUAUCAAUUAUUG
 GAACAUGCAGAUAAUACACCGCAUUAUUGUCUGGGAUUAUCUCAUUAUGGAGCCUAGCUUUGGU
 CUAAUCUUACACGACGGGGGACCACGUUAAAGUUUGUAGAUACACCCGAGAGUUUGUCGGGA
 35 UUAUACGUUUUUGUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUGUUGUAUCC
 ACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUCAGCCA
 CCGGCGACUACUAAACCCAAGGAAUUAACCCCGUAAACCCCGGAACGUCACCACUUCUACGAU
 AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUUUUGUCUCGUAAUUAUUUUAAUCU
 GUACGGCUAAACGAUAGAGGGUUAAGCCUUAAGGGUAGACAAGUCCCGUAUAACCAAAGCA
 40 UGUAAUACGUGGCCUUCAGUGGACGAUUUCGAGGACGCCGAAGCCGCCGAUGCCGAAGAAG
 AGUUUGGUAACGCGAUUGGAGGGAGUCACGGGGGUUCGAGUUAACGUGUGUAUAUAGAUAGA
 CCCGGUGAUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCA
 GCCCUCCUCCCCUUCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC
 (SEQ ID NO: 129)

VZV-GE-full-AEAADA (SEQ ID NO: 58)-Y582G

TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTATAGGGAAATAAGAGAGAAAAGA
 AGAGTAAGAAGAAATATAAGAGCCACCATGGGGACAGTTAATAAACCTGTGGTGGGGGTATTGAT
 50 GGGGTTTCGGAATTATCACGGGAACGTTGCGTATAACGAATCCGGTCAGAGCATCCGTCTTGCGATA
 CGATGATTTTACATCGATGAAGACAACTGGATACAACTCCGTATATGAGCCTTACTACCATTC
 AGATCATGCGGAGTCTTCATGGGTAAATCGGGGAGAGTCTTCGCGAAAAGCGTACGATCATAACT
 CACCTTATATATGGCCACGTAATGATTATGATGGATTTTTAGAGAACGCACACGAACACCATGGGG
 55 TGTATAATCAGGGCCGTGGTATCGATAGCGGGGAACGGTTAATGCAACCCACACAAATGTCTGCA
 CAGGAGGATCTTGGGGACGATACGGGCATCCACGTTATCCCTACGTTAAACGGCGATGACAGACA
 TAAAATTGTAAATGTGGACCAACGTCAATACGGTGACGTGTTTAAAGGAGATCTTAATCCAAAAC
 CCCAAGGCCAAAGACTCATTGAGGTGTCAGTGGAAAGAAAATCACCCGTTTACTTTACGCGCACCG
 ATTCAGCGGATTTATGGAGTCCGGTACACCGAGACTTGGAGCTTTTTGCCGTCATTAACTGTACG
 GGAGACGCAGCGCCCGCCATCCAGCATATATGTTTAAACATACAACATGCTTTCAAGACGTGGT
 60 GGTGGATGTGGATTGCGCGGAAAATACTAAAGAGGATCAGTTGGCCGAAATCAGTTACCGTTTTC
 AAGGTAAGAAGGAAGCGGACCAACCGTGGATTGTTGTAAACACGAGCACACTGTTTGATGAACCT

GAATTAGACCCCCCGAGATTGAACCGGGTGTCTTGAAAGTACTTCGGACAGAAAAACAATACTT
GGGTGTGTACATTTGGAACATGCGCGGCTCCGATGGTACGTCTACCTACGCCACGTTTTTGGTCAC
CTGGAAAGGGGATGAAAAACAAGAAACCCTACGCCCGCAGTAACTCCTCAACCAAGAGGGGCT
GAGTTTCATATGTGGAATTACCACTCGCATGTATTTTCAGTTGGTGATACGTTTAGCTTGGCAATGC
5 ATCTTCAGTATAAGATACATGAAGCGCCATTTGATTTGCTGTTAGAGTGGTTGTATGTCCCCATCG
ATCCTACATGTCAACCAATGCGGTTATATTCTACGTGTTTGTATCATCCCAACGCACCCCAATGCCT
CTCTCATATGAATTCGGTTGTACATTTACCTCGCCACATTTAGCCCAGCGTGTGCAAGCACAGTG
TATCAAATTTGTGAACATGCAGATAACTACACCGCATATTGTCTGGGAATATCTCATATGGAGCCT
10 AGCTTTGGTCTAATCTTACACGACGGGGGCACCACGTTAAAGTTTGTAGATACACCCGAGAGTTTG
TCGGGATTATACGTTTTTGTGGTGTATTTTAACGGGCATGTTGAAGCCGTAGCATACACTGTTGTAT
CCACAGTAGATCATTTTTGTAAACGCAATTGAAGAGCGTGGATTTCGCCAACGGCCGTCAGCCAC
CGGCGACTACTAAACCCAAGGAAATTACCCCCGTAAACCCCGGAACGTCACCACTTCTACGATAT
GCCGCATGGACCGGAGGGCTTGCAGCAGTAGTACTTTTATGTCTCGTAATATTTTAATCTGTACG
GCTAAACGAATGAGGGTTAAAGCCTATAGGGTAGACAAGTCCCCGTATAACCAAAGCATGTATGG
15 CGCTGGCCTTCCAGTGGACGATTTTCGAGGACGCCGAAGCCGCCGATGCCGAAGAAGAGTTTGGTA
ACGCGATTGGAGGGAGTCACGGGGGTTTCGAGTTACACGGTGTATATAGATAAGACCCGGTGATGA
TAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCCCCTGGGCCTCCCCCAGCCCCCTCCTCCCT
TCCTGCACCCGTACCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGGC (SEQ ID NO: 8)

20 VZV-GE-full-AEAADA (SEQ ID NO: 58)-Y582G (mRNA)

UCAAGCUUUUGGACCCUCGUACAGAAGCUAAUACGACUCACUUAUAGGGAAAUAGAGAGAAAA
GAAGAGUAAGAAGAAAUUAAGAGCCACCAUGGGGACAGUUAUAAACCUGUGGUGGGGUGAU
UGAUGGGGUUCGGAUUUAUCACGGGAACGUUGCGUAUAACGAUCCGGUCAGAGCAUCCGUCU
25 UGCGAUACGAUGAUUUUCACAUUGAUGAAGACAAACUGGAUACAAACUCCGUUAUAGAGCCUU
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55 (SEQ ID NO: 130)

VZV_gE_Oka_hlgkappa

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 20 ACCCTGAAGTTCGTTGATACGCCAGAATCTCTGTCTGGGCTCTATGTTTTCTGGTCTACTTCAATG
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 25 GGACAAGTCCCCCTACAATCAGTCAATGTACTACGCCGGCCTTCCCGTTGATGATTTTGAGGATTC
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 ACACGGTTTACATTGACAAGACACGCTGATAATAGGCTGGAGCCTCGGTGGCCATGCTTCTTGCCC
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VZV_gE_Oka_hIlgkappa (mRNA)

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UUUGAAUAAAGUCUGAGUGGGCGGC (SEQ ID NO: 131)

5 Table 2. Sequences of Variant VZV gE Constructs

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:9	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VZV_gE_ Oka	TCAAGCTTTTGG ACCCTCGTACAG AAGCTAATACGA CTCACATAGGG AAATAAGAGAGA AAAGAAGAGTAA GAAGAAATATAA GAGCCACCATGG GGACAGTGAATA AGCCGGTTGTGG GCGTGCTTATGG GCTTTGGGATTA TTACCGGTACAT TACGAATTACCA ATCCAGTGCGCG CCAGTGTGCTGC GTTACGACGACT TTCACATTGACG AGGATAAGCTGG ATACTAACAGCG TGTACGAACCTT ATTACCACTCAG ATCATGCCGAAT CAAGCTGGGTAA ATAGAGGAGAAA GCAGCCGAAAAG CCTACGACCACA ACTCACCTTATA TTTGGCCAGAA ACGATTATGACG GTTTCCTGGAAA ACGCACATGAAC ACCATGGAGTCT ACAACCAAGGCA GGGGAATCGACA GTGGCGAGCGTC TTATGCAGCCAA CACAGATGTCGG CACAGGAGGATC TCGGTGATGACA CCGGCATACACG TGATTCCACAT TAAACGGCGACG ACAGACATAAGA TCGTCAATGTGG ATCAGCGTCAGT ATGGGGATGTCT TTAAAGCGGATT TGAATCCAAAGC CCAAGGACAGA GACTGATCGAGG TCTCTGTAGAAG AAAATCACCCCT TCACTTTGCGCG CTCCAATCCAGA GGATTTACGGGG	MGTVNKPVVGLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAES SWVNRG ESSRKAYDHNSPYIWPR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLDGDTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHTTCFQDVVDVDCAE NTKEDQLAEISYRFQ GK KEADQPWIVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLGVIWNMRGS DGTSTYATFLVTWKGDE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGDTF SLAMHLQYKIHEAPFDL LLEWLYVPIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTSPHLAQRVA STVYQNCHEADNYTAYC LGISHMEPSFGLILHDG GTTLKFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGFP PTAGQPPATTKPKEITP VNPGTSPLLRYAAWTGG LAAVVLLCLVIFLICTA KRMRVKAYRVDKSPYNQ SMYYAGLPVDDFEDSES TDTEEEFGNAIGGSHGG SSYTVYIDKTR	ATGGGGACAGTGAATAA GCCGGTTGTGGGCGTGC TTATGGGCTTTGGGATT ATTACCGGTACATTACG AATTACCAATCCAGTGC GCGCCAGTGTGCTGCGT TACGACGACTTTCACAT TGACGAGGATAAGCTGG ATACTAACAGCGTGTAC GAACCTTATTACCACTC AGATCATGCCGAATCAA GCTGGGTTAATAGAGGA GAAAGCAGCCGAAAAGC CTACGACCACAACACAC CTTATATTTGGCCCAGA AACGATTATGACGGTTT CCTGGAAAACGCACATG AACACCATGGAGTCTAC AACCAAGGCAGGGGAAT CGACAGTGGCGAGCGTC TTATGCAGCCAACACAG ATGTCCGGCACAGGAGGA TCTCGGTGATGACACCG GCATACACGTGATTCCC ACATTAAACGGCGACGA CAGACATAAGATCGTCA ATGTGGATCAGCGTCAG TATGGGGATGTCTTTAA AGGCGATTTGAATCCAA AGCCCCAAGGACAGAGA CTGATCGAGGTCTCTGT AGAAGAAAATCACCCCT TCACTTTGCGCGCTCCA ATCCAGAGGATTACGG GGTGCGTTATACCGAAA CTTGGAGTTTCTTGCCG TCACTGACGTGTACGGG GGATGCCGCCCCCGCAA TCCAGCACATCTGTCTG AAACACACCACATGCTT TCAGGACGTGGTTGTGG ATGTGGATTGCGCGGAA AACACAAAAGAAGACCA ACTCGCCGAAATCAGCT ATCGTTTTTCAGGGTAAA AAAGAGGCCGACCAACC GTGGATTGTTGTGAATA CGAGCACGCTCTTCGAT GAGCTTGAACCTCGATCC CCCGGAAATCGAGCCTG GGGTTCTAAAAGTGTTG AGGACCGAGAAGCAGTA CCTCGGGGTTTATATCT GGAATATGAGAGGCTCC GATGGCACCTCTACCTA CGCAACGTTTCTGGTTA	G*GGGAAATAAG AGAGAAAAGAAG AGTAAGAAGAAA TATAAGAGCCAC CATGGGGACAGT GAATAAGCCGGT TGTGGGCGTGTCT TATGGGCTTTTGG GATTATTACCGG TACATTACGAAT TACCAATCCAGT GCGCGCCAGTGT GCTGCGTTACGA CGACTTTACAT TGACGAGGATAA GCTGGATACTAA CAGCGTGACGA ACCTTATTACCA CTCAGATCATGC CGAATCAAGCTG GGTAAATAGAGG AGAAAGCAGCCG AAAAGCCTACGA CCACAACCTACC TTATATTTGGCC CAGAAACGATTA TGACGGTTTTCCT GGAAAACGCACA TGAACACCATGG AGTCTACAACCA AGGCAGGGGAAT CGACAGTGGCGA GCGTCTTATGCA GCCAACACAGAT GTCGGCACAGGA GGATCTCGGTGA TGACACCGGCAT ACACGTGATTCC CACATTAAACGG CGACGACAGACA TAAGATCGTCAA TGTGGATCAGCG TCAGTATGGGGA GTCTTTTAAAGG CGATTTGAATCC AAAGCCCCAAGG ACAGAGATGAT CGAGGTCTCTGT AGAAGAAAATCA CCCCTTCACTTT GCGCGCTCCAAT CCAGAGGATTTA CGGGGTGCGTTA TACCGAACTTG GAGTTTCTTGCC GTCAGTACGCTG

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	TGCGTTATACCG AAACTTGGAGTT TCTTGCCGTCAC TGACGTGTACGG GGGATGCCGCCC CCGCAATCCAGC ACATCTGTCTGA AACACACCACAT GCTTTCAGGACG TGGTTGTGGATG TGGATTGCGCGG AAAACACAAAAG AAGACCAACTCG CCGAAATCAGCT ATCGTTTTCAGG GTAAAAAAGAGG CCGACCAACCGT GGATTGTTGTGA ATACGAGCACGC TCTTCGATGAGC TTGAACTCGATC CCCCGGAAATCG AGCCTGGGGTTC TAAAAGTGTTGA GGACCGAGAAGC AGTACCTCGGGG TTTATATCTGGA ATATGAGAGGCT CCGATGGCACCT CTACCTACGCAA CGTTTCTGGTTA CCTGGAAGGGAG ACGAGAAGACAC GGAATCCAACGC CCGCTGTGACCC CTCAGCCTAGGG GAGCCGAATTCC ACATGTGGAAC ATCACTCCCATG TATTCAGTGTGG GTGACACTTTCA GCCTGGCCATGC ACCTGCAGTATA AGATTCACGAGG CACCCTTCGACC TCCTGCTGGAGT GGTTGTACGTAC CTATTGATCCCA CTTGTCAGCCCCA TGCGCCTGTACT CCACTTGCTTGT ACCACCCCAATG CACCACAGTGTC TATCACACATGA ACTCCGGGTGTA CCTTTACTTCAC CCCATCTTGCCC AGCGGGTCGCCA GCACAGTGTATC AGAACTGTGAGC ATGCTGACAACT ATACTGCTTATT GCCTCGGAATAT CCCATATGGAGC CAAGCTTCGGGC TCATACTGCACG		CCTGGAAGGGAGACGAG AAGACACGGAATCCAAC GCCCCTGTGACCCCTC AGCCTAGGGGAGCCGAA TTCCACATGTGGAACATA TCACTCCCATGTATTCA GTGTGGGTGACACTTTC AGCCTGGCCATGCACCT GCAGTATAAGATTACAG AGGCACCCTTCGACCTC CTGCTGGAGTGGTTGTA CGTACCTATTGATCCCA CTTGTCAGCCCATGCGC CTGTACTCCACTTGCTT GTACCACCCCAATGCAC CACAGTGTCTATCACAC ATGAACTCCGGGTGTAC CTTTACTTCACCCCATC TTGCCCAGCGGGTCGCC AGCACAGTGTATCAGAA CTGTGAGCATGTGACA ACTATACTGCTTATTGC CTCGGAATATCCCATAT GGAGCCAAGCTTCGGGC TCATACTGCACGATGGT GGTACGACACTCAAGTT CGTGGACACCCCGAAA GCCTTTCTGGCTTGAC GTGTTCTGGTCTACTT CAATGGACATGTGGAGG CAGTGGCTTACACAGTG GTTTCGACAGTTGATCA CTTTGTAAATGCCATTG AGGAACGCGGCTTCCCG CCTACAGCGGGCCAGCC CCCTGCGACAACAAAAC CAAAAGAGATTACGCCC GTTAATCCTGGGACTAG TCCATTGCTGAGGTATG CCGCCTGGACTGGCGGT CTGGCGGCCGTGGTACT TCTGTGTTTAGTCATAT TTCTGATCTGTACCGCT AAACGTATGCGGGTCAA GGCTTACCGTGTGACA AGTCTCCTTACAATCAG TCAATGTACTATGCAGG ACTCCCTGTTGACGATT TCGAAGACTCAGAGAGT ACAGACACAGAAGAAGA ATTGGAACGCTATAG GTGGCTCTCACGGAGGT AGCTCGTATACAGTGTA CATCGATAAAACCAGA	TACGGGGGATGTC CGCCCCCGCAAT CCAGCACATCTG TCTGAAACACAC CACATGCTTTCA GGACGTGGTTGT GGATGTGGATTG CGCGGAAAACAC AAAAGAAGACCA ACTCGCCGAAAT CAGCTATCGTTT TCAGGGTAAAAA AGAGCCCGACCA ACCGTGGATTGT TGTGAATACGAG CAGCTCTTCGA TGAGCTTGAAC CGATCCCCCGGA AATCGAGCCTGG GGTCTAAAAGT GTTGAGGACCGA GAAGCAGTACCT CGGGGTTTATAT CTGGAATATGAG AGGCTCCGATGG CACCTCTACCTA CGCAACGTTTCT GGTTACCTGGAA GGGAGACGAGAA GACACGGAATCC AACGCCCCGTGT GACCCCTCAGCC TAGGGGAGCCGA ATTCCACATGTG GAACTATCACTC CCATGTATTGAG TGTGGGTGACAC TTTCAGCCTGGC CATGCACCTGCA GTATAAGATTCA CGAGGCACCCCTT CGACCTCCTGCT GGAGTGGTTGTA CGTACCTATTGA TCCCCTTGTCA GCCCATGCGCCT GTACTCCACTTG CTTGTAACACCC CAATGCACCACA GTGTCTATCACA CATGAACTCCGG GTGTACCTTTAC TTCACCCCATCT TGCCAGCGGGT CGCCAGCACAGT GTATCAGAAGT TGAGCATGCTGA CAACTATACTGC TTATTGCCTCGG AATATCCCATAT GGAGCCAAGCTT CGGGCTCATACT GCACGATGGTGG TACGACACTCAA GTTCTGTGGACAC CCCCGAAAGCCT

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	ATGGTGGTACGA CACTCAAGTTCTG TGGACACCCCCG AAAGCCTTTCTG GCTTGTTACGTGT TCGTGGTCTACT TCAATGGACATG TGGAGGCAGTGG CTTACACAGTGG TTTCGACAGTTG ATCACTTTGTAA ATGCCATTGAGG AACGCGGCTTCC CGCCTACAGCGG GCCAGCCCCCTG CGACAACAAAAC CAAAAGAGATTA CGCCCCGTTAATC CTGGGACTAGTC CATTGCTGAGGT ATGCCGCTTGA CTGGCGGTCTGG CGGCCGTGGTAC TTCTGTGTTTAG TCATATTTCTGA TCTGTACCGCTA AACGTATGCGGG TCAAGGCTTACC GTGTTGACAAGT CTCCTTACAATC AGTCAATGTACT ATGCAGGACTCC CTGTTGACGATT TCGAAGACTCAG AGAGTACAGACA CAGAAGAAGAAT TCGGAAACGCTA TAGGTGGCTCTC ACGGAGGTAGCT CGTATACAGTGT ACATCGATAAAA CCAGATGATAAT AGGCTGGAGCCT CGGTGGCCATGC TTCTTGCCCCCTT GGGCCTCCCCC AGCCCCCTCTCC CCTTCCTGCACC CGTACCCCCGTG GTCTTTGAATAA AGTCTGAGTGGG CGGC			TTCTGGCTTGTA CGTGTTTCGTGGT CTACTTCAATGG ACATGTGGAGGC AGTGGCTTACAC AGTGGTTTCGAC AGTTGATCACTT TGTAATGCCAT TGAGGAACGCGG CTTCCCGCCTAC AGCGGGCCAGCC CCCTGCGACAAC AAAACCAAAGA GATTACGCCCCGT TAATCCTGGGAC TAGTCCATTGCT GAGGTATGCCGC CTGGACTGGCGG TCTGGCGGCCGT GGTACTTCTGTG TTAGTCATATT TCTGATCTGTAC CGCTAAACGTAT GCGGGTCAAGGC TTACCGTGTTGA CAAGTCTCCTTA CAATCAGTCAAT GTACTATGCAGG ACTCCCTGTTGA CGATTTCAAGA CTCAGAGAGTAC AGACACAGAAGA AGAATTCGGA CGCTATAGGTGG CTCTCACGGAGG TAGCTCGTATAC AGTGTACATCGA TAAAACCAGATG ATAATAGGCTGG AGCCTCGGTGGC CATGCTTCTTGC CCCTTGGGCCTC CCCCAGCCCCCT CCTCCCCCTTCT GCACCCGTACCC CCGTGGTCTTTG AATAAAGTCTGA GTGGGCGGCAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA ATCTAG
mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:13	SEQ ID NO:14	SEQ ID NO:15	SEQ ID NO:16

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
VZV_gE_Oka_hIg_kappa	TCAAGCTTTTGG ACCCTCGTACAG AAGCTAATACGA CTCACTATAGGG AAATAAGAGAGA AAAGAAGAGTAA GAAGAAATATAA GAGCCACCATGG AGACTCCCGCTC AGCTACGATTCC TCCTGCTCCTTT GGCTGCCTGATA CTACAGGCTCTG TTTTGCGGTACG ACGACTTTCACA TCGATGAGGACA AGCTCGACACTA ATAGCGTGTATG AGCCCTACTACC ATTGATGATCAG CCGAGTCCTCTT GGGTGAACAGGG GTGAAAGTTCTA GGAAAGCCTATG ATCACAACAGCC CTTATATTTGGC CACGGATGATT ACGACGGATTTT TCGAAATGCCCC ACGAGCATCAGC GAGTGTACAACC AGGGCCGTGGAA TCGACTCTGGGG AGAGATTGATGC AACCTACACAGA TGAGCGCCAGG AAGATCTCGGGG ATGATACAGGAA TTCACGTTATCC CTACATTAAACG GAGATGACCGCC ACAAAATCGTCA ATGTCGATCAAA GACAGTATGGAG ATGTGTTCAAAG GCGATCTCAACC CTAAGCCGACAG GCCAGAGACTCA TTGAGGTGTCTG TCGAAGAGAACC ACCCTTTCACTC TGCGCGCTCCCA TTCAGAGAATCT ATGGAGTTTCGCT ATACGGAGACTT GGTCATTCCCTT CTTCCCTGACAT GCACCGGAGACG CCGCCCTGCCA TTCAGCACATAT GCCTGAAACATA CCACCTGTTTCC AGGATGTGGTGG TTGATGTTGATT GTGCTGAAAATA CCAAGGAAGACC	METPAQLLFLLLWLPD TTGSLVRYDDFHIDEDK LDTNSVYEPYYHSDHAE SSWVNRGESSRKAYDHN SPYIWPRNDYDGFLENA HEHHGVYNQGRGIDSGE RLMQPTQMSAQEDLGDD TGIHVIPTLNGDDRHKI VNVDQRQYGDVFKGDLN PKPQGQRLIEVSVEENH PFTLRAPIQRIYGVRYT ETWSFLPSLTCTGDAAP AIQHICLKHTTCFQDVV VDVDCAEENTKEDQLAEI SYRFQKGKEADQFWIVV NTSTLFDELELDPPEIE PGVLKVLRTKQYLGVI IWNMRGSDGTSTYATFL VTWKGDEKTRNPTPAVT PQPRGAEFHMWNYHSHV FSVGDTFSLAMHLQYKI HEAPFDLLEWLYVPID PTCQPMRLYSTCLYHPN APQCLSHMNSGCTFTSP HLAQRVASTVYQNCHEA DNYTAYCLGISHMEPSF GLILHDGGTTLKFVDTP ESLSGLYVFVVFNGHV EAVAYTVVSTVDHFVNA IEERGFPPTAGOPPATT KPKEITPVNPGTSPLLR YAAWTGGLAAVLLCLV IFLICTAKMRVKAYRV DKSPYNQSMYAGLPVD DFEDSESTDTEEEFGNA IGGSHGSSYTVYIDKTR	ATGGAGACTCCCGCTCA GCTACTGTTCCCTCTGC TCCTTTGGCTGCCTGAT ACTACAGGCTCTGTTTT GCGGTACGACGACTTTC ACATCGATGAGGACAAG CTCGACACTAATAGCGT GTATGAGCCCTACTACC ATTCAGATCACGCCGAG TCCTCTTGGGTGAACAG GGGTGAAAGTTCTAGGA AAGCCTATGATCACAAC AGCCCTTATATTTGGCC ACGGAATGATTACGACG GATTTCTCGAAAATGCC CACGAGCATCACGGAGT GTACAACACAGGCGGTG GAATCGACTCTGGGGAG AGATTGATGCAACCTAC ACAGATGAGCGCCAGG AAGATCTCGGGGATGAT ACAGGAATTCACGTTAT CCCTACATTAAACGGAG ATGACCGCCACAAAATC GTCAATGTCGATCAAAG ACAGTATGGAGATGTGT TCAAAGGCGATCTCAAC CCTAAGCCGACAGGGCCA GAGACTCATTGAGGTGT CTGTGCAAGAGAACAC CCTTTCACTCTGCGCGC TCCCATTCAGAGAATCT ATGGAGTTTCGCTATACG GAGACTTGGTCATTCTCT TCCTTCCCTGACATGCA CCGGAGACGCCGCCCT GCCATTACGACATATG CCTGAAACATACCACCT GTTTCCAGGATGTGGTG GTTGATGTTGATTGTGC TGAAAATACCAAGGAAG ACCAACTGGCCGAGATT AGTTACCGGTTCCAAGG GAAAAAGGAAGCCGACC AGCCATGGATTGTGGTT AATACAAGCACTCTGTT CGATGAGCTCGAGCTGG ATCCCCCGAGATAGAA CCCGGAGTTCTGAAAGT GCTCCGGACAGAAAAAC AATATCTGGGAGTCTAC ATATGGAACATGCGCGG TTCCGATGGGACCTCCA CTTATGCAACCTTTCTC GTCACGTGGAAGGGAGA TGAGAAAAC TAGGAATC CCACACCGCTGTCACA CCACAGCCAAGAGGGGC TGAGTTCCATATGTGGA ACTATCATAGTCACGTG TTTAGTGTCGGAGATAC GTTTTTCATTGGCTATGC ATCTCCAGTACAAGATT CATGAGGCTCCCTTCGA TCTGTTGCTTGAGTGGT GTACGTCCTCGATTGAC	G*GGGAAATAAG AGAGAAAAGAAG AGTAAGAAGAAA TATAAGAGCCAC CATGGAGACTCC CGCTCAGCTACT GTTCTCTCTGCT CCTTTGGCTGCC TGATACTACAGG CTCTGTTTTGCG GTACGACGACTT TCACATCGATGA GGACAAGCTCGA CACTAATAGCGT GTATGAGCCCTA CTACCATTGAGA TCACGCCCTGATC CTCTTGGGTGAA CAGGGGTGAAAG TTCTAGGAAAGC CTATGATCACAA CAGCCCTTATAT TTGGCCACGGAA TGATTACGACGG ATTTCTCGAAAA TGCCCACGAGCA TCACGGAGTGTA CAACCAGGGCCG TGGAATCGACTC TGGGGAGGAGAT GATGCAACCTAC ACAGATGAGCGC CCAGGAAGATCT CGGGGATGATAC AGGAATTCACGT TATCCCTACATT AAACGGAGATGA CCGCCACAAAAT CGTCAATGTCGA TCAAAGACAGTA TGGAGATGTGTT CAAAGGCGATCT CAACCCTAAGCC GCAGGGCCAGAG ACTCATTGAGGT GTCTGTGCAAGA GAACCACCCTTT CACTCTGCGCGC TCCCATTCAGAG AATCTATGGAGT TCGCTATACGGA GACTTGGTCATT CCTTCTTCCCT GACATGCACCGG AGACGCCGCCCC TGCCATTGACGA CATATGCCTGAA ACATACCACCTG TTTCCAGGATGT GGTGGTTGATGT TGATTGTGCTGA AAATACCAAGGA AGACCAACTGGC CGAGATTAGTTA CCGGTTCCAAGG GAAAAAGGAAGC

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	AACTGGCCGAGA TTAGTTACCGGT TCCAAGGGAAAA AGGAAGCCGACC AGCCATGGATTG TGGTTAATACAA GCACTCTGTTCG ATGAGCTCGAGC TGGATCCCCCG AGATAGAACCCG GAGTTCTGAAAG TGCTCCGGACAG AAAAACAATATC TGGGAGTCTACA TATGGAACATGC GCGGTTCCGATG GGACCTCCACTT ATGCAACCTTTC TCGTCACGTGGA AGGGAGATGAGA AACTAGGAATC CCACACCCGCTG TCACACCACAGC CAAGAGGGGCTG AGTTCCATATGT GGAACATATCATA GTCACGTGTTTA GTGTCGGAGATA CGTTTTTCATTGG CTATGCATCTCC AGTACAAGATTG ATGAGGCTCCCT TCGATCTGTTGC TTGAGTGGTTGT ACGTCCCGATTG ACCCGACCTGCC AGCCCATGCGAC TGTACAGCACCT GTCTCTACCATC CAAACGCTCCGC AATGTCTGAGCC ACATGAACTCTG GGTGTACTTTCA CCAGTCCCCACC TCGCCCAGCGGG TGGCCTCTACTG TTTACCAGAACT GTGAGCACGCCG ACAACTACACCG CATACTGCCTCG GTATTTCTCACA TGGAACCCTCCT TCGGACTCATCC TGCACGATGGGG GCACTACCCTGA AGTTCGTTGATA CGCCAGAATCTC TGTCTGGGCTCT ATGTTTTCGTGG TCTACTTCAATG GCCATGTCGAGG CCGTGCCCTATA CTGTCTGTTCTA CCGTGGATCATT TTGTGAACGCCA TCGAAGAACGGG		CCGACCTGCCAGCCCAT GCGACTGTACAGCACCT GTCTCTACCATCCAAC GCTCCGCAATGTCTGAG CCACATGAACTCTGGGT GTACTTTACCAGTCCC CACCTCGCCCAGCGGGT GGCTCTACTGTTTACC AGAACTGTGAGCACGCC GACAACCTACACGCATA CTGCCTCGGTATTTCTC ACATGGAACCCCTCCTTC GGACTCATCCTGCACGA TGGGGGCACTACCCTGA AGTTCGTTGATACGCCA GAATCTCTGTCTGGGCT CTATGTTTTCTGTTCT ACTTCAATGGCCATGTC GAGGCCGTGGCCTATAC TGTCGTTTTCTACCGTGG ATCATTGTTGTGAACGCC ATCGAAGAACGGGGATT CCCCCCTACGGCAGGCC AGCCGCTGCAACCACC AAGCCCAAGGAAATAAC ACCAGTGAACCCCTGGCA CCTCACCTCTCCTAAGA TATGCCGCGTGGACAGG GGGACTGGCGGCAGTGG TGCTCCTCTGTCTCGTG ATCTTTCTGATCTGTAC AGCCAAGAGGATGAGGG TCAAGGCTTATAGAGTG GACAAGTCCCCCTACAA TCAGTCAATGTACTACG CCGGCCTTCCCGTTGAT GATTTTGAGGATTCCGA GTCCACAGATACTGAGG AAGAGTTCGGTAACGCT ATAGGCGGCTCTCACGG GGGTTCAAGCTACACGG TTTACATTGACAAGACA CGC	CGACCAGCCATG GATTGTGGTTAA TACAAGCACTCT GTTCGATGAGCT CGAGCTGGATCC CCCCAGATAGA ACCCGGAGTTCT GAAAGTGCTCCG GACAGAAAAACA ATATCTGGGAGT CTACATATGGAA CATGCGCGGTTTC CGATGGGACCTC CACTTATGCAAC CTTTCTCGTCAC GTGGAAGGGAGA TGAGAAAACTAG GAATCCCACACC CGCTGTCACACC ACAGCCAAGAGG GGCTGAGTTCCA TATGTGGAACATA TCATAGTCACGT GTTTAGTGTCTGG AGATACGTTTTTC ATTGGCTATGCA TCTCCAGTACAA GATTCATGAGGC TCCCTTCGATCT GTTGCTTGAGTG GTTGATCGTCCC GATTGACCCGAC CTGCCAGCCCAT GCGACTGTACAG CACCTGTCTCTA CCATCCAAACGC TCCGCAATGTCT GAGCCACATGAA CTCTGGGTGTAC TTTACACAGTCC CCACCTCGCCCCA GCGGGTGGCCTC TACTGTTTACCA GAACTGTGAGCA CGCCGACAACATA CACCGCATACTG CCTCGGTATTTTC TCACATGGAACC CTCCTTCGGACT CATCCTGCACGA TGGGGGCACTAC CCTGAAGTTCGT TGATACGCCAGA ATCTCTGTCTGG GCTCTATGTTTT CGTGGTCTACTT CAATGGCCATGT CGAGGCCGTGGC CTATACTGTCGT TTCTACCGTGGA TCATTTTGTGAA CGCCATCGAAGA ACGGGGATTCCC CCCTACGGCAGG CCAGCCGCTGTC AACCACCAAGCC

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	GATTCCCCCCTA CGGCAGGCCAGC CGCCTGCAACCA CCAAGCCCAAGG AAATAACACCAG TGAACCCTGGCA CCTCACCTCTCC TAAGATATGCCG CGTGGACAGGGG GACTGGCGGCAG TGGTGCTCCTCT GTCTCGTGATCT TTCTGATCTGTA CAGCCAAGAGGA TGAGGGTCAAGG CTTATAGAGTGG ACAAGTCCCCCT ACAATCAGTCAA TGTACTACGCCG GCCTTCCCGTTG ATGATTTTGAGG ATTCCGAGTCCA CAGATACTGAGG AAGAGTTCGGTA ACGCTATAGGCG GCTCTCACGGGG GTTCAAGCTACA CGGTTTACATTG ACAAGACACGCT GATAATAGGCTG GAGCCTCGGTGG CCATGCTTCTTG CCCCCTGGGCCT CCCCCCAGCCCC TCCTCCCCTTCC TGCACCCGTACC CCCGTGGTCTTT GAATAAAGTCTG AGTGGGCGGC			CAAGGAAATAAC ACCAGTGAACCC TGGCACCTCACC TCTCCTAAGATA TGCCGCGTGGAC AGGGGGACTGGC GGCAGTGGTGCT CCTCTGTCTCGT GATCTTTCTGAT CTGTACAGCCAA GAGGATGAGGGT CAAGGCTTATAG AGTGGACAAGTC CCCCTACAATCA GTCAATGTACTA CGCCGGCCTTCC CGTTGATGATTT TGAGGATTCCGA GTCCACAGATAC TGAGGAAGAGTT CGGTAACGCTAT AGGCGGCTCTCA CGGGGGTTCAAG CTACACGGTTTA CATTGACAAGAC ACGCTGATAATA GGCTGGAGCCTC GGTGGCCATGCT TCTTGCCCCTTG GGCCTCCCCCCA GCCCCCTCCTCC CTTCCTGCACCC GTACCCCGTGG TCTTTGAATAAA GTCTGAGTGGGC GGCAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAATCTAG
mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:17	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
VZV-GE-delete-562	TCAAGCTTTTGG ACCCTCGTACAG AAGCTAATACGA CTCACTATAGGG AAATAAGAGAGA AAAGAAGAGTAA GAAGAAATATAA GAGCCACCATGG GGACAGTTAATA AACCTGTGGTGG GGGTATTGATGG GGTTCGGAATTA TCACGGGAACGT TGCCTATAACGA ATCCGGTCAGAG CATCCGTCTTGC GATACGATGATT TTCACATCGATG AAGACAACTGG ATACAACTCCG TATATGAGCCTT ACTACCATTCAG ATCATGCGGAGT CTTCATGGGTAA ATCGGGGAGAGT CTTCGCGAAAAG CGTACGATCATA ACTCACCTTATA TATGGCCACGTA ATGATTATGATG GATTTTATAGAGA ACGCACACGAAC ACCATGGGGTGT ATAATCAGGGCC GTGGTATCGATA GCGGGGAACGGT TAATGCAACCCA CACAAATGTCTG CACAGGAGGATC TTGGGGACGATA CGGGCATCCACG TTATCCCTACGT TAAACGGCATG ACAGACATAAAA TTGTAAATGTGG ACCAACGTCAAT ACGGTGACGTGT TTAAAGGAGATC TTAATCCAAAAC CCCAAGGCCAAA GACTCATTGAGG TGTCAGTGGAAG AAAATCACCCGT TTACTTTACGCG CACCGATTACAG GGATTTATGGAG TCCGGTACACCG AGACTTGAGACT TTTTGCCGTCAT TAACCTGTACGG GAGACGCAGCGC CCGCCATCCAGC ATATATGTTTAA AACATACAACAT GCTTTCAAGACG TGGTGGTGGATG	MGTVNKPVVGVLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWPR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHHTTCFQDVVVDVCAE NTKEDQLAEISYRFQKG KEADQPWIVVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLGVIWNMRGS DGTSTYATFLVTWKGE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGDTF SLAMHLQYKIHAEFPDL LLEWLYVPIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTSPHLAQRVA STVYQNCHEADNYTAYC LGI SHMEPSFGLILHDG GTTLKFFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGFP PTAGQPPATTKPKEITP VNPGTSPLLRYYAAWTGG LAAVLLCLVIFLICTA *	ATGGGGACAGTTAATAA ACCTGTGGTGGGGGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAACTGG ATACAACTCCGTATAT GAGCCTTACTACCATT AGATCATGCGGAGTCTT CATGGGTAAATCGGGGA GAGTCTTCGCGAAAAGC GTACGATCATAACTCAC CTTATATATGGCCACGT AATGATTATGATGGATT TTTAGAGAACGCACACG AACACCATGGGGTGTAT AATCAGGGCCGTGGTAT CGATAGCGGGGAACGGT TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTAAACGGCGATGA CAGACATAAAATTGTAA ATGTGGACCAACGTCAA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AACCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAAAATCACCCGT TTACTTTACGCGCACCG ATTACGCGGATTATGG AGTCCGGTACACCGAGA CTTGGAGCTTTTGGCCG TCATTAACCTGTACGGG AGACGCAGCGCCCGCCA TCCAGCATATATGTTTA AAACATACAACATGCTT TCAAGACGTGGTGGTGG ATGTGGATTGCGCGGAA AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTCAAGGTAAG AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACTCGAATTAGACCC CCCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAAAAACAATA CTTGGGTGTGTACATTT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTTTTGGTCA CCTGGAAAGGGGATGAA AAAACAAGAAACCCCTAC GCCCGCAGTAACTCCTC AACCAAGAGGGGCTGAG TTTCATATGTGGAATTA CCTACTCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTTGATTTG	G*GGGAAATAAG AGAGAAAAGAAG AGTAAGAAGAAA TATAAGAGCCAC CATGGGGACAGT TAATAAACCTGT GGTGGGGGTATT GATGGGGTTCGG AATTATCACGGG CTCCGTGCGTAT AACGAATCCGGT CAGAGCATCCGT CTTGCGATACGA TGATTTTTCACAT CGATGAAGACAA ACTGGATACAAA CTCCGTATATGA GCCTTACTACCA TTCAGATCATGC GGAGTCTTCATG GGTAAATCGGGG AGAGTCTTCGCG AAAAGCGTACGA TCAATACTACC TTATATATGGCC ACGTAATGATTA TGATGGATTTTT AGAGAACGCACA CGAACACCATGG GGTGATATAATCA GGGCCGTGGTAT CGATAGCGGGGA ACGTTAATGCA ACCCACACAAAT GTCTGCACAGGA GGATCTTGGGGA CGATACGGGCAT CCACGTTATCCC TACGTTAAACGG CGATGACAGACA TAAATTGTAA TGTGGACCAACG TCAATACGGTGA CGTGTTTAAAGG AGATCTTAATCC AAAACCCCAAGG CCAAAGACTCAT TGAGGTGTCAGT GGAAGAAAATCA CCCGTTTACTTT ACGCGCACCGAT TCAGCGGATTTA TGGAGTCCGGTA CACCGAGACTTG GAGCTTTTTGCC GTCATTAACTCG TACGGGAGACGC AGCGCCCGCCAT CCAGCATATATG TTTAAACATAC AACATGCTTTCA AGACGTGGTGGT GGATGTGGATTG CGCGGAAAATAC TAAAGAGGATCA GTTGGCCGAAAT

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	TGGATTGCGCGG AAAATACTAAAG AGGATCAGTTGG CCGAAATCAGTT ACCGTTTTCAG GTAAGAAGGAAG CGGACCAACCGT GGATTGTTGTAA ACACGAGCACAC TGTTTGATGAAC TCGAATTAGACC CCCCCGAGATTG AACCGGGTGTCT TGAAAGTACTTC GGACAGAAAAAC AATACTTGGGTG TGTACATTGGA ACATGCGCGGCT CCGATGGTACGT CTACCTACGCCA CGTTTTTGGTCA CCTGGAAAGGGG ATGAAAAAACAA GAAACCCCTACGC CCGCAGTAACTC CTCAACCAAGAG GGGCTGAGTTTC ATATGTGGAATT ACCACTCGCATG TATTTTCAGTTG GTGATACGTTTA GCTTGGAATGCA ATCTTCAGTATA AGATACATGAAG CGCCATTGATT TGCTGTTAGAGT GGTTGTATGTCC CCATCGATCCTA CATGTCAACCAA TGCGGTATATTT CTACGTGTTTGT ATCATCCCAACG CACCCCAATGCC TCTCTCATATGA ATTCCGGTTGTA CATTTACCTCGC CACATTTAGCCC AGCGTGTTGCAA GCACAGTGTATC AAAATTGTGAAC ATGCAGATAACT ACACCGCATATT GTCTGGGAATAT CTCATATGGAGC CTAGCTTTGGTC TAATCTTACACG ACGGGGGCACCA CGTTAAAGTTTG TAGATACACCCG AGAGTTTGTCGG GATTATACGTTT TTGTGGTGTATT TTAACGGGCATG TTGAAGCCGTAG CATACACTGTTG TATCCACAGTAG		CTGTTAGAGTGGTTGTA TGTCCCCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCAACGCAC CCCAATGCCTCTCTCAT ATGAATTCCGGTTGTAC ATTTACCTCGCCACATT TAGCCCAGCGTGTGCA AGCACAGTGTATCAAAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGG GGCACCACGTTAAAGTT TGTAGATACACCCGAGA GTTTGTGCGGATTATAC GTTTTTGTGGTGTATTT TAACGGGCATGTTGAAG CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTGTAAACGCAATTG AAGAGCGTGGATTTCGG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACCCCC GTAAACCCCGGAACGTC ACCACTTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT TTTTAATCTGTACGGCT TGA	CAGTTACCGTTT TCAAGGTAAGAA GGAAGCGGACCA ACCGTGGATTGT TGTAACACGAG CACACTGTTTGA TGAACTCGAATT AGACCCCCCGGA GATTGAACCGGG TGTCTTGAAAGT ACTTCGGACAGA AAAACAATACTT GGGTGTGTACAT TTGGAACATGCG CGGCTCCGATGG TACGTCTACCTA CGCCACGTTTTT GGTCACCTGGAA AGGGGATGAAAA AACAAGAAACCC TACGCCCCGAGT AACTCCTCAACC AAGAGGGCTGA GTTTCATATGTG GAATTACCACTC GCATGTATTTTC AGTTGGTGATAC GTTTAGCTTGGC AATGCATCTTCA GTATAAGATACA TGAAGCGCCATT TGATTTGCTGTT AGAGTGGTTGTA TGTCCCCATCGA TCCTACATGTCA ACCAATGCGGTT ATATTCTACGTG TTTGTATCATCC CAACGCACCCCA ATGCCTCTCTCA TATGAATTCCGG TTGTACATTTAC CTCGCCACATTT AGCCCAGCGTGT TGCAAGCACAGT GTATCAAAATTG TGAACATGCAGA TAACTACACCGC ATATTGTCTGGG AATATCTCATAT GGAGCCTAGCTT TGGTCTAATCTT ACACGACGGGGG CACCACGTTAAA GTTTGTAGATAC ACCCGAGAGTTT GTCGGGATTATA CGTTTTTGTGGT GTATTTTAAACGG GCATGTTGAAGC CGTAGCATACAC TGTGTATCCAC AGTAGATCATTT TGTAACGCAAT TGAAGAGCGTGG ATTTCGCCAAC

[illegible]

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	TAGAGAACGCAC ACGAACACCATG GGGTGTATAATC AGGGCCGTGGTA TCGATAGCGGGG AACGGTTAATGC AACCCACACAAA TGTCTGCACAGG AGGATCTTGGGG ACGATACGGGCA TCCACGTATATCC CTACGTTAAACG GCGATGACAGAC ATAAAATGTAA ATGTGGACCAAC GTCAATACGGTG ACGTGTTTAAAG GAGATCTTAATC CAAAACCCCAAG GCCAAAGACTCA TTGAGGTGTCAG TGGAAGAAAATC ACCCGTTTACTT TACGCGCACCGA TTCAGCGGATTT ATGGAGTCCGGT ACACCGAGACTT GGAGCTTTTTGC CGTCATTAACCT GTACGGGAGACG CAGCGCCCGCCA TCCAGCATATAT GTTTAAACATA CAACATGCTTTC AAGACGTGGTGG TGGATGTGGATT GCGCGGAAAATA CTAAAGAGGATC AGTTGGCCGAAA TCAGTTACCGTT TTCAAGGTAAGA AGGAAGCGGACC AACCGTGGATTG TTGTAAACACGA GCACACTGTTTG ATGAACTCGAAT TAGACCCCCCG AGATTGAACCGG GTGTCTTGAAG TACTTCGGACAG AAAAACAATACT TGGGTGTGTACA TTTGGAACATGC GCGGCTCCGATG GTACGTCTACCT ACGCCACGTTTT TGGTCACTGGA AAGGGGATGAAA AAACAAGAAACC CTACGCCCGCAG TAACTCCTCAAC CAAGAGGGGCTG AGTTTCATATGT GGAATTACCACT CGCATGTATTTT CAGTTGGTGATA	EAVAYTVVSTVDHFVNA IEERGFPPPTAGQFPATT KPKEITPVNPGTSPLLR YAAWTGGLAAVLLCLV IFLICTA*	AAGACTCATTGAGGTGT CAGTGGAAGAAAATCAC CCGTTTACTTTACGCGC ACCGATTACGCGGATTT ATGGAGTCCGGTACACC GAGACTTGGAGCTTTTT GCCGTCATTAACCTGTA CGGGAGACGCAGCGCCC GCCATCCAGCATATATG TTTAAACATACAACAT GCTTTCAAGACGTGGTG GTGGATGTGGATTGCGC GGAAAATACTAAAGAGG ATCAGTTGGCCGAAATC AGTTACCGTTTTCAAGG TAAGAAGGAAGCGGACC AACCGTGGATTGTTGTA AACACGAGCACACTGTT TGATGAACTCGAATTAG ACCCCCCGAGATTGAA CCGGGTGTCTTGAAAGT ACTTCGGACAGAAAAAC AATACTTGGGTGTGTAC ATTTGGAACATGCGCGG CTCCGATGGTACGTCTA CCTACGCCACGTTTTTG GTCACCTGGAAAGGGGA TGAAAAACAAGAAACC CTACGCCCCGAGTAACT CCTCAACCAAGAGGGGC TGAGTTTCATATGTGGA ATTACCACTCGCATGTA TTTTCAGTTGGTGATAC GTTTAGCTTGGCAATGC ATCTTCAGTATAAGATA CATGAAGCGCCATTTGA TTTGCTGTTAGAGTGGT TGTATGTCCCCATCGAT CCTACATGTCAACCAAT GCGGTTATATTCTACGT GTTTGTATCATCCCAAC GCACCCCAATGCCTCTC TCATATGAATTCCGGTT GTACATTTACCTCGCCA CATTTAGCCCAGCGTGT TGCAAGCACAGTGTATC AAAATTGTGAACATGCA GATAACTACACCGCATA TTGTCTGGGAATATCTC ATATGGAGCCTAGCTTT GGTCTAATCTTACACGA CGGGGGCACCACGTTAA AGTTTGTAGATACACCC GAGAGTTTGTCTGGGATT ATACGTTTTTGTGGTGT ATTTTAAACGGGCATGTT GAAGCCGTAGCATACAC TGTTGTATCCACAGTAG ATCATTTTGTAAACGCA ATTGAAGAGCGTGGATT TCCGCCAACGGCCGGTC AGCCACGGGCGACTACT AAACCCAAGGAAATTAC CCCCGTAAACCCCGGAA CGTCACCACTTCTACGA TATGCCGCATGGACCGG	TGGTATCGATAG CGGGGAACGGTT AATGCAACCCAC ACAAATGTCTGC ACAGGAGGATCT TGGGGACGATAC GGGCATCCACGT TATCCCTACGTT AAACGGCGATGA CAGACATAAAAT TGTAATGTGGA CCAACGTCAATA CGGTGACGTGTT TAAAGGAGATCT TAATCCAAAACC CCAAGGCCAAAG ACTCATTGAGGT GTCAGTGGAAGA AAATCACCCGTT TACTTTACGCGC ACCGATTACGCG GATTTATGGAGT CCGGTACACCGA GACTTGGAGCTT TTTGCCGTCATT AACCTGTACGGG AGACGCAGCGCC CGCCATCCAGCA TATATGTTTAAA ACATACAACATG CTTTCAGACGT GGTGGTGGATGT GGATTGCGCGGA AAATACTAAAGA GGATCAGTTGGC CGAAATCAGTTA CCGTTTTCAAGG TAAGAAGGAAGC GGACCAACCGTG GATTGTTGTAAA CACGAGCACACT GTTTGATGAACT CGAATTAGACCC CCCCGAGATTGA ACCGGGTGTCTT GAAAGTACTTCG GACAGAAAAACA ATACTTGGGTGT GTACATTTGGAA CATGCGCGGCTC CGATGGTACGTC TACCTACGCCAC GTTTTTGGTCAC CTGGAAAGGGGA TGAAAAACAAG AAACCCACGCC CGCAGTAACTCC TCAACCAAGAGG GGCTGAGTTTCA TATGTGGAATTA CCACTCGCATGT ATTTTCAGTTGG TGATACGTTTAG CTTGGCAATGCA TCTTCAGTATAA GATACATGAAGC

[illegible]

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
				AAAATCTAG
mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:25	SEQ ID NO:26	SEQ ID NO:27	SEQ ID NO:28
VZV-GE-full_wi th_AEAA DA (SEQ ID NO: 58)	TCAAGCTTTTGG ACCCTCGTACAG AAGCTAATACGA CTCACTATAGGG AAATAAGAGAGA AAAGAAGAGTAA GAAGAAATATAA GAGCCACCATGG GGACAGTTAATA AACCTGTGGTGG GGGTATTGATGG GGTTCGGAATTA TCACGGGAACGT TGCGTATAACGA ATCCGGTCAGAG CATCCGTCTTGC GATACGATGATT TTCACATCGATG AAGACAACTGG ATACAACTCCG TATATGAGCCTT ACTACCATTCAG ATCATGCGGAGT CTTCATGGGTAA ATCGGGGAGAGT CTTCGCGAAAAG CGTACGATCATA ACTCACCTTATA TATGGCCACGTA ATGATTATGATG GATTTTTAGAGA ACGCACACGAAC ACCATGGGGTGT ATAATCAGGGCC GTGGTATCGATA GCGGGGAACGGT TAATGCAACCCA CACAAATGTCTG CACAGGAGGATC TTGGGGACGATA CGGGCATCCACG TTATCCCTACGT TAAACGGCGATG ACAGACATAAAA TTGTAAATGTGG ACCAACGTCAAT ACGGTGACGTGT TTAAAGGAGATC TTAATCCAAAAC CCCAAGGCCAAA GACTCATTGAGG TGTCAGTGAAG AAAATCACCCGT TTACTTTACGCG CACCGATTCAGC GGATTTATGGAG TCCGGTACACCG	MGTVNKPVVGVLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHHTTCFQDVVDVDCAE NTKEDQLAEISYRFQGK KEADQPWIVNTSTLFD ELELDPPPEIEPGVLKVL RTEKQYLGVIWNMRGS DGTSTYATFLVTWKDE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGDTF SLAMHLQYKIHEAPFDL LLEWLYVPI DPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTS PHLAQRVA STVYQNCHEADNYTAYC LGI SHMEPSFGLILHDG GTTLKFVDTPESLSGLY VFVVFYFNHVEAVAYTV VSTVDHFVNAIEERGFP PTAGQPPATTKPKEITP VNPGTSPLLRYYAATGG LAADVLLCLVIFLICTA KRMRVKAYRVDKSPYNQ SMYYAGLPVDDFEDAEE ADAEFEFGNAIGGSHGG SSYTVYIDKTR*	ATGGGGACAGTTAATAA ACCTGTGGTGGGGGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAACTGG ATACAACTCCGTATAT GAGCCTTACTACCATT AGATCATGCGGAGTCTT CATGGGTAAATCGGGGA GAGTCTTCGCGAAAAGC GTACGATCATAACTCAC CTTATATATGGCCACGT AATGATTATGATGGATT TTTAGAGAACGCACACG AACACCATGGGGTGTAT AATCAGGGCCGTGGTAT CGATAGCGGGGAACGGT TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTAAACGCGCATGA CAGACATAAAATGTAA ATGTGGACCAACGTCAA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AACCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAAAATCAGCCGT TTACTTTACGCGCACCG ATTGAGCGGATTTATGG AGTCCGGTACACCGAGA CTTGGAGCTTTTGGCCG TCATTAACCTGTACGGG AGACGACGCGCCCGCA TCCAGCATATATGTTTA AAACATACAACATGCTT TCAAGACGTGGTGGTGG ATGTGGATTGCGCGGAA AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTCAAGGTAAG AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACTCGAATTGACCCC CCCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAAAAACAATA CTTGGGTGTGTACATTT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTTTTGGTCA CCTGGAAAGGGGATGAA	G*GGGAAATAAG AGAGAAAAGAAG AGTAAGAAGAAA TATAAGAGCCAC CATGGGGACAGT TAATAAACCTGT GGTGGGGGTATT GATGGGGTTCGG AATTATCACGGG AACGTTGCGTAT AACGAATCCGGT CAGAGATCCCGT CTTGCGATACGA TGATTTTCACAT CGATGAAGACAA ACTGGATACAAA CTCCGTATATGA GCCTTACTACCA TTCAGATCATGC GGAGTCTTCATG GGTAAATCGGGG AGAGTCTTCGCG AAAAGCGTACGA TCATAACTCACC TTATATATGGCC ACGTAATGATTA TGATGGATTTTT AGAGAACGCACA CGAACACCATGG GGTGTATAATCA GGGCCGTGGTAT CGATAGCGGGGA ACGGTTAATGCA ACCCACACAAAT GTCTGCACAGGA GGATCTTGGGGA CGATACGGGCAT CCACGTTATCCC TACGTTAAACGG CGATGACAGACA TAAAATTGTAAA TGTGGACCAACG TCAATACGGTGA CGTGTTTAAAGG AGATCTTAATCC AAAACCCCAAGG CCAAAGACTCAT TGAGGTGTCAGT GGAAGAAAATCA CCCGTTTACTTT ACGCGCACCGAT TCAGCGGATTTA TGGAGTCCGGTA CACCGAGACTTG GAGCTTTTTTGCC GTCATTAACCTG TACGGGAGACGC

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	AGACTTGGAGCT TTTTGCCGTCAT TAACCTGTACGG GAGACGCAGCGC CCGCCATCCAGC ATATATGTTTAA AACATACAACAT GCTTTCAGACG TGGTGGTGGATG TGGATTGCGCGG AAAATACTAAAG AGGATCAGTTGG CCGAAATCAGTT ACCGTTTCAAG GTAAGAAGGAAG CGGACCAACCGT GGATTGTTGTAA ACACGAGCACAC TGTTTGATGAAC TCGAATTAGACC CCCCCGAGATTG AACCGGGTGTCT TGAAAGTACTTC GGACAGAAAAAC AATACTTGGGTG TGTACATTTGGA ACATGCGCGGCT CCGATGGTACGT CTACCTACGCCA CGTTTTTGGTCA CCTGGAAAGGGG ATGAAAAACAA GAAACCTACGC CCGCAGTAACTC CTCAACCAAGAG GGGCTGAGTTTC ATATGTGGAATT ACCACTCGCATG TATTTTCAGTTG GTGATACGTTTA GCTTGGCAATGC ATCTTCAGTATA AGATACATGAAG CGCCATTGATT TGCTGTTAGAGT GGTTGTATGTCC CCATCGATCCTA CATGTCAACCAA TGCGGTTATATT CTACGTGTTTGT ATCATCCCAACG CACCCCAATGCC TCTCTCATATGA ATTCCGGTTGTA CATTTACCTCGC CACATTAGCCC AGCGTGTGCAA GCACAGTGTATC AAAATTGTGAAC ATGCAGATAACT ACACCGCATATT GTCTGGGAATAT CTCATATGGAGC CTAGCTTTGGTC TAATCTTACACG ACGGGGGCACCA		AAAACAAGAAACCCTAC GCCCCGAGTAACTCCTC AACCAAGAGGGGCTGAG TTTCATATGTGGAATTA CCACTCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTGATTTG CTGTTAGAGTGGTTGTA TGTCCCCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCAACGCAC CCCAATGCCTCTCTCAT ATGAATTCGGTTGTAC ATTTACCTCGCCACATT TAGCCCAGCGTGTGCA AGCACAGTGTATCAAAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGG GGCACCACGTTAAAGTT TGTAAGTACACCCGAGA GTTTGTGCGGATTATAC GTTTTTGTGGTGTATTT TAACGGGCATGTTGAAG CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTGTAAACGCAATTG AAGAGCGTGGATTTCGG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACCCCC GTAAACCCCGGAACGTC ACCACTTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT TTTTAATCTGTACGGCT AAACGAATGAGGGTTAA AGCCTATAGGGTAGACA AGTCCCCGTATAACCAA AGCATGTATTACGCTGG CCTTCCAGTGGACGATT TCGAGGACGCCGAAGCC GCCGATGCCGAAGAAGA GTTTGGTAACGCGATTG GAGGGAGTCACGGGGGT TCGAGTTACACGGTGTA TATAGATAAGACCCGGT GA	AGCGCCCCGCAT CCAGCATATATG TTTAAACATAC AACATGCTTTCA AGACGTGGTGGT GGATGTGGATTG CGCGGAAAATAC TAAAGAGGATCA GTTGGCCGAAAT CAGTTACCGTTT TCAAGGTAAGAA GGAAGCGGACCA ACCGTGGATTGT TGTAACACAGAG CACACTGTTTGA TGAATCGAATT AGACCCCCCGGA GATTGAACCGGG TGTCTTGAAAGT ACTTCGGACAGA AAAACAATACTT GGGTGTGTACAT TTGGAACATGCG CGGCTCCGATGG TACGTCTACCTA CGCCACGTTTTT GGTCACCTGGAA AGGGGATGAAAA ACAAGAAACCC TACGCCCGCAGT AACTCCTCAACC AAGAGGGGCTGA GTTTCATATGTG GAATTACCACTC GCATGTATTTTC AGTTGGTGATAC GTTTAGCTTGGC AATGCATCTTCA GTATAAGATACA TGAAGCGCCATT TGATTTGCTGTT AGAGTGGTTGTA TGTCCTCCATCGA TCCTACATGTCA ACCAATGCGGTT ATATTCTACGTG TTTGTATCATCC CAACGCACCCCA ATGCCTCTCTCA TATGAATTCGGG TTGTACATTTAC CTCGCCACATTT AGCCAGCGTGT TGCAAGCACAGT GTATCAAAATTG TGAACATGCAGA TAACTACACCGC ATATTGTCTGGG AATATCTCATAT GGAGCCTAGCTT TGGTCTAATCTT ACACGACGGGGG CACCACGTTAAA GTTTGTAGATAC ACCCGAGAGTTT GTCGGGATTATA

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	CGTTAAAGTTTG TAGATACACCCG AGAGTTTGTCCG GATTATACGTTT TTGTGGTGTATT TTAACGGGCATG TTGAAGCCGTAG CATACACTGTTG TATCCACAGTAG ATCATTTTGTAA ACGCAATTGAAG AGCGTGGATTTC CGCCAACGGCCG GTCAGCCACCGG CGACTACTAAAC CCAAGGAAATTA CCCCCGTAAACC CCGGAACGTCAC CACTTCTACGAT ATGCCGCATGGA CCGGAGGGCCTTG CAGCAGTAGTAC TTTTATGTCTCG TAATATTTTTAA TCTGTACGGCTA AACGAATGAGGG TTAAAGCCTATA GGGTAGACAAGT CCCCGTATAACC AAAGCATGTATT ACGCTGGCCTTC CAGTGGACGATT TCGAGGACGCCG AAGCCGCCGATG CCGAAGAAGAGT TTGGTAACGCGA TTGGAGGGAGTC ACGGGGGTTCGA GTTACACGGTGT ATATAGATAAGA CCCGGTGATGAT AATAGGCTGGAG CCTCGGTGGCCA TGCTTCTTGCCC CTTGGGCCTCCC CCCAGCCCCCTCC TCCCCCTCCTGC ACCCGTACCCCC GTGGTCTTTGAA TAAAGTCTGAGT GGGCGGC			CGTTTTTGTGGT GTATTTTAACGG GCATGTTGAAGC CGTAGCATACAC TGTTGTATCCAC AGTAGATCATTT TGTAACGCAAT TGAAGAGCGTGG ATTTCCGCCAAC GGCCGGTCAGCC ACCGCGACTAC TAAACCAAGGA AATTACCCCCGT AAACCCCGAAC GTCACCACTTCT ACGATATGCCGC ATGGACCGGAGG GCTTGCAGCAGT AGTACTTTTATG TCTCGTAATATT TTTAATCTGTAC GGCTAAACGAAT GAGGGTTAAAGC CTATAGGGTAGA CAAGTCCCCGTA TAACCAAAGCAT GTATTACGCTGG CCTTCCAGTGGA CGATTTTCGAGGA CGCCGAAGCCGC CGATGCCGAAGA AGAGTTTGGTAA CGCGATTGGAGG GAGTCACGGGGG TTCGAGTTACAC GGTGTATATAGA TAAGACCCGGTG ATGATAATAGGC TGGAGCCTCGGT GGCCATGCTTCT TGCCCCCTGGGC CTCCCCCAGCC CCTCCTCCCCTT CCTGCACCCGTA CCCCCGTGGTCT TTGAATAAAGTC TGAGTGGGCGGC AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAATCTAG
mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:29	SEQ ID NO:30	SEQ ID NO:31	SEQ ID NO:32

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
VZV-GE-full_wi th_AEAA DA (SEQ ID NO: 58)_and _Y582G	TCAAGCTTTTGG ACCCTCGTACAG AAGCTAATACGA CTCACTATAGGG AAATAAGAGAGA AAAGAAGAGTAA GAAGAAATATAA GAGCCACCATGG GGACAGTTAATA AACCTGTGGTGG GGGTATTGATGG GGTTCGGAATTA TCACGGGAACGT TGCGTATAACGA ATCCGGTCAGAG CATCCGTCTTGC GATACGATGATT TTCACATCGATG AAGACAACTGG ATACAACTCCG TATATGAGCCTT ACTACCATTCAG ATCATGCGGAGT CTTCATGGGTAA ATCGGGGAGAGT CTTCGCGAAAAG CGTACGATCATA ACTCACCTTATA TATGGCCACGTA ATGATTATGATG GATTTTATAGAGA ACGCACACGAAC ACCATGGGGTGT ATAATCAGGGCC GTGGTATCGATA GCGGGGAACGGT TAATGCAACCCA CACAAATGTCTG CACAGGAGGATC TTGGGGACGATA CGGGCATCCACG TTATCCCTACGT TAAACGGGATG ACAGACATAAAA TTGTAAATGTGG ACCAACGTCAAT ACGGTGACGTGT TTAAAGGAGATC TTAATCCAAAAC CCCAAGGCCAAA GACTCATTGAGG TGTCAGTGGAAG AAAATCACCCGT TTACTTTACGCG CACCGATTACAG GGATTTATGGAG TCCGGTACACCG AGACTTGAGACT TTTTGCCGTCAT TAACCTGTACGG GAGACGCAGCGC CCGCCATCCAGC ATATATGTTTAA AACATACAACAT GCTTTCAAGACG TGGTGGTGGATG	MGTVNKPVVGVLMGF ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWPR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHHTTCFQDVVVDVCAE NTKEDQLAEISYRFQK KEADQPWIVVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLVYIWNMRGS DGTSTYATFLVTWKGE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGDTF SLAMHLQYKIHAEFPDL LLEWLYVPIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTSPHLAQRVA STVYQNCHEADNYTAYC LGI SHMEPSFGLILHDG GTTLLKFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGFP PTAGQPPATTKPKEITP VNPPTSPLLRYAATWG LAAVLLCLVIFLICTA KRMRVKAYRVDKSPYNQ SMYGAGLPVDDFEDAEE ADAEEEFNGAIGGSHGG SSYTVYIDKTR*	ATGGGGACAGTTAATAA ACCTGTGGTGGGGGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAACTGG ATACAACTCCGTATAT GAGCCTTACTACCATT AGATCATGCGGAGTCTT CATGGGTAATCGGGGA GAGTCTTCGCGAAAAGC GTACGATCATAACTCAC CTTATATATGGCCACGT AATGATTATGATGGATT TTTAGAGAACGCACACG AACACCATGGGGTGTAT AATCAGGGCCGTGGTAT CGATAGCGGGGAACGGT TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTAAACGGCGATGA CAGACATAAAATTGTAA ATGTGGACCAACGTCAA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AACCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAAAATCACCCGT TTACTTTACGCGCACCG ATTACGCGGATTATGG AGTCCGGTACACCGAGA CTTGGAGCTTTTGGCG TCATTAACCTGTACGGG AGACGCAGCGCCCGCCA TCCAGCATATATGTTTA AAACATACAACATGCTT TCAAGACGTGGTGGTGG ATGTGGATTGCGCGGAA AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTCAAGGTAAG AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACTCGAATTAGACCC CCCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAAAAACAATA CTTGGGTGTGTACATTT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTTTTGGTCA CCTGGAAAGGGGATGAA AAAACAAGAAACCCCTAC GCCCGCAGTAACTCCTC AACCAAGAGGGGCTGAG TTTCATATGTGGAATTA CCAATCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTTGATTTG	G*GGGAAATAAG AGAGAAAAGAAG AGTAAGAAGAAA TATAAGAGCCAC CATGGGGACAGT TAATAAACCTGT GGTGGGGGTATT GATGGGGTTCGG AATTATCACGGG CTCCGTGCGTAT AACGAATCCGGT CAGAGCATCCGT CTTGCATACGA TGATTTTTCACAT CGATGAAGACAA ACTGGATACAAA CTCCGTATATGA GCCTTACTACCA TTCAGATCATGC GGAGTCTTCATG GGTAAATCGGGG AGAGTCTTCGCG AAAAGCGTACGA TCAATACTACC TTATATATGGCC ACGTAATGATTA TGATGGATTTTT AGAGAACGCACA CGAACACCATGG GGTGATATAATCA GGGCCGTGGTAT CGATAGCGGGGA ACGGTTAATGCA ACCCACACAAAT GTCTGCACAGGA GGATCTTGGGGA CGATACGGGCAT CCACGTTATCCC TACGTTAAACGG CGATGACAGACA TAAATTGTAA TGTGGACCAACG TCAATACGGTGA CGTGTTTAAAGG AGATCTTAATCC AAAACCCCAAGG CCAAAGACTCAT TGAGGTGTCAGT GGAAGAAAATCA CCCGTTTACTTT ACGCGCACCGAT TCAGCGGATTTA TGGAGTCCGGTA CACCGAGACTTG GAGCTTTTTGCC GTCATTAACCTG TACGGGAGACGC AGCGCCCGCCAT CCAGCATATATG TTTAAACATAC AACATGCTTTCA AGACGTGGTGGT GGATGTGGATTG CGCGGAAAATAC TAAAGAGGATCA GTTGGCCGAAAT

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	TGGATTGCGCGG AAAATACTAAAG AGGATCAGTTGG CCGAAATCAGTT ACCGTTTTCAG GTAAGAAGGAAG CGGACCAACCGT GGATTGTTGTAA ACACGAGCACAC TGTTTGTATGAAC TCGAATTAGACC CCCCCGAGATTG AACCGGGTGTCT TGAAAGTACTTC GGACAGAAAAAC AATACTTGGGTG TGTACATTTGGA ACATGCGCGGCT CCGATGGTACGT CTACCTACGCCA CGTTTTTGGTCA CCTGGAAAGGGG ATGAAAAAACAA GAAACCCACGC CCGCAGTAACTC CTCAACCAAGAG GGGCTGAGTTTC ATATGTGGAATT ACCACTCGCATG TATTTTCAGTTG GTGATACGTTTA GCTTGGCAATGC ATCTTCAGTATA AGATACATGAAG CGCCATTTGATT TGCTGTTAGAGT GGTTGTATGTCC CCATCGATCCTA CATGTCAACCAA TGCGGTTATATT CTACGTGTTTGT ATCATCCCAACG CACCCCAATGCC TCTCTCATATGA ATTCCGGTTGTA CATTTACCTCGC CACATTTAGCCC AGCGTGTTGCAA GCACAGTGTATC AAAATTGTGAAC ATGCAGATAACT ACACCGCATATT GTCTGGGAATAT CTCATATGGAGC CTAGCTTTGGTC TAATCTTACACG ACGGGGGCACCA CGTTAAAGTTTG TAGATACACCCG AGAGTTTGTCTG GATTATACGTTT TTGTGGTGTATT TTAACGGGCATG TTGAAGCCGTAG CATACACTGTTG TATCCACAGTAG		CTGTTAGAGTGGTTGTA TGTCCCCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCAACGCAC CCCAATGCCTCTCTCAT ATGAATTCCGGTTGTAC ATTTACCTCGCCACATT TAGCCCAGCGTGTGCA AGCACAGTGTATCAAAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGG GGCACCACGTTAAAGTT TGTAGATACACCGGAGA GTTTGTCTGGGATTATAC GTTTTTGTGGTGTATTT TAACGGGCATGTTGAAG CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTGTAAACGCAATTG AAGAGCGTGGATTTCCG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACCCCC GTAAACCCCGGAACGTC ACCACTTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT TTTTAATCTGTACGGCT AAACGAATGAGGGTTAA AGCCTATAGGGTAGACA AGTCCCCGTATAACCAA AGCATGTATGGCGCTGG CCTTCCAGTGGACGATT TCGAGGACGCCGAAGCC GCCGATGCCGAAGAAGA GTTTGGTAACGCGATTG GAGGGAGTCACGGGGGT TCGAGTTACACGGTGTA TATAGATAAGACCCGGT GA	CAGTTACCGTTT TCAAGGTAAGAA GGAAGCGGACCA ACCGTGGATTGT TGTAACACGAG CACACTGTTTGA TGAACTCGAATT AGACCCCCCGGA GATTGAACCGGG TGTCTTGAAAGT ACTTCGGACAGA AAAACAATACTT GGGTGTGTACAT TTGGAACATGCG CGGCTCCGATGG TACGTCTACCTA CGCCACGTTTTT GGTCACCTGGAA AGGGGATGAAAA AACAAGAAACCC TACGCCCCGAGT AACTCCTCAACC AAGAGGGCTGA GTTTCATATGTG GAATTACCACTC GCATGTATTTTC AGTTGGTGATAC GTTTAGCTTGGC AATGCATCTTCA GTATAAGATACA TGAAGCGCCATT TGATTTGCTGTT AGAGTGGTTGTA TGTCCTCATCGA TCCTACATGTCA ACCAATGCGGTT ATATTCTACGTG TTTGTATCATCC CAACGCACCCCA ATGCCTCTCTCA TATGAATTCCGG TTGTACATTTAC CTCGCCACATTT AGCCCAGCGTGT TGCAAGCACAGT GTATCAAAATTG TGAACATGCAGA TAACTACACCGC ATATTGTCTGGG AATATCTCATAT GGAGCCTAGCTT TGGTCTAATCTT ACACGACGGGGG CACCACGTTAAA GTTTGTAGATAC ACCCGAGAGTTT GTCGGGATTATA CGTTTTTGTGGT GTATTTTAACGG GCATGTTGAAGC CGTAGCATACAC TGTGTATCCAC AGTAGATCATTT TGTAACGCAAT TGAAGAGCGTGG ATTCCGCCAAC

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	ATCATTTTGTAA ACGCAATTGAAG AGCGTGGATTTC CGCCAACGGCCG GTCAGCCACCGG CGACTACTAAAC CCAAGGAAATTA CCCCCGTAAACC CCGGAACGTCAC CACTTCTACGAT ATGCCGCATGGA CCGGAGGGCTTG CAGCAGTAGTAC TTTTATGTCTCG TAATATTTTAA TCTGTACGGCTA AACGAATGAGGG TTAAAGCCTATA GGGTAGACAAGT CCCCCGTATAACC AAAGCATGTATG GCGCTGGCCTTC CAGTGGACGATT TCGAGGACGCCG AAGCCGCCGATG CCGAAGAAGAGT TTGGTAACGCGA TTGGAGGGAGTC ACGGGGGTTCGA GTTACACGGTGT ATATAGATAAGA CCCGGTGATGAT AATAGGCTGGAG CCTCGGTGGCCA TGCTTCTTGCCC CTTGGGCCCTCCC CCCAGCCCCCTCC TCCCCTTCCTGC ACCCGTACCCCC GTGGTCTTTGAA TAAAGTCTGAGT GGGCGGC			GGCCGGTCAGCC ACCGGCGACTAC TAAACCCAAGGA AATTACCCCGT AAACCCCGGAAC GTCACCACTTCT ACGATATGCCGC ATGGACCGGAGG GCTTGCAGCAGT AGTACTTTTATG TCTCGTAATATT TTTAATCTGTAC GGCTAAACGAAT GAGGGTTAAAGC CTATAGGGTAGA CAAGTCCCGTA TAACCAAGCAT GTATGGCGCTGG CCTTCCAGTGGA CGATTTTCGAGGA CGCCGAAGCCGC CGATGCCGAAGA AGAGTTTGGTAA CGCGATTGGAGG GAGTCACGGGGG TTCGAGTTACAC GGTGTATATAGA TAAGACCCGGTG ATGATAATAGGC TGGAGCCTCGGT GGCCATGCTTCT TGCCCCCTTGGGC CTCCCCCAGCC CCTCCTCCCCTT CCTGCACCCGTA CCCCCGTGGTCT TTGAATAAAGTC TGAGTGGGCGGC AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAATCTAG
mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:33	SEQ ID NO:34	SEQ ID NO:35	SEQ ID NO:36

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
VZV-GE-truncated-deleted-from_574	TCAAGCTTTTGG ACCCTCGTACAG AAGCTAATACGA CTCACTATAGGG AAATAAGAGAGA AAAGAAGAGTAA GAAGAAATATAA GAGCCACCATGG GGACAGTTAATA AACCTGTGGTGG GGGTATTGATGG GGTTCGGAATTA TCACGGGAACGT TGCCTATAACGA ATCCGGTCAGAG CATCCGTCTTGC GATACGATGATT TTCACATCGATG AAGACAACTGG ATACAACTCCG TATATGAGCCTT ACTACCATTCAG ATCATGCGGAGT CTTCATGGGTAA ATCGGGGAGAGT CTTCGCGAAAAG CGTACGATCATA ACTCACCTTATA TATGGCCACGTA ATGATTATGATG GATTTTATAGAGA ACGCACACGAAC ACCATGGGGTGT ATAATCAGGGCC GTGGTATCGATA GCGGGGAACGGT TAATGCAACCCA CACAAATGTCTG CACAGGAGGATC TTGGGGACGATA CGGGCATCCACG TTATCCCTACGT TAAACGGGATG ACAGACATAAAA TTGTAAATGTGG ACCAACGTCAAT ACGGTGACGTGT TTAAAGGAGATC TTAATCCAAAAC CCCAAGGCCAAA GACTCATTGAGG TGTCAGTGGAAG AAAATCACCCGT TTACTTTACGCG CACCGATTACAG GGATTTATGGAG TCCGGTACACCG AGACTTGAGACT TTTTGCCGTCAT TAACCTGTACGG GAGACGCAGCGC CCGCCATCCAGC ATATATGTTTAA AACATACAACAT GCTTTCAAGACG TGGTGGTGGATG	MGTVNKPVVGVLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWPR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHHTTCFQDVVVDVCAE NTKEDQLAEISYRFQKG KEADQPWIVVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLVYIWNMRGS DGTSTYATFLVTWKGE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGDTF SLAMHLQYKIHEAPFDL LLEWLYVPIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTSPHLAQRVA STVYQNCHEADNYTAYC LGI SHMEPSFGLILHDG GTTLLKFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGFP PTAGQPPATTKPKEITP VNPGTSPLLRYAAWTGG LAAVVLLCLVIFLICTA KRMRVKAYRVDK*	ATGGGGACAGTTAATAA ACCTGTGGTGGGGGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAACTGG ATACAACTCCGTATAT GAGCCTTACTACCATT AGATCATGCGGAGTCTT CATGGGTAAATCGGGGA GAGTCTTCGCGAAAAGC GTACGATCATAACTCAC CTTATATATGGCCACGT AATGATTATGATGGATT TTTAGAGAACGCACACG AACACCATGGGGTGTAT AATCAGGGCCGTGGTAT CGATAGCGGGGAACGGT TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTAAACGGCGATGA CAGACATAAAATTGTAA ATGTGGACCAACGTCAA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AACCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAAAATCACCCGT TTACTTTACGCGCACCG ATTACGCGGATTATGG AGTCCGGTACACCGAGA CTTGGAGCTTTTGGCCG TCATTAACCTGTACGGG AGACGCAGCGCCCGCCA TCCAGCATATATGTTTA AAACATACAACATGCTT TCAAGACGTGGTGGTGG ATGTGGATTGCGCGGAA AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTCAAGGTAAG AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACTCGAATTAGACCC CCCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAAAAACAATA CTTGGGTGTGTACATTT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTTTTGGTCA CCTGGAAAGGGGATGAA AAAACAAGAAACCCCTAC GCCCGCAGTAACTCCTC AACCAAGAGGGGCTGAG TTTCATATGTGGAATTA CCAATCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTTGATTTG	G*GGGAAATAAG AGAGAAAAGAAG AGTAAGAAGAAA TATAAGAGCCAC CATGGGGACAGT TAATAAACCTGT GGTGGGGGTATT GATGGGGTTCGG AATTATCACGGG CTCCGTGCGTAT AACGAATCCGGT CAGAGCATCCGT CTTGCGATACGA TGATTTTTCACAT CGATGAAGACAA ACTGGATACAAA TCCGTATATGA GCCTTACTACCA TTCAGATCATGC GGAGTCTTCATG GGTAAATCGGGG AGAGTCTTCGCG AAAAGCGTACGA CTATAACTACC TTATATATGGCC ACGTAATGATTA TGATGGATTTTT AGAGAACGCACA CGAACACCATGG GGTGATATAATCA GGGCCGTGGTAT CGATAGCGGGGA ACGGTTAATGCA ACCCACACAAAT GTCTGCACAGGA GGATCTTGGGGA CGATACGGGCAT CCACGTTATCCC TACGTTAAACGG CGATGACAGACA TAAATTTGTAA TGTGGACCAACG TCAATACGGTGA CGTGTTTAAAGG AGATCTTAATCC AAAACCCCAAGG CCAAAGACTCAT TGAGGTGTCAGT GGAAGAAAATCA CCCGTTTACTTT ACGCGCACCGAT TCAGCGGATTTA TGGAGTCCGGTA CACCGAGACTTG GAGCTTTTTGCC GTCATTAACCTG TACGGGAGACGC AGCGCCCGCCAT CCAGCATATATG TTTAAACATAC AACATGCTTTCA AGACGTGGTGGT GGATGTGGATTG CGCGGAAAATAC TAAAGAGGATCA GTTGGCCGAAAT

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	TGGATTGCGCGG AAAATACTAAAG AGGATCAGTTGG CCGAAATCAGTT ACCGTTTTCAG GTAAGAAGGAAG CGGACCAACCGT GGATTGTTGTAA ACACGAGCACAC TGTTTGATGAAC TCGAATTAGACC CCCCCGAGATTG AACCGGGTGTCT TGAAAGTACTTC GGACAGAAAAAC AATACTTGGGTG TGTACATTTGGA ACATGCGCGGCT CCGATGGTACGT CTACCTACGCCA CGTTTTTGGTCA CCTGGAAAGGGG ATGAAAAAACAA GAAACCCCTACGC CCGCAGTAACTC CTCAACCAAGAG GGGCTGAGTTTC ATATGTGGAATT ACCACTCGCATG TATTTTCAGTTG GTGATACGTTTA GCTTGGAATGCA ATCTTCAGTATA AGATACATGAAG CGCCATTTGATT TGCTGTTAGAGT GGTTGTATGTCC CCATCGATCCTA CATGTCAACCAA TGCGGTTATATT CTACGTGTTTGT ATCATCCCAACG CACCCCAATGCC TCTCTCATATGA ATTCCGGTTGTA CATTTACCTCGC CACATTTAGCCC AGCGTGTTGCAA GCACAGTGTATC AAAATTGTGAAC ATGCAGATAACT ACACCGCATATT GTCTGGGAATAT CTCATATGGAGC CTAGCTTTGGTC TAATCTTACACG ACGGGGGCACCA CGTTAAAGTTTG TAGATACACCCG AGAGTTTGTCGG GATTATACGTTT TTGTGGTGTATT TTAACGGGCATG TTGAAGCCGTAG CATACACTGTTG TATCCACAGTAG		CTGTTAGAGTGGTTGTA TGTCCCCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCAACGCAC CCCAATGCCTCTCTCAT ATGAATTCCGGTTGTAC ATTTACCTCGCCACATT TAGCCCAGCGTGTGCA AGCACAGTGTATCAAAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGG GGCACCACGTTAAAGTT TGTAGATACACCCGAGA GTTTGTGCGGATTATAC GTTTTTGTGGTGTATTT TAACGGGCATGTTGAAG CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTGTAAACGCAATTG AAGAGCGTGGATTTCCG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACCCCC GTAAACCCCGGAACGTC ACCACTTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT TTTTAATCTGTACGGCT AAACGAATGAGGGTTAA AGCCTATAGGGTAGACA AGTGA	CAGTTACCGTTT TCAAGGTAAGAA GGAAGCGGACCA ACCGTGGATTGT TGTAACACGAG CACACTGTTTGA TGAACTCGAATT AGACCCCCCGGA GATTGAACCGGG TGTCTTGAAAGT ACTTCGGACAGA AAAACAATACTT GGGTGTGTACAT TTGGAACATGCG CGGCTCCGATGG TACGTCTACCTA CGCCACGTTTTT GGTCACCTGGAA AGGGGATGAAAA AACAAGAAACCC TACGCCCCGCAGT AACTCCTCAACC AAGAGGGCTGA GTTTCATATGTG GAATTACCACTC GCATGTATTTTC AGTTGGTGATAC GTTTAGCTTGGC AATGCATCTTCA GTATAAGATACA TGAAGCGCCATT TGATTTGCTGTT AGAGTGGTTGTA TGTCCTCCATCGA TCCTACATGTCA ACCAATGCGGTT ATATTCTACGTG TTTGTATCATCC CAACGCACCCCA ATGCCTCTCTCA TATGAATTCCGG TTGTACATTTAC CTCGCCACATTT AGCCCAGCGTGT TGCAAGCACAGT GTATCAAAATTG TGAACATGCAGA TAACTACACCGC ATATTGTCTGGG AATATCTCATAT GGAGCCTAGCTT TGGTCTAATCTT ACACGACGGGGG CACCACGTTAAA GTTTGTAGATAC ACCCGAGAGTTT GTCGGGATTATA CGTTTTTGTGGT GTATTTTAACGG GCATGTTGAAGC CGTAGCATACAC TGTGTATCCAC AGTAGATCATTT TGTAACGCAAT TGAAGAGCGTGG ATTTCGCCAAC

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	ATCATTTTGTA ACGCAATTGAAG AGCGTGGATTTC CGCCAACGGCCG GTCAGCCACCGG CGACTACTAAAC CCAAGGAAATTA CCCCCGTAAACC CCGGAACGTCAC CACTTCTACGAT ATGCCGCATGGA CCGGAGGGCTTG CAGCAGTAGTAC TTTTATGTCTCG TAATATTTTAA TCTGTACGGCTA AACGAATGAGGG TTAAAGCCTATA GGGTAGACAAGT GATGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCCTTGGG CCTCCCCCAGC CCCTCCTCCCT TCCTGCACCCGT ACCCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG C			GGCCGGTCAGCC ACCGGCGACTAC TAAACCCAAGGA AATTACCCCGT AAACCCCGAAC GTCACCACTTCT ACGATATGCCGC ATGGACCGGAGG GCTTGACGAGT AGTACTTTTATG TCTCGTAATATT TTAAATCTGTAC GGCTAAACGAAT GAGGGTTAAAGC CTATAGGGTAGA CAAGTGATGATA ATAGGCTGGAGC CTCGGTGGCCAT GCTTCTTGCCCC TTGGGCCTCCCC CCAGCCCCCTCCT CCCCTTCCTGCA CCGGTACCCCG TGGTCTTTGAAT AAAGTCTGAGTG GGCGGCAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAATC TAG

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:37	SEQ ID NO:38	SEQ ID NO:39	SEQ ID NO:40
VZV-GE-truncated-from_574_Y569A	TCAAGCTTTTGG ACCCTCGTACAG AAGCTAATACGA CTCACTATAGGG AAATAAGAGAGA AAAGAAGAGTAA GAAGAAATATAA GAGCCACCATGG GGACAGTTAATA AACCTGTGGTGG GGGTATTGATGG GGTTCGGAATTA TCACGGGAACGT TGCGTATAACGA ATCCGGTCAGAG CATCCGTCTTGC GATACGATGATT TTCACATCGATG AAGACAACTGG ATACAACTCCG TATATGAGCCTT ACTACCATTCAG ATCATGCGGAGT CTTCATGGGTAA ATCGGGGAGAGT CTTCGCGAAAAG CGTACGATCATA ACTCACCTTATA TATGGCCACGTA ATGATTATGATG GATTTTTAGAGA ACGCACACGAAC ACCATGGGGTGT ATAATCAGGGCC GTGGTATCGATA GCGGGGAACGGT TAATGCAACCCA CACAAATGTCTG CACAGGAGGATC TTGGGGACGATA CGGGCATCCACG TTATCCCTACGT TAAACGGCGATG ACAGACATAAAA TTGTAAATGTGG ACCAACGTCAAT ACGGTGACGTGT TTAAAGGAGATC TTAATCCAAAC CCCAAGGCCAAA GACTCATTGAGG TGTCAGTGGAAG AAAATCACCCGT TTACTTTACGCG CACCGATTTCAGC GGATTTATGGAG TCCGGTACACCG AGACTTGAGAGCT TTTTGCCGTCAT TAACCTGTACGG	MGTVNKPVVGVLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWPR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHHTTCFQDVVDVDCAE NTKEDQLAEISYRFQ GK KEADQPWIVVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLGVIWNMRGS DGTSTYATFLVTWKGDE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGDTF SLAMHLQYKIHEAPFDL LLEWLYVPIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTS PHLAQRVA STVYQNCHEADNYTAYC LGI SHMEPSFGLILHDG GTTCLKFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGFP PTAGQPPATTKPKEITP VNPGTSPLLRYAAWTGG LAADVLLCLVIFLICTA KRMRVKAARVDK*	ATGGGGACAGTTAATAA ACCTGTGGTGGGGGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAACTGG ATACAACTCCGTATAT GAGCCTTACTACCATT AGATCATGCGGAGTCTT CATGGGTAAATCGGGGA GAGTCTTCGCGAAAAGC GTACGATCATAACTCAC CTTATATATGGCCACGT AATGATTATGATGGATT TTTAGAGAACGCACACG AACACCATGGGGTGTAT AATCAGGGCCGTGGTAT CGATAGCGGGGAACGGT TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTAAACGGCGATGA CAGACATAAAATGTAA ATGTGGACCAACGTCAA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AACCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAAAATCACCCGT TTACTTTACGCGCACCG ATTACGCGGATTATGG AGTCCGGTACACCGAGA CTTGGAGCTTTTGGCCG TCATTAACCTGTACGGG AGACGCAGCGCCCGCCA TCCAGCATATATGTTTA AAACATACAACATGCTT TCAAGACGTGGTGGTGG ATGTGGATTGCGCGGAA AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTCAAGGTAAG AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACTCGAATTAGACCC CCCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAAAAACAATA CTTGGGTGTGTACATT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTTTTGGTCA CCTGGAAAGGGGATGAA AAAACAAGAAACCCTAC GCCCGCAGTAACTCCTC AACCAAGAGGGGCTGAG	G*GGGAAATAAG AGAGAAAAGAAG AGTAAGAAGAAA TATAAGAGCCAC CATGGGGACAGT TAATAAACCTGT GGTGGGGGTATT CGATGGGGTTCGG AATTATCACGGG AACGTTGCGTAT AACGAATCCGGT CAGAGCATCCGT CTTGCGATACGA TGATTTTCACAT CGATGAAGACAAA ACTGGATACAAA CTCCGTATATGA GCCTTACTACCA TTCAGATCATGC GGAGTCTTCATG GGTAAATCGGGG AGAGTCTTCGCG AAAAGCGTACGA TCATAACTCACC TTATATATGGCC ACGTAATGATTA TGATGGATTTTT AGAGAACGCACA CGAACACCATGG GGTGTATAATCA GGGCCGTGGTAT CGATAGCGGGGA ACGGTTAATGCA ACCCACACAAAT GTCTGCACAGGA GGATCTTGGGGA CGATACGGGCAT CCACGTTATCCC TACGTTAAACGG CGATGACAGACA TAAAATTGTAAA TGTGGACCAACG TCAATACGGTGA CGTGTTTAAAGG AGATCTTAATCC AAAACCCCAAGG CCAAAGACTCAT TGAGGTGTCAGT GGAAGAAAATCA CCCGTTTACTTT ACGCGCACCGAT TCAGCGGATTTA TGGAGTCCGGTA CACCGAGACTTG GAGCTTTTGGC GTCATTAACCTG TACGGGAGACGC AGCGCCCGCCAT CCAGCATATATG TTTAAACATAC

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	GAGACGCAGCGC CCGCCATCCAGC ATATATGTTTAA AACATACAACAT GCTTTC AAGACG TGGTGGTGGATG TGGATTGCGCGG AAAATACTAAAG AGGATCAGTTGG CCGAAATCAGTT ACCGTTTTCAAG GTAAGAAGGAAG CGGACCAACCGT GGATTGTTGTAA ACACGAGCACAC TGTTTTGATGAAC TCGAATTAGACC CCCCCGAGATTG AACCGGGTGTCT TGAAAGTACTTC GGACAGAAAAAC AATACTTGGGTG TGTACATTTGGA ACATGCGCGGCT CCGATGGTACGT CTACCTACGCCA CGTTTTTGGTCA CCTGGAAAGGGG ATGAAAAAACAA GAAACCCCTACGC CCGCAGTAACTC CTCAACCAAGAG GGGCTGAGTTTC ATATGTGGAATT ACCACTCGCATG TATTTTCAGTTG GTGATACGTTTA GCTTGGCAATGC ATCTTCAGTATA AGATACATGAAG CGCCATTTGATT TGCTGTTAGAGT GGTTGTATGTCC CCATCGATCCTA CATGTCAACCAA TGCGGTATATTT CTACGTGTTTGT ATCATCCCAACG CACCCCAATGCC TCTCTCATATGA ATTCCGGTTGTA CATTTACCTCGC CACATTTAGCCC AGCGTGTGCAA GCACAGTGTATC AAAATTGTGAAC ATGCAGATAACT ACACCGCATATT GTCTGGGAATAT CTCATATGGAGC CTAGCTTTGGTC TAATCTTACACG ACGGGGGCACCA CGTTAAAGTTTG TAGATACACCCG AGAGTTTGTCGG		TTTCATATGTGGAATTA CCACTCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTTGATTTG CTGTTAGAGTGGTTGTA TGTCCCCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCACGCAC CCCAATGCCTCTCTCAT ATGAATTCGGTTGTAC ATTTACCTCGCCACATT TAGCCCAGCGTGTGCA AGCACAGTGTATCAAAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGG GGCACCACGTTAAAGTT TGTAGATACACCCGAGA GTTTGTGCGGATTATAC GTTTTTGTGGTGTATTT TAACGGGCATGTTGAAG CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTGTAAACGCAATTG AAGAGCGTGGATTTCGG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACCCCC GTAAACCCCGGAACGTC ACCACTTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT TTTTAATCTGTACGGCT AAACGAATGAGGGTTAA AGCCGCCAGGGTAGACA AGTGA	AACATGCTTTTCA AGACGTGGTGGT GGATGTGGATTG CGCGGAAAAATAC TAAAGAGGATCA GTTGGCCGAAAT CAGTTACCGTTT TCAAGGTAAGAA GGAAGCGGACCA ACCGTGGATTGT TGTAACACGAG CACACTGTTTGA TGAATCGAATT AGACCCCCCGGA GATTGAACCGGG TGTCTTGAAAGT ACTTCGGACAGA AAAACAATACTT GGGTGTGTACAT TTGGAACATGCG CGGCTCCGATGG TACGTCTACCTA CGCCACGTTTTT GGTCACCTGGAA AGGGGATGAAAA AACAGAAACCC TACGCCCGCAGT AACTCCTCAACC AAGAGGGGCTGA GTTTCATATGTG GAATTACCACTC GCATGTATTTTC AGTTGGTGATAC GTTTAGCTTGGC AATGCATCTTCA GTATAAGATACA TGAAGCGCCATT TGATTTGCTGTT AGAGTGGTTGTA TGTCCCCATCGA TCCTACATGTCA ACCAATGCGGTT ATATTCTACGTG TTGTATCATCC CAACGCACCCCA ATGCCTCTCTCA TATGAATTCCGG TTGTACATTTAC CTCGCCACATTT AGCCCAGCGTGT TGCAAGCACAGT GTATCAAAATTG TGAACATGCAGA TAATAACACCGC ATATTGTCTGGG AATATCTCATAT GGAGCCTAGCTT TGGTCTAATCTT ACACGACGGGGG CACCACGTTAAA GTTTGTAGATAC ACCCGAGAGTTT GTCGGGATTATA CGTTTTTGTGGT GTATTTTAACGG GCATGTTGAAGC

[illegible]

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	ATAATTATACAG AGATAAAAGGAC AGCTTGTCTTTA TTGGAGAGCAAC TACCTACCGGGA CAAACTATAGCG GAACACTGGAAC TGTTATACGCGG ATACGGTGGCGT TTTGTTTCCGGT CAGTACAAGTAA TAAGATACGACG GATGTCCCCGGA TTAGAACGAGCG CTTTTATTTCTG GTAGGTACAAAC ATTCGTGGCATT ATGGTAACTCAA CGGATCGGATAT CAACAGAGCCGG ATGCTGGTGTA TGTTGAAAATTA CCAAACCGGGAA TAAATGATGCTG GTGTGTATGTAC TTCTTGTTCGGT TAGACCATAGCA GATCCACCGATG GTTTCATTCTTG GTGTAAATGTAT ATACAGCGGGCT CGCATCACAACA TTCACGGGGTTA TCTACACTTCTC CATCTCTACAGA ATGGATATTCTA CAAGAGCCCTTT TTCAACAAGCTC GTTTGTGTGATT TACCCGCGACAC CCAAAGGTCCTG GTACCTCCCTGT TTCAACATATGC TTGATCTTCGTG CCGGTAAATCGT TAGAGGATAACC CTTGGTTACATG AGGACGTTGTTA CGACAGAACTA AGTCCGTTGTTA AGGAGGGGATAG AAAATCACGTAT ATCCAACGGATA TGTCCACGTTAC CCGAAAAGTCCC TTAATGATCCTC CAGAAAATCTAC TTATAATTATTC CTATAGTAGCGT CTGTCATGATCC TCACCGCCATGG TTATTGTTATTG TAATAAGCGTTA AGCGACGTAGAA TTAAAAACATC CAATTTATCGCC	ESDVMLEAAIAQLATIR EESPPHSVVPFVK*	CAAACATTTCGTGGCATT ATGGTAACTCAACGGAT CGGATATCAACAGAGCC GGATGCTGGTGTAATGT TGAAAATTACCAAACCG GGAATAAATGATGCTGG TGTGTATGTACTTCTTG TTCGGTTAGACCATAGC AGATCCACCGATGGTTT CATTCTTGGTGTAATG TATATACAGCGGGCTCG CATCACAACATTACGG GGTTATCTACACTTCTC CATCTCTACAGAATGGA TATTCTACAAGAGCCCT TTTTCAACAAGCTCGTT TGTGTGATTTACCCGCG ACACCCAAAGGGTCCGG TACCTCCCTGTTTCAAC ATATGCTTGATCTTCGT GCCGGTAAATCGTTAGA GGATAACCCTTGGTTAC ATGAGGACGTTGTTACG ACAGAACTAAGTCCGT TGTTAAGGAGGGGATAG AAAATCACGTATATCCA ACGGATATGTCCACGTT ACCCGAAAAGTCCCTTA ATGATCCTCCAGAAAAT CTACTTATAATTATTCC TATAGTAGCGTCTGTCA TGATCCTCACCGCCATG GTTATTGTTATTGTAAT AAGCGTTAAGCGACGTA GAATTAATAAACATCCA ATTTATCGCCCAAATAC AAAAAACAAGAAGGGGCA TACAAAATGCGACACCA GAATCCGATGTGATGTT GGAGGCCGCCATTGCAC AACTAGCAACGATTCGC GAAGAATCCCCCCCACA TTCCGTTGTAAACCCGT TTGTTAAATAG	GCAACTACCTAC CGGGACAAACTA TAGCGGAACACT GGAACGTGTATA CGCGGATACGGT GGCGTTTTGTTT CCGGTCAGTACA AGTAATAAGATA CGACGGATGTCC CCGGATTAGAAC GAGCGCTTTTAT TTCGTGTAGGTA CAAACATTTCGTG GCATTATGGTAA CTCAACGGATCG GATATCAACAGA GCCGGATGCTGG TGTAAATGTTGAA AATTACCAAACC GGAATAAATGA TGCTGGTGTGTA TGACTTCTTGT TCGGTTAGACCA TAGCAGATCCAC CGATGGTTTCAT TCTTGGTGTAAA TGTATATACAGC GGGCTCGCATCA CAACATTCACGG GGTATCTACAC TTCTCCATCTCT ACAGAATGGATA TTCTACAAGAGC CCTTTTTCAACA AGCTCGTTTGTG TGATTTACCCGC GACACCCAAAGG GTCCGGTACCTC CCTGTTTCAACA TATGCTTGATCT TCGTGCCGGTAA ATCGTTAGAGGA TAACCCTTGTT ACATGAGGACGT TGTTACGACAGA AACTAAGTCCGT TGTTAAGGAGGG GATAGAAAATCA CGTATATCCAAC GGATATGTCCAC GTTACCCGAAAA GTCCCTTAATGA TCCTCCAGAAAA TCTACTTATAAT TATTCCCTATAGT AGCGTCTGTCAT GATCCTCACCGC CATGGTTATTGT TATTGTAATAAG CGTTAAGCGACG TAGAATTAATAA ACATCCAATTTA TCGCCCAAATAC AAAAAACAAGAAG GGGCATACAAAA TGCGACACCAGA

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	CAAATACAAAAA CAAGAAGGGGCA TACAAAATGCGA CACCAGAATCCG ATGTGATGTTGG AGGCCGCCATTG CACAAC TAGCAA CGATTCGCGAAG AATCCCCCCCAC ATTCCGTTGTAA ACCCGTTTGTTA AATAGTGATAAT AGGCTGGAGCCT CGGTGGCCATGC TTCTTGCCCCCTT GGGCCTCCCCCC AGCCCCCTCCTCC CCTTCCTGCACC CGTACCCCCGTG GTCTTTGAATAA AGTCTGAGTGGG CGGC			ATCCGATGTGAT GTTGGAGGCCGC CATTGACAACCT AGCAACGATTCCG CGAAGAATCCCC CCCACATTCCGT TGTAAACCCGTT TGTTAAATAGTG ATAATAGGCTGG AGCCTCGGTGGC CATGCTTCTTGC CCCTTGGGCCTC CCCCCAGCCCCCT CCTCCCCCTTCTT GCACCCGTACCC CCGTGGTCTTTG AATAAAGTCTGA GTGGGCGGCAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA ATCTAG
mRNA Name (s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:60	SEQ ID NO:61	SEQ ID NO:62	SEQ ID NO:63
VZV-GE-truncated-delete_from_574_Y569A Variant 1	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGCACCGTGA ACAAGCCCGTCG TGGGCGTGCTGA TGGGCTTCGGCA TCATCACCGGCA CCCTGCGGATCA CCAATCCTGTGC GGGCCAGCGTGC TGAGATACGACG ACTTCCACATCG ACGAGGACAAGC TGGACACCAACA GCGTGTACGAGC CCTACTACCACA GCGACCACGCCG AGAGCAGCTGGG TCAACAGAGGCG AGTCCAGCCGGA AGGCCTACGACC ACAACAGCCCTT ACATCTGGCCCC GGAACGACTACG ACGGCTTCTTGG AAAATGCCACG AGCACCACGGCG TGTACAACAGG GCAGAGGCATCG ACAGCGGCGAGA GACTGATGCAGC	MGTVNKPVVGVLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWPR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHTTCFQDVVVDVDCAE NTKEDQLAEISYRFQ GK KEADQPWIVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLGVIWNMRGS DGTSTYATFLVTWKGDE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGDTF SLAMHLQYKIHEAPFDL LLEWLYVPIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTS PHLAQRVA STVYQNCHEADNYTAYC LGI SHMEPSFGLILHDG GTTLKFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGF PTAGQPPATTKPKEITP VNPPTSPLLRYYAAWTGG LAAVVLLCLVIFLICTA	ATGGGACACCGTGAACAA GCCCGTCGTGGGCGTGC TGATGGGCTTCGGCATC ATCACCGGCACCGTGCG GATCACCAATCCTGTGC GGGCCAGCGTGCTGAGA TACGACGACTTCCACAT CGACGAGGACAAGCTGG ACACCAACAGCGTGTAC GAGCCCTACTACCACAG CGACCACGCCGAGAGCA GCTGGGTCAACAGAGGC GAGTCCAGCCGGAAGGC CTACGACCACAACAGCC CCTACATCTGGCCCCGG AACGACTACGACGGCTT CCTGGAAAATGCCACG AGCACCACGGCGTGTAC AACCAGGGCAGAGGCAT CGACAGCGGCGAGAGAC TGATGCAGCCCACCCAG ATGAGCGCCCAGGAAGA TCTGGGCGACGACACCG GCATCCACGTGATCCCT ACCCTGAACGGCGACGA CCGGCACAAGATCGTGA ACGTGGACCAGCGGCAG TACGGCGACGTGTTCAA GGGCGACCTGAACCCCA AGCCCCAGGGACAGCGG CTGATTGAGGTGTCCGT GGAAGAGAACCACCCCT TCACCCTGAGAGCCCCT	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGCACCGTGA ACAAGCCCGTCG TGGGCGTGCTGA TGGGCTTCGGCA TCATCACCGGCA CCCTGCGGATCA CCAATCCTGTGC GGGCCAGCGTGC TGAGATACGACG ACTTCCACATCG ACGAGGACAAGC TGGACACCAACA GCGTGTACGAGC CCTACTACCACA GCGACCACGCCG AGAGCAGCTGGG TCAACAGAGGCG AGTCCAGCCGGA AGGCCTACGACC ACAACAGCCCTT ACATCTGGCCCC GGAACGACTACG ACGGCTTCTTGG AAAATGCCACG AGCACCACGGCG TGTACAACAGG GCAGAGGCATCG ACAGCGGCGAGA GACTGATGCAGC

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	CCACCCAGATGA GCGCCCAAGGAA ATCTGGGCGACG ACACCGGCATCC ACGTGATCCCTA CCCTGAACGGCG ACGACCGGCACA AGATCGTGAACG TGGACCAGCGGC AGTACGGCGACG TGTTCAGGGCG ACCTGAACCCCA AGCCCCAGGGAC AGCGGCTGATTG AGGTGTCCGTGG AAGAGAACCACC CCTTCACCCCTGA GAGCCCCATATCC AGCGGATCTACG GCGTGCGCTATA CCGAGACTTGGA GCTTCCTGCCCCA GCCTGACCTGTA CTGGCGACGCCG CTCCTGCCATCC AGCACATCTGCC TGAAGCACACCA CCTGTTTCCAGG ACGTGGTGGTGG ACGTGGACTGCG CCGAGAACACCA AAGAGGACCAGC TGGCCGAGATCA GCTACCGGTTCC AGGGCAAGAAAG AGGCCGACCAGC CCTGGATCGTCG TGAACACCAGCA CCCTGTTTCGACG AGCTGGAACCTGG ACCCTCCCGAGA TCGAACC CGGGG TGCTGAAGGTGC TGCGGACCGAGA AGCAGTACCTGG GAGTGTACATCT GGAACATGCGGG GCAGCGACGGCA CCTCTACCTACG CCACCTTCCTCG TGACCTGGAAGG GCGACGAGAAAA CCCGGAACCCCTA CCCCTGCCGTGA CCCCTCAGCCTA GAGGCGCCGAGT TTCACATGTGGA ATTACCACAGCC ACGTGTTCAGCG TGGGCGACACCT TCTCCCTGGCCA TGCATCTGCAGT ACAAGATCCACG AGGCCCTTTTCG ACCTGCTGCTGG AATGGCTGTACG	KRMRVKAARVDK	ATCCAGCGGATCTACGG CGTGCGCTATAACCGAGA CTTGGAGCTTCCTGCCC AGCCTGACCTGTACTGG CGACGCCGCTCCTGCCA TCCAGCACATCTGCCTG AAGCACACCACCTGTTT CCAGGACGTGGTGGTGG ACGTGGACTGCGCCGAG AACACCAAAGAGGACCA GCTGGCCGAGATCAGCT ACCGGTTCAGGGCAAG AAAGAGGCCGACCAGCC CTGGATCGTCGTGAACA CCAGCACCCCTGTTTCGAC GAGCTGGAACCTGGACCC TCCCGAGATCGAACC CG GGGTGCTGAAGGTGCTG CGGACCGAGAAGCAGTA CCTGGGAGTGTACATCT GGAACATGCGGGGCGAGC GACGGCACCTCTACCTA CGCCACCTTCCTCGTGA CCTGGAAGGGCGACGAG AAAACCCGGAACCCCTAC CCCTGCCGTGACCCCTC AGCCTAGAGGCGCCGAG TTTACATGTGGAATTA CCACAGCCACGTGTTCA GCGTGGGCGACACCTTC TCCCTGGCCATGCATCT GCAGTACAAGATCCACG AGGCCCTTTTCGACCTG CTGCTGGAATGGCTGTA CGTGCCCATCGACCCTA CCTGCCAGCCCATGCGG CTGTACTCCACCTGTCT GTACCACCCCAACGCC CTCAGTGCCTGAGCCAC ATGAATAGCGGCTGCAC CTTACACAGCCCTCACC TGGCTCAGAGGGTGGCC AGCACCGTGTACCAGAA TTGCGAGCACGCCGACA ACTACACCGCCTACTGC CTGGGCATCAGCCACAT GGAACCCAGCTTCGGCC TGATCCTGCACGATGGC GGCACACCCCTGAAGTT CGTGGACACCCCTGAGT CCCTGAGCGGCCTGTAC GTGTTCTGTGGTGTACTT CAACGGCCACGTGGAAG CCGTGCCCTACACCGTG GTGTCCACCGTGGACCA CTTCTGTGAACGCCATCG AGGAACGGGGCTTCCCT CCAACTGCTGGACAGCC TCCTGCCACCACCAAGC CCAAAGAAATCACCCCT GTGAACCCCGGCACCAAG CCCACTGCTGCGCTATG CTGCTTGGACAGGCGGA CTGGCTGCTGTGGTGCT GCTGTGCCTCGTGATTT TCCTGATCTGCACCGCC	CCACCCAGATGA GCGCCCAAGGAA ATCTGGGCGACG ACACCGGCATCC ACGTGATCCCTA CCCTGAACGGCG ACGACCGGCACA AGATCGTGAACG TGGACCAGCGGC AGTACGGCGACG TGTTCAGGGCG ACCTGAACCCCA AGCCCCAGGGAC AGCGGCTGATTG AGGTGTCCGTGG AAGAGAACCACC CCTTCACCCCTGA GAGCCCCATATCC AGCGGATCTACG GCGTGCGCTATA CCGAGACTTGGA GCTTCCTGCCCCA GCCTGACCTGTA CTGGCGACGCCG CTCCTGCCATCC AGCACATCTGCC TGAAGCACACCA CCTGTTTCCAGG ACGTGGTGGTGG ACGTGGACTGCG CCGAGAACACCA AAGAGGACCAGC TGGCCGAGATCA GCTACCGGTTCC AGGGCAAGAAAG AGGCCGACCAGC CCTGGATCGTCG TGAACACCAGCA CCCTGTTTCGACG AGCTGGAACCTGG ACCCTCCCGAGA TCGAACC CGGGG TGCTGAAGGTGC TGCGGACCGAGA AGCAGTACCTGG GAGTGTACATCT GGAACATGCGGG GCAGCGACGGCA CCTCTACCTACG CCACCTTCCTCG TGACCTGGAAGG GCGACGAGAAAA CCCGGAACCCCTA CCCCTGCCGTGA CCCCTCAGCCTA GAGGCGCCGAGT TTCACATGTGGA ATTACCACAGCC ACGTGTTCAGCG TGGGCGACACCT TCTCCCTGGCCA TGCATCTGCAGT ACAAGATCCACG AGGCCCTTTTCG ACCTGCTGCTGG AATGGCTGTACG

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	TGCCCATCGACC CTACCTGCCAGC CCATGCGGCTGT ACTCCACCTGTC TGTACCACCCCA ACGCCCCTCAGT GCCTGAGCCACA TGAATAGCGGCT GCACCTTCACCA GCCCTCACCTGG CTCAGAGGGTGG CCAGCACCGTGT ACCAGAATTGCG AGCACGCCGACA ACTACACCGCCT ACTGCCTGGGCA TCAGCCACATGG AACCCAGCTTCG GCCTGATCCTGC ACGATGGCGGCA CCACCCTGAAGT TCGTGGACACCC CTGAGTCCCTGA GCGGCCTGTACG TGTTCTGTGGTGT ACTTCAACGGCC ACGTGGAAGCCG TGGCCTACACCG TGGTGTCCACCG TGGACCACTTCG TGAACGCCATCG AGGAACGGGGCT TCCCTCCAACCTG CTGGACAGCCTC CTGCCACCACCA AGCCCAAAGAAA TCACCCCTGTGA ACCCCGGCACCA GCCCCACTGCTGC GCTATGCTGCTT GGACAGGCGGAC TGGCTGCTGTGG TGCTGCTGTGCC TCGTGATTTTCC TGATCTGCACCG CCAAGCGGATGA GAGTGAAGGCCG CCAGAGTGGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCCCTGGG CCTCCCCCAGC CCCTCCTCCCT TCCTGCACCCGT ACCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG C		AAGCGGATGAGAGTGAA GGCCGCCAGAGTGGACA AG	TGCCCATCGACC CTACCTGCCAGC CCATGCGGCTGT ACTCCACCTGTC TGTACCACCCCA ACGCCCCTCAGT GCCTGAGCCACA TGAATAGCGGCT GCACCTTCACCA GCCCTCACCTGG CTCAGAGGGTGG CCAGCACCGTGT ACCAGAATTGCG AGCACGCCGACA ACTACACCGCCT ACTGCCTGGGCA TCAGCCACATGG AACCCAGCTTCG GCCTGATCCTGC ACGATGGCGGCA CCACCCTGAAGT TCGTGGACACCC CTGAGTCCCTGA GCGGCCTGTACG TGTTCTGTGGTGT ACTTCAACGGCC ACGTGGAAGCCG TGGCCTACACCG TGGTGTCCACCG TGGACCACTTCG TGAACGCCATCG AGGAACGGGGCT TCCCTCCAACCTG CTGGACAGCCTC CTGCCACCACCA AGCCCAAAGAAA TCACCCCTGTGA ACCCCGGCACCA GCCCCACTGCTGC GCTATGCTGCTT GGACAGGCGGAC TGGCTGCTGTGG TGCTGCTGTGCC TCGTGATTTTCC TGATCTGCACCG CCAAGCGGATGA GAGTGAAGGCCG CCAGAGTGGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCCCTGGG CCTCCCCCAGC CCCTCCTCCCT TCCTGCACCCGT ACCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG CAAAAAAAAAAAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA AAAAAAAAAAAAAA

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
				AAAAATCTAG
mRNA Name (s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:64	SEQ ID NO:65	SEQ ID NO:66	SEQ ID NO:67
VZV-GE-truncated-deleted-from_574_Y569A Variant 2	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AAGCGTACGATC ATAACTCACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTATG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AAATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AACCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAAAATCACC CGTTTACTTTAC GCGCACCGATTTC AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTTTTGCCGT CATTAACCTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT	MGTVNKPVVGLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDNNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWPR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIYNVDQRQ YGDVFKGDLNPKPQQQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHTTFCQDVVDVDCAE NTKEDQLAEISYRFQKG KEADQPWIVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLGVIWNMRGS DGTSTYATFLVTWKGDE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGDTF SLAMHLQYKIHEAPFDL LLEWLYVPIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTSPHLAQRVA STVYQNCHEADNYTAYC LGI SHMEPSFGLILHDG GTTLLKFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGFP PTAGQPPATTKPKEITP VNPPTSPLLRYAATWGG LAAVVLLCLVIFLICTA KRMrvKAARVDK	ATGGGGACAGTTAATAA ACCTGTGGTGGGGGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAACTGG ATACAAACTCCGTATAT GAGCCTTACTACCATTC AGATCATGCGGAGTCTT CATGGGTAAATCGGGGA GAGTCTTCGCGAAAAGC GTACGATCATAACTCAC CTTATATATGGCCACGT AATGATTATGATGGATT TTTAGAGAACGCACACG AACACCATGGGGTGTAT AATCAGGGCCGTGGTAT CGATAGCGGGGAACGGT TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTAAACGGCGATGA CAGACATAAAATGTAA ATGTGGACCAACGTCAA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AACCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAAAATCACCCGT TTACTTTACGCGCACCG ATTGAGCGGATTATGG AGTCCGGTACACCGAGA CTTGGAGCTTTTGGCCG TCATTAACCTGTACGGG AGACGCAGCGCCGCCA TCCAGCATATATGTTTA AAACATACAACATGCTT TCAAGACGTGGTGGTGA ATGTGGATTGCGCGGAA AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTCAAGGTAAG AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACTCGAATTAGACCC CCCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAGAAACAATA CTTGGGTGTGTACATTT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTTTTGGTCA CCTGGAAAGGGGATGAG AAGACAAGAAACCCTAC GCCCGCAGTAACTCCTC	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AAGCGTACGATC ATAACTCACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTATG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AAATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AACCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAAAATCACC CGTTTACTTTAC GCGCACCGATTTC AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTTTTGCCGT CATTAACCTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	TAAAACATACAA CATGCTTTCAAG ACGTGGTGGTGG ATGTGGATTGCG CGGAAAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTT AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCCCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA AACAATACTTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAAG GGGATGAGAAGA CAAGAAACCCCTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT ATCAAAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTTTGTGGTGT ATTTTAACGGGC		AACCAAGAGGGGCTGAG TTTCATATGTGGAATTA CCACTCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTTGATTTG CTGTAGAGTGGTTGTA TGTCCCCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCAACGCAC CCCAATGCCTCTCTCAT ATGAATTCGGTGTGTAC ATTTACCTCGCCACATT TAGCCCAGCGTGTGCA AGCACAGTGTATCAAAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGG GGCACCACGTTAAAGTT TGTAAGATACACCGAGA GTTTGTGCGGATTATAC GTTTTTGTGGTGTATTT TAACGGGCATGTTGAAG CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTTGTAACGCAATTG AAGAGCGTGGATTTCCG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACCCCC GTAAACCCCGGAACGTC ACCACTTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT TTTTAATCTGTACGGCT AAACGAATGAGGGTTAA AGCCGCCAGGGTAGACA AG	TAAAACATACAA CATGCTTTCAAG ACGTGGTGGTGG ATGTGGATTGCG CGGAAAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTT AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCCCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA AACAATACTTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAAG GGGATGAGAAGA CAAGAAACCCCTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT ATCAAAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTTTGTGGTGT ATTTTAACGGGC

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAGGAAA TTACCCCCGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCCTTGGG CCTCCCCCAGC CCCTCCTCCCCCT TCCTGCACCCGT ACCCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG C			ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAGGAAA TTACCCCCGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCCTTGGG CCTCCCCCAGC CCCTCCTCCCCCT TCCTGCACCCGT ACCCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG CAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAATCTAG
mRNA Name (s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:68	SEQ ID NO:69	SEQ ID NO:70	SEQ ID NO:71
VZV-GE-truncated-delete_from_574_Y569A Variant 3	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG	MGTVNKPVVGLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWPR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHTTCFQDVVVDVCAE NTKEDQLAEISYRFQGK KEADQPWIVVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLGVIWNMRGS DGTSTYATFLVTWKGE	ATGGGGACAGTTAATAA ACCTGTGGTGGGGGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAACTGG ATACAACTCCGTATAT GAGCCTTACTACCATTC AGATCATGCGGAGTCTT CATGGGTAAATCGGGGA GAGTCTTCGCGAAAAGC GTACGATCATAACTCAC CTTATATATGGCCACGT AATGATTATGATGGATT TTTAGAGAACGCACACG AACACCATGGGGTGTAT AATCAGGGCCGTGGTAT	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AAGCGTACGATC ATAACTCACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTTAG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AAATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AACCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAAAATCACC CGTTTACTTTAC GCGCACCGATTG AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTTTTTGCCGT CATTAACCTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT TAAAACATACAA CATGCTTTCAAG ACGTGGTGTTGG ATGTGGATTGCG CGGAAAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTG AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCACCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA AACAATACTTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAAG GGGATGAGAAGA	KTRNPPTPAVTPQPRGAE FHMWNYHSHVFSVGDFT SLAMHLQYKIHEAPFDL LLEWLYVPIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTSPHLAQRVA STVYQNCEHADNYTAYC LGI SHMEPSFGLILHDG GTTCLKFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGF PTAGQPPATTKPKEITP VNPPTSPLLRYAATWGG LAAVVLLCLVIFLICTA KRM RVKAARVDK	CGATAGCGGGGAACGGT TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTAAACGCGCATGA CAGACATAAAATGTAA ATGTGGACCAACGTCAA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AACCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAAAATCACCCGT TTACTTTACGCGCACCG ATTCAGCGGATTTATGG AGTCCGGTACACCGAGA CTTGGAGCTTTTGGCCG TCATTAACCTGTACGGG AGACGCAGCGCCCGCCA TCCAGCATATATGTTTA AAACATACAACATGCTT TCAAGACGTGGTGGTGG ATGTGGATTGCGCGGAA AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTCAAGGTAAG AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACCTCGAATTAGACCC ACCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAGAAACAATA CTTGGGTGTGTACATTT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTTTTGGTCA CCTGGAAAGGGGATGAG AAGACAAGAAACCCTAC GCCCAGAGTAACCTCCTC AACCAAGAGGGGCTGAG TTTCATATGTGGAATTA CCACTCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTTGATTTG CTGTAGAGTGTTGTA TGTCCCCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCAACGCAC CCCAATGCCCTCTCAT ATGAATTCGGTTGTAC ATTTACCTCGCCACATT TAGCCCAGCGTGTGCA AGCACAGTGTATCAAAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGG GGCACCACGTTAAAGTT TGTAAGTACACCCGAGA GTTTGTGCGGATTATAC GTTTTTGTGGTGTATTT	AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AAGCGTACGATC ATAACTCACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTTAG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AAATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AACCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAAAATCACC CGTTTACTTTAC GCGCACCGATTG AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTTTTTGCCGT CATTAACCTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT TAAAACATACAA CATGCTTTCAAG ACGTGGTGTTGG ATGTGGATTGCG CGGAAAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTG AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCACCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA AACAATACTTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAAG GGGATGAGAAGA

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	CAAGAAACCCTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT ATCAAAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTTGTGGTGT ATTTTAACGGGC ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAGGAAA TTACCCCGGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCTTGGG		TAACGGGCATGTTGAAG CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTGTAACGCAATTG AAGAGCGTGGATTTCCG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACCCCC GTAAACCCCGGAACGTC ACCACTTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT TTTTAATCTGTACGGCT AAACGAATGAGGGTTAA AGCCGCCAGGGTAGACA AG	CAAGAAACCCTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT ATCAAAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTTGTGGTGT ATTTTAACGGGC ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAGGAAA TTACCCCGGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCTTGGG

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	CCTCCCCCAGC CCCTCCTCCCCT TCCTGCACCCGT ACCCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG C			CCTCCCCCAGC CCCTCCTCCCCT TCCTGCACCCGT ACCCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG CAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAATCTAG
mRNA Name (s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:72	SEQ ID NO:73	SEQ ID NO:74	SEQ ID NO:75
VZV-GE-truncated-deleted-from_574_Y569A Variant 4	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AGGCGTACGATC ATAACTCACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTATG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AGATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA	MGTVNKPVVGLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWPR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDGTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHTTCFQDVVDVDCAE NTKEDQLAEISYRFQ GK KEADQPWIVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLVGYIWNMRGS DGTSTYATFLVTWKGE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGDTF SLAMHLQYKIHEAPFDL LLEWLYVPI DPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTSPHLAQ RVA STVYQNCEHADNYTAYC LGI SHMEPSFGLILHDG GTTLKFDVTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGFP PTAGQPPATTKPKEITP VNPPTSPLLRyAAWTGG LAAVVLLCLVIFLIC TA KRMRVKAARVDK	ATGGGGACAGTTAATAA ACCTGTGGTGGGGGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAACTGG ATACAACTCCGTATAT GAGCCTTACTACCATT AGATCATGCGGAGTCTT CATGGGTAAATCGGGGA GAGTCTTCGCGAAAGGC GTACGATCATAACTCAC CTTATATATGGCCACGT AATGATTATGATGGATT TTTAGAGAACGCACACG AACACCATGGGGTGTAT AATCAGGGCCGTGGTAT CGATAGCGGGGAACGGT TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTTAAACGGCGATGA CAGACATAAGATTGTAA ATGTGGACCAACGTC AA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AGCCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAGAATCACCCGT TTACTTTACGCGCACCG ATTTCAGCGGATTTATGG AGTCCGGTACACCGAGA CTTGGAGCTTTTGCCG TCATTAACCTGTACGGG AGACGCAGCGCCCGCCA TCCAGCATATATGTTTA AAGCATAACAACATGCTT TCAAGACGTGGTGGTGG ATGTGGATTGCGCGGAG AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTCAAGGTAAG	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AGGCGTACGATC ATAACTCACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTATG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AGATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	AGCCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAGAATCACC CGTTTACTTTAC GCGCACCGATTG AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTTTTTGCCGT CATTAACCTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT TAAAGCATACAA CATGCTTTCAAG ACGTGGTGCGTGG ATGTGGATTGCG CGGAGAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTTC AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCCCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA AACAATACTTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAG GGGATGAGAAGA CAAGAAACCCTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT		AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACTCGAATTAGACCC CCCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAGAAACAATA CTTGGGTGTGTACATTT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTTTTGGTCA CCTGGAAGGGGATGAG AAGACAAGAAACCCTAC GCCCCGAGTAACTCCTC AACCAAGAGGGGCTGAG TTTCATATGTGGAATTA CCACTCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTTGATTTG CTGTAGAGTGGTTGTA TGTCCTCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCAACGCAC CCCAATGCCTCTCTCAT ATGAATTCGGTTGTAC ATTTACCTCGCCACATT TAGCCCAGCGTGTGCA AGCACAGTGTATCAGAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGG GGCACCACGTTAAAGTT TGTAAGATACACCCGAGA GTTTGTGCGGATTATAC GTTTTTGTGGTGTATTT TAACGGGCATGTTGAAG CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTGTAAACGCAATTG AAGAGCGTGGATTTCGG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACCCCC GTAAACCCCGGAACGTC ACCACTTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT TTTTAATCTGTACGGCT AAACGAATGAGGGTTAA AGCCCGCAGGGTAGACA AG	AGCCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAGAATCACC CGTTTACTTTAC GCGCACCGATTG AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTTTTTGCCGT CATTAACCTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT TAAAGCATACAA CATGCTTTCAAG ACGTGGTGCGTGG ATGTGGATTGCG CGGAGAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTTC AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCCCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA AACAATACTTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAG GGGATGAGAAGA CAAGAAACCCTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	ATCAGAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTGTGGTGT ATTTTAACGGGC ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCCAAGGAAA TTACCCCGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCCCTGGG CCTCCCCCAGC CCCTCCTCCCCT TCCTGCACCCGT ACCCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG C			ATCAGAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTGTGGTGT ATTTTAACGGGC ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCCAAGGAAA TTACCCCGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCCCTGGG CCTCCCCCAGC CCCTCCTCCCCT TCCTGCACCCGT ACCCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG CAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAATCTAG
mRNA Name (s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:76	SEQ ID NO:77	SEQ ID NO:78	SEQ ID NO:79
VZV-GE-truncated-delete from 57	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA	MGTVNKPVVGLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWPR	ATGGGGACAGTTAATAA ACCTGTGGTGGGGGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
4 - Y569A Variant 5	ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AGGCGTACGATC ATAACCTACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTATG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTAAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AGATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AGCCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAGAATCACC CGTTTACTTTAC GCGCACCGATTG AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGG GCTTTTTGCCGT CATTAACCTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT TAAAGCATACAA CATGCTTTCAAG ACGTGGTGGTGG ATGTGGATTGCG CGGAGAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTT AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA	NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHTTCFQDVVDVDCAE NTKEDQLAEISYRFQ GK KEADQPWIVVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLGVIWNMRGS DGTSTYATFLVTWKGDE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGDTF SLAMHLQYKIHEAPFDL LLEWLYVPIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTS PHLAQRVA STVYQNCHEADNYTAYC LGISHMEPSFGLILHDG GTTLKFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGFP PTAGQPPATTKPKEITP VNPGTSPLLRYAAWTGG LAAVVLLCLVIFLICTA KRMRVKAARVDK	GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAAACTGG ATACAAACTCCGTATAT GAGCCTTACTACCATTTC AGATCATGCGGAGTCTT CATGGGTAAATCGGGGA GAGTCTTCGCGAAAGGC GTACGATCATAACTCAC CTTATATATATGGCCACGT AATGATTATGATGGATT TTTAGAGAACGCACACG AACACCATGGGGTGTAT AATCAGGGCCGTGGTAT CGATAGCGGGGAACGGT TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTTAAACGGCGATGA CAGACATAAGATTGTAA ATGTGGACCAACGTCAA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AGCCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAGAATCACCCTG TTAATTTACGCGCACCG ATTCAGCGGATTATATGG AGTCCGGTACACCGAGA CTTGGAGCTTTTGGCCG TCATTAACCTGTACGGG AGACGCAGCGCCCGCCA TCCAGCATATATGTTTA AAGCATACAACATGCTT TCAAGACGTGGTGGTGG ATGTGGATTGCGCGGAG AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTTCAAGGTAAG AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACTCGAATTAGACCC ACCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAGAAACAATA CTTGGGTGTGTACATT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTTTTGGTCA CCTGGAAAGGGGATGAG AAGACAAGAAACCTAC GCCCGCAGTAACTCCTC AACCAGAGGGGCTGAG TTCATATGTGGAATTA CCACTCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTTGATTTG CTGTTAGAGTGGTTGTA TGTCCCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCAACGCAC	ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AGGCGTACGATC ATAACCTACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTATG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTAAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AGATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AGCCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAGAATCACC CGTTTACTTTAC GCGCACCGATTG AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGG GCTTTTTGCCGT CATTAACCTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT TAAAGCATACAA CATGCTTTCAAG ACGTGGTGGTGG ATGTGGATTGCG CGGAGAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTT AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	CACTGTTTGATG AACTCGAATTAG ACCCACCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA AACAATACTTGG GTGTGTACATTT GGAACATGCGCG GTCCTCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAAG GGGATGAGAAGA CAAGAAACCCTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGCCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT ATCAGAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTGTGGTGT ATTTTAACGGGC ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAGGAAA TTACCCCGTAA ACCCCGGAACGT		CCCAATGCCTCTCTCAT ATGAATTCGGTTGTAC ATTTACCTCGCCACATT TAGCCCAGCGTGTGCA AGCACAGTGTATCAGAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGG GGCACCACGTTAAAGTT TGTAAGATACACCCGAGA GTTTGTGCGGATTATAC GTTTTTGTGGTGTATTT TAACGGGCATGTTGAAG CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTGTAACGCAATTG AAGAGCGTGGATTTCGG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACCCCG GTAAACCCCGGAACGTC ACCACTTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT TTTTAATCTGTACGGCT AAACGAATGAGGGTTAA AGCCGCCAGGGTAGACA AG	CACTGTTTGATG AACTCGAATTAG ACCCACCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA AACAATACTTGG GTGTGTACATTT GGAACATGCGCG GTCCTCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAAG GGGATGAGAAGA CAAGAAACCCTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGCCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT ATCAGAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTGTGGTGT ATTTTAACGGGC ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAGGAAA TTACCCCGTAA ACCCCGGAACGT

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCCCTGGG CCTCCCCCAGC CCCTCCTCCCCCT TCCTGCACCCGT ACCCCGTGGTTC TTTGAATAAAGT CTGAGTGGGCGG C			CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCCCTGGG CCTCCCCCAGC CCCTCCTCCCCCT TCCTGCACCCGT ACCCCGTGGTTC TTTGAATAAAGT CTGAGTGGGCGG CAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAATCTAG
mRNA Name (s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:80	SEQ ID NO:81	SEQ ID NO:82	SEQ ID NO:83
VZV-GE-truncated-deleted from 574_Y569A Variant 6	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AAGCGTACGATC ATAACTACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTTAG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC	MGTVNKPVVGVLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDTSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWPR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHTTCFQDVVVDVDCAE NTKEDQLAEISYRFQ GK KEADQPWIVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLGVIWNMRGS DGTSTYATFLVTWKGE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGDTF SLAMHLQYKIHAEFPDL LLEWLYVPIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTSPHLAQRVA STVYQNCHEADNYTAYC LGI SHMEPSFGLILHDG GTTLLKFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGF PTAGQPPATTKPKEITP VNPGTSPLLRYA AWTGG	ATGGGGACAGTTAATAA ACCTGTGGTGGGGGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAACTGG ATACAACTCCGTATAT GAGCCTTACTACCATT AGATCATGCGGAGTCTT CATGGGTAAATCGGGGA GAGTCTTCGCGAAAAGC GTACGATCATAACTCAC CTTATATATGGCCACGT AATGATTATGATGGATT TTTAGAGAACGCACACG AACACCATGGGGTGTAT AATCAGGGCCGTGGTAT CGATAGCGGGGAACGGT TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTAAACGGCGATGA CAGACATAAAATTGTAA ATGTGGACCAACGTCAA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AACCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAAAATCACCCGT	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AAGCGTACGATC ATAACTACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTTAG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AAATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AACCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAAAATCACC CGTTTACTTTAC GCGCACCGATTTC AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTTTTTGCCGT CATTAACCTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT TAAAGCATACAA CATGCTTTCAAG ACGTGGTGGTGG ATGTGGATTGCG CGGAAAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTTC AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCCCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA ACAATACTTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAG GGGATGAGAAGA CAAGAAACCTTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG	LAADVLLCLVIFLICTA KRMRVKAARVDK	TTACTTTACGCGCACCG ATTCAGCGGATTATGG AGTCCGGTACACCGAGA CTTGGAGCTTTTGGCG TCATTAACCTGTACGGG AGACGCAGCGCCCGCCA TCCAGCATATATGTTTA AAGCATACAACATGCTT TCAAGACGTGGTGGTGG ATGTGGATTGCGCGGAA AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTCAAGGTAAG AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACTCGAATTAGACCC CCCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAGAAACAATA CTTGGGTGTGTACATTT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTTTTGGTCA CCTGGAAAGGGGATGAG AAGACAAGAAACCCTAC GCCCCGAGTAACCTCCTC AACCAAGAGGGGCTGAG TTTCATATGTGGAATTA CCACTCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTTGATTTG CTGTAGAGTGGTTGTA TGTCCCCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCAACGCAC CCCAATGCCTCTCTCAT ATGAATTCGGTTGTAC ATTTACCTCGCCACATT TAGCCCAGCGTTTGCA AGCACAGTGTATCAGAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGG GGCACCACGTTAAAGTT TGTAAGATACACCCGAGA GTTTGTCTGGGATTATAC GTTTTTGTGGTGTATTT TAACGGGCATGTTGAAG CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTTGTAACGCAATTG AAGAGCGTGGATTTCCG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACCCCC GTAAACCCCGGAACGTC ACCACCTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT	GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AAATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AACCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAAAATCACC CGTTTACTTTAC GCGCACCGATTTC AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTTTTTGCCGT CATTAACCTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT TAAAGCATACAA CATGCTTTCAAG ACGTGGTGGTGG ATGTGGATTGCG CGGAAAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTTC AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCCCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA ACAATACTTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAG GGGATGAGAAGA CAAGAAACCTTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT ATCAGAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTTGTGGTGT ATTTTAACGGGC ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAAGGAAA TTACCCCCGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCCCTGGG CCTCCCCCAGC CCCTCCTCCCCCT TCCTGCACCCGT ACCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG C		TTTTAATCTGTACGGCT AAACGAATGAGGGTTAA AGCCGCCAGGGTAGACA AG	AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT ATCAGAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTTGTGGTGT ATTTTAACGGGC ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAAGGAAA TTACCCCCGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCCCTGGG CCTCCCCCAGC CCCTCCTCCCCCT TCCTGCACCCGT ACCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG CAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
				AAAAAAAAAAAA AAAAATCTAG
mRNA Name (s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:84	SEQ ID NO:85	SEQ ID NO:86	SEQ ID NO:87
VZV-GE-truncated-deleted-from_574_Y569A Variant 7	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AAGCGTACGATC ATAACTCACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTATG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AAATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AACCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAAAATCACC CGTTTACTTTAC GCGCACCGATTG AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTTTTGCCGT CATTAACTGTGA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT	MGTVNKPVVGVLMGF ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWP NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIVNVDQ YGDVFKGDLNPKPQGR LIEVSVEENHPTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHTTFCQDVVVDVCAE NTKEDQLAEISYRFQ KEADQPWIVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLGVIWNMRGS DGTSTYATFLVTWKGDE KTRNPTPAVTPQPRGAE FHMWNYHSHVFSVGD SLAMHLQYKIHAEFDFL LLEWLYVPIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTSPHLAQRVA STVYQNCHEADNYTAYC LGI SHMEPSFGLILHDG GTTLKFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGFP PTAGQPPATTKPKAITP VNPPTSPLLRYAATWG LAAVVLLCLVIFLICTA KRMRVKAARVDK	ATGGGGACAGTTAATAA ACCTGTGGTGGGGGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAACTGG ATACAACTCCGTATAT GAGCCTTACTACCATT AGATCATGCGGAGTCTT CATGGGTAAATCGGGGA GAGTCTTCGCGAAAAGC GTACGATCATAACTCAC CTTATATATGGCCACGT AATGATTATGATGGATT TTTAGAGAACGCACACG AACACCATGGGGTGAT AATCAGGGCCGTGGTAT CGATAGCGGGGAACGGT TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTTAAACGGCGATGA CAGACATAAAATTGTAA ATGTGGACCAACGTCAA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AACCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAAAATCACCCGT TTACTTTACGCGACCG ATTCAGCGGATTATGG AGTCCGGTACACCGAGA CTTGGAGCTTTTGGCG TCATTAACCTGTACGGG AGACGCAGCGCCCGCCA TCCAGCATATATGTTTA AAGCATACAACATGCTT TCAAGACGTGGTGGTGG ATGTGGATTGCGCGGAA AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTCAAGGTAAG AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACTCGAATTAGACCC ACCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAGAAACAATA CTTGGGTGTGTACATTT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTTTTGGTCA CCTGGAAAGGGGATGAG AAGACAAGAAACCCCTAC GCCCGCAGTAACTCCTC	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGGGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG TAAATCGGGGAG AGTCTTCGCGAA AAGCGTACGATC ATAACTCACCTT ATATATGGCCAC GTAATGATTATG ATGGATTTTATG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AAATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AACCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAAAATCACC CGTTTACTTTAC GCGCACCGATTG AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTTTTGCCGT CATTAACTGTGA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	TAAAGCATACAA CATGCTTTCAAG ACGTGGTGGTGG ATGTGGATTGCG CGGAAAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTT AAGGTAAGAAGG AAGCGGAACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCACCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA ACAATACTTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAAG GGGATGAGAAGA CAAGAAACCCCTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT ATCAGAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTTTGTGGTGT ATTTTAACGGGC		AACCAAGAGGGGCTGAG TTTCATATGTGGAATTA CCACTCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTTGATTTG CTGTTAGAGTGGTTGTA TGTCCCCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCAACGCAC CCCAATGCCTCTCTCAT ATGAATTCGGTGTGAC ATTTACCTCGCCACATT TAGCCCAGCGTGTGCA AGCACAGTGTATCAGAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGG GGCACCACGTTAAAGTT TGTAAGATACCCGAGA GTTTGTGCGGATTATAC GTTTTTGTGGTGTATTT TAACGGGCATGTTGAAG CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTTGTAACGCAATTG AAGAGCGTGGATTTCCG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACCCCC GTAAACCCCGGAACGTC ACCACTTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT TTTTAATCTGTACGGCT AAACGAATGAGGGTTAA AGCCGCCAGGGTAGACA AG	TAAAGCATACAA CATGCTTTCAAG ACGTGGTGGTGG ATGTGGATTGCG CGGAAAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTT AAGGTAAGAAGG AAGCGGAACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCACCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA ACAATACTTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTTTTGG TCACCTGGAAAG GGGATGAGAAGA CAAGAAACCCCTA CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTTG CAAGCACAGTGT ATCAGAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGGGGCA CCACGTTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TTTTTTGTGGTGT ATTTTAACGGGC

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAGGAAA TTACCCCGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCTTGGG CCTCCCCCAGC CCCTCCTCCCCT TCCTGCACCCGT ACCCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG C			ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAGGAAA TTACCCCGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTTT TAATCTGTACGG CTAAACGAATGA GGGTTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCTTGGG CCTCCCCCAGC CCCTCCTCCCCT TCCTGCACCCGT ACCCCCGTGGTC TTTGAATAAAGT CTGAGTGGGCGG CAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAAAAAAAAA AAAAAATCTAG
mRNA Name (s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	SEQ ID NO:88	SEQ ID NO:89	SEQ ID NO:90	SEQ ID NO:91
VZV-GE-truncated-delete from_574_Y569A Variant 8	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGCGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG	MGTVNKPVVGVLMGFGI ITGTLRITNPVRASVLR YDDFHIDEDKLDNSVY EPYYHSDHAESSWVNRG ESSRKAYDHNSPYIWR NDYDGFLENAHEHHGVY NQGRGIDSGERLMQPTQ MSAQEDLGDDTGIHVIP TLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQR LIEVSVEENHPFTLRAP IQRIYGVRYTETWSFLP SLTCTGDAAPAIQHICL KHTTCFQDVVDVDCAE NTKEDQLAEISYRFQGK KEADQPWIVNTSTLFD ELELDPPEIEPGVLKVL RTEKQYLGVIWNMRGS DGTSTYATFLVTWKGE KTRNPTPAVTPQPRGAE	ATGGGGACAGTTAATAA ACCTGTGGTGGGCGTAT TGATGGGGTTCGGAATT ATCACGGGAACGTTGCG TATAACGAATCCGGTCA GAGCATCCGTCTTGCGA TACGATGATTTTCACAT CGATGAAGACAACTGG ATACAAACTCCGTATAT GAGCCTTACTACCATTTC AGATCATGCGGAGTCTT CATGGGTAAATCGGGGA GAGTCTTCGCGAAAGGC GTACGATCATAACTCAC CTTATATATGGCCACGT AATGATTATGATGGATT CTTAGAGAACGCACACG AACACCATGGGGTGTAT AATCAGGGCCGTGGTAT CGATAGCGGGGAACGGT	GGGAAATAAGAG AGAAAAGAAGAG TAAGAAGAAATA TAAGAGCCACCA TGGGGACAGTTA ATAAACCTGTGG TGGGCGTATTGA TGGGGTTCGGAA TTATCACGGGAA CGTTGCGTATAA CGAATCCGGTCA GAGCATCCGTCT TGCGATACGATG ATTTTCACATCG ATGAAGACAAAC TGGATACAAACT CCGTATATGAGC CTTACTACCATT CAGATCATGCGG AGTCTTCATGGG

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	TAAATCGGGGAG AGTCTTCGCGAA AGGCGTACGATC ATAACTCACCTT ATATATGGCCAC GTAATGATTATG ATGGATTCTTAG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AGATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AGCCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAGAATCACC CGTTTACTTTAC GCGCACCGATTG AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTCTTGCCGT CATTAACTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT TAAAGCATACAA CATGCTTTCAAG ACGTGGTGGTGG ATGTGGATTGCG CGGAGAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTT AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCACCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA AACAACTACTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTCTTGG TCACCTGGAAAG GGGATGAGAAGA CAAGAAACCCCTA	FHMWNYHSHVFSVGDTF SLAMHLQYKIHEAPFDL LLEWLYVPIIDPTCQPMR LYSTCLYHPNAPQCLSH MNSGCTFTSPHLAQRVA STVYQNCHEADNYTAYC LGI SHMEPSFGLILHDG GTTLKFVDTPESLSGLY VFVVYFNGHVEAVAYTV VSTVDHFVNAIEERGFP PTAGQPPATTKPKEITP VNPGTSPLLRYYAAWTGG LAAVLLCLVIFLICTA KRMRVKAARVDK	TAATGCAACCCACACAA ATGTCTGCACAGGAGGA TCTTGGGGACGATACGG GCATCCACGTTATCCCT ACGTTAAACGGCGATGA CAGACATAAGATTGTAA ATGTGGACCAACGTCAA TACGGTGACGTGTTTAA AGGAGATCTTAATCCAA AGCCCCAAGGCCAAAGA CTCATTGAGGTGTCAGT GGAAGAGAATCACCCGT TTACTTTACGCGCACCG ATTCAGCGGATTATGG AGTCCGGTACACCGAGA CTTGGAGCTTCTTGCCG TCATTAACCTGTACGGG AGACGCAGCGCCCGCCA TCCAGCATATATGTTTA AAGCATACAACATGCTT TCAAGACGTGGTGGTGG ATGTGGATTGCGCGGAG AATACTAAAGAGGATCA GTTGGCCGAAATCAGTT ACCGTTTTCAAGGTAAG AAGGAAGCGGACCAACC GTGGATTGTTGTAAACA CGAGCACACTGTTTGAT GAACTCGAATTAGACCC ACCCGAGATTGAACCGG GTGTCTTGAAAGTACTT CGGACAGAGAAACAATA CTTGGGTGTGTACATTT GGAACATGCGCGGCTCC GATGGTACGTCTACCTA CGCCACGTTCTTGGTCA CCTGGAAGGGGATGAG AAGACAAGAAACCCTAC GCCCGCAGTAACTCCTC AACCAAGAGGGGCTGAG TTTCATATGTGGAATTA CCACTCGCATGTATTTT CAGTTGGTGATACGTTT AGCTTGGCAATGCATCT TCAGTATAAGATACATG AAGCGCCATTTGATTTG CTGTAGAGTGGTTGTA TGTCCCCATCGATCCTA CATGTCAACCAATGCGG TTATATTCTACGTGTTT GTATCATCCCAACGCAC CCCAATGCCTCTCTCAT ATGAATTCCGGTTGTAC ATTTACCTCGCCACATT TAGCCCAGCGTGTTGCA AGCACAGTGTATCAGAA TTGTGAACATGCAGATA ACTACACCGCATATTGT CTGGGAATATCTCATAT GGAGCCTAGCTTTGGTC TAATCTTACACGACGGA GGCACCACGTTAAAGTT TGTAAGATACACCGAGA GTTTGTGCGGATTATAC GTCTTTGTGGTGTATTT TAACGGGCATGTTGAAG	TAAATCGGGGAG AGTCTTCGCGAA AGGCGTACGATC ATAACTCACCTT ATATATGGCCAC GTAATGATTATG ATGGATTCTTAG AGAACGCACACG AACACCATGGGG TGTATAATCAGG GCCGTGGTATCG ATAGCGGGGAAC GGTTAATGCAAC CCACACAAATGT CTGCACAGGAGG ATCTTGGGGACG ATACGGGCATCC ACGTTATCCCTA CGTTAAACGGCG ATGACAGACATA AGATTGTAAATG TGGACCAACGTC AATACGGTGACG TGTTTAAAGGAG ATCTTAATCCAA AGCCCCAAGGCC AAAGACTCATTG AGGTGTCAGTGG AAGAGAATCACC CGTTTACTTTAC GCGCACCGATTG AGCGGATTTATG GAGTCCGGTACA CCGAGACTTGGA GCTTCTTGCCGT CATTAACTGTA CGGGAGACGCAG CGCCCGCCATCC AGCATATATGTT TAAAGCATACAA CATGCTTTCAAG ACGTGGTGGTGG ATGTGGATTGCG CGGAGAATACTA AAGAGGATCAGT TGGCCGAAATCA GTTACCGTTTTT AAGGTAAGAAGG AAGCGGACCAAC CGTGGATTGTTG TAAACACGAGCA CACTGTTTGATG AACTCGAATTAG ACCCACCCGAGA TTGAACCGGGTG TCTTGAAAGTAC TTCGGACAGAGA AACAACTACTGG GTGTGTACATTT GGAACATGCGCG GCTCCGATGGTA CGTCTACCTACG CCACGTTCTTGG TCACCTGGAAAG GGGATGAGAAGA CAAGAAACCCCTA

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTG CAAGCACAGTGT ATCAGAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGAGGCA CCACGTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TCTTTGTGGTGT ATTTTAACGGGC ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAGGAAA TTACGCCCGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTCT TAATCTGTACGG CTAAACGAATGA GGGTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCTTGGG CCTCCCCCAGC		CCGTAGCATACACTGTT GTATCCACAGTAGATCA TTTTGTAAACGCAATTG AAGAGCGTGGATTTCG CCAACGGCCGGTCAGCC ACCGGCGACTACTAAAC CCAAGGAAATTACGCCC GTAAACCCCGGAACGTC ACCACTTCTACGATATG CCGCATGGACCGGAGGG CTTGCAGCAGTAGTACT TTTATGTCTCGTAATAT TCTTAATCTGTACGGCT AAACGAATGAGGGTTAA AGCCGCCAGGGTAGACA AG	CGCCCGCAGTAA CTCCTCAACCAA GAGGGGCTGAGT TTCATATGTGGA ATTACCACTCGC ATGTATTTTCAG TTGGTGATACGT TTAGCTTGGCAA TGCATCTTCAGT ATAAGATACATG AAGCGCCATTTG ATTTGCTGTTAG AGTGGTTGTATG TCCCCATCGATC CTACATGTCAAC CAATGCGGTTAT ATTCTACGTGTT TGTATCATCCCA ACGCACCCCAAT GCCTCTCTCATA TGAATTCCGGTT GTACATTTACCT CGCCACATTTAG CCCAGCGTGTG CAAGCACAGTGT ATCAGAATTGTG AACATGCAGATA ACTACACCGCAT ATTGTCTGGGAA TATCTCATATGG AGCCTAGCTTTG GTCTAATCTTAC ACGACGGAGGCA CCACGTAAAGT TTGTAGATACAC CCGAGAGTTTGT CGGGATTATACG TCTTTGTGGTGT ATTTTAACGGGC ATGTTGAAGCCG TAGCATACACTG TTGTATCCACAG TAGATCATTTTG TAAACGCAATTG AAGAGCGTGGAT TTCCGCCAACGG CCGGTCAGCCAC CGGCGACTACTA AACCCAAGGAAA TTACGCCCGTAA ACCCCGGAACGT CACCACTTCTAC GATATGCCGCAT GGACCGGAGGGC TTGCAGCAGTAG TACTTTTATGTC TCGTAATATTCT TAATCTGTACGG CTAAACGAATGA GGGTAAAGCCG CCAGGGTAGACA AGTGATAATAGG CTGGAGCCTCGG TGGCCATGCTTC TTGCCCTTGGG CCTCCCCCAGC

mRNA Name(s)	Sequence, NT (5' UTR, ORF, 3' UTR)	ORF Sequence, AA	ORF Sequence, NT	mRNA Sequence (assumes T100 tail)
	CCCTCCTCCCT TCCTGCACCCGT ACCCCGTGCTC TTTGAATAAAGT CTGAGTGGGCGG C			CCCTCCTCCCT TCCTGCACCCGT ACCCCGTGCTC TTTGAATAAAGT CTGAGTGGGCGG CAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAAAAAAAAA AAAAATCTAG

VZV mRNA Sequences

mRNA Name(s)	mRNA Sequence (assumes T100 tail)	SEQ ID NO
VZV_gE_ Oka	G*GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAUAUAAGA GCCACCAUGGGGACAGUGAAUAAGCCGGUUGUGGGCGUGCUUUAU GGGCUUUGGGAUUAUUAACCGGUACAUAACGAUUACCAUCCAG UGCGCGCCAGUGUGCUGCGUUAACGACGACUUAACAUAUGACGAG GAUAAGCUGGAUACUAACAGCGUGUACGAACCUUAUUAACACUC AGAUCAUGCCGAAUCAAGCUGGGUUAUAAGAGGAGAAAGCAGC CGAAAAGCCUACGACCACAACUCACCUUAUAUUUGGCCAGAAA CGAUUAUGACGGUUAUCCUGGAAAACGCACAUGAACACCAUGGAG UCUACAACCAAGGCAGGGGAUUCGACAGUGGCGAGCGUCUUAUG CAGCCAACACAGAUGUCGGCACAGGAGGAUCUCGGUGAUGACAC CGGCAUACACGUGAUUCCCAUAUAAACGGCGACGACAGACAUUA AGAUCGUCAAUGUGGAUCAGCGUCAGUAUGGGGAUGUCUUUAA AGGCGAUUUGAAUCCAAAGCCCCAAGGACAGAGACUGAUCGAGG UCUCUGUAAGAAGAAAUACACCCUUCACUUUGCGCGCUCCAUA CAGAGGAUUUAACGGGGUGCGUUAUACCGAAACUUGGAGUUUCU UGCCGUCACUGACGUGUACGGGGGAUGCCGCCCCCGCAAUCCAG CACAUCUGUCUGAAACACACCACAUGCUUUCAGGACGUGGUUGU GGAUGUGGAUUGCGCGGAAAACACAAAAGAAGACCAACUCGCCG AAAUCAGCUAUCGUUUUCAGGGUAAAAAGAGGCCGACCAACCG UGGAUUGUUGUGAAUACGAGCACGCUCUUCGAUGAGCUUGAAC UCGAUCCCCCGAAAUCGAGCCUGGGGUUCUAAAAGUGUUGAGG ACCGAGAAGCAGUACCUUCGGGUUUUAUAUCUGGAUAUAGAGAG GCUCCGAUGGCACCUCUACCUACGCAACGUUUCUGGUUACCUGG AAGGGAGACGAGAAGACACGGAAUCCAACGCCCCGUGUGACCCC UCAGCCUAGGGGAGCCGAUUCCACAUGUGGAACUAUCACUCCC AUGUAUUCAGUGUGGGUGACACUUUCAGCCUGGCCAUGCACCUG CAGUAUAAGAUUCACGAGGCACCCUUCGACCUCUGCUGGAGUG GUUGUACGUACCUAUUGAUCCCAUUGUCAGCCCAUGCGCCUGU ACUCCACUUGCUUGUACCAACCCCAUUGCACCACAGUGUCUAUCA CACAUGAACUCCGGGUGUACCUUUAUUCACCCCAUCUUGCCCA GCGGGUCGCCAGCACAGUGUAUCAGAACUGUGAGCAUGCUGACA ACUAUACUGCUUAUUGCCUCGGAAUAUCCCAUAUGGAGCCAAGC UUCGGGCUCAUACUGCACGAUGGUGGUACGACACUCAAGUUCGU GGACACCCCCGAAAGCCUUUCUGGCUUGUACGUGUUCGUGGUCU ACUUCAAUGGACAUGUGGAGGCAGUGGCUUACACAGUGGUUUC GACAUUGAUCACUUUGUAAAUGCCAUAUGAGGAACGCGGUUCC CGCCUACAGCGGGCCAGCCCCUGCGACAACAAAACCAAAGAG AUUACGCCCCGUUAAUCCUGGGACUAGUCCAUAUGCUGAGGUUAGC CGCCUGGACUGGCGGUCUGGCGGCCGUGGUACUUCUGUGUUUAG UCAUAUUUCUGAUCUGUACCGCUAAACGUAUGCGGGUCAAGGCU UACCGUGUUGACAAGUCUCCUUAACAUCAGUCAUUGUACUAGC AGGACUCCCUUGUUGACGAUUUCGAAGACUCAGAGAGUACAGACA CAGAAGAAGAAUUCGGAACGCUAUAGGUGGCUCUCACGGAGG	92

	UAGCUCGUUAACAGUGUACAUCGAUAAAACCAGAUGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCC CCAGCCCCUCCUCCCCUUCUGCACC CGUACCCCCGUGGUCUUUG AAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAA AAA AAUUCUAG	
VZV_gE full_indel _fixed	G*GGGAAAUAAAGAGAGAAAAGAAGAGUAAGAAGAAAUAAAGA GCCACCAUGGGGACAGUUAAUAAACCUUGUGGUGGGGUAAUUGA UGGGGUUCGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCC GGUCAGAGCAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUG AAGACAAACUGGAUACAAACUCCGUUAUAGAGCCUUAUACCAU UCAGAUCAUGCGGAGUCUUCUUGGGUAAAUCGGGGAGAGUCUU CGCGAAAAGCGUACGAUCAUAACUCACCUUAUUAUUGGCCACGU AAUGAUUAUGAUGGAUUUUUAGAGAACGCACACGAACACCAUG GGGUGUAUAAUCAGGGCCGUGGUUAUCGAUAGCGGGGAACGGUU AAUGCAACCCACACAAAUGUCUGCACAGGAGGAUCUUGGGGACG AUACGGGCAUCCACGUUAUCCCUACGUUAAACGGCGAUGACAGA CAUAAA AUUGUAAAUGUGGACCAACGUCAAUACGGUGACGUGU UUAAAGGAGAUUUAAUCCAAAACCCCAAGGCCAAAGACUCAU GAGGUGUCAGUGGAAGAAAUCACCCGUUUACUUUACGCGCACC GAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGAGCU UUUUGCCGUCAUUAACCUGUACGGGAGACGCAGCGCCCGCCAUC CAGCAUAUAGUUUAAAGCAUACAACAUUGCUUUAAGACGUGG UGGUGGAUGUGGAUUUGCGCGGAAAUAUACUAAAGAGGAUCAGU GGCCGAAAUCAGUUACCGUUUUAAGGUAAGAAGGAAGCGGACC AACCGUGGAUUUGUUGUAAACACGAGCACACUGUUUGAUGAACUC GAAUUAAGACCCACCCGAGAUUGAACCGGGUGUCUUGAAAGUACU UCGGACAGAGAAACAUAUACUUGGGUGUGUACAUUUGGAACAUG CGCGGCUCCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCAC CUGGAAAGGGGAUGAGAAGACAAGAAACCCUACGCCCCGAGUAA CUCCUCAACCAAGAGGGGCGUGAGUUUCAUAUGUGGAUUUACCAC UCGCAUGUAUUUUCAGUUGGUGAUACGUUUAGCUUGGCAAUGC AUCUUCAGUAUAAGAUACAUGAAGCGCCAUUUGAUUUGCUGUU AGAGUGGUUGUAUGUCCCCAUUGAUCCUACAUGUCAACCAAUGC GGUUAUAUUCUACGUGUUUGUAUCAUCCCAACGCACCCCAAUGC CUCUCUCAUAUGAAUUCGGUUGUACAUUUACCUCCGCCACAUUU AGCCCAGCGUGUUGCAAGCACAGUGUAUCAGAAUUGUGAACAU CAGAUAAUCUACACCGCAUAUUGUCUGGGAAUAUCUCAUAUGGAG CCUAGCUUUGGUCUAAUCUUAACACGACGGGGGCACCACGUUAAA GUUUGUAUGAUACACCCGAGAGUUUGUCGGGAUUUAACGUUUU GUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUG UUGUAUCCACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCG UGGAUUUCCGCCAACGGCCGGUCAGCCACCGGCGACUACUAAAC CCAAGGAAAUUACCCCGUAACCCCGGAACGUCACCACUUCUA CGAU AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUU AUGUUCUGUAUAUUAUUUUAUCUGUACGGCUAAACGAUGAGG GUUAAAGCCUACAGGGUAGACAAGUCUCCUUAACAUCAGUCAU GUACUAUGCAGGACUCCUGUUGACGAUUUCGAAGACUCAGAGA GUACAGACACAGAAGAAGAAUUCGGAACGCUAUAGGUGGCUC UCACGGAGGUAGCUCGUUAUACAGUGUACAUCGAUAAAACCAGA UGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUG GGCCUCCCCCAGCCCCUCCUCCCCUUCUGCACCCCGUACCCCG UGGUCUUUGAAUAAAGUCUGAGUGGGCGGC	135
VZV_gE Oka_hlgk appa	G*GGGAAAUAAAGAGAGAAAAGAAGAGUAAGAAGAAAUAAAGA GCCACCAUGGAGACUCCCGCUCAGCUACUGUCCUCCUGCUCCU UUGGCUGCCUGAUACUACAGGCUCUGUUUUGCGGUACGACGACU UUCACAUCGAUGAGGACAAGCUCGACACUAAUAGCGUGUAUGAG CCCUACUACCAUUCAGAUACGCGGAGUCCUCUUGGGUGAACAG GGGUGAAAGUUCUAGGAAAGCCUAUGAUCACAACAGCCCUUAUA UUUGGCCACGGAAUGAUUACGACGGAUUUCUGGAAAUGCCAC GAGCAUCACGGAGUGUACAACCAGGGCCGUGGAAUCGACUCUGG GGAGAGAUUGAUGCAACCUACACAGAUGAGCGCCCAGGAAGAUC	93

	UCGGGGAUGAUACAGGAAUUCACGUUAUCCCUACAUAACGGA GAUGACCGCCACAAAUCGUCAAUGUCGAUCAAAGACAGUAUGG AGAUGUGUUCAAAGGCGAUCUCAACCCUAAAGCCGAGGGCCAGA GACUCAUUGAGGUGUCUGUCGAAGAGAACCACCCUUUCACUCUG CGCGCUCCCAUUCAGAGAAUCUAUGGAGUUCGCUAUACGGAGAC UUGGUCAUUCUCCUCCUCCUGACAUGCACCGGAGACGCCGCC CUGCCAUUCAGCACAUAGCCUGAAACAUACCACCUGUUUCCAG GAUGUGGUGGUUGAUGUUGAUUGUGCUGAAAAUACCAAGGAAG ACCAACUGGCCGAGAUUAGUUACCGGUUCCAAGGGAAAAAGGAA GCCGACCAGCCAUGGAUUGUGGUUAAUACAAGCACUCUGUUCGA UGAGCUCGAGCUGGAUCCCCCGAGAUAGAACCCGGAGUUCUGA AAGUGCUCCGGACAGAAAAACAAUAUCUGGGAGUCUACAUAUG GAACAUGCGCGGUUCCGAUGGGACCUCACUUAUGCAACCUUUC UCGUCACGUGGAAGGGAGAUAGAGAAAACUAGGAAUCCACACCC GCUGUCACACCACAGCCAAGAGGGGCGUGAGUCCAUAUGUGGAA CUAUCAUAGUCACGUGUUUAGUGUCGGAGAUACGUUUUCAUUG GCUAUGCAUCUCCAGUACAAGAUUCAUGAGGCUCCCUUCGAUCU GUUGCUUGAGUGGUUGUACGUCCCGAUUGACCCGACCUGCCAGC CCAUGCGACUGUACAGCACCUGUCUCUACCAUCCAAACGCUCCG CAAUGUCUGAGCCACAUGAACUCUGGGUGUACUUUCACCAGUCC CCACCUCGCCCAGCGGGUGGCCUCUACUGUUUACCAGAACUGUG AGCACGCCGACAACUACACCGCAUACUGCCUCGGUAUUUCAC AUGGAACCCUCCUUCGGACUCAUCCUGCAGAUUGGGGGCACUAC CCUGAAGUUCGUUGAUACGCCAGAAUCUCUGUCUGGGCUCUAUG UUUUCGUGGUCUACUCAAUGGCCAUGUCGAGGCCGUGGCCUAU ACUGUCGUUUUCUACCGUGGAUCAUUUUGUGAACGCCAUCGAAGA ACGGGGAUUCUUUUUACGGCAGGCCAGCCGCCUGCAACCACCA AGCCCAAGGAAUAACACCAGUGAACCCUGGCACCUCACCUCUC CUAAGAU AUGCCGCGUGGACAGGGGGACUGGCGGCAGUGGUGCU CCUCUGUCUGUGAUUUUCUGAUCUGUACAGCCAAGAGGAUGA GGGUCAAGGCUUAUAGAGUGGACAAGUCCCCCUACAAUCAGUCA AUGUACUACGCCGGCCUUCGGUUGAUGAUUUUGAGGAUCCGA GUCCACAGAUACUGAGGAAGAGUUCGGUAACGCUAUAGGCGGCU CUCACGGGGGUUCAAGCUACACGGUUUACAUAUGACAAGACACGC UGAUAAUAGGCUUGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUG GGCCUCCCCCAGCCCCUCCUCCCCUUCUGCACCCGUACCCCCG UGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAA AA AA AUCUAG	
VZV-GE- delete-562	G*GGGAAUAAGAGAGAAAAAGAGUAAGAAGAAUAUAAGA GCCACCAUGGGGACAGUUAUAUAAACCUUGUGGUGGGGUUAUGA UGGGGUUCGGAAUUAUCACGGGAACGUUGCGUAUAACGAAUCC GGUCAGAGCAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUG AAGACAAACUGGAUACAAACUCCGUUAUAGAGCCUUAUACCAU UCAGAUCAUGCGGAGUCUUAUGGGUAAAUCGGGGAGAGUCUU CGCGAAAAGCGUACGAUAACUACCUUAUAUAUGGCCACGU AAUGAUUAUGAUGGAUUUUAGAGAACGCACACGAACACCAUG GGGUGUAUAUAUCAGGGCCGUGGUUAUCGAUAGCGGGGAACGGUU AAUGCAACCCACACAAUUGUCUGCACAGGAGGAUCUUGGGGACG AUACGGGCAUCCACGUUAUCCCUACGUUAAACGGCGAUGACAGA CAUAAAUAUGUAAAUGUGGACCAACGUCAAUACGGUGACGUGU UUAAGGAGAUUUUAUCCAAAACCCCAAGGCCAAAGACUCAUU GAGGUGUCAGUGGAAGAAAAUCACCCGUUUACUUUACGCGCACC GAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGAGCU UUUUGCCGUCAUUAACCUGUACGGGAGACGCAGCGCCCGCCAUC CAGCAUAUAUGUUUAAACAUACAACAUGCUUUAAGACGUGG UGGUGGAUGUGGAUUGCGCGGAAAAUACUAAAGAGGAUCAGUU GGCCGAAAUCAGUUACCGUUUUAAGGUAGAAGGAAGCGGACC AACCGUGGAUUGUUGUAAACACGAGCACACUGUUUGAUGAACUC GAAUAGACCCCCCGAGAUUGAACCGGGUGUCUUGAAAGUACU UCGGACAGAAAAACAAUACUUGGGUGUGUACAUAUUGGAACAUG	94

	CGCGGCUCCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCAC CUGGAAAGGGGAUGAAAAACAAGAAACCCUACGCCCGCAGUAA CUCCUCAACCAAGAGGGGCGAGUUUCAUAUGUGGAAUUACCAC UCGCAUGUAUUUUCAGUUGGUGAUACGUUUAGCUUGGCAAUGC AUCUUCAGUAUAAGAUACAUGAAGCGCCAUUUGAUUUGCUGUU AGAGUGGUUGUAUGUCCCCAUCGAUCCUACAUGUCAACCAAUGC GGUUUAUUAUCUACGUGUUUGUAUCAUCCCAACGCACCCCAAUGC CUCUCUCAUAUGAAUUCGGGUUGUACAUUUACCUCGCCACAUUU AGCCCAGCGUGUUGCAAGCACAGUGUAUCAAUUUGUGAACAUG CAGAUAAUCUACACCGCAUAUUGUCUGGGAAUAUCUCAUAUGGAG CCUAGCUUUGGUCUAAUCUACACGACGGGGGCACCACGUUAAA GUUUGUAGAUACACCCGAGAGUUUGUCGGGAUUUAACGUUUUU GUGGUGUAUUUUAAACGGGCAUGUUGAAGCCGUAGCAUACACUG UUGUAUCCACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCG UGGAUUUCCGCCAACGGCCGGUCAGCCACCGGCGACUACUAAAC CCAAGGAAAUUACCCCGUAACCCCGGAACGUCACCACUUCUA CGAU AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUUU AUGUCUCGUAAUAUUUUUAAUCUGUACGGCUUGAUGAUAAUAG GCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCC CCAGCCCCUCCUCCCCUCCUGCACCCCGUACCCCGUGGUCUUUG AAUAAAGUCUGAGUGGGCGCAAAAAAAAAAAAAAAAAAAAAA AA AAAUCUA	
VZV-GE- delete- 562- replacedS P- withIgKap pa	G*GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAUAUAAGA GCCACCAUGGAAACCCCGGCGCAGCUGCUGUUUCUGCUGCUGCU GUGGCUGCCGGAUACCACCGGCUCUGUCUUGCGAUACGAUGAUU UUCACAU CGAUGAAGACAAACUGGAUACAAACUCCGUUAUAGAG CCUUACUACCAUUCAGAUCAUGCGGAGUCUUCUUGGGUAAAUCG GGGAGAGUCUUCGCGAAAAGCGUACGAUCAUAACUCACCUUAUA UAUGGCCACGUAAUGAUUAUGAUGGAUUUUUAGAGAACGCACA CGAACACCAUGGGGUGUAUAAUCAGGGCCGUGGUUAUCGAUAGCG GGGAACGGUUAUGCAACCCACACAAAUGUCUGCACAGGAGGAU CUUGGGGACGAUACGGGCAUCCACGUUAUCCCUACGUUAAACGG CGAUGACAGACAUAUUUUUGUAAAUGUGGACCAACGUCAAUAC GGUGACGUGUUUAAAGGAGAUUUUAUCCAAAACCCCAAGGCCA AAGACUCAUUGAGGUGUCAGUGGAAGAAAUCACCCGUUUACU UUACGCGCACCGAUUCAGCGGAUUUAUGGAGUCCGGUACACCGA GACUUGGAGCUUUUUGCCGUAUUAAACUGUACGGGAGACGCAG CGCCCGCAUCCAGCAUAUAUGUUUAAAACAUACAACAUAGCUUU CAAGACGUGGUGGUGGAUGUGGAUUGCGCGGAAAUAUCAAAG AGGAUCAGUUGGCCGAAAUCAGUUACCGUUUUAAGGUAAGAA GGAAGCGGACCAACCGUGGAUUGUUGUAAACACGAGCACACUGU UUGAUGAACUCGAAUUAGACCCCCCGAGAUUGAACCGGGUGUC UUGAAAGUACUUCGGACAGAAAAACAUAUUGGGUGUGUACA UUUGGAACAUGCGCGGCUCGGAUGGUACGUCUACCUACGCCACG UUUUUGGUCACCUGGAAAGGGGAUGAAAAACAAGAAACCCUA CGCCCCAGUAACUCCUCAACCAAGAGGGGCUGAGUUUCAUUG UGGAUUUACCACUCGCAUGUAUUUUCAGUUGGUGAUACGUUUUA GCUUGGCAAU GCAUCUUCAGUAUAAGAUACAUGAAGCGCCAUUU GAUUUGCUGUUAGAGUGGUUGUAUGUCCCCAUCGAUCCUACAUG UCAACCAAUGCGGUUAUAUUCUACGUGUUUGUAUCAUCCCAACG CACCCCAAUGCCUCUCUCAUAUGAAUUCGGUUGUACAUUUACC UCGCCACAUUUAGCCCAGCGUGUUGCAAGCACAGUGUAUCAA UUUGAACAUGCAGAUAAUACACCGCAUAUUGUCUGGGAAUA UCUCAUAUGGAGCCUAGCUUUGGUCUAAUCUUAACACGACGGGGG CACCACGUUAAAGUUUGUAGAUACACCCGAGAGUUUGUCGGGAU UAUACGUUUUUGUGGUGUAUUUUAAACGGGCAUGUUGAAGCCGU AGCAUACACUGUUGUAUCCACAGUAGAUAUUUUUGUAAACGCAA UUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUCAGCCACCGGCG ACUACUAAACCCAAGGAAAUUACCCCGUAACCCCGGAACGUC ACCACUUCUACGAUUGCCGCAUGGACCGGAGGGCUUGCAGCAG UAGUACUUUUUUGUCUCGUAAUAUUUUUAAUCUGUACGGCUUG	95

	AUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCU GGGCCUCCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCCCC GUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAA AA AA AAUCUAG	
VZV-GE- full_with_ AEAADA (SEQ ID NO: 58)	G*GGGAAUAAGAGAGAAAAAGAAGAGUAAGAAGAAUAUAAGA GCCACCAUGGGGACAGUUAUAUAACCUUGUGGUGGGGUUAUGA UGGGGUUCGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCC GGUCAGAGCAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUG AAGACAAACUGGAUACAAACUCCGUUAUAUGAGCCUUAUACCAU UCAGAUCAUGCGGAGUCUUAUGGGUAAAUCGGGGAGAGUCUU CGCGAAAAGCGUACGAUCAUAACUCACCUUAUAUAUGGCCACGU AAUGAUUAUGAUGGAUUUUAGAGAACGCACACGAACACCAUG GGGUGUAUAUAUCAGGGCCGUGGUUAUCGAUAGCGGGGAACGGUU AAUGCAACCCACACAAAUGUCUGCACAGGAGGAUCUUGGGGACG AUACGGGCAUCCACGUUAUCCCUACGUUAAACGGCGAUGACAGA CAUAAAAUUGUAAUUGUGGACCAACGUCAAUACGGUGACGUGU UUAAGGAGAUUUUAUCCAAAACCCCAAGGCCAAAGACUCAU GAGGUGUCAGUGGAAGAAAAUCACCCGUUUACUUUACGCGCACC GAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGAGCU UUUUGCCGUCAUUAACCUGUACGGGAGACGCAGCGCCCGCCAUC CAGCAUAUAUGUUUAAAACAUACAACAUUGCUUUAAGACGUGG UGGUGGAUGUGGAUUGCGCGGAAAAUACUAAAGAGGAUCAGU GGCCGAAAUCAGUUAACGUUUUAAGGUAAGAAGGAAGCGGACC AACCGUGGAUUGUUGUAAACACGAGCACACUGUUUGAUGAACUC GAAUUAAGCCCCCGAGAUUGAACCGGGUGUCUUGAAAGUACU UCGGACAGAAAAACAUAUUGGUGUGUACAUUUGGAACAUG CGCGGCUCCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCAC CUGGAAAGGGGAUGAAAAACAAGAAACCCUACGCCCCGAGUAA CUCCUCAACCAAGAGGGGCGAGUUUCAUAUGUGGAUUUACCAC UCGCAUGUAUUUUCAGUUGGUGAUACGUUUAGCUUGGCAAUGC AUCUUCAGUAUAAGAUACAUGAAGCGCCAUUUGAUUUGCUGUU AGAGUGGUUGUAUGUCCCCAUCAUCCUACAUGUCAACCAAUGC GGUUAUAUUCUACGUGUUUGUAUCAUCCCAACGCACCCCAAUGC CUCUCUCAUAUGAAUUCGGUUGUACAUUUACCUCCGCCACAUU AGCCCAGCGUGUUGCAAGCACAGUGUAUCAAUUUGUGAACAU CAGAUAAUACACCGCAUAUUGUCUGGGAAUAUCUCAUAUGGAG CCUAGCUUUGGUCUAAUCUUAACACGACGGGGGCACCACGUUAAA GUUUGUAGAUACACCCGAGAGUUUGUCGGGAUUUAACGUUUU GUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUG UUGUAUCCACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCG UGGAUUUCCGCCAACGGCCGGUCAGCCACCGGCGACUACUAAAC CCAAGGAAAUUACCCCCGUAAACCCCGGAACGUCACCACUUCUA CGAU AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUU AUGUUCUGUAUAUUAUUUUAUUCUGUACGGCUAAACGAUAGAG GUUAAAGCCUAUAGGGUAGACAAGUCCCCGUUAACCAAAGCAU GUAUUACGCUGGCCUUCAGUGGACGAUUUCGAGGACGCCGAAG CCGCCGAUGCCGAAGAAGAGUUUGGUAACGCGAUUGGAGGGAG UCACGGGGGUUCGAGUUAACCGGUGUAUAUAGAUAAAGACCCGG UGAUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCC UUGGGCCUCCCCCAGCCCCUCCUCCCCUCCUGCACCCGUACCC CCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAA AA AA AAAAUCUAG	96
VZV-GE- full_with_ AEAADA (SEQ ID NO: 58) and	G*GGGAAUAAGAGAGAAAAAGAAGAGUAAGAAGAAUAUAAGA GCCACCAUGGGGACAGUUAUAUAACCUUGUGGUGGGGUUAUGA UGGGGUUCGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCC GGUCAGAGCAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUG AAGACAAACUGGAUACAAACUCCGUUAUAUGAGCCUUAUACCAU UCAGAUCAUGCGGAGUCUUAUGGGUAAAUCGGGGAGAGUCUU	97

Y582G	CGCGAAAAGCGUACGAUCAUAACUCACCUUAUAUAUGGCCACGU AAUGAUUAUGAUGGAUUUUUAGAGAACGCACACGAACACCAUG GGGUGUAUAAUCAGGGCCGUGGUUUCGAUAGCGGGGAACGGUU AAUGCAACCCACACAAAUGUCUGCACAGGAGGAUCUUGGGGACG AUACGGGCAUCCACGUUAUCCCUACGUUAAACGGCGAUGACAGA CAUAAAAUUGUAAAUGUGGACCAACGUCAAUACGGUGACGUGU UUAAGGAGAUUUAAUCCAAAACCCCAAGGCCAAAGACUCAUU GAGGUGUCAGUGGAAGAAAAUCACCCGUUUACUUUACGCGCACC GAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGAGCU UUUUGCCGUCAUUAACCGUACGGGAGACGCAGCGCCCGCCAUC CAGCAUAUAUGUUUAAAACAUACAACAUUCUUUCAAGACGUGG UGGUGGAUGUGGAUUGCGCGGAAAUAACUAAAGAGGAUCAGUU GGCCGAAAUCAGUUACCGUUUUAAGGUAAGAAGGAAGCGGACC AACCGUGGAUUGUUGUAAACACGAGCACACUGUUUGAUGAACUC GAAUAGACCCCCCGAGAUUGAACCGGGUGUCUUGAAAGUACU UCGGACAGAAAAACAUAUACUUGGGUGUGUACAUUUGGAACAUG CGCGGCUCCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCAC CUGGAAAGGGGAUGAAAAACAAGAAACCCUACGCCCCGAGUAA CUCCUCAACCAAGAGGGGCGUGAUUUCAUAUGUGGAUUUACCAC UCGCAUGUAUUUCAGUUGGUGAUACGUUUAGCUUGGCAAUGC AUCUUCAGUAUAAGAUACAUGAAGCGCCAUUUGAUUUGCUGUU AGAGUGGUUGUAUGUCCCCAUCGAUCCUACAUGUCAACCAAUGC GGUUAUAUUCUACGUGUUUGUAUCAUCCCAACGCACCCCAUUGC CUCUCUCAUAUGAAUCCGGUUGUACAUUUACCUCGCCACAUUU AGCCCAGCGUGUUGCAAGCACAGUGUAUCAAUUUGUGAACAUUG CAGAUAAUCUACACCGCAUAUUGUCUGGGAAUAUCUCAUAUGGAG CCUAGCUUUUGGUCUAAUCUUAACACGACGGGGGCACCACGUUAAA GUUUGUAGAUACACCCGAGAGUUUGUCGGGAUUUAACGUUUUU GUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUG UUGUAUCCACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCG UGGAUUUCCGCCAACGGCCGGUCAGCCACCGGCGACUACUAAAC CCAAGGAAAUUACCCCCGUAAACCCCGGAACGUCACCACUUCUA CGAUUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUUU AUGUCUCGUAAUAUUUUUAAUCUGUACGGCUAAACGAAUGAGG GUUAAAGCCUAUAGGGUAGACAAGUCCCCGUUAACCAAAGCAU GUAUGGCGCUGGCCUUCAGUGGACGAUUUCGAGGACGCCGAAG CCGCCGAUGCCGAAGAAGAGUUUGGUAACGCGAUUGGAGGGAG UCACGGGGGUUCGAGUUAACCGGUGUAUAUAGAUAAAGACCCGG UGAUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCC UUGGGCCUCCCCCAGCCCCUCCUCCCCUCCUGCACCCCGUACCC CCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAA AA AA AAAAUCUAG	
VZV-GE- Truncated - delete_fro m_574	G*GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGA GCCACCAUGGGGACAGUUAUAAACCUUGUGGUGGGGUAAUGA UGGGGUUCGGAUUUAUCAGGGGAACGUUGCGUAUAACGAUCC GGUCAGAGCAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUG AAGACAAACUGGAUACAAACUCCGUUAUAUGAGCCUUAUACCAU UCAGAUCAUGCGGAGUCUUCUUGGGUAAAUCGGGGAGAGUCUU CGCGAAAAGCGUACGAUCAUAACUCACCUUAUAUAUGGCCACGU AAUGAUUAUGAUGGAUUUUUAGAGAACGCACACGAACACCAUG GGGUGUAUAAUCAGGGCCGUGGUUUCGAUAGCGGGGAACGGUU AAUGCAACCCACACAAAUGUCUGCACAGGAGGAUCUUGGGGACG AUACGGGCAUCCACGUUAUCCCUACGUUAAACGGCGAUGACAGA CAUAAAAUUGUAAAUGUGGACCAACGUCAAUACGGUGACGUGU UUAAGGAGAUUUAAUCCAAAACCCCAAGGCCAAAGACUCAUU GAGGUGUCAGUGGAAGAAAAUCACCCGUUUACUUUACGCGCACC GAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGAGCU UUUUGCCGUCAUUAACCGUACGGGAGACGCAGCGCCCGCCAUC CAGCAUAUAUGUUUAAAACAUACAACAUUCUUUCAAGACGUGG UGGUGGAUGUGGAUUGCGCGGAAAUAACUAAAGAGGAUCAGUU	98

	GGCCGAAAUCAGUUACCGUUUUAAGGUAAGAAGGAAGCGGACC AACCGUGGAUUGUUGUAAAACACGAGCACACUGUUUGAUGAACUC GAAUUAAGACCCCCCGAGAUUGAACCGGGUGUCUUGAAAGUACU UCGGACAGAAAAACAUAUACUUGGGUGUGUACAUUUGGAACAUG CGCGGCUCCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCAC CUGGAAAGGGGAUGAAAAACAAGAAACCCUACGCCCCGAGUAA CUCCUCAACCAAGAGGGGCUGAGUUUCAUAUGUGGAAUUAACCAC UCGCAUGUAUUUUCAGUUGGUGAUACGUUUAGCUUGGCAAUGC AUCUUCAGUAUAAGAUACAUGAAGCGCCAUUUUGAUUUGCUGUU AGAGUGGUUGUAUGUCCCCAUCAUCCUACAUGUCAACCAAUGC GGUUAUAUUCUACGUGUUUGUAUCAUCCCAACGCACCCCAAUGC CUCUCUCAUAUGAAUUCGGUUGUACAUUUACCUCCGCCACAUUU AGCCCAGCGUGUUGCAAGCACAGUGUAUCAAUUGUGAACAUG CAGAUAAUAACACCCGCAUAUUGUCUGGGAAUAUCUCAUAUGGAG CCUAGCUUUGGUCUAAUCUUAACACGACGGGGGCACCACGUUAAA GUUUGUAGAUACACCCGAGAGUUUGUCGGGAUUAUACGUUUUU GUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUG UUGUAUCCACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCG UGGAUUUCCGCCAACGGCCGGUCAGCCACCGGCGACUACUAAAC CCAAGGAAAUUACCCCGUAACCCCGGAACGUCACCACUUCUA CGAU AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUUU AUGUCUCGUAAUAUUUUUAAUCUGUACGGCUAAACGAAUGAGG GUUAAAGCCUAUAGGGUAGACAAGUGAUGAUAAUAGGCUGGAG CCUCGGUGGCCAUGCUUCUUGCCCUUGGGCCUCCCCCAGCCCC UCCUCCCCUUCUGCACCCGUACCCCGUGGUUUUGAAUAAAG UCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUCUAG	
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	GUGGUGUAUUUUAAACGGGCAUGUUGAAGCCGUAGCAUACACUG UUGUAUCCACAGUAGAUCAUUUUGUAAAACGCAAUUGAAGAGCG UGGAUUUCCGCCAACGGCCGGUCAGCCACCGGCGACUACUAAAC CCAAGGAAAUUACCCCGUAACCCCGGAACGUCACCACUUCUA CGAU AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUUU AUGUCUCGUAAUAUUUUUAAUCUGUACGGCUAAACGAAUGAGG GUUAAAGCCGCCAGGGUAGACAAGUGAUGAUAAUAGGCUGGAG CCUCGGUGGCCAUGCUUCUUGCCCUUGGGCCUCCCCCAGCCCC UCCUCCCCUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAG UCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAA AAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUCUAG	
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VZV-GI- full	G*GGGAAUAAGAGAGAAAAAGAAGAGUAAGAAGAAAUUAAGA GCCACCAUGUUUUUAAUCCAAUGUUUGAUUACGGCCGUUAUAU UUACAUACAAGUGACCAACGCUUUGAUCUUAAGGGCGACCACG UGAGCUUGCAAGUUAACAGCAGUCUCACGUCUAUCCUUAUUC AUGCAAAUAGAUAAUUAACAGAGAUAAAAGGACAGCUUGUCU UUAUUGGAGAGCAACUACCUACCGGACAAACUAUAGCGGAACA CUGGAACUGUUAUACGCGGAUACGGUGGCGUUUUGUUUCCGGUC	100

	AGUACAAGUAUAAGAUACGACCGGAUGUACCCCGGAUUAAGAACG AGCGCUUUUAAUUCGUGUAGGUACAAACAUUCGUGGCAUUAUG GUAACUCAACGGAUCGGAUAUCAACAGAGCCGGAUGCUGGUGUA AUGUUGAAAAUUAACAAACCGGGAAUAAAUGAUGCUGGUGUGU AUGUACUUCUUGUUCGGUUAAGACCAUAGCAGAUCCACCGAUGGU UUCAUUCUUGGUGUAAAUGUAUAUACAGCGGGCUCGCAUCACAA CAUUCACGGGGUUAUCUACACUUCUCCAUCUCUACAGAAUGGAU AUUCUACAAGAGCCCUUUUUAACAAGCUCGUUUGUGUGAUUUA CCC GCGACACCCAAAGGGUCCGGUACCUCUCCUGUUUCAACAUU GCUUGAUCUUCGUGCCGGUAAAUCGUUAGAGGAUAACCCUUGGU UACAUGAGGACGUUGUACGACAGAAACUAAGUCCGUUGUUA GGAGGGGAUAGAAAUCACGUUAUCCAACGGAUAUGUCCACG UUACCCGAAAAGUCCCUAAUGAUCCUCCAGAAAAUCUACUUAU AAUUAAUCCUUAAGUAGCGUCUGUCAUGAUCCUCACCGCCAUGG UUAUUGUUAUUGUAAUAAGCGUUAAGCGACGUAGAAUAAAAA ACAUCCA AUUUAUCGCCCAAAUACAAAAACAAGAAGGGGCAUAC AAAAUGCGACACCAGAAUCCGAUGUGAUGUUGGAGGCCGCCAUU GCACAACUAGCAACGAUUCGCGAAGAAUCCCCCCCACAUUCCGU UGUAAACCCGUUUGUUAUAAUAGUGAUAAUAGGCUGGAGCCUCG GUGGCCAUGCUCUUGCCCCUUGGGCCUCCCCCAGCCCCUCCUC CCCUUCCUGCACCCGUACCCCGUGGUCUUGAAUAAAGUCUGA GUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AA AA	
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	GACAGGCGGACUGGCUGCUGUGGUGCUGCUGUGCCUCGUGAUUU UCCUGAUUCUGCACCGCCAAGCGGAUGAGAGUGAAGGCCGCCAGA GUGGACAAGUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCU UGCCCCUUGGGCCUCCCCCAGCCCCUCCUCCCCUCCUGCACCC GUACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAAA AA AA AAAAAAAAAAUCUAG	
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[illegible]

	CUCCUCAACCAAGAGGGGCGUGAGUUUCAUAUGUGGAAUUAACCAU UCGCAUGUAUUUUCAGUUGGUGAUACGUUUAGCUUGGCAAUGC AUCUUCAGUAUAAGAUACAUGAAGCGCCAUUUGAUUUGCUGUU AGAGUGGUUGUAUGUCCCCAUCGAUCCUACAUGUCAACCAAUGC GGUUAUAUUCUACGUGUUUGUAUCAUCCCAACGCACCCCAAUGC CUCUCUCAUAUGAAUUCGGUUGUACAUUUACCUCGCCACAUUU AGCCCAGCGUGUUGCAAGCACAGUGUAUCAGAAUUGUGAACAU CAGAUAAUAACACCGCAUAUUGUCUGGGAAUAUCUCAUAUGGAG CCUAGCUUUGGUCUAAUCUACACGACGGGGGCACCACGUUAAA GUUUGUAGAUACACCCGAGAGUUUGUCGGGAUUAUACGUUUUU GUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUG UUGUAUCCACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCG UGGAUUUCCGCCAACGGCCGGUCAGCCACCGGCGACUACUAAAC CCAAGGAAAUUACCCCCGUAAACCCCGGAACGUCACCACUUCUA CGAU AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUUU AUGUCUCGUAAUAUUUUUAAUCUGUACGGCUAAACGAAUGAGG GUUAAAGCCGCCAGGGUAGACAAGUGAUAAUAGGCUGGAGCCUC GGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCCUCCU CCCC UUCUGCACCCGUACCCCCGUGGUCUUUGAAUAAAGUCUG AGUGGGCGGCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAA AA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUCUAG	
VZV-GE- Truncated - delete_fro m_574_- _Y569A Variant 5	G*GGGAAUAAGAGAGAAAAAGAAGAGUAAGAAGAAAUUAAGA GCCACCAUGGGGACAGUUAUAAACCGUGGGUGGGGGUUAUGA UGGGGUUCGGAAUUAUCACGGGAACGUUGCGUAUAACGAAUCC GGUCAGAGCAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUG AAGACAAACUGGAUACAACUCCGUUAUAGAGCCUUAUACCAU UCAGAUACUGCGGAGUCUUAUGGGUAAAUCGGGAGAGUCUU CGCGAAAGGCGUACGAUUAACUCACCUUAUAUAGGGCCAGU AAUGAUUAUGAUUGGAUUUUUAGAGAACGCACACGAACACCAUG GGGUGUAUAUACAGGGCCGUGGUUAUCGAUAGCGGGGAACGGUU AAUGCAACCCACACAAAUGUCUGCACAGGAGGAUCUUGGGGACG AUACGGGCAUCCACGUUAUCCCUACGUUAAACGGCGAUGACAGA CAUAAGAUAUGUAAAUGUGGACCAACGUCAAUACGGUGACGUGU UUAAGGAGAUUUAAUCCAAAGCCCCAAGGCCAAAGACUCAUU GAGGUGUCAGUGGAAGAGAAUACCCGUUUACUUUACGCGCACC GAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGAGCU UUUUGCCGUCAUUAACCGUACGGGAGACGCAGCGCCCGCCAUC CAGCAUAUAUGUUUAAAGCAUACAACAUAGCUUUAAGACGUGG UGGUGGAUGUGGAUUGCGCGGAGAAUACUAAAGAGGAUCAGUU GGCCGAAAUACAGUUAACCGUUUUAAGGUAAGAAGGAAGCGGACC AACCGUGGAUUGUUGUAAACACGAGCACACUGUUUGAUGAACUC GAAUUAAGACCCACCCGAGAUUGAACC GGGUGUCUUGAAAGUACU UCGGACAGAGAAACAUAUACUUGGGUGUGUACAUUUGGAACAUG CGCGGCUCCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCAC CUGGAAAGGGGAUGAGAAGACAAGAAACCCUACGCCCGCAGUAA CUCCUCAACCAAGAGGGGCGUGAGUUUCAUAUGUGGAAUUAACCA UCGCAUGUAUUUUCAGUUGGUGAUACGUUUAGCUUGGCAAUGC AUCUUCAGUAUAAGAUACAUGAAGCGCCAUUUGAUUUGCUGUU AGAGUGGUUGUAUGUCCCCAUCGAUCCUACAUGUCAACCAAUGC GGUUAUAUUCUACGUGUUUGUAUCAUCCCAACGCACCCCAAUGC CUCUCUCAUAUGAAUUCGGUUGUACAUUUACCUCGCCACAUUU AGCCCAGCGUGUUGCAAGCACAGUGUAUCAGAAUUGUGAACAU CAGAUAAUAACACCGCAUAUUGUCUGGGAAUAUCUCAUAUGGAG CCUAGCUUUGGUCUAAUCUACACGACGGGGGCACCACGUUAAA GUUUGUAGAUACACCCGAGAGUUUGUCGGGAUUAUACGUUUUU GUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUG UUGUAUCCACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCG UGGAUUUCCGCCAACGGCCGGUCAGCCACCGGCGACUACUAAAC CCAAGGAAAUUACCCCCGUAAACCCCGGAACGUCACCACUUCUA CGAU AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUUU AUGUCUCGUAAUAUUUUUAAUCUGUACGGCUAAACGAAUGAGG	105

[illegible]

	AAUGCAACCCACACAAAUGUCUGCACAGGAGGAUCUUGGGGACG AUACGGGCAUCCACGUUAUCCCUACGUUAAACGGCGAUGACAGA CAUAAAAUUGUAAAUGUGGACCAACGUCAAUACGGUGACGUGU UAAAAGGAGAUUCUAAUCCAAAACCCCAAGGCCAAAGACUCAU GAGGUGUCAGUGGAAGAAAAUCACCCGUUUACUUUACGCGCACC GAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGAGCU UUUUGCCGUCAUUAACCUGUACGGGAGACGCAGCGCCCGCCAUC CAGCAUAUAUGUUUAAAGCAUACAACAUGCUUUAAGACGUGG UGGUGGAUGUGGAUUGCGCGGAAAAUACUAAAGAGGAUCAGUU GGCCGAAAUCAGUUACCGUUUUAAGGUAAGAAGGAAGCGGACC AACCGUGGAUUGUUGUAAACACGAGCACACUGUUUGAUGAACUC GAAUAGACCCCCCGAGAUUGAACCGGGUGUCUUGAAAGUACU UCGGACAGAGAAACAUAUACUUGGGUGUGUACAUUUGGAACAUG CGCGGCUCCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCAC CUGGAAAGGGGAUGAGAAGACAAGAAACCCUACGCCCCGAGUAA CUCCUCAACCAAGAGGGGCGUGAGUUUCAUAUGUGGAAUUACCAC UCGCAUGUAUUUUCAGUUGGUGAUACGUUAGCUUGGCAAUGC AUCUUCAGUAUAAGAUACAUGAAGCGCCAUUUGAUUUGCUGUU AGAGUGGUUGUAUGUCCCCAUCAUCCUACAUGUCAACCAAUGC GGUUAUAUUCUACGUGUUUGUAUCAUCCCAACGCACCCCAAUGC CUCUCUCAUAUGAAUUCGGUUGUACAUUUACCUCGCCACAUUU AGCCCAGCGUUGCAAGCACAGUGUAUCAGAAUUGUGAACAUC CAGUAACUACACCGCAUAUUGUCUGGGAAUAUCUCAUUGGAG CCUAGCUUUGGUCUAAUCUACACGACGGGGGCACCACGUUAAA GUUUGUAGAUACACCCGAGAGUUUGUCGGGAUUUAACGUUUU GUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUG UUGUAUCCACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCG UGGAUUUCCGCCAACGGCCGGUCAGCCACCGGCGACUACUAAAC CCAAGGAAAUUACCCCGUAACCCCGGAACGUCACCACUUCUA CGAUUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUU AUGUCUCGUAAUAUUUUUAUCUGUACGGCUAAACGAAUGAGG GUUAAAGCCGCCAGGGUAGACAAGUGAUAAUAGGCUGGAGCCUC GGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCCUCCU CCCCUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUCUG AGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUCUAG	
VZV-GE- Truncated - delete from m_574 - _Y569A Variant 7	G*GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGA GCCACCAUGGGGACAGUUAUAUAAACCUUGUGGUGGGGGUAUUGA UGGGGUUCGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCC GGUCAGAGCAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUG AAGACAAACUGGAUACAAACUCCGUUAUUGAGCCUUAUACCAU UCAGAUCAUGCGGAGUCUUAUGGGUAAAUCGGGGAGAGUCUU CGCGAAAAGCGUACGAUCAUAACUCACCUUAUAUAUGGCCACGU AAUGAUUAUGAUGGAUUUUUAGAGAACGCACACGAACACCAUG GGGUGUAUAAUCAGGGCCGUGGUUAUCGAUAGCGGGGAACGGUU AAUGCAACCCACACAAAUGUCUGCACAGGAGGAUCUUGGGGACG AUACGGGCAUCCACGUUAUCCCUACGUUAAACGGCGAUGACAGA CAUAAAAUUGUAAAUGUGGACCAACGUCAAUACGGUGACGUGU UAAAAGGAGAUUCUAAUCCAAAACCCCAAGGCCAAAGACUCAU GAGGUGUCAGUGGAAGAAAAUCACCCGUUUACUUUACGCGCACC GAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGAGCU UUUUGCCGUCAUUAACCUGUACGGGAGACGCAGCGCCCGCCAUC CAGCAUAUAUGUUUAAAGCAUACAACAUGCUUUAAGACGUGG UGGUGGAUGUGGAUUGCGCGGAAAAUACUAAAGAGGAUCAGUU GGCCGAAAUCAGUUACCGUUUUAAGGUAAGAAGGAAGCGGACC AACCGUGGAUUGUUGUAAACACGAGCACACUGUUUGAUGAACUC GAAUAGACCCACCCGAGAUUGAACCGGGUGUCUUGAAAGUACU UCGGACAGAGAAACAUAUUGGGUGUGUACAUAUUGGAACAUG CGCGGCUCCGAUGGUACGUCUACCUACGCCACGUUUUUGGUCAC CUGGAAAGGGGAUGAGAAGACAAGAAACCCUACGCCCCGAGUAA CUCCUCAACCAAGAGGGGCGUGAGUUUCAUAUGUGGAAUUACCAC	107

[illegible]

	UCCUGACCCCGUACCCCGUGGUCUUUGAAUAAAGUCUGAGUGG GCGGC	
VZV-GE- Truncated - delete_fro m_574_- Y569A Variant 8	G*AGAAGAAAUAUAAGAGCCACCAUGGGGACAGUUAUAUAACCU GUGGUGGGCGUAUUGAUGGGGUUCGGAAUUAUCACGGGAACGU UGCGUAUAACGAAUCCGGUCAGAGCAUCCGUCUUGCGAUACGAU GAUUUUCACAUCGAUGAAGACAAACUGGAUACAAACUCCGUUA UGAGCCUUAUACCAUUCAGAUCAUGCGGAGUCUUAUGGGUAA AUCGGGGAGAGUCUUCGCGAAAGGCGUACGAUCAUAACUCACCU UAUAUAUGGCCACGUAAUGAUUAUGAUGGAUUCUUAAGAGAACG CACACGAACACCAUGGGGUGUAUAAUCAGGGCCGUGGUUAUCGAU AGCGGGGAACGGUUAUUGCAACCCACACAAUGUCUGCACAGGA GGAUCUUGGGGACGAUACGGGCAUCCACGUUAUCCCUACGUUAA ACGGCGAUGACAGACAUAAAGAUUGUAAAUGUGGACCAACGUCA AUACGGUGACGUGUUUAAAGGAGAUUUAAUCCAAAGCCCCAAG GCCAAAGACUCAUUGAGGUGUCAGUGGAAGAGAAUACCCCGUUU ACUUUACGCGCACCGAUUCAGCGGAUUUAUGGAGUCCGGUACAC CGAGACUUGGAGCUUCUUGCCGUCAUUAACCGUACGGGAGACG CAGCGCCCGCCAUCCAGCAUAUAUGUUUAAAGCAUACAACAU UUUCAAGACGUGGUGGUGGAUGUGGAUUGCGCGGAGAAUACUA AAGAGGAUCAGUUGGCCGAAAUACGUUACCGUUUUAAGGUAA GAAGGAAGCGGACCAACCGUGGAUUGUUGUAAACACGAGCACAC UGUUUGAUGAACUCGAAUUAAGACCCACCCGAGAUUGAACCGGU GUCUUGAAAGUACUUCGGACAGAGAAACAAUACUUGGGUGUGU ACAUUUGGAACAUGCGCGGCUCCGAUGGUACGUCUACCUACGCC ACGUUCUUGGUCACCUGGAAAGGGGAUGAGAAGACAAGAAACCC UACGCCCAGUAACUCCUCAACCAAGAGGGGCUGAGUUUCAUA UGUGGAAUUAACACUCGCAUGUAUUUUCAGUUGGUGAUACGUU UAGCUUUGGCAUUGCAUUCAGUAUAAGAUACAUGAAGCGCCAU UUGAUUUGCUGUUAAGAGUGGUUGUAUGUCCCAUGCAUCCACA UGUCAACCAUUGCGGUUAUAUUCUACGUGUUUGUAUCAUCCAA CGCACCCCAUUGCCUCUCUCAUAUGAAUUCGGUUGUAUUAUUA CCUCGCCACAUAUUAAGCCAGCGUGUUGCAAGCACAGUGUAUCAG AAUUGUGAACAUGCAGAUAAUACACCGCAUAUUGUCUGGGAA UAUCUCAUAUGGAGCCUAGCUUUGGUCUAAUCUUAACGACGGA GGCACACGUUAAAGUUUGUAGAUACACCCGAGAGUUUGUCGGG AUUAUACGUCUUGUGGGUGUAUUUUAACGGGCAUGUUGAAGCC GUAGCAUACACUGUUGUAUCCACAGUAGAUCAUUUUGUAAACGC AAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUCAGCCACCGG CGACUACUAAACCCAAGGAAAUACGCCCUGAAACCCCGGAACG UCACCACUUCUACGAUAUGCCGCAUGGACCGGAGGGCUUGCAGC AGUAGUACUUUUUGUCUCGUAAUAUUCUUAUCUGUACGGCU AAACGAAUGAGGGUUAAGCCGCCAGGGUAGACAAGUGAUAAU AGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCC CCCCAGCCCCUCCUCCCCUUCUGCACCCGUACCCCGUGGUCUU UGAAUAAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAA AAA AAUCUAG	108
VZV-GE- Truncated - delete_fro m_574_- Y569A Variant 8 (5'UTR includes promoter region)	G*GGGAAUAAGAGAGAAAAAGAGUAAGAAGAAAUAUAAGA GCCACCAUGGGGACAGUUAUAUAACCUUGGUGGGCGUAUUGA UGGGGUUCGGAAUUAUCACGGGAACGUUGCGUAUAACGAAUCC GGUCAGAGCAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUG AAGACAAACUGGAUACAAACUCCGUUAUUGAGCCUUAUACCAU UCAGAUCAUGCGGAGUCUUAUGGGUAAAUCGGGGAGAGUCUU CGCGAAAGGCGUACGAUCAUAACUCACCUUAUAUAUGGCCACGU AAUGAUUAUGAUGGAUUCUUAAGAGAACGCACACGAACACCAUG GGGUGUAUAAUCAGGGCCGUGGUUAUCGAUAGCGGGGAACGGU AAUGCAACCCACACAAUUGUCUGCACAGGAGGAUUCUUGGGACG AUACGGGCAUCCACGUUAUCCCUACGUUAACACGGCGAUGACAGA CAUAAGAUUGUAAAUGUGGACCAACGUAUACGGUGACGUGU UUAAGGAGAUUUUAUCCAAAGCCCCAAGGCCAAAGACUCAU GAGGUGUCAGUGGAAGAGAAUACCCGUUUACUUUACGCGCACC GAUUCAGCGGAUUUAUGGAGUCCGGUACACCGAGACUUGGAGCU	141

	UCUUGCCGUCUAUUAACCGUACGGGAGACGCGGACGCCGCCGCCCAUC CAGCAUAUAUGUUUAAAAGCAUACAACAUGCUIUCAAGACGUGG UGGUGGAUGUGGAUUGCGCGGAGAAUACUAAAGAGGAUCAGUU GGCCGAAAUCAGUUAACCGUUUUAAGGUAAGAAGGAAGCGGACC AACCGUGGAUUGUUGUAAACACGAGCACACUGUUUGAUGAACUC GAAUUAAGACCCACCCGAGAUUGAACCGGGUGUCUUGAAAGUACU UCGGACAGAGAAACAUAUACUUGGGUGUGUACAUIUGGAACAUG CGCGGCUCCCGAUGGUACGUCUACCUACGCCACGUUCUUGGUCAC CUGGAAAGGGGAUGAGAAGACAAGAAACCCUACGCCCGCAGUAA CUCCUCAACCAAGAGGGGCUGAGUUUCAUUAUGUGGAAUUAACCAC UCGCAUGUAUUUUCAGUUGGUGAUACGUUUAAGCUUGGCAAUGC AUCUUCAGUAUAAGAUACAUGAAGCGCCAUIUGAUUUGCUGUU AGAGUGGUUGUAUGUCCCCAUCGAUCCUACAUGUCAACCAAUGC GGUUAUAUUCUACGUGUUUGUAUCAUCCCAACGCACCCCAAUGC CUCUCUCAUAUGAAUUCGGUUGUACAUIUUAACUCGCCACAUIU AGCCCAGCGUGUUGCAAGCACAGUGUAUCAGAAUUGUGAACAU CAGAUAAUAACACCCGCAUAUUGUCUGGGAAUAUCUCAUAUGGAG CCUAGCUUUGGUCUAAUCUUAACGACGCGAGGCACACGUUAAA GUUUGUAGAUACACCCGAGAGUUUGUCGGGAUUAUACGUCUUU GUGGUGUAUUUUAACGGGCAUGUUGAAGCCGUAGCAUACACUG UUGUAUCCACAGUAGAUCAUUUUGUAAACGCAAUUGAAGAGCG UGGAUUUCCGCCAACGGCCGUCAGCCACCGGCGACUACUAAAC CCAAGGAAAUACGCCCGUAAACCCCGGAACGUCACCACUUCUA CGAU AUGCCGCAUGGACCGGAGGGCUUGCAGCAGUAGUACUUU AUGUCUCGUAAUAUUCUUAUUCUGUACGGCUAAACGAAUGAGG GUUAAAGCCGCCAGGUGAGACAAGUGAUAAUAGGCUAGGCCUC GGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCCCCUCCU CCCCUCCUGCACCCCGUACCCCGUGGUCUUUGAAUAAAGUCUG AGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AA AA	
VZV-GE- Truncated - delete_from_574_Y569A Variant 9	G*GGGAAUAAGAGAGAAAAAGAGUAAGAAGAAUAUAAGA GCCACCAUGGGCACCCGUGAACAAAGCCUGUUGUGGGCGUGCUGAU GGGCUUCGGCAUCAUCACAGGCACCCUGCGGAUCACCAAUCCUG UGCGGGCUAGCGUGCUGAGAUACGACGACUUCACAUAGCAGAG GACAAGCUGGACACCAACAGCGUGUACGAGCCCUACUACCACAG CGAUCACGCCGAGUCUAGCUGGGUCAACAGAGGCGAGAGCAGCA GAAAGGCCUACGACCACAACAGCCCUUACAUCUGGCCCAGAAAC GACUACGACGGCUUCCUCGAGAAUGCCCACGAACACCACGGCGU GUACAUAUAAGGCAGAGGCAUCGACAGCGGCGAGAGACUGAUGC AGCCUACACAGAUGAGCGCCCAAGAGGACCUGGGAGAUGAUACC GGCAUCCACGUGAUCCCUACACUGAACGGCGACGACCGGCACAA GAUCGUGAACGUGGACCAGAGACAGUACGGCGACGUGUUAAGG GCGACCUGAAUCCUAAGCCUCAGGGCCAGCGCCUGAUCGAGGUU UCCGUGGAAGAGAAUACCCCUUACACUUGCGGGCUCCCAUCCA GAGAAUCUACGGCGUGCGCUAUACCGAGACAUGGUCCUUUCUGC CCAGCCUGACAUGUACCGGCGACGCCGCUCCUGCCAUCCAGCAC AUUUGUCUGAAGCACACCACCUUUAUCCAGGACGUGGUGGUGGA UGUGGACUGCGCCGAGAACACCAAAGAGGAUCAGCUGGCCGAGA UCAGCUACCGGUUCCAGGGAAAGAAAGAGGCCGACCAGCCUUGG AUCGUGGUCAACACCAGCACACUGUUCGACGAGCUGGAACUGGA CCUCCUGAGAUUGAACCCGGCGUCCUGAAGGUGCUGAGAACCG AGAAGCAGUACCUGGGAGUGUACAUCUGGAACAUGAGAGGCAG CGACGGCACCUUACCUACGCCACCUUUCUGGUCACAUGGAAGG GCGACGAGAAGACCAGAAAUCCACACCAGCCGUGACACCUCAG CCUAGAGGCGCCGAUUUCACAUGUGGAACUACCACUCUCACGU GUUCAGCGUGGGCGAUACCUACGCCUGGCCAUGCAUCUGCAGU ACAAGAUCCACGAGGCUCCUUCGACCCUUGCUGGAUUGGCUG UACGUGCCCAUCGAUCCUACCUGCCAGCCUUGCGGCUGUACUC CACCUGUCUGUAUACCCUAACGCUCUCCUAGUGCCUGAGCCACA UGAAUAGCGGCUGCACCUUACAAAGCCUACCUUGGCUCAGCGA GUGGCCAGCACAGUGUACCAGAAUUGCGAGCACGCCGACAAUA	137

	CACCGCCUACUGUCUGGGCAUCAGCCACAUGGAACCUAGCUUCG GCCUGAUCCUGCACGAUGGCGGCACCACACUGAAGUUCGUGGAC ACACCUGAGAGCCUGAGCGGCCUGUAUGUGUUUGUGGUGUACUU CAACGGCCACGUGGAAGCCGUGGCCUACACCGUGGUGUCUACCG UGGACCACUUCGUGAACGCCAUCGAGGAAAAGAGGCUUCCCUCCA ACUGCUGGACAGCCUCCUGGCCACCACCAAGCCUAAAGAAAUCAC ACCCGUGAAUCCCGGCACUAGCCCUUGCUUAGAUACGCCGCUU GGACAGGCGGACUGGCUGCUGUUGUUCUGCUGUGCCUGGUAUC UUCUGAUCUGCACCGCCAAGCGGAUGAGAGUGAAAGCCGCCAG AGUGGACAAGUGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUC UUGCCCCUUGGGCCUCCCCCAGCCCCUCCUCCCUUCCUGCACC CGUACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGCAAAA AA AA AAAAAAAAAAUCUAG	
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G* represents a 5' terminal cap, e.g., 7mG(5')ppp(5')NimpNp

All mRNAs contains a 5'-UTR, a 3'UTR, and a polyA tail.

5'-UTR: GGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGAGCCACC (SEQ ID NO: 138)

5 3'-UTR: UGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCC
AGCCCCUCCUCCCUUCCUGCACCCGUACCCCCGUGGUCUUUGAAUAAAGUCUGAGUGGGCGGC
(SEQ ID NO: 139)

polyA tail: AA
AAAUCUAG (SEQ ID NO:
10 140)

It should also be understood that the 5' and/or 3' UTR for each construct may be omitted, modified or substituted for a different UTR sequences in any one of the vaccines as provided herein.

Example 14: Variant gE Antigen Distribution in Vero and Mewo Cells

The expression and trafficking of VZV gE antigens having different C terminal variants was investigated in Vero cells and Mewo cells.

Vero cells are lineages of cells used in cell cultures. The 'Vero' lineage was isolated from kidney epithelial cells extracted from an African green monkey. MeWo cells are human malignant melanoma cells that are susceptible to VZV infection. Vero cells or Mewo cells were transfected with the constructs indicated below in Table 3. The transfected cells were stained with antibodies for gE and for golgi markers GM 130 and golgin. Confocal microscopy was used to visualize the stained cells. The results for the constructs are described in Table 3 ("Cellular localization" column). Fig. 9 provides an exemplary experiment, which shows the results of the following transfected constructs: (1) VZV gE mRNA encoding a VZV gE polypeptide having a 62 amino acid deletion at the C-terminus (encoded by SEQ ID NO: 3); (2) full-length VZV gE mRNA encoding a VZV gE polypeptide having the AEAADA sequence (SEQ ID NO: 58) (encoded by SEQ ID NO: 7); or (3) PBS (as negative control). Using an anti-gE antibody, Fig. 9 shows that the truncated VZV gE polypeptide (having the 62 amino acid C-terminal deletion) localizes to a perinuclear location and organelles. The full-length VZV gE polypeptide having AEAADA

sequence (SEQ ID NO: 58) was localized to the golgi and a perinuclear location.

Importantly, several of the constructs, *e.g.*, gE-truncated-delete_from_574_Y569A, gE full length with AEAADA (SEQ ID NO: 58), gE full length with AEAADA (SEQ ID NO: 58)

and Y582C mutation, gE-truncated-delete_from_574, and gE-truncated-delete_from_574

- 5 with Y569A mutation each encoded polypeptides that localized to the cell membrane, indicating that these polypeptides may have enhanced antigenicity.

Table 3. Summary of Results for Cellular Trafficking of Variant VZV gE Polypeptides

Construct	Experimental conditions	Expression	Cellular localization
Full length gE	Vero cells- 500ng/well, transfected 24 h transfection (C8) Construct =Full length GE	+	shows Golgi localization
GE-full with AEAADA (SEQ ID NO: 58)	Vero cells- 500ng/well, transfected 24 h transfection (C1)- Construct = VZV-GE-full with AEAADA (SEQ ID NO: 58)	+++	shows Golgi localization and diffuse perinuclear
GE-full with AEAADA (SEQ ID NO: 58) and Y582C	Vero cells- 500ng/well, transfected 24 h transfection (C6) Construct = VZV-GE full_with_AEAADA (SEQ ID NO: 58) and_Y582G	low	shows organelles and cytoplasmic localization
GE-delete-562	Vero cells- 500ng/well, transfected 24 h transfection (C2)- Construct =C2 VZV-GE-delete-562	+	shows perinuclear and organelles
GE-delete-562-replaced SP-with IgKappa	Vero cells- 500ng/well, transfected 24 h transfection (C5) VZV-GE-delete-562-replacedSignal Peptide-with IgKappa	+++	shows golgi localization and cytoplasmic
GE-truncated-delete_from_574	Vero cells- 500ng/well, transfected 24 h transfection (C4) - Construct = VZV-GE-truncated-delete_from_574	++	shows Golgi and cytoplasmic localization
GE-truncated-delete_from_574_Y569A	Vero cells- 500ng/well, transfected 24 h transfection (C3)- Construct = VZV-GE-truncated-delete_from_574_Y569A	+++	shows Golgi and cell membrane localization
Full length gE	MeWo cells- 500ng/well, transfected 24 h transfection (C8) Construct =Full length GE	+++	shows Golgi localization
GE-full with AEAADA (SEQ ID NO: 58)	MeWo cells- 500ng/well, transfected 24 h transfection (C1)- Construct = VZV-GE-full with AEAADA (SEQ ID NO: 58)	+++	shows Golgi and Membrane localization
GE-full with AEAADA (SEQ ID NO: 58) and Y582C	MeWo cells- 500ng/well, transfected 24 h transfection (C6) Construct = SE-VZV-GE full_with_AEAADA (SEQ ID NO: 58) and_Y582G	++	shows golgi and cell membrane localization

Construct	Experimental conditions	Expression	Cellular localization
GE-delete-562	MeWo cells- 500ng/well, transfected 24 h transfection (C2)- Construct =C2 VZV-GE-delete-562	+++	shows perinuclear and cytoplasmic localization
GE-delete-562-replaced SP-with Ig Kappa	MeWo cells- 500ng/well, transfected 24 h transfection (C5) VZV-GE-delete-562-replacedSignal Peptide with IgKappa	+++	shows golgi localization and cytoplasmic
GE-truncated-delete_from_574	MeWo cells- 500ng/well, transfected 24 h transfection (C4) - Construct = -VZV-GE-truncated-delete_from_574	+++	shows Golgi and cell membrane localization
GE-truncated-delete_from_574_Y569A	MeWo cells- 500ng/well, transfected 24 h transfection (C3)- Construct = VZV-GE-truncated-delete_from_574_Y569A	+++	shows Golgi and cell membrane localization

Example 15: Immunization of BALB/C Mice with MC3 formulated mRNA encoded VZV gE antigens

5 An immunization study was conducted as an initial evaluation of the effect of MC3-formulated mRNAs encoding VZV antigens as vaccine candidates to achieve immunization in BALB/C mice post intramuscular or intradermal administration.

The candidate vaccines were as follows:

(1) MC3 formulated VZVgE-hIg kappa mRNA having 5' cap: m7G(5')ppp(5')G-2'-O-methyl, N1-methylpseudouridine chemical modification, and the additional hIg Kappa
10 sequence.

(2) MC3 formulated VZV gE mRNA having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification.

(3) MC3 formulated VZVgE mRNA having 5' cap: m7G(5')ppp(5')G-2'-O-methyl
15 and no chemical modification.

All of the VZV gE mRNAs were strain Oka.

BALB/C mice were given a single 10 µg dose or two 10 µg doses (at day 28) of MC3 formulated VZV gE mRNA (either vaccine (1), (2), or (3) described above) either intramuscularly or intradermally. G5 refers mRNA having N1-methylpseudouridine
20 chemical modification. G0 refers to unmodified mRNA. Cap 1 refers to 5' cap: m7G(5')ppp(5')G-2'-O-methyl. Each treatment group contained eight mice. The positive control was VARIVAX[®] vaccine and the negative control was PBS.

Blood samples were taken to determine the presence/level of serum protein and antibodies. Western blots were performed to detect VZV-gE protein expression at six hours

and ELISAs were performed to detect mouse IgGs. Schematics of the constructs encoding VZV gE (strain Oka) are shown in Fig. 2. Schematics of the study's design and schedule of injection are shown in Fig. 3 and Table 3. Table 4 shows the various time points for collection of different samples. Blood was collected for serum protein and antibody determination, while VZV protein expression was surveyed 6 hours post-dosing on day 0 for groups 1-4, 13, and 14, and 6 hours post-dosing on day 28 for groups 2, 4, and 14. Antibody detection assays were performed on day -3, day 14, day 27, day 42, and day 56.

Table 4: Injection Schedule

G#	Antigen	Route	N=	Dose (µg)	Dose Vol (µl)	1 st dose	2 nd dose	LNP	mRNA Conc. (mg/ml)	Volume +Overage
1	VZV-gE-oka-hIgkappa (G5; cap1) (SEQ ID NO: 93)	IM	8	10	50	Day 0		MC3	0.2	1x 600 µl
2	VZV-gE-oka-hIgkappa (G5; cap1)	IM	8	10	50	Day 0	Day 28	MC3	0.2	2 x600 µl
3	VZV-gE-oka-hIgkappa (G5; cap1)	ID	8	10	50	Day 0		MC3	0.2	1x 600 µl
4	VZV-gE-oka-hIgkappa (G5; cap1)	ID	8	10	50	Day 0	Day 28	MC3	0.2	2 x600 µl
5	VZV-gE-oka (G0; cap1) (SEQ ID NO: 92)	IM	8	10	50	Day 0		MC3	0.2	1x 600 µl
6	VZV-gE-oka (G0; cap1)	IM	8	10	50	Day 0	Day 28	MC3	0.2	2 x600 µl
7	VZV-gE-oka (G0; cap1)	ID	8	10	50	Day 0		MC3	0.2	1x 600 µl
8	VZV-gE-oka (G0; cap1)	ID	8	10	50	Day 0	Day 28	MC3	0.2	2 x600 µl
9	VZV-gE-oka (G5; cap1)	IM	8	10	50	Day 0		MC3	0.2	1x 600 µl
10	VZV-gE-oka (G5; cap1)	IM	8	10	50	Day 0	Day 28	MC3	0.2	2 x600 µl
11	VZV-gE-oka (G5; cap1)	ID	8	10	50	Day 0		MC3	0.2	1x 600 µl
12	VZV-gE-oka (G5; cap1)	ID	8	10	50	Day 0	Day 28	MC3	0.2	2 x600 µl
13	Negative control (PBS)	IM	6	/	50	Day 0		PBS	/	1x 600 µl
14	Negative control (PBS)	IM	6	/	50	Day 0	Day 28	PBS	/	2 x600 µl

G#	Antigen	Route	N=	Dose (µg)	Dose Vol (µl)	1 st dose	2 nd dose	LNP	mRNA Conc. (mg/ml)	Volume +Overage
15	Positive control (VARIVAX [®])	SC	6	54 (pfu)	50	Day 0			/	1x1250µl
16	Positive control (VARIVAX [®])	SC	6	54 (pfu)	50	Day 0	Day 28		/	
17	Positive control (VARIVAX [®])	SC	4	675 (pfu)	100	Day 0			/	4x220µl
18	Positive control (VARIVAX [®])	SC	4	675 (pfu)	100	Day 0	Day 28		/	

Table 5: Schedule of Sample Collection

G#	Antigen	Pre-bleed	Day0 +6h	Day 14	Day27	Day28 +6h	Day 42	Day 56
1	VZV-gE-oka-hlgkappa (G5; cap1)	√	√	√	√		√	√
2	VZV-gE-oka-hlgkappa (G5; cap1)	√	√	√	√	√	√	√
3	VZV-gE-oka-hlgkappa (G5; cap1)	√	√	√	√		√	√
4	VZV-gE-oka-hlgkappa (G5; cap1)	√	√	√	√	√	√	√
5	VZV-gE-oka (G0; cap1)	√		√	√		√	√
6	VZV-gE-oka (G0; cap1)	√		√	√		√	√
7	VZV-gE-oka (G0; cap1)	√		√	√		√	√
8	VZV-gE-oka (G0; cap1)	√		√	√		√	√
9	VZV-gE-oka (G5; cap1)	√		√	√		√	√
10	VZV-gE-oka (G5; cap1)	√		√	√		√	√
11	VZV-gE-oka (G5; cap1)	√		√	√		√	√
12	VZV-gE-oka (G5; cap1)	√		√	√		√	√
13	PBS	√	√	√	√		√	√
14	PBS	√	√	√	√	√	√	√
15	Positive control	√		√	√		√	√
16	Positive control	√		√	√		√	√
17	Positive control	√		√	√		√	√
18	Positive control	√		√	√		√	√

Example 16: Immunogenicity Study - ELISA

The instant studies were designed to test the immunogenicity in BALB/C mice of candidate VZV vaccines comprising a mRNA polynucleotide encoding glycoprotein gE from VZV. Mice were immunized with various VZV mRNA vaccine formulations at set intervals, and sera were collected after each immunization. The immunization schedule is provided in Table 2 of Example 15. The sera collection schedule is set forth in Table 4 of Example 15.

Enzyme-linked immunosorbent assay (ELISA)

Serum antibody titers against VZV glycoprotein E were determined by Enzyme-linked immunosorbent assay (ELISA) using standard methods. In one study, the amount of anti-VZV gE mouse IgG was measured in the pre-bleed and in serum collected at day 14 and day 42 post vaccination in mice vaccinated intramuscularly with two 10µg doses of either: (1) VZV-gE-hIgkappa (SEQ ID NO: 93) having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification (#1 in Tables 2 and 3); (2) VZV-gE (SEQ ID NO: 135) having 5' cap: m7G(5')ppp(5')G-2'-O-methyl, and no chemical modification (#6 in Tables 2 and 3) (SEQ ID NO: 135); (3) VZV-gE having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification (#10 in Tables 2 and 3); (4) VARIVAX[®] vaccine (positive control); or (5) PBS (negative control).

Figs. 5-7 show that there was a very strong immune response with all mRNA encoded VZV-gE vaccines tested relative to the current VARIVAX[®] vaccine. Fig. 5 shows that at day 14, the titer for anti-VZV-gE IgG was about 10µg/mL in the serum of mice vaccinated with vaccine candidate (1) VZV-gE-hIgkappa having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification and about 50 µg/mL in the serum of mice vaccinated with vaccine candidate (3) VZV-gE having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification. The level of anti-VZV-gE IgG in the serum of mice vaccinated with VARIVAX[®] was not detectable at day 14. At day 42, the amount of anti-VZV-gE IgG present in the serum of mice vaccinated with vaccine candidate (1) VZV-gE-hIgkappa having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification or vaccine candidate (3) VZV-gE having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification was almost 1000-fold greater than the amount of anti-VZV-gE IgG present in the serum of mice vaccinated with VARIVAX[®]. The amount of anti-VZV gE IgG present in the serum of mice vaccinated with vaccine candidate (2) VZV-gE having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and no chemical modification was almost 100-fold greater than the amount of anti-VZV-gE IgG present in the serum of mice vaccinated with VARIVAX[®]. This study indicates that

each of the VZV gE mRNA vaccines tested is a more immunogenic vaccine than the current VARIVAX[®] VZV vaccine.

Example 17: Immunogenicity Study- ELISA

The instant studies were designed to test the immunogenicity in BALB/C mice of candidate VZV vaccines comprising a mRNA polynucleotide encoding glycoprotein gE from VZV. Mice were immunized with various VZV mRNA vaccine formulations at set intervals, and sera were collected after each immunization. The immunization schedule is provided in Table 4 of Example 15. The sera collection schedule is set forth in Table 5 of Example 15.

Serum antibody titers against VZV glycoprotein E was determined by Enzyme-linked immunosorbent assay (ELISA) using standard methods. In a second expanded study, the serum samples were serially diluted to bring the signal within the scope of detectability using ELISA. The amount of anti-VZV gE mouse IgG was measured in serum collected at day 42 post vaccination in mice vaccinated intramuscularly with two 10µg doses of either: (1) VZV-gE-hIgkappa having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification (#1 in Tables 2 and 3); (2) VZV-gE having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and no chemical modification (#6 in Tables 2 and 3); (3) VZV-gE having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification (#10 in Tables 2 and 3); (4) VARIVAX[®] vaccine (positive control); or (5) PBS (negative control).

The concentration of anti-VZV-gE mouse IgG was measured in 10-fold serial dilutions.

Fig 6 shows that the strongest immune response was found in mice vaccinated with vaccine candidate (1) VZV-gE-hIgkappa having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification. The second strongest response was found in mice vaccinated with vaccine candidate (3) VZV-gE having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification. The third strongest response was found in mice vaccinated with vaccine candidate (2) VZV-gE having 5' cap: m7G(5')ppp(5')G-2'-O-methyl and no chemical modification. All three VZV gE mRNA vaccines generated a significantly greater immune response than VARIVAX[®] vaccine.

Fig. 7 shows the amount of anti-VZV-gE mouse IgG present in mice vaccinated with vaccines (1) - (4) and (5) negative control at day 3, day 14, and day 42 post-vaccination.

Example 18: Immunogenicity Study

The instant studies are designed to test the immunogenicity in BALB/C mice of candidate VZV vaccines comprising a mRNA polynucleotide encoding variant glycoprotein gE from VZV. Mice were immunized with various VZV mRNA vaccine formulations at set

intervals, and sera were collected after each immunization at indicated time points. The immunization schedule is provided in Table 6 below. The sera collection schedule is set forth in Table 7 below.

The amount of anti-VZV gE mouse IgG is measured in serum collected at the times indicated in Table 7 post vaccination in mice vaccinated intramuscularly with two 10 µg or 2 µg doses of the indicated constructs. All mRNAs used have the 5' cap: m7G(5')ppp(5')G-2'-O-methyl and N1-methylpseudouridine chemical modification. ZOSTAVAX[®] was used as a positive control and was injected into mice intramuscularly with twice clinical dose of 19400 pfu SC. PBS was used as negative control.

Antibody titers against the VZV gE variant polypeptides in the sera of mice immunized with VZV gE variant mRNA vaccines indicated in Table 6 were determined by enzyme-linked immunosorbent assay (ELISA). To perform the ELISA, wells of a plate were coated with VZV gE antigen (Abcam: ab43050) in PBS. 100 µl of the VZV gE antigen at a concentration of 1, 2, or 4 µg/ml were used for coating overnight at 4 °C. The wells were then washed with 300 µl of PBST (PBS with 0.05% tween) 3 times. The VZV gE-coated wells were blocked with 200 µl of blocking butter containing 1% Blotto in PBS for 30 minutes at room temperature. Mice sera containing anti-VZV gE antibodies were diluted 1:2000 and then subject to 1:3 serial dilutions using PBST. The diluted sera were added to the VZV gE-coated wells and incubated for 1 hour at room temperature. A secondary antibody, rabbit anti-mouse conjugated to horseradish peroxidase (HRP, Abcam: ab6728) was diluted 1:1000 in PBST and 100 µl of the secondary antibody containing solution was added to the wells and incubated for 45 minutes at room temperature. 100 µl of HRP substrates, KPL TMB, were added the wells and incubated for 3 minutes at room temperature before 100 µl of a stop solution (2M H₂SO₄) was added to stop the HRP reaction. Signals generated from the HRP substrates were measured at A450. The results were shown in Figs. 11A, 11B, 12A, 12B, 13 and Tables 8 and 9.

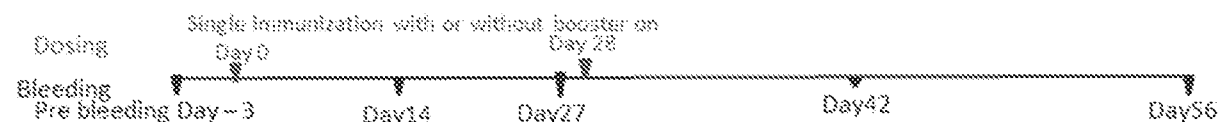
Fig. 11A shows that all gE variants induced much stronger immune response than ZOSTAVAX[®] after the two 10 µg doses. Fig. 11B show that all gE variants induced much stronger immune response than ZOSTAVAX[®] after the two 2 µg doses. With both dosages, the gE variants GE-del_574_Y569A and GE-del-562-IgKappaSP induced the strongest immune response and the antibody titer measured in the sera of mice immunized with this gE variant is over 10 times more than the antibody titer measured in the sera of mice immunized with ZOSTAVAX[®], indicating that the GE-del_574_Y569A and GE-del_562-IgKappaSP mRNAs are superior vaccine candidates against VZV.

Fig. 12A shows the amount of antibodies in titrated sera collected from mice immunized twice with 10 µg of VZV gE mRNA variants described in Table 6. Fig. 12B shows the amount of antibodies in titrated sera collected from mice immunized twice with 2 µg of VZV gE mRNA variants described in Table 6. When the sera were diluted more than 100 fold, the antibody titer is higher in VZV gE variants vaccinated mice sera than in ZOSTAVAX[®] vaccinated mice sera, suggesting that the VZV gE mRNA variants induced much stronger immune response than ZOSTAVAX[®] in mice. All the VZV gE mRNA variants tested showed comparable ability in inducing immune response in mice.

Fig. 13 is a graph showing the anti-VZV gE immune response induced by the VZV gE variant mRNA vaccines compared to ZOSTAVAX[®]. The VZV gE variant GE-delete_from_574-Y569A induced immune response in mice that is about 1 log greater than ZOSTAVAX[®].

Table 9 summarizes the reciprocal IgG titer (IC50) in the sera collected from mice immunized with 10 µg or 2 µg of the respective VZV gE mRNAs twice. GE-delete_from_574-Y569A induced strong immune response with either 10 µg or 2 µg dosages. The Geometric Mean Titer (GMT) was used to indicate the immunogenic potential of the VZV gE variant mRNA vaccines. GE-delete_from_574-Y569A showed the highest GMT value, indicating that it is the most efficacious in inducing immune response against VZV gE.

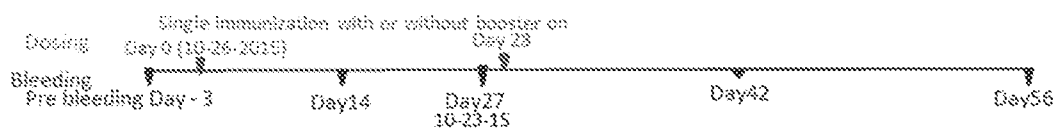
Table 6: Injection Schedule



G#	Antigen	Route	N	Dose ge (µg)	Dose Vol (µl)	1 st dose	2 nd dose	MC3/ conc	
1	SE-VZV-GE- full_with_AEAADA (SEQ ID NO: 58)	IM	5	10	50	Day 0	Day 28	0.2 mg/ml	2 x 500
2	SE-VZV-GE- full_with_AEAADA (SEQ ID NO: 58)	IM	5	2	50	Day 0	Day 28	0.04 mg/ml	2 x 500
3	SE-VZV-GE- full_with_AEAADA (SEQ ID NO: 58) and_Y582G	IM	5	10	50	Day 0	Day 28	0.2 mg/ml	2 x 500
4	SE-VZV-GE- full_with_AEAADA (SEQ ID NO: 58) and_Y582G	IM	5	2	50	Day 0	Day 28	0.04 mg/ml	2 x 500
5	SE-VZV-GE-delete-562	IM	5	10	50	Day 0	Day 28	0.2 mg/ml	2 x 500

G#	Antigen	Route	N	Dose ge (μ g)	Dose Vol (μ l)	1 st dose	2 nd dose	MC3/ conc	
6	SE-VZV-GE-delete-562	IM	5	2	50	Day 0	Day 28	0.04 mg/ml	2 x 500
7	SE-VZV-GE-delete-562- replacedSP-withIgKappa	IM	5	10	50	Day 0	Day 28	0.2 mg/ml	2 x 500
8	SE-VZV-GE-delete-562- replacedSP-withIgKappa	IM	5	2	50	Day 0	Day 28	0.04 mg/ml	2 x 500
9	SE-VZV-GE-truncated- delete from 574	IM	5	10	50	Day 0	Day 28	0.2 mg/ml	2 x 500
10	SE-VZV-GE-truncated- delete from 574	IM	5	2	50	Day 0	Day 28	0.04 mg/ml	2 x 500
11	SE-VZV-GE-truncated- delete from 574 - Y569A	IM	5	10	50	Day 0	Day 28	0.2 mg/ml	2 x 500
12	SE-VZV-GE-truncated- delete from 574 - Y569A	IM	5	2	50	Day 0	Day 28	0.04 mg/ml	2 x 500
13	KB_VZV_gE_Oka_hIgkappa	IM	5	10	50	Day 0	Day 28	0.2 mg/ml	2 x 500
14	KB_VZV_gE_Oka_hIgkappa	IM	5	2	50	Day 0	Day 28	0.04 mg/ml	2 x 500
15	KB_VZV_gE_Oka	IM	5	10	50	Day 0	Day 28	0.2 mg/ml	2 x 500
16	KB_VZV_gE_Oka	IM	5	2	50	Day 0	Day 28	0.04 mg/ml	2 x 500
17	SE-VZV-GI-full	IM	5	10	50	Day 0	Day 28	0.2 mg/ml	2 x 500
18	SE-VZV-GI-full	IM	5	2	50	Day 0	Day 28	0.04 mg/ml	2 x 500
19	SE-VZV-GE- full_with_AEAADA (SEQ ID NO: 58)+SE-VZV-GI-full	IM	5	10	50	Day 0	Day 28	0.2 mg/ml	2 x 500
20	SE-VZV-GE- full_with_AEAADA (SEQ ID NO: 58)+SE-VZV-GI-full	IM	5	2	50	Day 0	Day 28	0.04 mg/ml	2 x 500
21	SE-VZV-GE-truncated- delete_from_574_-_Y569A	IM	5	10	50	Day 0	No dosing	0.2 mg/ml	500
22	PBS	IM	5	-	50	Day 0	Day 28		2 x 500
23	Positive control	SC	5	1940 0 PFU s	100	Day 0	Day 28		2 x 500

Table 7: Schedule of Sample Collection



G#	Antigen	Pre- bleed	Day 14	Day 27	Day 42	Day 56
1	SE-VZV-GE-full_with_AEAADA (SEQ ID NO: 58)	✓	✓	✓	✓	✓

G#	Antigen	Pre-bleed	Day 14	Day 27	Day 42	Day 56
2	SE-VZV-GE-full_with_AEAADA (SEQ ID NO: 58)	✓	✓	✓	✓	✓
3	SE-VZV-GE-full_with_AEAADA (SEQ ID NO: 58) and Y582G	✓	✓	✓	✓	✓
4	SE-VZV-GE-full_with_AEAADA (SEQ ID NO: 58) and Y582G	✓	✓	✓	✓	✓
5	SE-VZV-GE-delete-562	✓	✓	✓	✓	✓
6	SE-VZV-GE-delete-562	✓	✓	✓	✓	✓
7	SE-VZV-GE-delete-562-replacedSP-withIgKappa	✓	✓	✓	✓	✓
8	SE-VZV-GE-delete-562-replacedSP-withIgKappa	✓	✓	✓	✓	✓
9	SE-VZV-GE-truncated-delete_from_574	✓	✓	✓	✓	✓
10	SE-VZV-GE-truncated-delete_from_574	✓	✓	✓	✓	✓
11	SE-VZV-GE-truncated-delete_from_574_-_Y569A	✓	✓	✓	✓	✓
12	SE-VZV-GE-truncated-delete_from_574_-_Y569A	✓	✓	✓	✓	✓
13	KB_VZV_gE_Oka_hlgkappa	✓	✓	✓	✓	✓
14	KB_VZV_gE_Oka_hlgkappa	✓	✓	✓	✓	✓
15	KB_VZV_gE_Oka	✓	✓	✓	✓	✓
16	KB_VZV_gE_Oka	✓	✓	✓	✓	✓
17	SE-VZV-GI-full	✓	✓	✓	✓	✓
18	SE-VZV-GI-full	✓	✓	✓	✓	✓
19	SE-VZV-GE-full_with_AEAADA (SEQ ID NO: 58)+SE-VZV-GI-full	✓	✓	✓	✓	✓
20	SE-VZV-GE-full_with_AEAADA (SEQ ID NO: 58)+SE-VZV-GI-full	✓	✓	✓	✓	✓
21	SE-VZV-GE-truncated-delete_from_574_-_Y569A	✓	✓	✓	✓	✓
22	PBS	✓	✓	✓	✓	✓
23	Positive control	✓	✓	✓	✓	✓

Table 8. Summary of IC₅₀ of the different VZV constructs

Reciprocal IgG titer (IC50)

Name	10ug	2ug				
GE-FULL_AEAADA	5741	6378				
GE-FULL_AEAADA_&_Y582G	10306	3556				
GE-del-562	11872	6445				
GE-del-562-IgKappaSP	16490	7939				
GE-del_574	9031	4082				
GE-del_574_Y569A	11704	7291				
GE_Oka_hlgkappa	11708	6448				
GE_Oka_hlgkappa	7045	3672				
GE-full_AEAADA_GI-full	4457	8242				
GE-del_574_Y569A	NA					
PBS	NA	NA				
Zostavax	960	860				
Assay controls	plate 1	plate 2	%CV	std	mean	CV
VZV_gE_Oka_hlgkappa	13809	11078	7.22	862.5	11940.5	0.07

Table 9. Reciprocal anti-gE IgG titer (IC50) measured by ELISA

Name	IC50	GMT	Name	IC50	GMT
GE-full_AEAADA	1188.5	4291.4	GE-del_574_Y569A	20941.1	
	31915.4			48865.2	
	1261.8			8810.5	
	30408.9			43351.1	
	1000			68076.9	
GE-full_AEAADA_&_Y582G	1150.8	3181.3	GE_Oka_hlgkappa	24266.1	6763.9
	25351.3			3026.9	
	1000			13213	
	1000			5236	
	11168.6			2786.1	
GE-del-562	1000	6921.5	GE_Oka	27227	9078.2
	47752.9			30903	
	12676.5			7638.4	
	12912.2			1000	
	2032.4			9594	
GE-del-562-IgKappaSP	1000	13140.1	Zostavax	6397.3	2228.4
	13122			1000	
	51760.7			1000	
	84918.1			2660.7	
	6792			3228.5	
GE-del_574	13091.8	13795.9			
	6760.8				
	14223.3				
	28774				

Example 19: VZV in vitro neutralization assay

5 A VZV *in vitro* neutralization assay was performed to evaluate the anti-VZV gE antibodies in neutralizing VZV. The anti-VZV gE antibodies were obtained by collecting the sera of mice vaccinated with VZV gE variant mRNA vaccines. Mice were vaccinated with VZV gE variant mRNA vaccines at dosages of 10 µg or 2 µg as described in Table 6 and sera were collected 2 weeks post 2nd immunization.

10 To perform the assay, mice sera were diluted 1:5 and then subjected to 1:2 serial dilutions. VZV virus were added to the sera and neutralization was allowed to continue for 1 hour at room temperature. ARPE-19 cells were seeded in 96-wells one day before and the virus/serum mixtures were added to ARPE-19 cells at 50-100 pfu per well. The ARPE-19 cells were fixed on the next day and VZV-specific staining was performed. The plates were scanned and analyzed. Results of the VZV in vitro neutralization assay were summarized in

15 Table 10. Values in Table 10 are serum dilutions showing 50% reduction in well-area coverage by VZV virus plaques. No reduction in plaque number was observed. As shown in Table 10, one replicate of serum from mice immunized with GE-delete_from_574-Y569A variant mRNA vaccine was able to reduce well-area coverage by VZV virus plaques at 1:80

20 dilution.

Table 10. *In vitro* neutralization assay

Antigen	10ug		2ug	
	Replicate1	Replicate2	Replicate1	Replicate2
SE-VZV-GE-full_with_AEAADA	20	10	10	10
SE-VZV-GE-full_with_AEAADA_and_Y582G	40	20		10
SE-VZV-GE-delete-562	40	20	20	10
SE-VZV-GE-delete-562-replacedSP-withIgKappa	40	40	20	40
SE-VZV-GE-truncated-delete_from_574	80	40	40	20
SE-VZV-GE-truncated-delete_from_574_-_Y569A	20	80	10	10
KB_VZV_gE_Oka_hlgkappa	40	<20	20	10
KB_VZV_gE_Oka	160	10	10	10
SE-VZV-GI-full	<10	<10	<20	<20
SE-VZV-GE-full_with_AEAADA+SE-VZV-GI-full	20	10	-	-
SE-VZV-GE-truncated-delete_from_574_-_Y569A	<10	<10	-	-
PBS	<20	<20		
Positive control	20	20		

Example 20: Immunogenicity in Mice

Herpes zoster (HZ) or shingles is a debilitating disease characterized by a vesicular rash, with the most common complication being post-herpetic neuralgia (PHN). PHN is a constant and severe pain that develops after clearance of the cutaneous outbreak, and can last for several years, thereby contributing to the high morbidity of affected individuals. HZ is caused by reactivation of latent varicella-zoster virus (VZV) from the sensory ganglia.

Immune responses generated during primary VZV infection (chickenpox) have been shown to prevent the reactivation of latent VZV. However, the incidence of HZ is strongly associated with advancing age. Several investigations have shown that T cell-mediated immune responses decline with increasing age and during immunosuppression, resulting in reactivation of VZV. Nonetheless, the levels of anti-VZV antibodies remain relatively stable with increasing age, demonstrating that the humoral immune response may not be sufficient for the prevention of HZ. Several studies have reported the induction of VZV-specific CD4⁺ and CD8⁺ T cells, with CD4⁺ T cells dominating the memory response.

The approved vaccine ZOSTAVAX[®] demonstrates around 60-70% efficacy in 50-60 years adults and declines with age. Recently a subunit adjuvanted vaccine (Shingrix: gE protein +AS01B) was shown to have ~90% efficacy in all age group of adults 50+. However, this vaccine demonstrated grade 3 severe AE's in 10% of the vaccinated subjects. Shingrix demonstrated about a log fold better T and B cell response after two doses to ZOSTAVAX[®]. In the present studies mRNA immunization with a gE construct was investigated for immunogenicity in mice and NHP.

The instant study was defined to test the immunogenicity in BALB/C mice of candidate VZV vaccines comprising a mRNA polynucleotide encoding glycoprotein gE from

VZV. Mice were immunized with various VZV mRNA vaccine formulations as set forth below in Table 11. Groups 1 to 5 were primed with ZOSTAVAX[®] to mimic a primary Varicella exposure and group 6 was primed with mRNA construct VZV-gE-del_574_Y569A. All groups (groups 1-6) were boosted on day 28, as shown in Table 11. The animals were bled on days -3, 21, 38 and 42. Blood and spleens were collected for serological and T cell analysis on days 38 and 42. As shown in Fig. 14A, all groups gave comparable anti-gE antibody responses to mRNA vaccination with the animal receiving mRNA for prime and boost (group 6) trending higher. As shown in Fig. 15A, all groups demonstrated CD4 T-cell responses with mRNA prime and boost (group 6) trending towards a higher response. As shown in Fig. 15B, variable CD8 T-cell responses were noted with the mRNA prime and boost (group 6) demonstrating highest CD8 T-cell frequencies.

Table 11: Injection Schedule

G#	Primary Immunization (Day 0) 10 µg	Boost (Day 28) 10 µg	Localization (VERO / MeWo) of Boost Construct	Number mice per group
1	ZOSTAVAX [®]	VZV-gE-del_574_Y569A	Golgi (high) & cell membrane (high)	10
2	ZOSTAVAX [®]	VZV-gE-Oka-hIgKappa	Golgi (low) & cytoplasmic	10
3	ZOSTAVAX [®]	VZV-gE-Oka	Golgi/Golgi	10
4	ZOSTAVAX [®]	VZV-gE full_with_AEAADA (SEQ ID NO: 58)_and_ Y582G	Organelles & cytoplasmic/cell membrane	10
5	ZOSTAVAX [®]	VZV-gE-full with AEAADA (SEQ ID NO: 58)	Golgi & diffuse perinuclear/cell membrane	10
6	VZV-gE- del_574_Y569A	VZV-gE-del_574_Y569A	Golgi (high) & cell membrane (high)	10

Example 21: Immunogenicity in non-human primates

Based on the data in Example 20, the mRNA construct VZV-gE-del_574_Y569A was further evaluated for immunogenicity in non-human primates. Three groups of Rhesus monkeys were primed with mRNA (VZV-gE-del_574_Y569A) or ZOSTAVAX[®] and boosted as set forth below in Table 12. The animals were bled at days 0, 14, 28, and 42 for serological and T-cell analysis. T-cell analysis was performed on days 0, 28, and 42. As shown in Fig. 16A and 16B, the mRNA prime and boost (group 1) gave the highest anti-gE titers which were followed by ZOSTAVAX[®] prime, mRNA boost (group 2). The latter group (group 2) anti-gE titers were approximately 10X better than the ZOSTAVAX[®] prime,

ZOSTAVAX® boost (group 3). As shown in Figs. 16C and 16D, no CD4-T cells producing IFN γ , IL-2 or TNF α were detected in the ZOSTAVAX® prime, ZOSTAVAX® boost group (group 3). In contrast, as shown in Figs. 16C and 16D, reasonable frequency of CD4 T-cells producing IFN γ , IL-2 or TNF α were detected in the mRNA prime, mRNA boost group (group 1) and the ZOSTAVAX® prime, mRNA boost group (group 2) and were statistically undifferentiated. These data indicate that one dose of mRNA vaccination after ZOSTAVAX® exposure was equivalent to two doses of mRNA vaccination in inducing comparable T-cell responses.

Table 12: Injection Schedule

G#	Primary Immunization (Day 0) 10 μ g	Boost (Day 28) 10 μ g	Number Rhesus macaques (male and female) per group
1	VZV-gE- del 574_Y569A	VZV-gE- del 574_Y569A	5
2	ZOSTAVAX®	VZV-gE- del 574_Y569A	5
3	ZOSTAVAX®	ZOSTAVAX®	5

Example 22: Expression and in vivo localization of VZV-gE-del_574_Y569A variants

Variants of mRNAs encoding the VZV-gE-del_574_Y569A (variant 7, SEQ ID NO: 107, ORF sequence: SEQ ID NO: 86) or VZV-gE-full length (SEQ ID NO: 135) were constructed, where the nucleotide sequence of the homopolymeric stretches (e.g., 6, 5, or 4 consecutive As) were altered (without altering the amino acid sequence) to reduce the frequency of potential indel formation. Next generation sequencing showed the absence of significant indels in VZV-gE-del_574_Y569A variant 7 (VZV-gE-del_574_Y569A-v7) (Fig. 17). Mass spectrometry detected very low levels of indels in a 4A stretch in nucleotides 760-771 of the ORF.

Next, the expression levels of VZV-gE-del_574_Y569A-v7 were tested in Mewo and HeLa cells and compared to the VZV-gE-del_574_Y569A mRNA construct. The mRNA constructs (200 ng) were transfected into Mewo or HeLa cells, using the Mirus TF transfection reagent in 96-well plates. NTIX control mRNA and PBS control were used in 30 K of MeWo cells. All cells were serially diluted (2 fold) 6 hours post transfection, permeabilized, stained, and analyzed by flow cytometry for FITC+ cells (cells with antigen expression). Results show that the VZV-gE-del_574_Y569A-v7 mRNA construct was expressed at a level comparable to the VZV-gE-del_574_Y569A mRNA construct in both Mewo cells (Fig. 18A) and HeLa cells (Fig. 18B).

Protein was then detected in cell lysates of HeLa cells transfected (lipoplexed with 3 μ l LF2000 per well in a 24-well plate) with either the VZV-gE-del_574_Y569A mRNA construct (500 ng) or the VZV-gE-del_574_Y569A-v7 mRNA construct (500 ng), serially diluted to 100 μ g or 20 μ g. Western blots for the VZV-gE antigen 16 hours post transfection showed that the VZV-gE-del_574_Y569A-v7 mRNA construct was expressed at a level comparable to the VZV-gE-del_574_Y569A mRNA construct in HeLa cells (Figs. 19A and 19B).

Next, the *in vivo* trafficking and localization of different VZV gE antigens were assessed. HeLa or MeWo cells were plated on 96 well plates and transfected with mRNA constructs encoding NTFIX control, full length VZV-gE, VZV-gE-del_574_Y569A, or VZV-gE-del_574_Y569A-v7 with Lipofectamine 2000 (100 ng mRNA /0.4 μ l LF per well). Twenty-four hours after transfection, the cells were fixed in 4 % PFA, permeabilized with 0.5 % TX-100, and co-stained with rabbit anti-GM130 (CST D6B1- Golgi marker) and mouse anti-gE (Abcam 52549) antibodies at room temperature for 1 h in blocking buffer (1% BSA in PBS). The samples were then washed to remove the primary antibody and were incubated with anti-rabbit Alexa 647 and anti-mouse Alexa 488 conjugated antibodies for 30 minutes at room temperature, followed by counterstaining with DAPI for nuclear localization and HCS blue mask for cell segmentation. Cells were imaged with the Opera Phenix high throughput spinning disk confocal.

The expression level and the localization of each antigen were quantified. The antigens expressed at comparable levels (Fig. 20A) but VZV-gE-del_574_Y569A encoded by the VZV-gE-del_574_Y569A mRNA construct or the VZV-gE-del_574_Y569A-v7 mRNA construct showed lower golgi localization compared to the full-length VZV-gE (Fig. 20B). A shift in the localization of VZV-gE-del_574_Y569A (encoded by either the VZV-gE-del_574_Y569A mRNA construct or the VZV-gE-del_574_Y569A-v7 mRNA construct) from golgi to the cell membrane, compared to the full-length VZV gE, was observed in HeLa cells (Fig. 21A) and in MeWo cells (Figs. 21B and 21C).

Table 13. Exemplary VZV gE mRNA constructs

mRNA Construct	ORF of mRNA Construct (excluding the stop codon)
SE_VZV_gE_full_indel_fixed	AUGGGGACAGUUAUAAACCUGUGGGGGUAAUUGAUGGGGUU CGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCCGGUCAGAG CAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUGAAGACAAA CUGGAUACAAACUCCGUAAUAGAGCCUUAUACCAUUCAGAUCA UGCGGAGUCUUAUGGGUAAAUCGGGGAGAGUCUUCGCGAAAAG CGUACGAUCAUAACUCACCUUAUAUAUGGCCACGUAAUGAUUAU GAUGGAUUUUUAGAGAACGCACACGAACACCAUGGGGUGUAUAA UCAGGGCCGUGGUUAUCGAUAGCGGGGAACGGUUAUUGCAACCCA CACAAUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUC

mRNA Construct	ORF of mRNA Construct (excluding the stop codon)
	CACGUUAUCCCUACGUUAAACGGCGAUGACAGACAUAAAAUUGU AAAUGUGGACCAACGUCAAUACGGUGACGUGUUUAAAGGAGAUC UUAUCCAAAACCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUG GAAGAAAAUCACCCGUUUACUUUACGCGCACC GAUUCAGCGGAU UUAUGGAGUCCGGUACACCGAGACUUGGAGCUUUUUGCCGUCAU UAACCUGUACGGGAGACGCAGCGCCCGCCAUCCAGCAUAUAUGUU UAAAGCAUACAACAUGCUUUAAGACGUGGUGGUGGAUGUGGAU UGCGCGGAAAAUACUAAAGAGGAUCAGUUGGCCGAAAUCAGUUA CCGUUUUCAAGGUAAGAAGGAAGCGGACCAACCGUGGAUUGUUG UAAACACGAGCACACUGUUUGAUGAACUCGAAUUAAGACCCACCC GAGAUUGAACCGGGUGUCUUGAAAGUACUUCGGACAGAGAAACA AUACUUGGGUGUGUACA UUUUGGAACAUGCGCGGCUC CCAUGGUA CGUCUACCUACGCCACGUUUUUGGUCACCUGGAAAGGGGAUGAG AAGACAAGAAACCCUACGCCCGCAGUAACUCCUCAACCAAGAGGG GCUGAGUUUCAUAUGUGGAAUUAACACUCGCAUGUAUUUUCAGU UGGUGAUACGUUUAGCUUGGCAAUGCAUCUUCAGUAUAAGAUAC AUGAAGCGCCAUUUGAUUUGCUGUUAGAGUGGUUGUAUGUCCCC AUCGAUCCUACAUGUCAACCA AUGCGGUUAUAUUCUACGUGUUU GUAUCAUCCCAACGCACCCCAUAGCCUCUCUCAUUGAAUUCGG UUGUACA UUUACCUCGCCACA UUUAGCCCAGCGUGUUGCAAGCAC AGUGUAUCAGAAUUGUGAACAUAGCAGAUAAUACACCGCAUAU GUCUGGGAAUAUCUCAUUGGAGCCUAGCUUUGGUCUAUUCUUA CACGACGGGGGCACCACGUUAAAGUUUGUAGUAACACCCGAGAG UUUGUCGGGAUUAUACGUUUUUGUGGUGUAUUUUAACGGGCAUG UUGAAGCCGUAGCAUACACUGUUGUAUCCACAGUAGAUCAUUUU GUAAACGCAAUUGAAGAGCGUGGAUUAUCCGCCAACGGCCGGUCA GCCACCGGCGACUACUAAACCCAAAGGAAAUUACCCCGUAAACCC CGGAACGUCACCACUUCUACGAUAUGCCGCAUGGACCGGAGGGCU UGCAGCAGUAGUACUUUAUGUCUCGUAUAUUUUUAAUCUGUA CGGCUAAACGAAUGAGGGUUAAAGCCUACAGGGUAGACAAGUCU CCUACA AUCAGUCA AUGUACU AUGCAGGACUCCUGUUGACGA UUUCGAAGACUCAGAGAGUACAGACACAGAAGAAGAAUUCGGAA ACGCUAUAGGUGGCUCUCACGGAGGUAGCUCGUUAACAGUGUAC AUCGAUAAAACCA (SEQ ID NO: 142)
SE-VZV-GE-574-Y569A-v2	AUGGGGACAGUUAUAAACCGUGUGGUGGGGUAUUGAUGGGGUU CGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCCGGUCAGAG CAUCCGUCUUGCGAUACGAUGAUUUUCACAU CGAUGAAGACAAA CUGGAUACAAACUCCGUUAUUGAGCCUUAUACCAUUCAGAUCA UGCGGAGUCUUAUGGGUAAAUCGGGGAGAGUCUUCGCGAAAAG CGUACGAUCAUAACUCACCUUAUAU AUGGCCACGUAAUGAUUAU GAUGGAUUUUUAGAGAACGCACACGAACACCAUGGGGUGUAUAA UCAGGGCCGUGGUUUCGAUAGCGGGGAACGGUUAUUGCAACCCA CACAAUUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUC CACGUUAUCCCUACGUUAAACGGCGAUGACAGACAUAAAAUUGU AAAUGUGGACCAACGUCAAUACGGUGACGUGUUUAAAGGAGAUC UUAUCCAAAACCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUG GAAGAAAAUCACCCGUUUACUUUACGCGCACC GAUUCAGCGGAU UUAUGGAGUCCGGUACACCGAGACUUGGAGCUUUUUGCCGUCAU UAACCUGUACGGGAGACGCAGCGCCCGCCAUCCAGCAUAUAUGUU UAAAACAUACAACAUGCUUUAAGACGUGGUGGUGGAUGUGGAU UGCGCGGAAAAUACUAAAGAGGAUCAGUUGGCCGAAAUCAGUUA CCGUUUUCAAGGUAAGAAGGAAGCGGACCAACCGUGGAUUGUUG UAAACACGAGCACACUGUUUGAUGAACUCGAAUUAAGACCCCCCG AGAUUGAACCGGGUGUCUUGAAAGUACUUCGGACAGAGAAACAA UACUUGGGUGUGUACA UUUUGGAACAUGCGCGGCUC CCAUGGUA GUCUACCUACGCCACGUUUUUGGUCACCUGGAAAGGGGAUGAGA AGACAAGAAACCCUACGCCCGCAGUAACUCCUCAACCAAGAGGGG CUGAGUUUCAUAUGUGGAAUUAACACUCGCAUGUAUUUUCAGUU GGUGAUACGUUUAGCUUGGCAAUGCAUCUUCAGUAUAAGAUACA UGAAGCGCCAUUUGAUUUGCUGUUAAGAGUGGUUGUAUGUCCCCA UCGAUCCUACAUGUCAACCA AUGCGGUUAUAUUCUACGUGUUUG

mRNA Construct	ORF of mRNA Construct (excluding the stop codon)
	UAUCAUCCCAACGCACCCCAUGCCUCUCUCAUAUGAAUUCGGU UGUACAUUUACCUCGCCACAUUUAGCCCAGCGUGUUGCAAGCACA GUGUAUCAAAAUUGUGAACAUUGCAGAUAAUACACCGCAUAUUG UCUGGGAAUAUCUCAUAUGGAGCCUAGCUUUGGUCUAAUCUAC ACGACGGGGGCACCACGUUAAAGUUUGUAGAUACACCCGAGAGU UUGUCGGGAUUAUACGUUUUUGUGGUGUAUUUUAACGGGCAUGU UGAAGCCGUAGCAUACACUGUUGUAUCCACAGUAGAUCAUUUUG UAAACGCAAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUCAG CCACCGGCGACUACUAAACCCAAGGAAAUUACCCCCGUAAACCC GGAACGUCACCACUUCUACGAUAUGCCGCAUGGACCGGAGGGCU UGCAGCAGUAGUACUUUUUUGUCUCGUAUUAUUUUUAAUCUGUA CGGCUAAACGAAUGAGGGUUAAAGCCGCCAGGGUAGACAAG (SEQ ID NO: 143)
SE-VZV-GE-574-Y569A-v3	AUGGGGACAGUUAUAAACCUGUGGGUGGGGUAAUUGAUGGGGU CGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCCGGUCAGAG CAUCCGUCUUGCGAUACGAUGAUUUUACAUCGAUGAAGACAAA CUGGAUACAAACUCCGUUAUUGAGCCUUAUACCAUUCAGAUCA UGCGGAGUCUUCUUGGGUAAAUCGGGGAGAGUCUUCGCGAAAG CGUACGAUCAUAACUCACCUUAUAUUGGCCACGUAAUGAUUAU GAUGGAUUUUUAGAGAACGCACACGAACACCAUGGGGUGUAUAA UCAGGGCCGUGGUUUCGAUAGCGGGGAACGGUUAUUGCAACCCA CACAAUUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUC CACGUUAUCCUACGUUAAACGGCGAUGACAGACAUAAAAUUGU AAAUGUGGACCAACGUCAAUACGGUGACGUGUUUAAAGAGAGU UUAUCCAAAACCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUG GAAGAAAUCACCCGUUUACUUUACGCGCACCGAUUCAGCGGAU UUAUGGAGUCCGGUACACCGAGACUUGGAGCUUUUUGCCGUCAU UAACCUGUACGGGAGACGCAGCGCCCGCCAUCCAGCAUAUAUGUU UAAAACAUACAACAUUCUUAAGACGUGGUGGUGGAUGUGGAU UGCGCGGAAAUAUAAAGAGGAUCAGUUGGCCGAAUUCAGUUA CCGUUUUAAGGUAAGAAGGAAGCGGACCAACCGUGGAUUGUUG UAAACACGAGCACACUGUUUGAUGAACUCGAAUUAAGACCCACCC GAGAUUGAACCGGGUGUCUUGAAAGUACUUCGGACAGAGAAACA AUACUUGGUGUGUACAUAUUGGAACAUGCGCGGCUCCGAUGGUA CGUCUACCUACGCCACGUUUUUGGUCACCUUGGAAAGGGGAUGAG AAGACAAGAAACCCUACGCCCGCAGUAACUCCUCAACCAAGAGGG GCUGAGUUUCAUAUGUGGAAUUAACACUCGCAUGUAUUUUCAGU UGGUGAUACGUUUAGCUUGGCAAUGCAUCUUCAGUAUAAGAUAC AUGAAGCGCCAUUUGAUUUUGCUGUUAGAGUGGUUGUAUGUCCCC AUCGAUCCUACAUGUCAACCAUUGCGGUUAUAUUCUACGUGUUU GUAUCAUCCCAACGCACCCCAUUGCCUCUCUCAUAUGAAUUCGG UUGUACAUUUACCUCGCCACAUUUAGCCCAGCGUGUUGCAAGCAC AGUGUAUCAAAAUUGUGAACAUUGCAGAUAAUACACCGCAUAU GUCUGGGAAUAUCUCAUAUGGAGCCUAGCUUUGGUCUAAUCUUA CACGACGGGGGCACCACGUUAAAGUUUGUAGAUACACCCGAGAG UUUGUCGGGAUUAUACGUUUUUGUGGUGUAUUUUAAACGGGCAUG UUGAAGCCGUAGCAUACACUGUUGUAUCCACAGUAGAUCAUUUU GUAAACGCAAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUCA GCCACCGGCGACUACUAAACCCAAGGAAAUUACCCCCGUAAACCC CGGAACGUCACCACUUCUACGAUAUGCCGCAUGGACCGGAGGGCU UGCAGCAGUAGUACUUUUUUGUCUCGUAUUAUUUUUAAUCUGUA CGGCUAAACGAAUGAGGGUUAAAGCCGCCAGGGUAGACAAG (SEQ ID NO: 144)
SE-VZV-GE-574-Y569A-v4	AUGGGGACAGUUAUAAACCUGUGGGUGGGGUAAUUGAUGGGGU CGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCCGGUCAGAG CAUCCGUCUUGCGAUACGAUGAUUUUACAUCGAUGAAGACAAA CUGGAUACAAACUCCGUUAUUGAGCCUUAUACCAUUCAGAUCA UGCGGAGUCUUCUUGGGUAAAUCGGGGAGAGUCUUCGCGAAAG CGUACGAUCAUAACUCACCUUAUAUUGGCCACGUAAUGAUUAU GAUGGAUUUUUAGAGAACGCACACGAACACCAUGGGGUGUAUAA UCAGGGCCGUGGUUUCGAUAGCGGGGAACGGUUAUUGCAACCCA

mRNA Construct	ORF of mRNA Construct (excluding the stop codon)
	CACAAAUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUC CACGUUAUCCCUACGUUAAACGGCGAUGACAGACAUAAAGAUUGU AAAUGUGGACCAACGUCAAUACGGUGACGUGUUUAAAGGAGAUC UUAUCCAAAGCCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUG GAAGAGAAUCACCCGUUUACUUUACGCGCACCGAUUCAGCGGAU UUAUGGAGUCCGGUACACCGAGACUUGGAGCUUUUUGCCGUCAU UAACCUGUACGGGAGACGCAGCGCCCGCCAUCCAGCAUAUAUGUU UAAAGCAUACAACAUGC UUUAAGACGUGGUGGUGGAUGUGGAU UGCGCGGAGAAUACUAAAGAGGAUCAGUUGGCCGAAAUCAGUUA CCGUUUUCAAGGUAAGAAGGAAGCGGACCAACCGUGGAUUGUUG UAAACACGAGCACACUGUUUGAUGAACUCGAAUUAAGACCCCCCG AGAUUGAACCGGGUGUCUUGAAAGUACUUCGGACAGAGAAACAA UACUUGGGUGUGUACAUUUGGAACAUGCGCGGCCUCCGAUGGUAC GUCUACCUACGCCACGUUUUUGGUCACCUGGAAAGGGGAUGAGA AGACAAGAAACCCUACGCCCCGAGUAACUCCUCAACCAAGAGGGG CUGAGUUUCAUAUGUGGAUUUACCACUCGCAUGUAUUUUCAGUU GGUGAUACGUUUAGCUUGGCAAUGCAUCUUCAGUAUAAGAUACA UGAAGCGCCAUUUGAUUUGCUGUUAGAGUGGUUGUAUGUCCCCA UCGAUCCUACAUGUCAACCAUUGCGGUUAUAUUCUACGUGUUUG UAUCAUCCCAACGCACCCCAUUGCCUCUCUCAUAUGAAUUCGGU UGUACAUUUACCUCGCCACAUUUAGCCCAGCGUGUUGCAAAGCACA GUGUAUCAGAAUUGUGAACAUAGCAGAUAAACUACCCGCAUAUUG UCUGGGAAUAUCUCAUAUGGAGCCUAGCUUUGUCAAUCUUAC ACGACGGGGGCACCACGUUAAAGUUUGUAGAUACACCCGAGAGU UUGUCGGGAUUUAACGUUUUUGUGGUGUAUUUUAACGGGCAUGU UGAAGCCGUAGCAUACACUGUUGUAUCCACAGUAGAUCAUUUUG UAAACGCAAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUCAG CCACCGGCGACUACUAAACCCAAGGAAAUUACCCCCGUAAACCCC GGAACGUCACCACUUCUACGAUAUGCCGCAUGGACCGGAGGGCU UGCAGCAGUAGUACUUUUAUGUCUCGUAUAUUUUUAAUCUGUA CGGCUAAACGAAUGAGGGUUAAAGCCGCCAGGGUAGACAA (SEQ ID NO: 145)
SE-VZV-GE-574-Y569A-v5	AUGGGGACAGUUAUAAACCUUGUGGUGGGGGUAUUGAUGGGGUU CGGAAUUUAUCACGGGAACGUUGCGUAUAACGAAUCCGGUCAGAG CAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUGAAGACAAA CUGGAUACAAACUCCGUUAUUGAGCCUUAUACCAUUCAGAUCA UGCGGAGUCUUCAUUGGGUAAAUCGGGGAGAGUCUUCGCGAAAGG CGUACGAUCAUAACUCACCUUAUAUAUGGCCACGUAAUGAUUAU GAUGGAUUUUUAGAGAACGCACACGAACACCAUGGGGUGUAUAA UCAGGGCCGUGGUUUCGAUAGCGGGGAACGGUUAAUGCAACCCA CACAAAUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUC CACGUUAUCCCUACGUUAAACGGCGAUGACAGACAUAAAGAUUGU AAAUGUGGACCAACGUCAAUACGGUGACGUGUUUAAAGGAGAUC UUAUCCAAAGCCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUG GAAGAGAAUCACCCGUUUACUUUACGCGCACCGAUUCAGCGGAU UUAUGGAGUCCGGUACACCGAGACUUGGAGCUUUUUGCCGUCAU UAACCUGUACGGGAGACGCAGCGCCCGCCAUCCAGCAUAUAUGUU UAAAGCAUACAACAUGC UUUAAGACGUGGUGGUGGAUGUGGAU UGCGCGGAGAAUACUAAAGAGGAUCAGUUGGCCGAAAUCAGUUA CCGUUUUCAAGGUAAGAAGGAAGCGGACCAACCGUGGAUUGUUG UAAACACGAGCACACUGUUUGAUGAACUCGAAUUAAGACCCACCC GAGAUUGAACCGGGUGUCUUGAAAGUACUUCGGACAGAGAAACA AUACUUGGGUGUGUACA UUUGGAACAUGCGCGGCUCCGAUGGUA CGUCUACCUACGCCACGUUUUUGGUCACCUGGAAAGGGGAUGAG AAGACAAGAAACCCUACGCCCCGAGUAACUCCUCAACCAAGAGGG GCUGAGUUUCAUAUGUGGAUUUACCACUCGCAUGUAUUUUCAGU UGGUGAUACGUUUAGCUUGGCAAUGCAUCUUCAGUAUAAGAUAC AUGAAGCGCCAUUUGAUUUGCUGUUAGAGUGGUUGUAUGUCCCC AUCGAUCCUACAUGUCAACCAUUGCGGUUAUAUUCUACGUGUUU GUAUCAUCCCAACGCACCCCAUUGCCUCUCUCAUAUGAAUUCGG UUGUACAUUUACCUCGCCACA UUUAGCCCAGCGUGUUGCAAGCAC

mRNA Construct	ORF of mRNA Construct (excluding the stop codon)
	AGUGUAUCAGAAUUGUGAACAUAGCAGAUAAACUACACCGCAUAUU GUCUGGGAAUAUCUCAUAUGGAGCCUAGCUUUGGUCUAAUCUUA CACGACGGGGGCACCACGUUAAAGUUUGUAGAUACACCCGAGAG UUUGUCGGGAUUAUACGUUUUUGUGGUGUAUUUUAAACGGGCAUG UUGAAGCCGUAGCAUACACUGUUGUAUCCACAGUAGAUAUUUU GUAAACGCAAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUCA GCCACCGGCGACUACUAAACCCAAGGAAAUUACCCCGUAAACCC CGGAACGUCACCACUUCUACGAUAUGCCGCAUGGACCGGAGGGCU UGCAGCAGUAGUACUUUUUUGUCUCGUAUUAUUUUUAAUCUGUA CGGCUAAACGAAUGAGGGUUAAAGCCGCCAGGGUAGACAAG (SEQ ID NO: 146)
SE-VZV-GE-574-Y569A-v6	AUGGGGACAGUUAUAAACCGUGUGGUGGGGGUAUUGAUGGGGUU CGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCCGGUCAGAG CAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUGAAGACAAA CUGGAUACAAACUCCGUUAUUGAGCCUUACUACCAUUCAGAUCA UGCGGAGUCUUCUUGGGUAAAUCGGGGAGAGUCUUCGCGAAAAG CGUACGAUCAUAACUCACCUUAUAUAUGGCCACGUAAUGAUUAU GAUGGAUUUUUAGAGAACGCACACGAACACCAUGGGGUGUAUAA UCAGGGCCGUGGUUUCGAUAGCGGGGAACGGUUAUUGCAACCCA CACAAUUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUC CACGUUAUCCCUACGUUAAACGGCGAUGACAGACAUAAAAUUGU AAAUGUGGACCAACGUCAAUACGGUGACGUGUUUAAAGGAGAU UUAUCCAAAACCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUG GAAGAAAUCACCCGUUUACUUUACGCGCACCGAAUUCAGCGGAU UUAUGGAGUCCGGUACACCGAGACUUGGAGCUUUUUUCCGUCAU UAACCGUACGGGAGACGCAGCGCCCGCCAUCCAGCAUAUAUGUU UAAAGCAUACAACAUGCUUUCAAGACGUGGUGGUGGAUGUGGAU UGCGCGGAAAUAACUAAAAGAGGAUCAGUUGGCCGAAAUCAGUUA CCGUUUUCAAGGUAAGAAGGAAGCGGACCAACCGUGGAUUGUUG UAAACACGAGCACACUGUUUGAUGAACUCGAAUUAGACCCCCCG AGAUUGAACCGGGUGUCUUGAAAGUACUUCGGACAGAGAAACAA UACUUGGGUGUGUACAUUUGGAACAUGCGCGGCUCGGAUGGUAC GUCUACCUACGCCACGUUUUUGGUCACCUGGAAAGGGGAUGAGA AGACAAGAAACCCUACGCCCCGAGUAACUCCUCAACCAAGAGGGG CUGAGUUUCAUAUGUGGAUUUACCACUCGCAUGUAUUUUUCAGUU GGUGAUACGUUUAGCUUGGCAAUGCAUCUUCAGUAUAAGAUACA UGAAGCGCCAUUUGAUUUGCUGUUAAGAGUGGUUGUAUGUCCCCA UCGAUCCUACAUGUCAACCAUUGCGGUUAUAUUCUACGUGUUUG UAUCAUCCCAACGCACCCCAUUGCCUCUCUCAUAUGAAUUCGGU UGUACAUUUACCUCGCCACAUUUAGCCCAGCGUGUUGCAAGCACA GUGUAUCAGAAUUGUGAACAUAGCAGAUAAUACACCGCAUAUUG UCUGGGAAUAUCUCAUAUGGAGCCUAGCUUUGGUCUAAUCUUA ACGACGGGGGCACCACGUUAAAGUUUGUAGAUACACCCGAGAGU UUGUCGGGAUUUAACGUUUUUGUGGUGUAUUUUAAACGGGCAUGU UGAAGCCGUAGCAUACACUGUUGUAUCCACAGUAGAUCAUUUUG UAAACGCAAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUACAG CCACCGGCGACUACUAAACCCAAGGAAAUUACCCCGUAAACCCC GGAACGUCACCACUUCUACGAUAUGCCGCAUGGACCGGAGGGCU UGCAGCAGUAGUACUUUUUUGUCUCGUAUUAUUUUUAAUCUGUA CGGCUAAACGAAUGAGGGUUAAAGCCGCCAGGGUAGACAAG (SEQ ID NO: 147)
SE-VZV-GE-574-Y569A-v7	AUGGGGACAGUUAUAAACCGUGUGGUGGGGGUAUUGAUGGGGUU CGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCCGGUCAGAG CAUCCGUCUUGCGAUACGAUGAUUUUCACAUCGAUGAAGACAAA CUGGAUACAAACUCCGUUAUUGAGCCUUACUACCAUUCAGAUCA UGCGGAGUCUUCUUGGGUAAAUCGGGGAGAGUCUUCGCGAAAAG CGUACGAUCAUAACUCACCUUAUAUAUGGCCACGUAAUGAUUAU GAUGGAUUUUUAGAGAACGCACACGAACACCAUGGGGUGUAUAA UCAGGGCCGUGGUUUCGAUAGCGGGGAACGGUUAUUGCAACCCA CACAAUUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUC CACGUUAUCCCUACGUUAAACGGCGAUGACAGACAUAAAAUUGU

mRNA Construct	ORF of mRNA Construct (excluding the stop codon)
	AAAUGUGGACCAACGUCAAUACGGUGACGUGUUUAAAGGAGAUC UUAUCCAAAACCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUG GAAGAAAUCACCCGUUUACUUUACGCGCACCGAUUCAGCGGAU UUAUGGAGUCCGGUACACCGAGACUUGGAGCUUUUUGCCGUCAU UAACCUGUACGGGAGACGCAGCGCCCGCCAUCCAGCAUAUAUGUU UAAAGCAUACAACAUGC UUUAAGACGUGGUGGUGGAUGUGGAU UGCGCGGAAAUAACUAAAGAGGAUCAGUUGGCCGAAAUCAGUUA CCGUUUUCAAGGUAAGAAGGAAGCGGACCAACCGUGGAUUGUUG UAAACACGAGCACACUGUUUGAUGAACUCGAAUUAAGACCCACCC GAGAUUGAACCGGGUGUCUUGAAAGUACUUCGGACAGAGAAACA AUACUUGGGUGUGUACA UUUUGGAACAUGCGCGGCUCCGAUGGUA CGUCUACCUACGCCACGUUUUUGGUCACCUUGGAAAGGGGAUGAG AAGACAAGAAACCCUACGCCCGCAGUAACUCCUCAACCAAGAGGG GCUGAGUUUCAUAUGUGGAAUUACCACUCGCAUGUAUUUUCAGU UGGUGAUACGUUUAGCUUGGCAAUGCAUCUUCAGUAUAAGAUAC AUGAAGCGCCAUUUGAUUUGCUGUUAGAGUGGUUGUAUGUCCCC AUCGAUCCUACAUGUCAACCA AUGCGGUUAUAUUCUACGUGUUU GUAUCAUCCCAACGCACCCCAUGGCCUCUCUCAUAUGAAUCCGG UUGUACA UUUACCUCGCCACAUUUAGCCCAGCGUGUUGCAAGCAC AGUGUAUCAGAAUUGUGAACAUAGCAGAUAAUACACCGCAUAUU GUCUGGGAAUAUCUCAUAUGGAGCCUAGCUUUGGUCUAUUCUUA CACGACGGGGGCACCACGUUAAAGUUUGUAGAUACACCCGAGAG UUUGUCGGGAUUUAACGUUUUUGUGGGUGUAUUUUAACGGGGCAG UUGAAGCCGUAGCAUACACUGUUGUAUCCACAGUAGAUCAUUUU GUAACCGCAAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUCA GCCACCGGCGACUACUAAACCCAAAGGAAAUUACCCCGUAACCCC CGGAACGUCACCACUUCUACGAUAUGCCGCAUGGACCGGAGGGCU UGCAGCAGUAGUACUUUU AUGUCUCGUAAUAUUUUUAAUCUGUA CGGCUAAACGAAUGAGGGUUAAAGCCGCCAGGGUAGACAAG (SEQ ID NO: 148)
SE-VZV-GE-574-Y569A-v8	AUGGGGACAGUUAUAAACCUGUGGUGGGCGUAUUGAUGGGGUU CGGAUUUAUCACGGGAACGUUGCGUAUAACGAAUCCGGUCAGAG CAUCCGUCUUGCGAUACGAUGAUUUUACAUCGAUGAAGACAAA CUGGAUACAAACUCCGUUAUUGAGCCUUAUACCAUUCAGAUCA UGC GGAGUCUUAUGGGUAAAUCGGGGAGAGUCUUCGCGAAAGG CGUACGAUCAUAACUCACCUUAUAUUGGCCACGUAAUGAUUAU GAUGGAUUCUUAAGAGAACGCACACGAACACCAUGGGGGUGUAUAA UCAGGGCCGUGGUUAUCGAUAGCGGGGAACGGUUAUUGCAACCCA CACAAAUUGUCUGCACAGGAGGAUCUUGGGGACGAUACGGGCAUC CACGUUAUCCCUACGUUAAACGGCGAUGACAGACAUAAAGAUUGU AAAUGUGGACCAACGUCAAUACGGUGACGUGUUUAAAGGAGAUC UUAUCCAAAGCCCCAAGGCCAAAGACUCAUUGAGGUGUCAGUG GAAGAGAAUCACCCGUUUACUUUACGCGCACCGAUUCAGCGGAU UUAUGGAGUCCGGUACACCGAGACUUGGAGCUUCUUGCCGUCAU UAACCUGUACGGGAGACGCAGCGCCCGCCAUCCAGCAUAUAUGUU UAAAGCAUACAACAUGC UUUAAGACGUGGUGGUGGAUGUGGAU UGC GCGGAGAAUACUAAAGAGGAUCAGUUGGCCGAAAUCAGUUA CCGUUUUCAAGGUAAGAAGGAAGCGGACCAACCGUGGAUUGUUG UAAACACGAGCACACUGUUUGAUGAACUCGAAUUAAGACCCACCC GAGAUUGAACCGGGUGUCUUGAAAGUACUUCGGACAGAGAAACA AUACUUGGGUGUGUACA UUUUGGAACAUGCGCGGCUCCGAUGGUA CGUCUACCUACGCCACGUUCUUGGUCACCUUGGAAAGGGGAUGAG AAGACAAGAAACCCUACGCCCGCAGUAACUCCUCAACCAAGAGGG GCUGAGUUUCAUAUGUGGAAUUACCACUCGCAUGUAUUUUCAGU UGGUGAUACGUUUAGCUUGGCAAUGCAUCUUCAGUAUAAGAUAC AUGAAGCGCCAUUUGAUUUGCUGUUAGAGUGGUUGUAUGUCCCC AUCGAUCCUACAUGUCAACCA AUGCGGUUAUAUUCUACGUGUUU GUAUCAUCCCAACGCACCCCA AUGGCCUCUCUCAUAUGAAUCCGG UUGUACA UUUACCUCGCCACAUUUAGCCCAGCGUGUUGCAAGCAC AGUGUAUCAGAAUUGUGAACAUAGCAGAUAAUACACCGCAUAUU GUCUGGGAAUAUCUCAUAUGGAGCCUAGCUUUGGUCUAUUCUUA

mRNA Construct	ORF of mRNA Construct (excluding the stop codon)
	CACGACGGAGGCACCACGUUAAAGUUUGUAGAUACACCCGAGAG UUUGUCGGGAUUUAUACGUCUUUGUGGUGUAUUUUAAACGGGCAUG UUGAAGCCGUAGCAUACACUGUUGUAUCCACAGUAGAUAUUUU GUAAACGCAAUUGAAGAGCGUGGAUUUCCGCCAACGGCCGGUCA GCCACCGGCGACUACUAAACCCAAGGAAAUUACGCCCUGAAACCC CGGAACGUCACCACUUCUACGAUAUGCCGCAUGGACCGGAGGGCU UGCAGCAGUAGUACUUUUUUGUCUCGUAUUAUUCUUAUUCUGUA CGGCUAAACGAAUGAGGGUUAAAGCCGCCAGGGUAGACAAG (SEQ ID NO: 149)
VZV-GE-Truncated-modified-V9	AUGGGCACCGUGAACAAGCCUGUUGUGGGCGUGCUGAUGGGCUU CGGCAUCAUCACAGGCACCCUGCGGAUACCAAUCCUGUGCGGGC UAGCGUGCUGAGAUACGACGACUCCACAUCGACGAGGACAAGC UGGACACCAACAGCGUGUACGAGCCCUACUACCACAGCGAUCACG CCGAGUCUAGCUGGGUCAACAGAGGCGAGAGCAGCAGAAAGGCC UACGACCACAACAGCCCUUACAUCUGGCCCAGAAACGACUACGAC GGCUUCCUCGAGAAUGCCCACGAACACCACGGCGUGUACAAUCAA GGCAGAGGCAUCGACAGCGGCGAGAGACUGAUGCAGCCUACACA GAUGAGCGCCCAAGAGGACCUGGGAGAUGAUACCGGCAUCCACG UGAUCCCUACACUGAACGGCGACGACCGGCACAAGAUUGAAGC UGGACCAGAGACAGUACGGCGACGUGUUAAGGGCGACCUGAAU CCUAAGCCUCAGGGCCAGCGCCUGAUCGAGGUUCCGUGGAAGA GAAUCACCCUUUCACACUGCGGGCUCCAUCCAGAGAAUUCUACGG CGUGCGCUAUACCGAGACAUGGUCCUUUCUGCCCAGCCUGACAGU UACCGGCGACGCGCGUCCUGCCAUCAGCAUUAUUGUGAAGCA CACCACCUGUUCCAGGACGUGGUGGUGGAUGUGGACUGCGCCG AGAACACCAAAGAGGAUCAGCUGGCCGAGAUACGCUACCGGUUC CAGGGAAAGAAAGAGGCCGACCAGCCUUGGAUCGUGGUCAACAC CAGCACACUGUUCGACGAGCUGGAACUGGACCCUCCUGAGAUUG AACCCGGCGUCCUGAAGGUGCUGAGAACCGAGAAGCAGUACCUG GGAGUGUACAUCUGGAACAUGAGAGGCAGCGACGGCACCUCUAC CUACGCCACCUUUCUGGUCACAUGGAAGGGCGACGAGAAGACCA GAAAUCCCACACCAGCCGUGACACCUCAGCCUAGAGGCGCCGAU UUCACAUGUGGAACUACCACUCUCACGUGUUCAGCGUGGGCGAU ACCUUCAGCCUGGCCAUGCAUCUGCAGUACAAGAUCACGAGGCU CCCUUCGACCUGCUGGAAUGGCUGUACGUGCCCAUCGAUCCU ACCGGCCAGCCUAGCGGCGUGUACUCCACCUGUCUGUAUACCCU AACGCUCCUCAGUGCCUGAGCCACAUGAAUAGCGGCUGCACCUUC ACAAGCCCUCACCUGGCUCAGCGAGUGGCCAGCACAGUGUACCAG AAUUGCGAGCACGCCGACAAUACACCGCCUACUGUCUGGGCAUC AGCCACAUGGAACCUAGCUUCGGCCUGAUCCUGCACGAUGGCGGC ACCACACUGAAGUUCGUGGACACACCUGAGAGCCUGAGCGGCCUG UAUGUGUUUGUGGUGUACUUAACGGCCACGUGGAAGCCGUGGC CUACACCGUGGUGUCUACCGUGGACCACUUCGUGAACGCCAUCCA GGAAAGAGGCUUCCCUCCAACUGCUGGACAGCCUCCUGCCACCAC CAAGCCUAAAGAAAUACACCCGUGAAUCCCGGCACUAGCCCUU GCUUAGAUACGCCGCUUGGACAGGCGGACUGGUCUGUUGUUC UGCUGUGCCUGGUAUCUUCUGAUCUGCACCGCCAAGCGGAUGA GAGUGAAAGCCGCCAGAGUGGACAAG (SEQ ID NO: 150)
Corresponding amino acid sequence	MGTVNKPVVGVLMGFGITGLRITNPVRASVLRYYDDFHIDEDKLDTN SVYEPYHSDHAESSWVNRGESSRKAYDHNSPYIWRNDYDGFLENA HEHHGVYNQGRGIDSGERLMQPTQMSAQEDLGDDTGIHVIPTLNGDD RHKIVNVDQRQYGDVFKGDLNPKPQGQRLIEVSVEENHPFTLRPIQRI YGVRYTETWSFLPSLTCTGDAAPAIQHICLKHTTCFQDVVDVDCEN TKEDQLAEISYRFQGKKEADQPWIVVNTSTLFDELELDPPEIEPGVLKV LRTEKQYLGVIWNMRGSDGTSTYATFLVTWKGDEKTRNPTPAVTPQ PRGAEFHMWNYHSHVFSVGDTFSLAMHLQYKIHEAPFDLLEWLYVP IDPTCQPMRLYSTCLYHPNAPQCLSHMNSGCTFTSPHLAQRVASTVYQ NCEHADNYTAYCLGISHMEPSFGLILHDGGTTLKFVDTPESLSGLYVVF VYFNGHVEAVAYTVVSTVDHFNVAIEERGFPPTAGQPPATTKPKEITP VNPGETSPLRLYAAWTGGLAAVLLCLVIFLICTAKRMRVKAARVDK (SEQ ID NO: 38)

mRNA Construct	ORF of mRNA Construct (excluding the stop codon)
5' UTR	GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCC ACC (SEQ ID NO: 138)
3' UTR	UGAUAUUAGGCUAGGCCUCGGUGGCCAUGCUUCUUGCCCCUUG GGCCUCCCCCAGCCCCUCCUCCCUUCCUGCACCCGUACCCCGU GGUCUUUGAAUAAAGUCUGAGUGGGCGGC (SEQ ID NO: 139)
PolyA Tail	100 nt (SEQ ID NO: 140)

Table 14. Varicella zoster virus Amino Acid Sequences

Protein	Name	GenBank Accession
glycoprotein B	envelope glycoprotein B [Human herpesvirus 3]	NP_040154.2
glycoprotein B	ORF31 [Human herpesvirus 3]	AKG57704.1
glycoprotein B	ORF 31 [Human herpesvirus 3]	AIT52967.1
glycoprotein B	envelope glycoprotein B [Human herpesvirus 3]	AFJ68532.1
glycoprotein B	ORF31 [Human herpesvirus 3]	AKG57414.1
glycoprotein B	ORF31 [Human herpesvirus 3]	AKG58507.1
glycoprotein B	RecName: Full=Envelope glycoprotein B; Short=gB; AltName: Full=Glycoprotein II; Flags: Precursor [Human herpesvirus 3 strain Oka vaccine]	Q4JR05.2
glycoprotein B	ORF31 [Human herpesvirus 3]	AEL30845.1
glycoprotein B	glycoprotein B [Human herpesvirus 3]	AAK01041.1
glycoprotein B	glycoprotein B [Human herpesvirus 3]	AEW89232.1
glycoprotein B	glycoprotein B [Human herpesvirus 3]	AEW88728.1
glycoprotein B	ORF31 [Human herpesvirus 3]	AAK19938.1
glycoprotein B	glycoprotein B [Human herpesvirus 3]	AAP32845.1
glycoprotein B	ORF 31 [Human herpesvirus 3]	AHJ08729.1
glycoprotein B	ORF31 [Human herpesvirus 3]	AAAY57715.1
glycoprotein B	ORF31 [Human herpesvirus 3]	AGY33726.1
glycoprotein B	ORF 31 [Human herpesvirus 3]	AHJ09321.1
glycoprotein B	ORF31 [Human herpesvirus 3]	AAAY57644.1
glycoprotein B	ORF 31 [Human herpesvirus 3]	AHJ09025.1
glycoprotein B	glycoprotein B [Human herpesvirus 3]	AEW88584.1
glycoprotein B	ORF 31 [Human herpesvirus 3]	AHJ09099.1
glycoprotein B	ORF31 [Human herpesvirus 3]	AGY33060.1
glycoprotein B	ORF 31 [Human herpesvirus 3]	AHJ09395.1
glycoprotein C	RecName: Full=Envelope glycoprotein C; Short=gC; AltName: Full=Glycoprotein V; Short=gpV	P09256.2
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABH08453.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AIH07125.1
glycoprotein C	unknown protein [Human herpesvirus 3]	AAA69563.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AIH07051.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AIJ28607.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AEL30828.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABE03032.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABE67122.1
glycoprotein C	envelope glycoprotein C [Human herpesvirus 3]	NP_040137.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW88351.1
glycoprotein C	envelope glycoprotein C [Human herpesvirus 3]	AFJ68515.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AAT07696.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABF22098.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW89287.1
glycoprotein C	glycoprotein C [Human herpesvirus 3]	AGC94505.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABF21514.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABF21879.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABF21587.1
glycoprotein C	ORF 14 [Human herpesvirus 3]	AIT53315.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW89215.1

Protein	Name	GenBank Accession
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABF21660.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW88567.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	CAI44857.1
glycoprotein C	envelope glycoprotein C [Human herpesvirus 3]	AHB80244.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AAAY57702.1
glycoprotein C	glycoprotein c [Human herpesvirus 3]	AGS32072.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	AGL50971.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AAT07772.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW88495.1
glycoprotein C	ORF 14 [Human herpesvirus 3]	AIT53461.1
glycoprotein C	ORF 14 [Human herpesvirus 3]	AIT52950.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AAAY57631.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABF21952.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW89143.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW88783.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW88999.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW88063.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW89071.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW88639.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW87991.1
glycoprotein C	ORF 14 [Human herpesvirus 3]	AIT53753.1
glycoprotein C	ORF 14 [Human herpesvirus 3]	AIT53096.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABF22025.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	AFO85518.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABF21733.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	ABF21806.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW89359.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW88855.1
glycoprotein C	envelope glycoprotein gC [Human herpesvirus 3]	AFO85591.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW89431.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW88711.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW88135.1
glycoprotein C	membrane glycoprotein C [Human herpesvirus 3]	AEW88927.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AKG56156.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AKG57178.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AKG58125.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AGY32970.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AKG56229.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AGY32896.1
glycoprotein C	ORF14 [Human herpesvirus 3]	AKG56521.1
glycoprotein C	ORF 14 [Human herpesvirus 3]	AHJ08712.1
glycoprotein E	unknown [Human herpesvirus 3]	ABE03086.1
glycoprotein E	glycoprotein E [Human herpesvirus 3]	AAK01047.1
glycoprotein E	RecName: Full=Envelope glycoprotein E; Short=gE; Flags: Precursor	Q9J3M8.1
glycoprotein E	membrane glycoprotein E [Human herpesvirus 3]	AEW88548.1
glycoprotein E	ORF68 [Human herpesvirus 3]	AGY33616.1
glycoprotein E	membrane glycoprotein E [Human herpesvirus 3]	AEW89124.1
glycoprotein E	ORF 68 [Human herpesvirus 3]	AIT53150.1
glycoprotein E	unnamed protein product [Human herpesvirus 3]	CAA25033.1
glycoprotein E	envelope glycoprotein E [Human herpesvirus 3]	NP_040190.1
glycoprotein E	ORF68 [Human herpesvirus 3]	AKG56356.1
glycoprotein E	membrane glycoprotein E [Human herpesvirus 3]	AEW89412.1
glycoprotein E	membrane glycoprotein gE [Human herpesvirus 3]	ABF21714.1
glycoprotein E	membrane glycoprotein E [Human herpesvirus 3]	AAT07749.1
glycoprotein E	membrane glycoprotein E [Human herpesvirus 3]	AEW88764.1
glycoprotein E	glycoprotein E [Human herpesvirus 3]	AAG48520.1
glycoprotein E	membrane glycoprotein E [Human herpesvirus 3]	AEW88980.1

Protein	Name	GenBank Accession
glycoprotein H	envelope glycoprotein H [Human herpesvirus 3]	NP_040160.1
glycoprotein H	glycoprotein H [Human herpesvirus 3]	AEW89454.1
glycoprotein H	ORF37 [Human herpesvirus 3 VZV-32]	AAK19252.1
glycoprotein H	RecName: Full=Envelope glycoprotein H; Short=gH; AltName: Full=Glycoprotein III; Short=GPIII; Flags: Precursor	Q775J3.1
glycoprotein H	glycoprotein H [Human herpesvirus 3]	AAK01042.1
glycoprotein H	ORF37 [Human herpesvirus 3]	AKG58587.1
glycoprotein H	ORF37 [Human herpesvirus 3]	AGY33215.1
glycoprotein H	glycoprotein H [Human herpesvirus 3]	AAP32857.1
glycoprotein H	envelope glycoprotein gH [Human herpesvirus 3]	ABE03056.1
glycoprotein H	ORF 37 [Human herpesvirus 3]	AHJ09328.1
glycoprotein H	glycoprotein H [Human herpesvirus 3]	AAP32862.1
glycoprotein H	ORF37 [Human herpesvirus 3]	AKG57421.1
glycoprotein H	ORF37 [Human herpesvirus 3]	AKG56618.1
glycoprotein H	ORF37 [Human herpesvirus 3]	AKG56545.1
glycoprotein H	glycoprotein H [Human herpesvirus 3]	AEW89382.1
glycoprotein H	glycoprotein H [Human herpesvirus 3]	AGC94548.1
glycoprotein I	envelope glycoprotein I [Human herpesvirus 3]	NP_040189.1
glycoprotein I	membrane glycoprotein I [Human herpesvirus 3]	AEW89195.1
glycoprotein I	ORF67 [Human herpesvirus 3]	AKG58616.1
glycoprotein I	ORF67 [Human herpesvirus 3]	AGY34059.1
glycoprotein I	membrane glycoprotein I [Human herpesvirus 3]	AEW89051.1
glycoprotein I	ORF67 [Human herpesvirus 3 VZV-32]	AAK19249.1
glycoprotein I	membrane glycoprotein I [Human herpesvirus 3]	AEW89483.1
glycoprotein K	envelope glycoprotein K [Human herpesvirus 3]	NP_040128.1
glycoprotein K	glycoprotein K [Human herpesvirus 3]	AEW88773.1
glycoprotein K	ORF 5 [Human herpesvirus 3]	AHJ09368.1
glycoprotein K	ORF5 [Human herpesvirus 3]	AKG58699.1
glycoprotein K	glycoprotein K [Human herpesvirus 3]	AEW88701.1
glycoprotein K	ORF5 [Human herpesvirus 3]	AKG56803.1
glycoprotein K	glycoprotein K [Human herpesvirus 3]	AEW88053.1
glycoprotein L	RecName: Full=Envelope glycoprotein L; Short=gL; Flags: Precursor	Q9J3N1.1
glycoprotein L	virion glycoprotein gL [Human herpesvirus 3]	ABE03078.1
glycoprotein L	glycoprotein L [Human herpesvirus 3]	AGM33094.1
glycoprotein L	ORF60 [Human herpesvirus 3]	AKG56786.1
glycoprotein L	envelope glycoprotein L [Human herpesvirus 3]	NP_040182.1
glycoprotein L	virion glycoprotein gL [Human herpesvirus 3]	ABF21706.1
glycoprotein M	envelope glycoprotein M [Human herpesvirus 3]	NP_040172.1
glycoprotein M	ORF 50 [Human herpesvirus 3]	AIT53351.1
glycoprotein M	ORF50 [Human herpesvirus 3]	AKG56119.1
glycoprotein M	ORF50 [Human herpesvirus 3]	AGY33080.1
glycoprotein M	envelope glycoprotein gM [Human herpesvirus 3]	ABE03068.1
glycoprotein M	virion membrane glycoprotein M [Human herpesvirus 3]	AEW88530.1
glycoprotein M	virion membrane glycoprotein M [Human herpesvirus 3]	AEW88674.1
glycoprotein N	envelope glycoprotein N [Human herpesvirus 3]	YP_068406.1
glycoprotein N	ORF9a [Human herpesvirus 3]	AGY33038.1
glycoprotein N	membrane protein [Human herpesvirus 3]	AAT07690.1
glycoprotein N	membrane protein [Human herpesvirus 3]	AEW88273.1
glycoprotein N	membrane protein [Human herpesvirus 3]	AEW88489.1

Table 15. VZV Polypeptide Sequences

Name	Sequence	SEQ ID NO:
gi 443500676 gb AGC94542.1 glycoprotein E [Human herpesvirus 3]	MGTVNKPVVGVLGMGFGIITGTLRITNPVRASVRLRYDDFH IDEDKLDTNSVYEPYHSDHAESSWVNRGESSRKAYDH NSPYIWPRNDYDGFLENAHEHHGVYNQGRGIDSGERLM QPTQMSAQEDLGDDTGIHVIPTLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQRLIEVSVEENHPFTLRAPIQRIYG VRYTETWSFLPSLTCTGDAAPAIQHICLKHTTCFQDVVV DVDCAENTKEDQLAEISYRFQGKKEADQPWIVVNTSTLF DELELDPPEIEPGVLKVLRTKQYLGVIWNMRGSDGTS TYATFLVTWKGDEKTRNPTPAVTPQPRGAEFHMWNYH SHVFSVGDTFSLAMHLQYKIHEAPFDLLEWLYVPIDPT CQPMRLYSTCLYHPNAQCLSHMNSGCTFTSPHLAQRV ASTVYQNCEHADNYTAYCLGISHMEPSFGLILHDGGTTL KFVDTPESLSGLYVFVVYFNGHVEAVAYTVVSTVDHVF NAIEERGFPTAGQPPATTKPKEITPVNPGTSPLLRYYAAW TGGLAAVLLCLVIFLICTAKRMRVKAYRVDKSPYNQS MYAGLPVDDFEDSESTDTEEEFGNAIGGSHGGSSYTVY IDKTR	45
gi 443500675 gb AGC94541.1 glycoprotein I [Human herpesvirus 3]	MFLIQCLISAVIFYIQVTNALIFKGDHVS LQVNSSLTSILIP MQNDNYTEIKGQLVFigEQLPTGTNYSGTLELLYADTVA FCFRSVQVIRYDGCPRIRTSAFISCRYKHSWHYGNSTDRI STEPDAGVMLKITKPGINDAGVYVLLVRLDHSRSTDGFI LGVNVYTAGSHHNIHGVIYTPSLQNGYSTRALFQQARL CDLPATPKGSGTSLFQHMLDLRAGKSLEDNPWLHEDVV TTETKSVVKEGIENHVYPTDMSTLPEKSLNDPPENLLIPI VASVMILTAMVIVIVISVKRRRIKKHPIYRPNTKTRRGIQ NATPESDVMLEAAIAQLATIREESPPHSVVPFVK	46
VZV-GE-delete-562	MGTVNKPVVGVLGMGFGIITGTLRITNPVRASVRLRYDDFH IDEDKLDTNSVYEPYHSDHAESSWVNRGESSRKAYDH NSPYIWPRNDYDGFLENAHEHHGVYNQGRGIDSGERLM QPTQMSAQEDLGDDTGVIPITLNGDDRHKIVNVDQRQY DVFKGDLNPKPQGQRLIEVSVEENHPFTLRAPIQRIYGV YTETWSFLPSLTCTGDAAPAIQHICLKHTTCFQDVVV DCAENTKEDQLAEISYRFQGKKEADQPWIVVNTTLFDEL ELDPPEIEPGVLKVLRTKQYLGVIWNMRGSDGTSTYA TFLVTWKGDEKTRNPTPAVTPQPRGAEFHMWNYHSHV SVGDTFSLAMHLQYKIHEAPFDLLEWLYVPIDPTCQPM RLYSTCLYHPNAQCLSHMNSGCTFTSPHLAQRVASTVY QNCEHADNYTAYCLGISHMEPSFGLILHDGGTTLKFVD TPESLSGLYVFVVYFNGHVEAVAYTVVSTVDHVFNAIE ERGFPTAGQPPATTKPKEITPVNPGTSPLLRYYAWTG GLAAVLLCLVIFLICTA	47
gi 46981496 gb AT07772.1 membrane glycoprotein C [Human herpesvirus 3]	MKRIQINLILTIACIQLSTESQPTPV SITELYTSAATRKPDP AVAP TSAATRKPDP AVAP TSAATRKPDP AVAP TSAATR KPDP AVAP TSAATRKPDP AVAP TSAATRKPDP AVAP TSAATRKPDP AVAP TSAATRKPDP AVAP TSAATRKP DPAANTQHSQPPFLYENIQCVHGGIQSIPYFHTFIMPCY MRLTTGQQAAFKQQQKTYEQYSLDPEGSNITRWKSLIR PDLHIEVWFTRHLIDPHRQLGNALIRMPDLPVMLYSNS ADLNLINNPEIFTHAKENYVIPDVKTTSDFSVTLSMDAT TEGTIYWRVNTKTKNVISEHSITVTYYRPNITVVGDPV LTGQTYAAYCNVSKYPPHSVRVRWTSRFGNIGKNFIT DAIQEYANGLFSYVSAVRIPQQKQMDYPPPAIQC NVLWIRGVSNMKYSAVVTPDVYFPNVSIGIIDGHIV CTAKCVPRGVVHFVWWVNDSPINHENSEITGVCDQ NKR FVNMQSSCPTSELDGPITYSCHLDGYPKKFP PFSAVYTYDASTYATTFSVVAVIIGVISILGTLGLI AVIATLCIRCCS	48

Name	Sequence	SEQ ID NO:
gi 9625934 ref NP_040182.1 envelope glycoprotein L [Human herpesvirus 3]	MASHKWLLQIVFLKTITIAAYCLHLQDDTPLFFGAKPLSD VSLIITEPCVSSVYEAWDYAAPVSNLSEALSGIVVKTKC PVPEVILWFKDKQMAYWTNPYVTLKGLAQSVGEEHKS GDIRDALLDALSGVWVDSTPSSTNIPENGCVWGADRLFQ RVCQ	49
gi 9625925 ref NP_040172.1 envelope glycoprotein M [Human herpesvirus 3]	MGTQKKGPRSEKVSPYDTTTPPEVEALDHQMDTLNWRI WIIQVMMFTLGAVMLLATLIAASSEYTGIPCFYAAVVDY ELFNATLDGGVWVSGNRGGYSAPVLFLEPHSVVAFTYYT ALTAMAMAVYTLITAAIIHRETKNQVRVQSSGVAVLWV DPTTLFWGLLSLWLLNAVVLLEYKQIGVAATLYLGHF ATSVIFTTYFCGRGKLDENIKAVANLRQQSVFLYRLAG PTRAVFVNLMAALMAICILFVSLMLELVVANHLHTGLW SSVSVAMSTFSTLSVVYLIVSELILAHYIHVLIGPSLGLTV ACATLGTAAHSYMDRLYDPISVQSPRLIPTTRGTLACLA VFSVVMLLLRLMRAVYVYHRQKRSRFYGAVRRVPERVR GYIRKVKPAHRNSRRTNYPSPQGYGVYVENDSTYETDRE DELLYERSNSGWE	50
gi 9625912 ref NP_040160.1 envelope glycoprotein H [Human herpesvirus 3]	MFALVLAVVILPLWTTANKSYVTPTPATRSIGHMSALLR EYSDRNMSLKLEAFYPTGFDEELIKSLHWGNDRKHVFL VIVKVNPTTHEGDVGLVIFPKYLLSPYHFKAEHRAPFPA GRFGFLSHPVTPDVSFDDSSFAPYLTTQHLVAFTTFPPNPL VWHLERAETAATAERPFGVSLPARPTVPKNTILEHKAH FATWDALARHTFFSAEAIITNSTLRIHVPLFGSVWPIRYW ATGSVLLTSDSGRVEVNIGVGFMSLSLSSGPPIELIVVP HTVKLNAVTSDDTWQFNPPGPDGPSYRVYLLGRGLD MNFSKHATVDICAYPEESLDYRYHLSMAHTEALRMTTK ADQHDINEESYYHIAARIATSIFALSEMGRTEYFLLDEIV DVQYQLKFLNYILMRIGAGAHNPNTISGTSDLIFADPSQLH DELSLLFGQVKPANVDYFISYDEARDQLKTAYALSRGQ DHVNALSLARRVIMSIYKGLLVKQNLNATERQALFFAS MILLNFREGLNSSRVLDGRTTLLMTSMCTAAHATQA ALNIQEGLAYLNPSKHMFTIPNVYSPCMGSLRTDLTEEIH VMNLLSAIPTRPGLNEVLHTQLDESEIFDAAFKTMIFTT WTAKDLHILHTHVPEVFTCQDAARNGEYVLILPAVQG HSYVITRNKPQRLVYSLADVDVYNPISVVYLSRDTCVS EHGVIETVALPHPDNLKECLYCGSVFLRYLTTGAIMDIII DSKDTERQLAAMGNSTIPFPNPDMHGDDSKAVLLFPNG TVVTLLGFERRQAIRMSGQYLGASLGGAFLLAVVGFGIIG WMLCGNSRLREYNKIPLT	51
gi 584403829 gb AHB80298.1 envelope glycoprotein E [Human herpesvirus 3]	MFYEALKAEVYTRAVHGFPRANCVVLSYIPRVACN MGTVNKPVVGVLMGFGIITGLRITNPVRASVLRYYDDFH IDEDKLDTNVYEPYYHSDHAESSWVNRGESSRKAYDH NSPYIWPRNDYDGFLENAHEHHGVYNQGRGIDSGERLM QPTQMSAQEDLGDDTGIHVIPTLNGDDRHKIVNVDQRQ YGDVFKGDLNPKPQGQRLIEVSVEENHPFTLRAPIQRIYG VRYTETWSFLPSLTCTGDAAPAIQHICLKHTTCFQDVVV DVDCAENTKEDQLAEISYRFQGKKEADQPWIVVNTSTLF DELELDPPEIEPGVLKVLRTKQYLGVIYNMRGSDGTS TYATFLVTWKGDEKTRNPTPAVTPQPRGAEFHMWNYH SHVFSVGDTFSLAMHLQYKIHEAPFDLLLEWLYVPIDPT CQPMRLYSTCLYHPNAPQCLSHMNSGCTFTSPHLAQRV ASTVYQNCHEADNYTAYCLGISHMEPSFGLILHDGGTTL KFVDTPESLSGLYVFVVFNGHVEAVAYTVVSTVDHFV NAIEERGFPPTAGQPPATTKPKEITPVNPGTSPLLRVAAW TGGLAAVVLLCLVIFLICTAKRMVRKAYRVDKSPYNQS MYYAGLPVDDFEDSESTDTEEEFGNAIGGSHGGSSYTVY IDKTR	52

Name	Sequence	SEQ ID NO:
gi 46981513 gb A AT07789.1 glycoprotein B [Human herpesvirus 3]	MFVTA VVS VSPSSFYESLQVEPTQSEDI TRSAHLGDGDEI REAIHKSQDAETKPTFYVCPPTGSTIVRLEPTRTCPDYH LGKNFTEGIAVVYKENIAAYKFKATVYYKDVIVSTAWA GSSYTQITNRYADRVPIPVSEITDTIDKFGKCSSKATYVR NNHKVEAFNEDKNPQDMPLIASKYNSVGSKAWHTTND TYMVAGTPGTYRTGTSVNCIIEEVEARSIFPYDSFGLSTG DIIYMSPFFGLRDGAYREHSNYAMDRFHQFEGYRQRDL DTRALLEPAARNFLVTPHLTVGWNWPKRTEVCSLVK WREVEDVVRDEYAHNFRFTMKTLSSTTFISETNEFNLNQI HLSQC VKEEARAIINRIYTTRYNSSHVRTGDIQTYLARGG FVVVFQPLLSNSLARLYLQELVRENTNHSPQKHPTNRTR SRRSVPVELRANRTITTTSSVEFAMLQFTYDHIQEHVNE MLARISSWCQLQNRERALWSGLFPINPSALASTILDQRV KARILGDVISVSNCP ELGSDTRILQNSMRVSGSTTRCYSR PLISIVSLNGSGTVEGQLGTDNELIMSRDLLEPCVANHKR YFLFGHHYVYYEDYRYVREIAVHDVGMISTYVDNLNLT LKDREFMPLQVYTRDEL RDTGLLDYSEIQR RNQMHS LRF YDIDKVVQYDSGTAIMQGM A QFFQGLGTAGQAVGHVV LGATGALLSTVHGFTTFLSNPFGALAVGLLVLAGLVAAF FAYRYVLKKTSPMKALYPLTTKGLKQLPEGMDPFAEK PNATDTPIEEIGDSQNT E P SVNSGFD PDKFREAQEMIKYM TLVSA AERQESKARKKNKTSALLTSRLTGLALRNRRGYS RVRTENVTVG	53
gi 46981487 gb A AT07763.1 glycoprotein K [Human herpesvirus 3]	MQALGIKTEHFIIMCLLSGHAVFTLWYTARVKFEHECVY ATTVINGGPVWGSYNNSLIYVTFVNHSTFLDGLSGYDY SCREENLLSGDTMVKTAISTPLHDKIRIVLGTRNCHAYFW CVQLKMIFFAWFVYGMYLQFRRIRRMFGPFRSSCELISPT SYSLNYVTRVISNILLGYPYTKLARLLCDVSMRRDGMSK VFNADPISFLYMHKGVTLLMLLEVIAHISSGCIVLLTLGV AYTPCALLYPTYIRILAWVVVCTLAIVELISYVRPKPTKD NHLNHINTGGIRGICTTCCATVMSGLAIKCFYIVIFAI VV IFMHYEQRVQVSLFGESENSQKH	54
gi 443500633 gb AGC94499.1 glycoprotein N [Human herpesvirus 3]	MGSITASFILITMQILFFCEDSSGEPNFAERNFWHASCSAR GVYIDGSMITTLFFYASLLGVCVALISLAYHACFRLFTRS VLRSTW	55
Ig heavy chain epsilon-1 signal peptide (IgE HC SP)	MDWTWILFLVAAATRVHS	56
IgGk chain V-III region HAH signal peptide (IgGk SP)	METPAQLLFLLLLWLPDTTG	57
Japanese encephalitis PRM signal sequence	MLGSNSGQRVVFTILLLLVAPAYS	109
VSVg protein signal sequence	MKCLLYLAFLFIGVNCA	110
Japanese encephalitis JEV signal sequence	MWLVSLAIVTACAGA	111

Table 16. Flagellin Nucleic Acid Sequences

Name	Sequence	SEQ ID NO:
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NT (5' UTR, ORF, 3' UTR)	TCAAGCTTTTGGACCCTCGTACAGAAGCTAATACGACTCACTAT AGGGAAATAAGAGAGAAAAAGAAGTAAGAAGAAATATAAG AGCCACCATGGCACAAGTCATTAATACAAACAGCCTGTCGCTG TTGACCCAGAATAACCTGAACAAATCCCAGTCCGCACTGGGCA CTGCTATCGAGCGTTTGTCTTCCGGTCTGCGTATCAACAGCGCG AAAGACGATGCGGCAGGACAGGCGATTGCTAACCGTTTTACCG CGAACATCAAAGGTCTGACTCAGGCTTCCCGTAACGCTAACGA CGGTATCTCCATTGCGCAGACCACTGAAGGCGCGCTGAACGAA ATCAACAACAACCTGCAGCGTGTGCGTGAAGTGGCGGTTCACT CTGCGAATGGTACTAACTCCCAGTCTGACCTCGACTCCATCCAG GCTGAAATCACCCAGCGCCTGAACGAAATCGACCGTGTATCCG GCCAGACTCAGTTCAACGGCGTGAAAGTCCTGGCGCAGGACAA CACCTGACCATCCAGGTTGGTGCCAACGACGGTGAAACTATC GATATTGATTTAAAAGAAATCAGCTCTAAAACACTGGGACTTG ATAAGCTTAATGTCCAAGATGCCTACACCCCGAAAGAACTGC TGTAACCGTTGATAAAACTACCTATAAAAAATGGTACAGATCCT ATTACAGCCCAGAGCAATACTGATATCCAAACTGCAATTGGCG GTGGTGCAACGGGGGTTACTGGGGCTGATATCAAATTTAAAGA TGGTCAATACTATTTAGATGTTAAAGGCGGTGCTTCTGCTGGTG TTTATAAAGCCACTTATGATGAAACTACAAAGAAAGTTAATAT TGATACGACTGATAAAACTCCGTTGGCAACTGCGGAAGCTACA GCTATTTCGGGGAACGGCCACTATAACCCACAACCAAATTGCTG AAGTAACAAAAGAGGGTGTGATACGACCACAGTTGCGGCTCA ACTTGCTGCAGCAGGGGTTACTGGCGCCGATAAGGACAATACT AGCCTTGTAAAACTATCGTTTGAGGATAAAAAACGGTAAGGTTA TTGATGGTGGCTATGCAGTGAAAATGGGCGACGATTTCTATGC CGCTACATATGATGAGAAAAACAGGTGCAATTACTGCTAAAACC ACTACTTATACAGATGGTACTGGCGTTGCTCAAACCTGGAGCTGT GAAATTTGGTGGCGCAAATGGTAAATCTGAAGTTGTTACTGCT ACCGATGGTAAGACTTACTTAGCAAGCGACCTTGACAAACATA ACTTCAGAACAGGCGGTGAGCTTAAAGAGGTAAATACAGATAA GACTGAAAACCCACTGCAGAAAATTGATGCTGCCTTGGCACAG GTTGATACACTTCGTTCTGACCTGGGTGCGGTTCAAGACCGTTT CAACTCCGCTATACCAACCTGGGCAATACCGTAAATAACCTG TCTTCTGCCCCGTAGCCGTATCGAAGATTCCGACTACGCAACCGA AGTCTCCAACATGTCTCGCGCGCAGATTCTGCAGCAGGCCGGT ACCTCCGTTCTGGCGCAGGCGAACCAGGTTCCGCAAAACGTCC TCTCTTTACTGCGTTGATAATAGGCTGGAGCCTCGGTGGCCATG CTTCTTGCCCCCTTGGGCCTCCCCCAGCCCCCTCCTCCCCCTCCTG CACCCGTACCCCGTGGTCTTTGAATAAAGTCTGAGTGGGCGG C	112
ORF Sequence, NT	ATGGCACAAGTCATTAATACAAACAGCCTGTCGCTGTTGACCC AGAATAACCTGAACAAATCCCAGTCCGCACTGGGCACTGCTAT CGAGCGTTTGTCTTCCGGTCTGCGTATCAACAGCGCGAAAGAC GATGCGGCAGGACAGGCGATTGCTAACCGTTTTACCGCGAACA TCAAAGTCTGACTCAGGCTTCCCGTAACGCTAACGACGGTAT CTCCATTGCGCAGACCACTGAAGGCGCGCTGAACGAAATCAAC AACAACCTGCAGCGTGTGCGTGAAGTGGCGGTTCACTGCGA ATGGTACTAACTCCCAGTCTGACCTCGACTCCATCCAGGCTGAA ATCACCCAGCGCCTGAACGAAATCGACCGTGTATCCGGCCAGA CTCAGTTCAACGGCGTGAAAGTCCTGGCGCAGGACAACACCCT GACCATCCAGGTTGGTGCCAACGACGGTGAAACTATCGATATT GATTTAAAAGAAATCAGCTCTAAAACACTGGGACTTGATAAGC TTAATGTCCAAGATGCCTACACCCCGAAAGAAACTGCTGTAAC CGTTGATAAAACTACCTATAAAAAATGGTACAGATCCTATTACA GCCAGAGCAATACTGATATCCAAACTGCAATTGGCGGTGGTG CAACGGGGGTTACTGGGGCTGATATCAAATTTAAAGATGGTCA ATACTATTTAGATGTTAAAGGCGGTGCTTCTGCTGGTGTATTATA AAGCCACTTATGATGAAACTACAAAGAAAGTTAATATTGATAC GACTGATAAAACTCCGTTGGCAACTGCGGAAGCTACAGCTATT CGGGGAACGGCCACTATAACCCACAACCAAATTGCTGAAGTAA CAAAAGAGGGTGTGATACGACCACAGTTGCGGCTCAACTTGC	113

	TGCAGCAGGGGTTACTGGCGCCGATAAGGACAATACTAGCCTT GTAAACTATCGTTTGAGGATAAAAACGGTAAGGTTATTGATG GTGGCTATGCAGTGAAAATGGGCGACGATTTCTATGCCGCTAC ATATGATGAGAAAACAGGTGCAATTACTGCTAAAACCACTACT TATACAGATGGTACTGGCGTTGCTCAAACCTGGAGCTGTGAAAT TTGGTGGCGCAAATGGTAAATCTGAAGTTGTTACTGCTACCGAT GGTAAGACTTACTTAGCAAGCGACCTTGACAAACATAACTTCA GAACAGGCGGTGAGCTTAAAGAGGTTAATACAGATAAGACTG AAAACCCACTGCAGAAAATTGATGCTGCCTTGGCACAGGTTGA TACACTTCGTTCTGACCTGGGTGCGGTTTCAGAACCGTTTCAACT CCGCTATCACCAACCTGGGCAATACCGTAAATAACCTGTCTTCT GCCCCGTAGCCGTATCGAAGATTCCGACTACGCAACCGAAGTCT CCAACATGTCTCGCGCGCAGATTCTGCAGCAGGCCGGTACCTC CGTTCTGGCGCAGGCGAACCAGGTTCCGCAAAACGTCTCTCTT TACTGCGT	
mRNA Sequence (assumes T100 tail)	G*GGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAA GAGCCACCAUGGCACAAGUCAUUAUACAAACAGCCUGUCGC UGUUGACCCAGAAUAACCUGAACAUAUCCAGUCCGCACUGG GCACUGCUAUCGAGCGUUUGUCUUCGGUCUGCGUAUCAACA GCGCGAAAGACGAUGCGGCAGGACAGGCGAUUGCUAACCGUU UUACCGCGAACAUCAAAGGUCUGACUCAGGCUUCCCGUAACG CUAACGACGGUAUCUCCAUUGCGCAGACCACUGAAGGCGCGC UGAACGAAAUCAACAACAACCUGCAGCGUGUGCGUGAACUGG CGGUUCAGUCUGCGAAUGGUACUAACUCCAGUCUGACCUCG ACUCCAUCCAGGCUGAAAUCACCCAGCGCCUGAACGAAAUCG ACCGUGUAUCCGGCCAGACUCAGUUAACGGCGUGAAAAGUCC UGGCGCAGGACAACACCCUGACCAUCCAGGUUGGUGCCAACG ACGGUGAAACUAUCGAUAUUGAUUUAAAAGAAAUCAGCUCU AAAACACUGGGACUUGAUAAAGCUUAAUGUCCAAGAUGCCUAC ACCCCGAAAGAAACUGCUGUAACCGUUGAUAAAACUACCUAU AAAAAUGGUACAGAUCCUAUACAGCCCAGAGCAAUACUGAU AUCCAAACUGCAAUUGGCGGUGGUGCAACGGGGGUUACUGG GGCUGAUUAUCAAUUUAAAAGAUGGUCAAUACUAAUUAAGAUG UUAAGGCGGUGCUUCUGCUGGUGUUUAUAAAGCCACUUAU GAUGAAACUACAAAGAAAGUAAUAUUGAUACGACUGAUAA AACUCCGUUGGCAACUGCGGAAGCUACAGCUAUUCGGGGAAC GGCCACUAUAACCCACAACCAAAUUGCUGAAGUAACAAAAGA GGGUGUUGAUACGACCACAGUUGCGGCUAACUUGCUGCAGC AGGGGUUACUGGCGCCGAUAAGGACAAUACUAGCCUUGUAA AACUAUCGUUUGAGGAUAAAAACGGUAAGGUUAUUGAUGGU GGCUAUGCAGUGAAAAUGGGCGACGAUUUCUAUGCCGCUACA UAUGAUGAGAAAACAGGUGCAAUACUGCUAAAACCACUAC UUUAACAGAUUGGUACUGGCGUUGCUCAAACUGGAGCUGUGA AAUUUGGUGGCGCAAUUGGUAAAUCUGAAGUUGUUAUCUGCU ACCGAUGGUAAAGACUUAUAGCAAGCGACCUUGACAAACAU AACUUCAGAACAGGCGGUGAGCUUAAAGAGGUUAAUACAGA UAAAGACUGAAAACCCACUGCAGAAAAAUUGAUGCUGCCUUGGC ACAGGUUGAUACACUUCGUUCUGACCUGGGUGCGGUUCAGAA CCGUUUCAACUCCGCUAUCACCAACCUGGGCAAUACCGUAAA UAACCGUCUUCUGCCCGUAGCCGUUUCGAAGAUUCCGACUA CGCAACCGAAGUCUCCAACAUGUCUCGCGCGCAGAUUCUGCA GCAGGCCGGUACCUCCGUUCUGGCGCAGGCGAACCCAGGUUCC GCAAAACGUCCUCUCUUUACUGCGUUGAUAAUAGGCUGGAGC CUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCCAGCC CCUCCUCCCCUUCUGCACCCGUACCCCGUGGUCUUUGAAU AAAGUCUGAGUGGGCGGCAAAAAAAAAAAAAAAAAAAAAAAAAA AAA AAAUCUAG	114

It should be understood that each of the ORF sequences provided herein may be combined with a 5' and/or 3' UTR, such as those described herein. It should also be understood that the 5' and/or 3' UTR for each construct may be omitted, modified or substituted for a different UTR sequences in any one of the vaccines as provided herein.

Table 17. Flagellin Amino Acid Sequences

Name	Sequence	SEQ ID NO:
ORF Sequence, AA	MAQVINTNSLSLLTQNNLNKSQSALGTAIERLSSGLRINSKDDAA GQAIANRFTANIKGLTQASRNANDGISIAQTTEGALNEINNNLQRV RELAVQSANGTNSQSDLSIQAEITQRLNEIDRVSGQTQFNGVKVL AQDNTLTIQVGANDGETIDIDLKEISSKTLGLDKLNVQDAYTPKET AVTVDKTTYKNGTDPITAQSNTDIQTAIGGGATGVTGADIKFKDG QYYLDVKGGASAGVYKATYDETTKKVNIDTTDKTPLATAEATAI RGTATITHNQIAEVTKEGVDTTTVAQAALAAAGVTGADKDNTSLV KLSFEDKNGKVIDGGYAVKMGDDFYAATYDEKTGAITAKTTTTYT DGTGVAQGTGAVKFGGANGKSEVVTATDGKTYLASDLKHNFR GGELKEVNTDKTENPLQKIDAALAQVDTLRSDLGAVQNRFN NSAITNLGNTVNNLSSARSRIEDSDYATEVSNMSRAQILQQAGTSVLAQA NQVPQNVLSLLR	115
Flagellin- <u>GS linker- circumspor ozoite protein (CSP)</u>	MAQVINTNSLSLLTQNNLNKSQSALGTAIERLSSGLRINSKDDAA GQAIANRFTANIKGLTQASRNANDGISIAQTTEGALNEINNNLQRV RELAVQSANSTNSQSDLSIQAEITQRLNEIDRVSGQTQFNGVKVL AQDNTLTIQVGANDGETIDIDLKQINSQTLGLDTLNVQQKYKVS DTAATVTGYADTTIALDNSTFKASATGLGGTDQKIDGDLKFDDTTG KYYAKVTVTGGTGKDGYYEVSVDKTNGEVTLAGGATSPLTGG LPATATEDVKNVQVANADLTEAKAALTAAGVTGTASVVKMSYTDN NGKTIDGGLAVKVGDDYYSATQNKDGSISINTTKYTADDGTSKTA LNKLGGADGKTEVVSIGGKTYAASKAEGHNFKAQPDLAEEAATT TENPLQKIDAALAQVDTLRSDLGAVQNRFNNSAITNLGNTVNNLTS ARSRIEDSDYATEVSNMSRAQILQQAGTSVLAQANQVPQNVLSLL RGGGGSGGGGSMAPDPNANPNANPNANPNANPNANPNANPNAN PNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNAN PNANPNANPNKNNQNGGQGHNMPPNDPNRNVDENANANNVKNNN NEEPSDKHIEQYLKKIKNSISTEWSPCSVTCGNGIQVRIKPGSANKP KDEL DYENDIEKKICKMEKCSSVFENVVNS	116
Flagellin- <u>RPVT linker- circumspor ozoite protein (CSP)</u>	MMAPDPNANPNANPNANPNANPNANPNANPNANPNANPNANPN ANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNKNN QNGGQGHNMPPNDPNRNVDENANANNVKNNNNEEPSDKHIEQY LKKIKNSISTEWSPCSVTCGNGIQVRIKPGSANKPKDEL DYENDIEK KICKMEKCSSVFENVVNSRPVTMAQVINTNSLSLLTQNNLNKSQSA LGTAIERLSSGLRINSKDDAAGQAIANRFTANIKGLTQASRNAND GISIAQTTEGALNEINNNLQRVRELAVQSANSTNSQSDLSIQAEIT QRLNEIDRVSGQTQFNGVKVLAQDNTLTIQVGANDGETIDIDLKQI NSQTLGLDTLNVQQKYKVSDTAATVTGYADTTIALDNSTFKASAT GLGGTDQKIDGDLKFDDTTGKYYAKVTVTGGTGKDGYYEVSVD KTNGEVTLAGGATSPLTGGLPATATEDVKNVQVANADLTEAKAAL TAAGVTGTASVVKMSYTDNNGKTIDGGLAVKVGDDYYSATQNK DGSISINTTKYTADDGTSKALTALNKLGGADGKTEVVSIGGKTYAA SKAEGHNFKAQPDLAEEAATTTENPLQKIDAALAQVDTLRSDLG AVQNRFNNSAITNLGNTVNNLTSARSRIEDSDYATEVSNMSRAQILQ OAGTSVLAOANOVPONVLSLLR	117

EQUIVALENTS

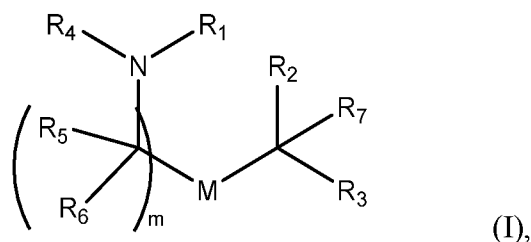
Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

- 5 All references, including patent documents, disclosed herein are incorporated by reference in their entirety.

What is claimed is:

CLAIMS

1. A vaccine comprising at least one RNA polynucleotide having an open reading frame encoding at least one varicella zoster virus (VZV) antigenic polypeptide formulated in a lipid nanoparticle comprising at least one cationic lipid selected from compounds of Formula (I):



or a salt or isomer thereof, wherein:

- R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';
- R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;
- R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a carbocycle, heterocycle, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -N(R)₂, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(R)N(R)₂C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5;
- each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;
- R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle; each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H; each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl; each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl; each Y is independently a C₃₋₆ carbocycle; each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

2. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those in which when R₄ is -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, or -CQ(R)₂, then (i) Q is not -N(R)₂ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

3. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms selected from N, O, and S which is substituted with one or more substituents selected from oxo (=O), OH, amino, mono- or di-alkylamino, and C₁₋₃ alkyl, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,
 -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,

5 and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R,
 -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

10 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,
 -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

15 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

20 4. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those
 in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and
 -R''M'R';

25 R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄
 alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
 attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR,
 -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a
 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S,

30 -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂,
 -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈,
 -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR,
 -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂,
 -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and

- C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a 5- to 14-membered heterocycle and (i) R₄ is -(CH₂)_nQ in which n is 1 or 2, or (ii) R₄ is -(CH₂)_nCHQR in which n is 1, or (iii) R₄ is -CHQR, and -CQ(R)₂, then Q is either a 5- to 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;
- 5 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;
- 10 R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle; R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle; each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- 15 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H; each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl; each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl; each Y is independently a C₃₋₆ carbocycle;
- 20 each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13, or salts or isomers thereof.

5. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those
25 in which

- R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';
- R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
30 attached, form a heterocycle or carbocycle;
- R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂,
35 -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈,

- O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5;
- 5 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;
- 10 R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H; R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle; R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle; each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- 15 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H; each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl; each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl; each Y is independently a C₃₋₆ carbocycle;
- 20 each X is independently selected from the group consisting of F, Cl, Br, and I; and m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13, or salts or isomers thereof.

6. The vaccine of claim 1, wherein subset of compounds of Formula (I) includes those in which

- 25 R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';
- R₂ and R₃ are independently selected from the group consisting of H, C₂₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
- 30 attached, form a heterocycle or carbocycle;
- R₄ is -(CH₂)_nQ or -(CH₂)_nCHQR, where Q is -N(R)₂, and n is selected from 3, 4, and 5;
- each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
- M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,

-C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

5 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

10 each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

7. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those
15 in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
20 attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, and -CQ(R)₂, where Q is -N(R)₂, and n is selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

25 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

30 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

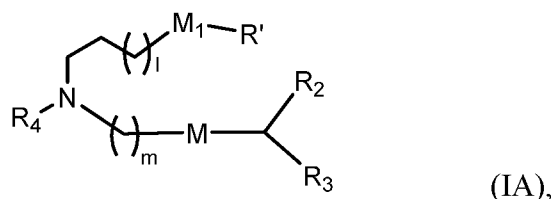
each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

35 each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
or salts or isomers thereof.

8. The vaccine of claim 1, wherein a subset of compounds of Formula (I) includes those
5 of Formula (IA):



or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6,
7, 8, and 9; M₁ is a bond or M'; R₄ is unsubstituted C₁₋₃ alkyl, or -(CH₂)_nQ, in which Q is
OH, -NHC(S)N(R)₂, -NHC(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)R₈,
10 -NHC(=NR₉)N(R)₂, -NHC(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, heteroaryl or
heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-,
-C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R₂ and R₃ are
independently selected from the group consisting of H, C₁₋₁₄ alkyl, and C₂₋₁₄ alkenyl.

9. The vaccine of any one of claims 1-8, wherein the at least one RNA polynucleotide
has an open reading frame encoding two or more VZV antigenic polypeptides.

10. The vaccine of any one of claims 1-9, wherein the at least one VZV antigenic
polypeptide is a VZV glycoprotein.

11. The vaccine of any one of claims 1-9, wherein the at least one VZV antigenic
polypeptide is a VZV tegument protein.

12. The vaccine of claim 10, wherein the VZV glycoprotein is selected from VZV gE, gI,
25 gB, gH, gK, gL, gC, gN, and gM.

13. The vaccine of claim 12, wherein the VZV glycoprotein is VZV gE.

14. The vaccine of claim 12, wherein the VZV glycoprotein is VZV gI.

15. The vaccine of claim 13, wherein the VZV glycoprotein is a variant VZV gE
polypeptide.

16. The vaccine of claim 15, wherein the variant VZV gE polypeptide is a truncated polypeptide lacking the anchor domain (ER retention domain).

17. The vaccine of claim 15, wherein the variant VZV gE polypeptide is a truncated polypeptide lacking the carboxy terminal tail domain.

18. The vaccine of claim 16, wherein the VZV antigenic polypeptide is a truncated VZV gE polypeptide comprising amino acids 1-561 of SEQ ID NO: 10.

19. The vaccine of claim 17, wherein the VZV antigenic polypeptide is a VZV gE variant polypeptide comprising amino acids 1-573 of SEQ ID NO: 18.

20. The vaccine of claim 15, wherein the variant VZV gE polypeptide has at least one mutation in at least one motif associated with ER retention, endocytosis, and/or localization to the golgi or trans-golgi network.

21. The vaccine of claim 20, wherein the variant VZV gE polypeptide has at least one mutation in at least one phosphorylated acidic motif(s).

22. The vaccine of claim 21, wherein the variant VZV gE polypeptide has a Y582G mutation with respect to SEQ ID NO: 10.

23. The vaccine of claim 21, wherein the variant VZV gE polypeptide has a Y569A mutation with respect to SEQ ID NO: 10.

24. The vaccine of claim 21, wherein the variant VZV gE polypeptide has a Y582G mutation and a Y569A mutation with respect to SEQ ID NO: 10.

25. The vaccine of claim 19, wherein the VZV antigenic polypeptide has a Y569A mutation with respect to SEQ ID NO: 10.

26. The vaccine of claim 13, or any of claims 20-24, wherein the variant VZV gE polypeptide has an A-E-A-A-D-A sequence (SEQ ID NO: 58).

27. The vaccine of claim 13 or any of claims 20-24, wherein the variant VZV gE polypeptide has an IgKappa sequence at the C-terminus.

28. The vaccine of claim 18 or 19, wherein the VZV gE antigenic polypeptide has an IgKappa sequence at the C-terminus.

29. The vaccine of claim 12, wherein the at least one RNA polynucleotide encodes VZV gE and gI antigenic polypeptides.

30. The vaccine of claim 13 or any of claims 15-28, wherein the vaccine comprises at least one RNA polynucleotide having an open reading frame encoding a gI antigenic polypeptide.

31. The vaccine of claim 13 or any of claims 15-28, wherein the vaccine comprises at least one RNA polynucleotide having an open reading frame encoding an antigenic polypeptide selected from VZV gB, gH, gK, gL, gC, gN, and gM.

32. The vaccine of claim 11, wherein at least one RNA polynucleotide has an open reading frame encoding a VZV antigenic polypeptide selected from VZV gE, gI, gB, gH, gK, gL, gC, gN, and gM.

33. The vaccine of any of claims 12-32, further comprising a live attenuated VZV, whole inactivated VZV, or VZV VLP.

34. The vaccine of any one of claims 1-10, wherein the at least one RNA polynucleotide is encoded by at least one nucleic acid sequence selected from SEQ ID NO: 1-8, 37, 39, 41, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, or 90, or wherein the at least one RNA polynucleotide is encoded by at least one nucleic acid sequence selected from SEQ ID NO: 6, 37, 39, 84, or 86.

35. The vaccine of any one of claims 1-10, wherein the at least one RNA polynucleotide comprises a sequence selected from SEQ ID NO: 63, 67, 71, 75, 79, 83, 87, 91, 92-108, 123-131, 134, and 142-150.

36. The vaccine of any one of claims 1-10, wherein the at least one RNA polynucleotide comprises at least one fragment of a nucleic acid sequence or at least one epitope of a mRNA sequence selected from SEQ ID NO: 63, 67, 71, 75, 79, 83, 87, 91, 92-108, 123-131, 134, and 142-150.

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37. The vaccine of claim 13, wherein the at least one RNA polynucleotide is encoded by SEQ ID NO: 1.

38. The vaccine of claim 14, wherein the at least one RNA polynucleotide is encoded by
10 SEQ ID NO: 2.

39. The vaccine of claim 18, wherein the at least one RNA polynucleotide is encoded by SEQ ID NO: 3.

15 40. The vaccine of claim 19, wherein the at least one RNA polynucleotide is encoded by SEQ ID NO: 5.

41. The vaccine of claim 25, wherein the at least one RNA polynucleotide is encoded by SEQ ID NO: 6, 37, 39, 84, or 86.

20

42. The vaccine of claim 26, wherein the at least one RNA polynucleotide is encoded by SEQ ID NO: 7.

43. The vaccine of claim 26, wherein the at least one RNA polynucleotide is encoded by
25 SEQ ID NO: 8.

44. The vaccine of claim 10, wherein the at least one RNA polynucleotide encodes an antigenic polypeptide having at least 90% identity to the amino acid sequence of any one of SEQ ID NO: 10, 14, 26, 30, 42 and 45-55.

30

45. The vaccine of claim 10, wherein the at least one RNA polynucleotide encodes an antigenic polypeptide having at least 95% identity to the amino acid sequence of any one of SEQ ID NO: 10, 14, 26, 30, 38, 42 and 45-55.

46. The vaccine of claim 10, wherein the at least one RNA polynucleotide encodes an antigenic polypeptide having at least 96% identity to the amino acid sequence of any one of SEQ ID NO: 10, 14, 26, 30, 38, 42 and 45-55.
- 5 47. The vaccine of claim 10, wherein the at least one RNA polynucleotide encodes an antigenic polypeptide having at least 97% identity to the amino acid sequence of any one of SEQ ID NO: 10, 14, 26, 30, 38, 42 and 45-55.
48. The vaccine of claim 10, wherein the at least one RNA polynucleotide encodes an
10 antigenic polypeptide having at least 98% identity to the amino acid sequence of any one of SEQ ID NO: 10, 14, 26, 30, 38, 42 and 45-55.
49. The vaccine of claim 10, wherein the at least one RNA polynucleotide encodes an antigenic polypeptide having at least 99% identity to the amino acid sequence of any one of
15 SEQ ID NO: 10, 14, 26, 30, 38, 42 and 45-55.
50. The vaccine of any one of claims 1-49, wherein the open reading frame from which the polynucleotide is encoded is codon-optimized.
- 20 51. The vaccine of any one of claims 1-50, wherein the vaccine is multivalent.
52. The vaccine of any one of claims 1-51, wherein the RNA polynucleotide comprises at least one chemical modification.
- 25 53. The vaccine of claim 52, further comprising a second chemical modification.
54. The vaccine of claim 52 or 53, wherein the chemical modification is selected from the group consisting of pseudouridine, N1-methylpseudouridine, N1-ethylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methoxyuridine, and 2'-O-methyl uridine.
- 30

55. The vaccine of claim 52, wherein 80% of the uracil in the open reading frame have a chemical modification.

56. The vaccine of claim 55, wherein 100% of the uracil in the open reading frame have a chemical modification.

57. The vaccine of any one of claims 52-56, wherein the chemical modification is in the 5-position of the uracil.

58. The vaccine of claim 57, wherein the chemical modification is N1-methylpseudouridine.

59. The vaccine of any one of claims 1-58, wherein the vaccine is formulated within a cationic lipid nanoparticle.

60. The vaccine of claim 59, wherein the lipid nanoparticle further comprises a PEG-modified lipid, a sterol and a non-cationic lipid.

61. The vaccine of claim 60, wherein the non-cationic lipid is a neutral lipid.

62. The vaccine of claim 60 or 61, wherein the sterol is a cholesterol.

63. The vaccine of any one of claims 1-62, wherein the lipid nanoparticle has a molar ratio of about 20-60% cationic lipid, about 5-25% non-cationic lipid, about 25-55% sterol, and about 0.5-15% PEG-modified lipid.

64. The vaccine of any one of claims 1-63, wherein the lipid nanoparticle has a polydispersity value of less than 0.4.

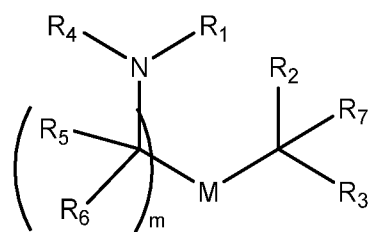
65. The vaccine of any one of claims 1-64, wherein the lipid nanoparticle has a net neutral charge at a neutral pH.

66. The vaccine of any one of claims 1-65, wherein the nanoparticle has a mean diameter of 50-200nm.

67. A VZV vaccine of any one of claims 1-66 for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering the vaccine to the subject in an effective amount to produce an antigen specific immune response.

68. Use of a VZV vaccine of any one of claims 1-66 in the manufacture of a medicament for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering the vaccine to the subject in an effective amount to produce an antigen specific immune response.

69. A VZV vaccine comprising at least one ribonucleic acid (RNA) polynucleotide, said RNA polynucleotide having (i) a 5' terminal cap, (ii) an open reading frame encoding at least one VZV antigenic polypeptide, and (iii) a 3' polyA tail formulated in a lipid nanoparticle comprising at least one cationic lipid selected from compounds of Formula (I):



(I),

or a salt or isomer thereof, wherein:

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';

R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a carbocycle, heterocycle, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -N(R)₂, -C(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(R)N(R)₂C(O)OR, and each n is independently selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;

- 5 R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 R_8 is selected from the group consisting of C_{3-6} carbocycle and heterocycle;
 R_9 is selected from the group consisting of H, CN, NO₂, C_{1-6} alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C_{2-6} alkenyl, C_{3-6} carbocycle and heterocycle;
each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
10 each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, -R*YR'', -YR'', and H;
each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
each R* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;
each Y is independently a C_{3-6} carbocycle;
15 each X is independently selected from the group consisting of F, Cl, Br, and I; and
m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13.

70. The vaccine of claim 69, wherein a subset of compounds of Formula (I) includes those in which when R_4 is $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, or -CQ(R)₂, then (i) Q is
20 not -N(R)₂ when n is 1, 2, 3, 4 or 5, or (ii) Q is not 5, 6, or 7-membered heterocycloalkyl when n is 1 or 2.

71. The vaccine of claim 69, wherein a subset of compounds of Formula (I) includes those in which
25 R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, -R*YR'', -YR'', and -R''M'R';
 R_2 and R_3 are independently selected from the group consisting of H, C_{1-14} alkyl, C_{2-14} alkenyl, -R*YR'', -YR'', and -R*OR'', or R_2 and R_3 , together with the atom to which they are attached, form a heterocycle or carbocycle;
30 R_4 is selected from the group consisting of a C_{3-6} carbocycle, $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, -CHQR, -CQ(R)₂, and unsubstituted C_{1-6} alkyl, where Q is selected from a C_{3-6} carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S,
-OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂,
-N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈,
35 -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR,

-N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂,
 -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)N(R)₂, -C(=NR₉)R,
 -C(O)N(R)OR, and a 5- to 14-membered heterocycloalkyl having one or more heteroatoms
 selected from N, O, and S which is substituted with one or more substituents selected from
 5 oxo (=O), OH, amino, mono- or di-alkylamino, and C₁₋₃ alkyl, and each n is independently
 selected from 1, 2, 3, 4, and 5;

each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,
 10 -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,
 and a heteroaryl group;

R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;

R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R,

15 -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;

each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;

each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,

-R*YR'', -YR'', and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;

20 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;

each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,

or salts or isomers thereof.

25

72. The vaccine of claim 69, wherein a subset of compounds of Formula (I) includes
 those in which

R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and
 -R''M'R';

30 R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄
 alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
 attached, form a heterocycle or carbocycle;

R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR,
 -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a

35 5- to 14-membered heterocycle having one or more heteroatoms selected from N, O, and S,

- OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂,
 -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈,
 -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR,
 -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂,
 5 -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and
 -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5; and when Q is a
 5- to 14-membered heterocycle and (i) R₄ is -(CH₂)_nQ in which n is 1 or 2, or (ii) R₄
 is -(CH₂)_nCHQR in which n is 1, or (iii) R₄ is -CHQR, and -CQ(R)₂, then Q is either a 5- to
 14-membered heteroaryl or 8- to 14-membered heterocycloalkyl;
 10 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-,
 -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group,
 and a heteroaryl group;
 15 R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;
 R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R,
 -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;
 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 20 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl,
 -R*YR'', -YR'', and H;
 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;
 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;
 each Y is independently a C₃₋₆ carbocycle;
 25 each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
 or salts or isomers thereof.

73. The vaccine of claim 69, wherein a subset of compounds of Formula (I) includes
 30 those in which
 R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and
 -R''M'R';
 R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, C₂₋₁₄
 alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
 35 attached, form a heterocycle or carbocycle;

- R₄ is selected from the group consisting of a C₃₋₆ carbocycle, -(CH₂)_nQ, -(CH₂)_nCHQR, -CHQR, -CQ(R)₂, and unsubstituted C₁₋₆ alkyl, where Q is selected from a C₃₋₆ carbocycle, a 5- to 14-membered heteroaryl having one or more heteroatoms selected from N, O, and S, -OR, -O(CH₂)_nN(R)₂, -C(O)OR, -OC(O)R, -CX₃, -CX₂H, -CXH₂, -CN, -C(O)N(R)₂,
 5 -N(R)C(O)R, -N(R)S(O)₂R, -N(R)C(O)N(R)₂, -N(R)C(S)N(R)₂, -CRN(R)₂C(O)OR, -N(R)R₈, -O(CH₂)_nOR, -N(R)C(=NR₉)N(R)₂, -N(R)C(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, -N(OR)C(O)R, -N(OR)S(O)₂R, -N(OR)C(O)OR, -N(OR)C(O)N(R)₂, -N(OR)C(S)N(R)₂, -N(OR)C(=NR₉)N(R)₂, -N(OR)C(=CHR₉)N(R)₂, -C(=NR₉)R, -C(O)N(R)OR, and -C(=NR₉)N(R)₂, and each n is independently selected from 1, 2, 3, 4, and 5;
 10 each R₅ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 each R₆ is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, -S(O)₂-, -S-S-, an aryl group, and a heteroaryl group;
 15 R₇ is selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 R₈ is selected from the group consisting of C₃₋₆ carbocycle and heterocycle;
 R₉ is selected from the group consisting of H, CN, NO₂, C₁₋₆ alkyl, -OR, -S(O)₂R, -S(O)₂N(R)₂, C₂₋₆ alkenyl, C₃₋₆ carbocycle and heterocycle;
 each R is independently selected from the group consisting of C₁₋₃ alkyl, C₂₋₃ alkenyl, and H;
 20 each R' is independently selected from the group consisting of C₁₋₁₈ alkyl, C₂₋₁₈ alkenyl, -R*YR'', -YR'', and H;
 each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;
 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₂₋₁₂ alkenyl;
 each Y is independently a C₃₋₆ carbocycle;
 25 each X is independently selected from the group consisting of F, Cl, Br, and I; and
 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
 or salts or isomers thereof.

74. The vaccine of claim 69, wherein subset of compounds of Formula (I) includes those
 30 in which

- R₁ is selected from the group consisting of C₅₋₃₀ alkyl, C₅₋₂₀ alkenyl, -R*YR'', -YR'', and -R''M'R';
 R₂ and R₃ are independently selected from the group consisting of H, C₂₋₁₄ alkyl, C₂₋₁₄ alkenyl, -R*YR'', -YR'', and -R*OR'', or R₂ and R₃, together with the atom to which they are
 35 attached, form a heterocycle or carbocycle;

R_4 is $-(CH_2)_nQ$ or $-(CH_2)_nCHQR$, where Q is $-N(R)_2$, and n is selected from 3, 4, and 5;
 each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')$, $-N(R')C(O)-$,
 5 $-C(O)-$, $-C(S)-$, $-C(S)S-$, $-SC(S)-$, $-CH(OH)-$, $-P(O)(OR')O-$, $-S(O)_2-$, $-S-S-$, an aryl group,
 and a heteroaryl group;
 R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl,
 10 $-R^*YR''$, $-YR''$, and H;
 each R'' is independently selected from the group consisting of C_{3-14} alkyl and C_{3-14} alkenyl;
 each R^* is independently selected from the group consisting of C_{1-12} alkyl and C_{1-12} alkenyl;
 each Y is independently a C_{3-6} carbocycle;
 each X is independently selected from the group consisting of F, Cl, Br, and I; and
 15 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
 or salts or isomers thereof.

75. The vaccine of claim 69, wherein a subset of compounds of Formula (I) includes those in which

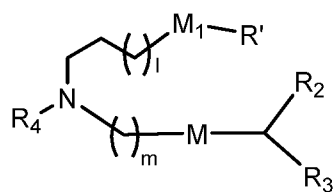
20 R_1 is selected from the group consisting of C_{5-30} alkyl, C_{5-20} alkenyl, $-R^*YR''$, $-YR''$, and $-R''M'R'$;
 R_2 and R_3 are independently selected from the group consisting of C_{1-14} alkyl, C_{2-14} alkenyl, $-R^*YR''$, $-YR''$, and $-R^*OR''$, or R_2 and R_3 , together with the atom to which they are attached, form a heterocycle or carbocycle;
 25 R_4 is selected from the group consisting of $-(CH_2)_nQ$, $-(CH_2)_nCHQR$, $-CHQR$, and $-CQ(R)_2$,
 where Q is $-N(R)_2$, and n is selected from 1, 2, 3, 4, and 5;
 each R_5 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 each R_6 is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 M and M' are independently selected from $-C(O)O-$, $-OC(O)-$, $-C(O)N(R')$, $-N(R')C(O)-$,
 30 $-C(O)-$, $-C(S)-$, $-C(S)S-$, $-SC(S)-$, $-CH(OH)-$, $-P(O)(OR')O-$, $-S(O)_2-$, $-S-S-$, an aryl group,
 and a heteroaryl group;
 R_7 is selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 each R is independently selected from the group consisting of C_{1-3} alkyl, C_{2-3} alkenyl, and H;
 each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl,
 35 $-R^*YR''$, $-YR''$, and H;

each R'' is independently selected from the group consisting of C₃₋₁₄ alkyl and C₃₋₁₄ alkenyl;
 each R* is independently selected from the group consisting of C₁₋₁₂ alkyl and C₁₋₁₂ alkenyl;
 each Y is independently a C₃₋₆ carbocycle;

each X is independently selected from the group consisting of F, Cl, Br, and I; and

- 5 m is selected from 5, 6, 7, 8, 9, 10, 11, 12, and 13,
 or salts or isomers thereof.

76. The vaccine of claim 69, wherein a subset of compounds of Formula (I) includes those of Formula (IA):



(IA),

or a salt or isomer thereof, wherein l is selected from 1, 2, 3, 4, and 5; m is selected from 5, 6, 7, 8, and 9; M₁ is a bond or M'; R₄ is unsubstituted C₁₋₃ alkyl, or -(CH₂)_nQ, in which Q is OH, -NHC(S)N(R)₂, -NHC(O)N(R)₂, -N(R)C(O)R, -N(R)S(O)₂R, -N(R)R₈, -NHC(=NR₉)N(R)₂, -NHC(=CHR₉)N(R)₂, -OC(O)N(R)₂, -N(R)C(O)OR, heteroaryl or heterocycloalkyl; M and M' are independently selected from -C(O)O-, -OC(O)-, -C(O)N(R')-, -P(O)(OR')O-, -S-S-, an aryl group, and a heteroaryl group; and R₂ and R₃ are independently selected from the group consisting of H, C₁₋₁₄ alkyl, and C₂₋₁₄ alkenyl.

77. The vaccine of any one of claims 69-76, wherein the at least one mRNA polynucleotide is encoded by a sequence comprising the sequence identified by SEQ ID NO: 6.

78. The vaccine of any one of claims 69-76, wherein the at least one mRNA polynucleotide is encoded by a sequence comprising the sequence identified by SEQ ID NO: 37.

79. The vaccine of any one of claims 69-76, wherein the at least one mRNA polynucleotide is encoded by a sequence comprising the sequence identified by SEQ ID NO: 39.

80. The vaccine of any one of claims 69-76, wherein the at least one mRNA polynucleotide is encoded by a sequence comprising the sequence identified by SEQ ID NO: 84.

5 81. The vaccine of any one of claims 69-76, wherein the at least one mRNA polynucleotide is encoded by a sequence comprising the sequence identified by SEQ ID NO: 86.

82. The vaccine of any one of claims 69-76, wherein the at least one mRNA
10 polynucleotide comprises a sequence identified by SEQ ID NO: 128.

83. The vaccine of any one of claims 69-76, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 99 or 133.

15 84. The vaccine of any one of claims 69-76, wherein the at least one mRNA polynucleotide comprises a sequence identified by SEQ ID NO: 107 or 134.

85. The vaccine of any one of claims 69-76, wherein the at least one VZV antigenic polypeptide comprises a sequence identified by SEQ ID NO: 38.

20 86. The vaccine of any one of claims 69-85, wherein the 5' terminal cap is or comprises 7mG(5')ppp(5')N1mpNp.

87. The vaccine of any one of claims 69-86 comprising at least one messenger ribonucleic
25 acid (mRNA) polynucleotide comprising a nucleic acid sequence identified by any one of SEQ ID NO: 142-150 having (i) a 5' terminal cap, (ii) an open reading frame encoding a VZV antigenic polypeptide, and (iii) a 3' polyA tail, wherein 5' terminal cap is or comprises 7mG(5')ppp(5')N1mpNp.

30 88. The vaccine of any one of claims 69-87, wherein 100% of the uracil in the open reading frame is modified to include N1-methyl pseudouridine at the 5-position of the uracil.

89. A method of preventing or treating VZV infection comprising administering to a subject the vaccine of any of claims 1-67 or 69-88.

90. A method of inducing an antigen-specific immune response in a subject, the method comprising administering to the subject the vaccine of any one of claims 1-67 or 69-89 in an amount effective to produce an antigen-specific immune response in the subject.

91. The method of claim 90, wherein the antigen specific immune response comprises a T cell response or a B cell response.

92. The method of claim 90 or 91, wherein the subject is administered a single dose of the vaccine.

93. The method of claim 90 or 91, wherein the subject is administered a first dose and a second (booster) dose of the vaccine.

94. The method of any one of claims 90-93, wherein the vaccine is administered to the subject by intradermal injection or intramuscular injection.

95. The method of any one of claims 90-94, wherein an anti-antigenic polypeptide antibody titer produced in the subject is increased by at least 1 log relative to a control.

96. The method of any one of claims 90-95, wherein an anti-antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a control.

97. The method of any one of claims 90-96, wherein the anti-antigenic polypeptide antibody titer produced in the subject is increased at least 2 times relative to a control.

98. The method of any one of claims 90-97, wherein the anti-antigenic polypeptide antibody titer produced in the subject is increased 2-10 times relative to a control.

99. The method of any one of claims 95-98, wherein the control is an anti-antigenic polypeptide antibody titer produced in a subject who has not been administered a vaccine against the virus.

100. The method of any one of claims 95-98, wherein the control is an anti-antigenic polypeptide antibody titer produced in a subject who has been administered a live attenuated vaccine or an inactivated vaccine against the virus.

101. The method of any one of claims 95-98, wherein the control is an anti-antigenic polypeptide antibody titer produced in a subject who has been administered a recombinant protein vaccine or purified protein vaccine against the virus.

5

102. The method of any one of claims 95-98, wherein the control is an anti-antigenic polypeptide antibody titer produced in a subject who has been administered a VLP vaccine against the virus.

10 103. The method of any one of claims 90-102, wherein the effective amount is a dose equivalent to an at least 2-fold reduction in the standard of care dose of a recombinant protein vaccine or a purified protein vaccine against the virus, and wherein an anti-antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-antigenic polypeptide antibody titer produced in a control subject administered the standard of care
15 dose of a recombinant protein vaccine or a purified protein vaccine against the virus, respectively.

104. The method of any one of claims 90-102, wherein the effective amount is a dose equivalent to an at least 2-fold reduction in the standard of care dose of a live attenuated
20 vaccine or an inactivated vaccine against the virus, and wherein an anti-antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a live attenuated vaccine or an inactivated vaccine against the virus, respectively.

25 105. The method of any one of claims 90-102, wherein the effective amount is a dose equivalent to an at least 2-fold reduction in the standard of care dose of a VLP vaccine against the virus, and wherein an anti-antigenic polypeptide antibody titer produced in the subject is equivalent to an anti-antigenic polypeptide antibody titer produced in a control subject administered the standard of care dose of a VLP vaccine against the virus.

30

106. The method of any one of claims 90-105, wherein the effective amount is a total dose of 50 µg-1000 µg.

107. The method of claim 105, wherein the effective amount is a dose of 25 µg, 100 µg,
35 400 µg, or 500 µg administered to the subject a total of two times.

108. The method of any one of claims 90-107, wherein the efficacy of the vaccine against the virus is greater than 65%.

5 109. The method of any one of claims 90-108, wherein the vaccine immunizes the subject against the virus for up to 2 years.

110. The method of any one of claims 90-108, wherein the vaccine immunizes the subject against the virus for more than 2 years.

10

111. The method of any one of claims 90-110, wherein the subject has an age of about 1 year old to about 10 years old.

112. The method of any one of claims 90-111, wherein the subject has been exposed to the virus, wherein the subject is infected with the virus, or wherein the subject is at risk of infection by the virus.

15

113. The method of any one of claims 90-112, wherein the subject is immunocompromised.

20

114. A pharmaceutical composition for use in vaccination of a subject comprising an effective dose of the vaccine of any one of claims 1-67 or 69-88, wherein the effective dose is sufficient to produce detectable levels of antigen as measured in serum of the subject at 1-72 hours post administration.

25

115. The composition of claim 114, wherein the cut off index of the antigen is 1-2.

116. A pharmaceutical composition for use in vaccination of a subject comprising an effective dose of the vaccine of any one of claims 1-67 or 69-88,

30

wherein the effective dose is sufficient to produce a 1,000-10,000 neutralization titer produced by neutralizing antibody against said antigen as measured in serum of the subject at 1-72 hours post administration.

PROPOSED VARICELLAR ZOSTER VIRUS PATHWAY

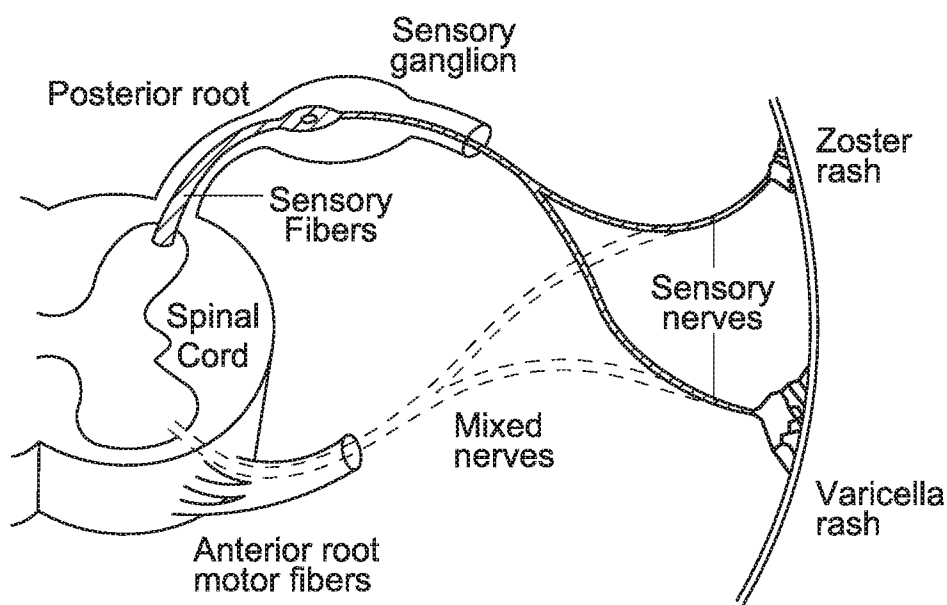


FIG. 1

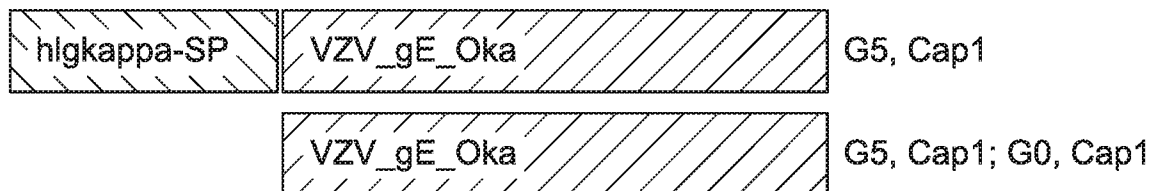


FIG. 2

Single immunization with or without booster on

Dosing Day 0 Day 28

Bleeding Day -2 Day 0 +6h Day 14 Day 27 Day 28 +6h Day 42 Day 56

G#	Antigen	Route	N=	Dosage (ug)	Dose Vol (ul)	1 st dose	2 nd dose	LNP	mRNA Conc. (mg/ml)	Volume +Overage
1	VZV-gE-oka-hlgkappa (G5; cap1)	IM	8	10	50	Day 0		MC3	0.2	1x600 ul
2	VZV-gE-oka-hlgkappa (G5; cap1)	IM	8	10	50	Day 0	Day 28	MC3	0.2	2x600 ul
3	VZV-gE-oka-hlgkappa (G5; cap1)	ID	8	10	50	Day 0		MC3	0.2	1x600 ul
4	VZV-gE-oka-hlgkappa (G5; cap1)	ID	8	10	50	Day 0	Day 28	MC3	0.2	2x600 ul
5	VZV-gE-oka (G0; cap1)	IM	8	10	50	Day 0		MC3	0.2	1x600 ul
6	VZV-gE-oka (G0; cap1)	IM	8	10	50	Day 0	Day 28	MC3	0.2	2x600 ul
7	VZV-gE-oka (G0; cap1)	ID	8	10	50	Day 0		MC3	0.2	1x600 ul
8	VZV-gE-oka (G0; cap1)	ID	8	10	50	Day 0	Day 28	MC3	0.2	2x600 ul
9	VZV-gE-oka (G5; cap1)	IM	8	10	50	Day 0		MC3	0.2	1x600 ul
10	VZV-gE-oka (G5; cap1)	IM	8	10	50	Day 0	Day 28	MC3	0.2	2x600 ul
11	VZV-gE-oka (G5; cap1)	ID	8	10	50	Day 0		MC3	0.2	1x600 ul
12	VZV-gE-oka (G5; cap1)	ID	8	10	50	Day 0	Day 28	MC3	0.2	2x600 ul
13	Negative control (PBS)	IM	6	/	50	Day 0		PBS	/	1x600 ul
14	Negative control (PBS)	IM	6	/	50	Day 0	Day 28	PBS	/	2x600 ul
15	Positive control (Varivax)	SC	6	54 (pfu)	50	Day 0			/	1x1250 ul
16	Positive control (Varivax)	SC	6	54 (pfu)	50	Day 0	Day 28		/	
17	Positive control (Varivax)	SC	4	675 (pfu)	100	Day 0			/	4x220 ul
18	Positive control (Varivax)	SC	4	675 (pfu)	100	Day 0	Day 28		/	

FIG. 3

Fig. 4

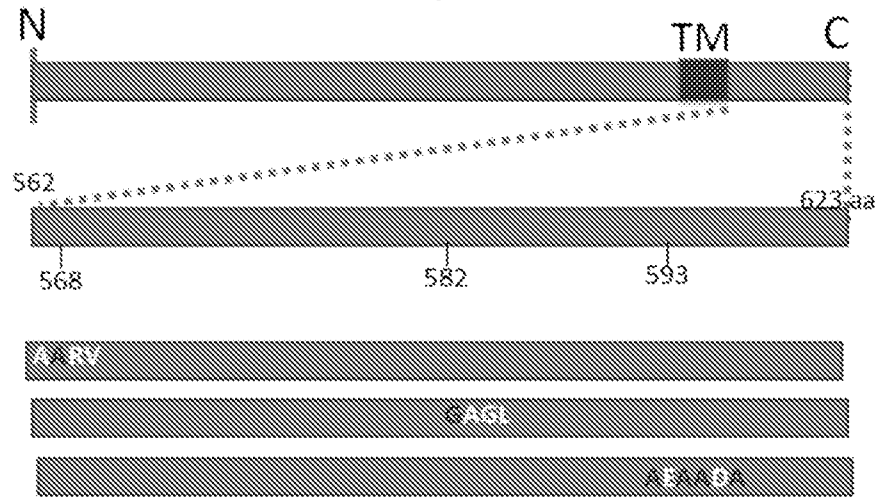


Fig. 5

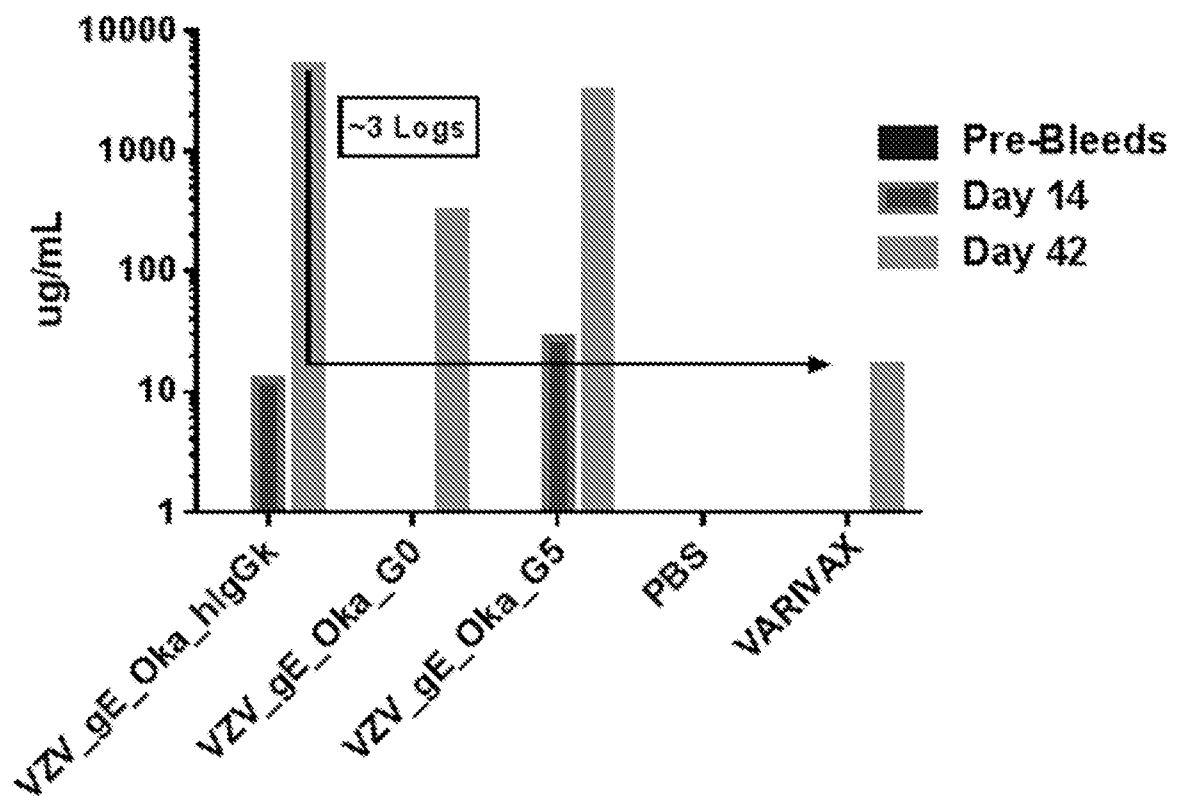


Fig. 6

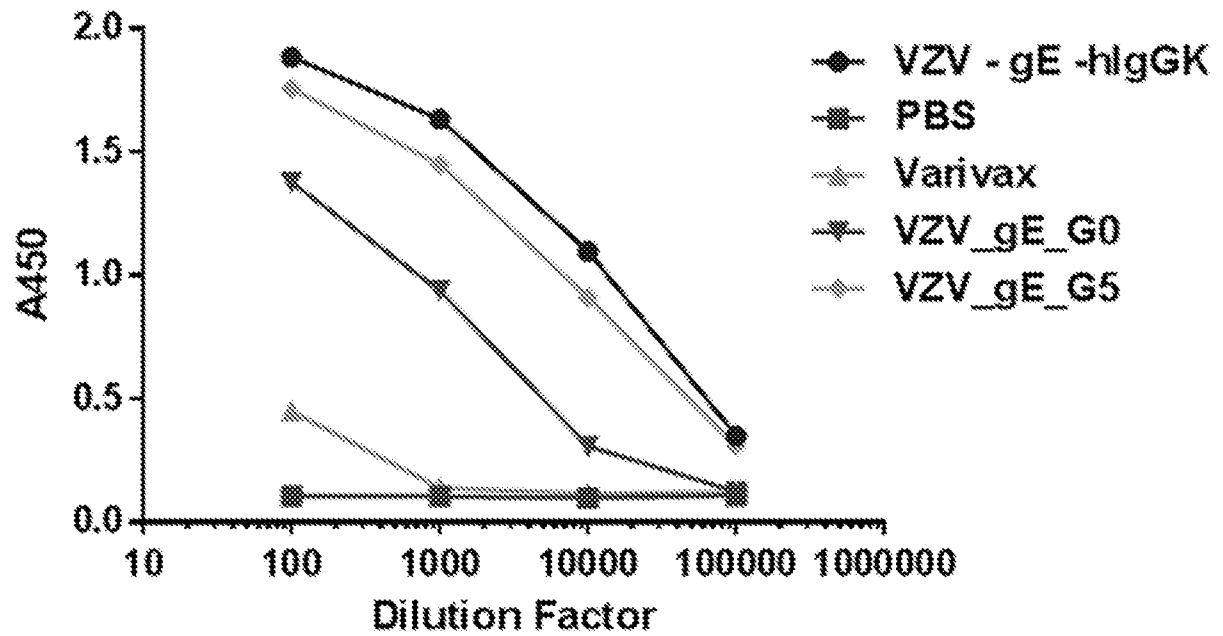


Fig. 7

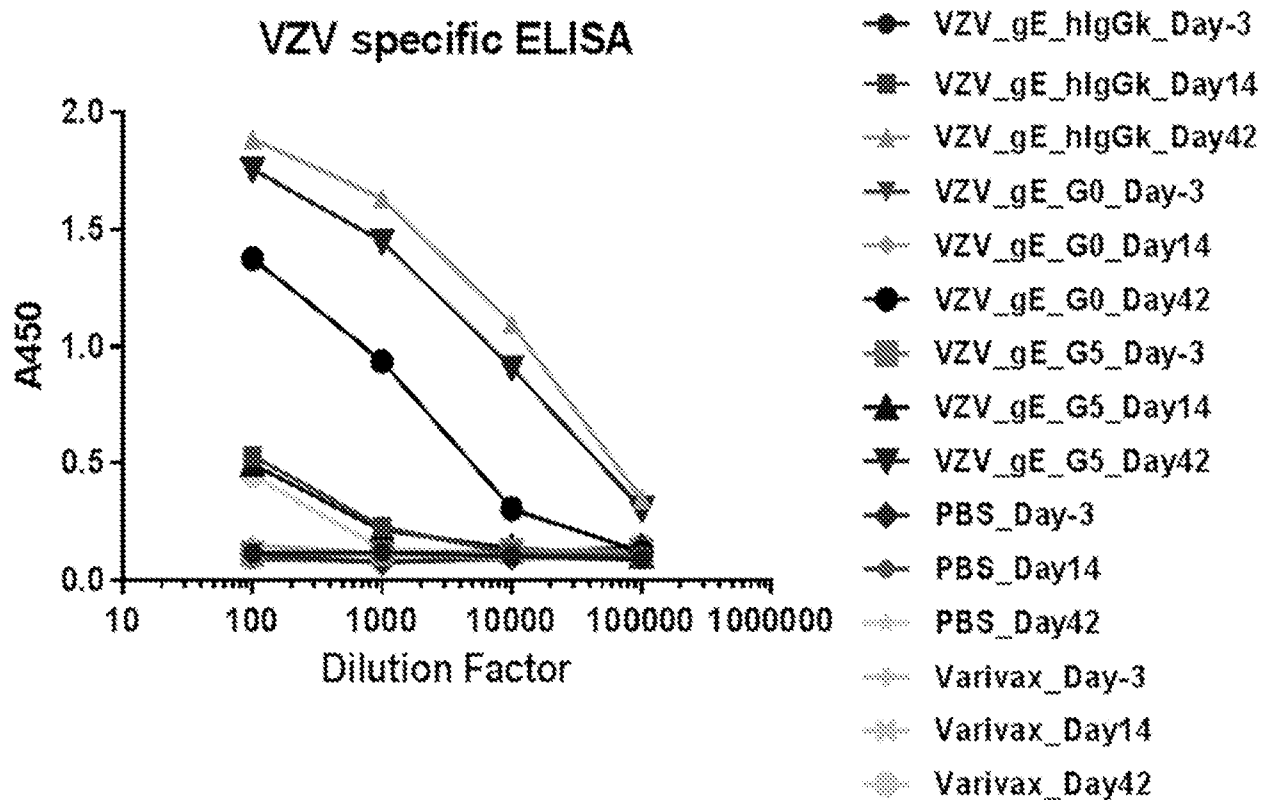


Fig. 8A

Anti GM130 (CST D6B1) - green

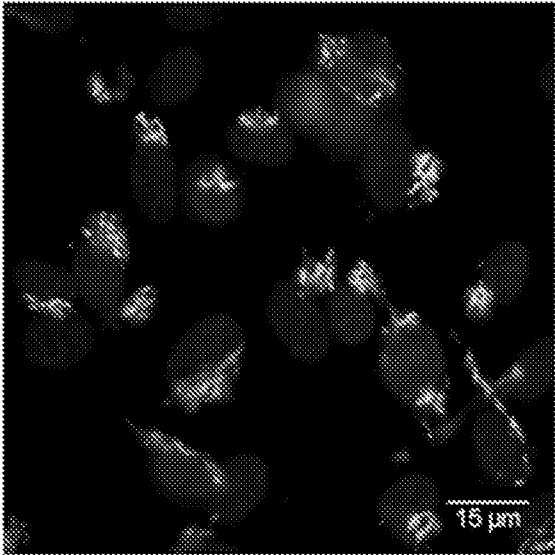


Fig. 8B

Anti Golgin-97 (CST D8P2K) - green

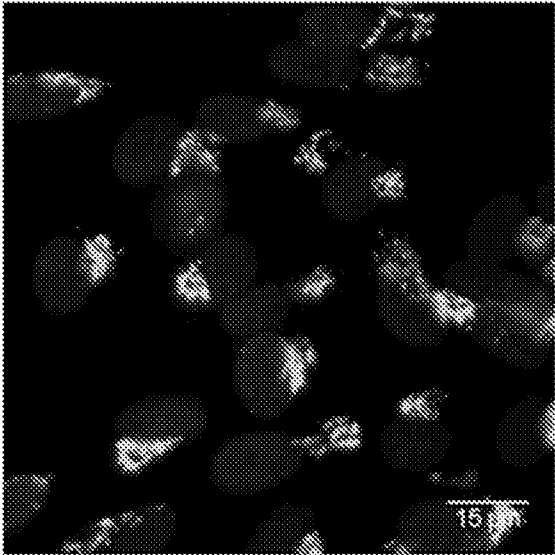


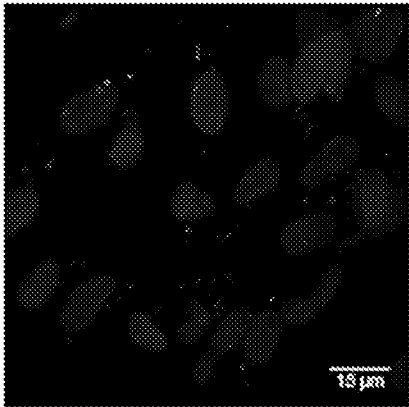
Fig. 9A

Fig. 9B

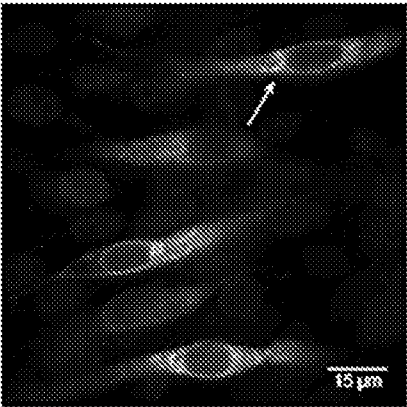
Fig. 9C

Anti GE Abcam 52549 - green

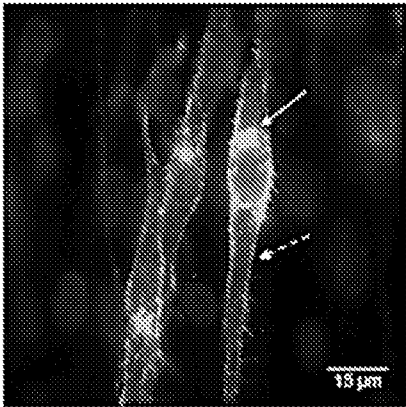
Untransfected Control



SE-VZV-GE-delete-562



SE-VZV-GE-full_with_AEAADA



————→ Perinuclear (Golgi ?) localization
-----→ Cell membrane localization

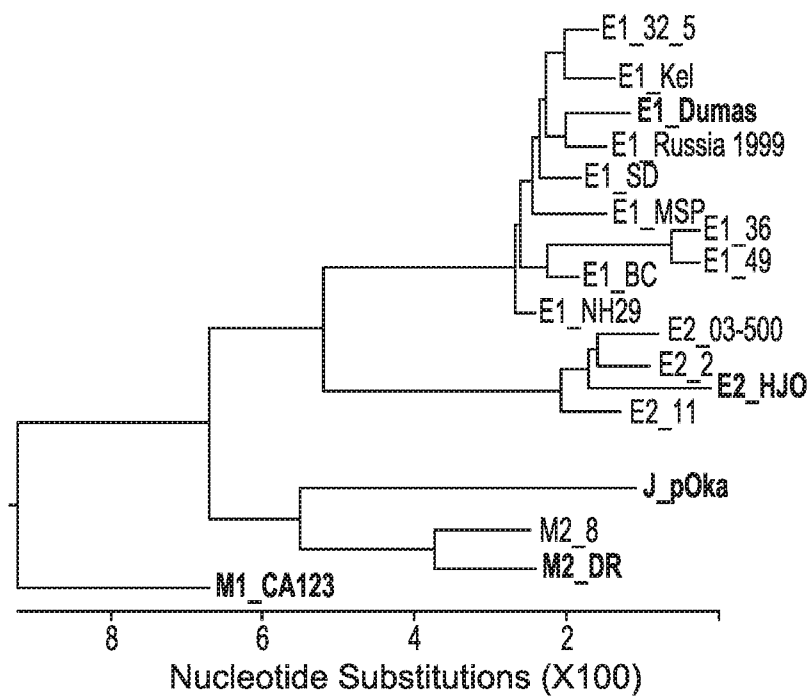


FIG. 10A

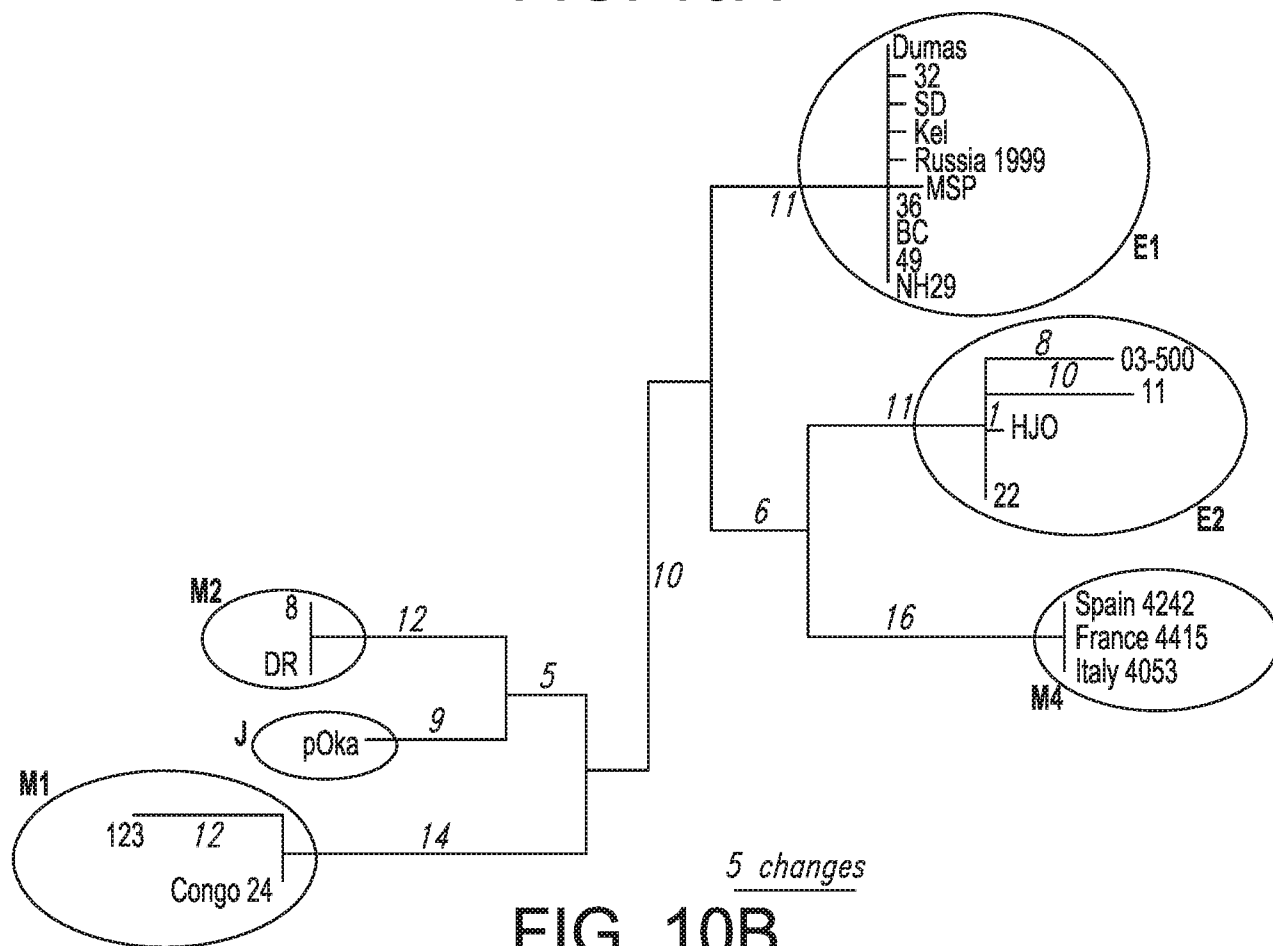


FIG. 10B

Fig. 11A

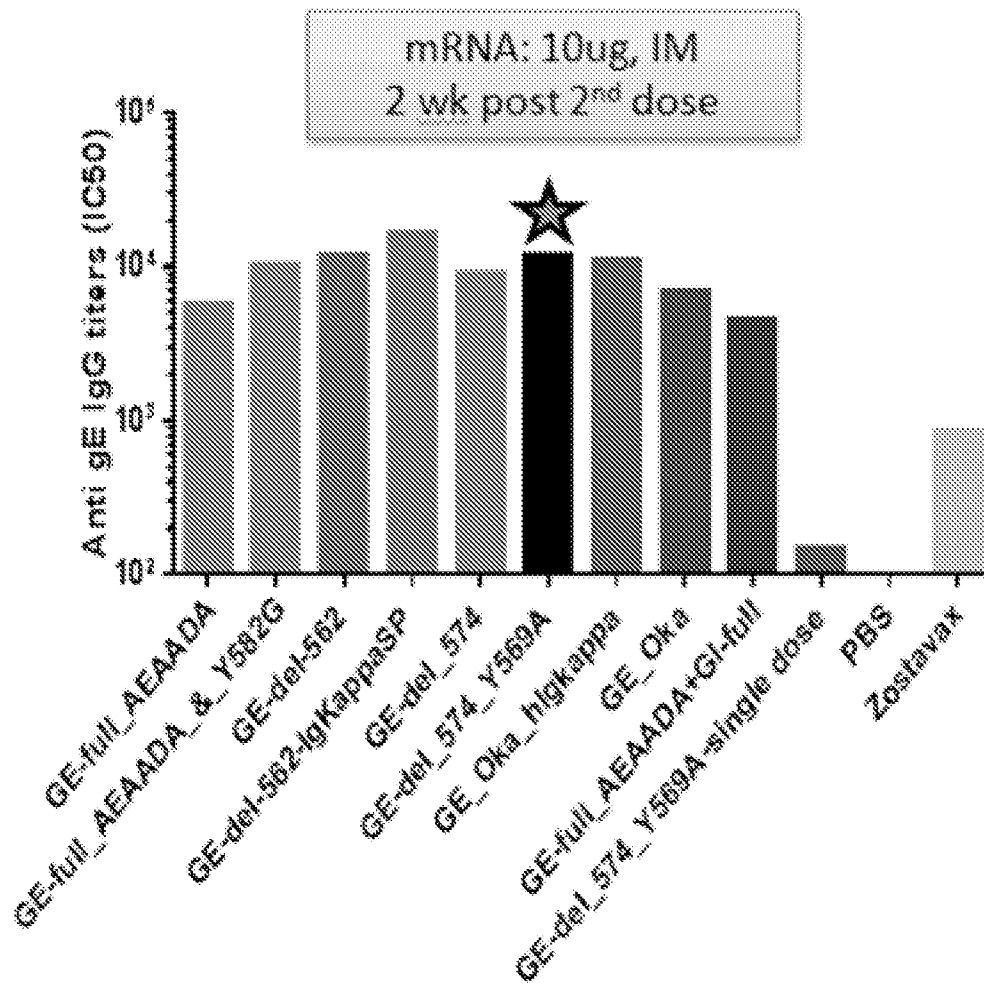


Fig. 11B

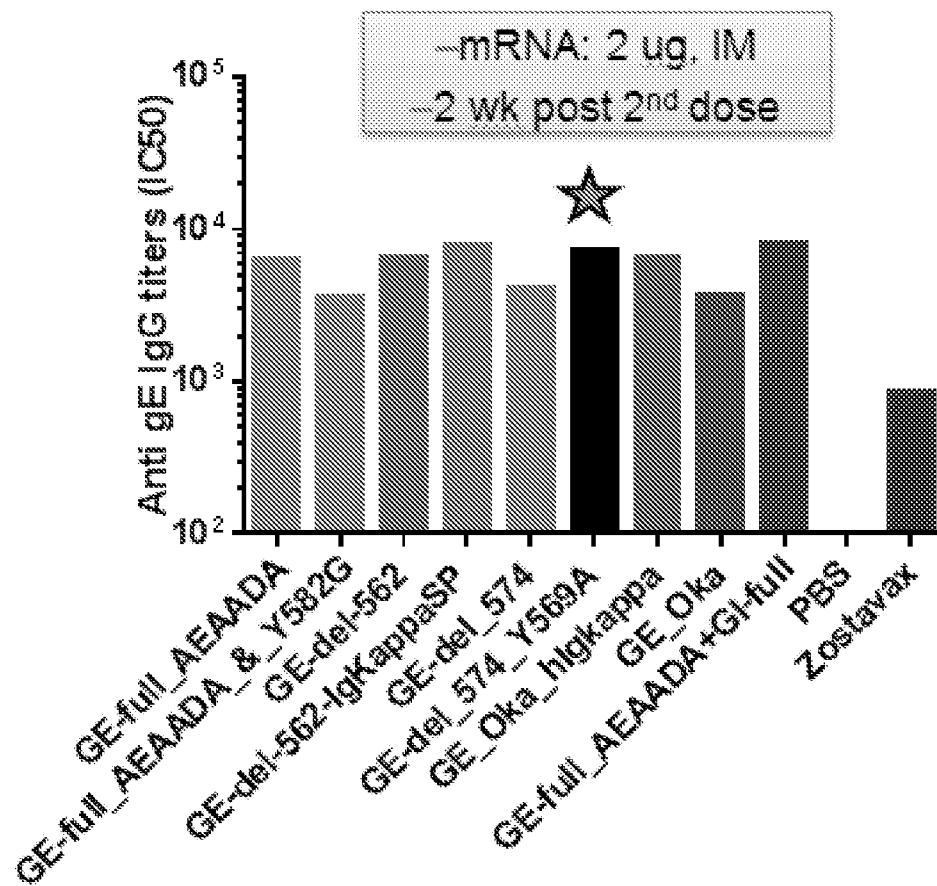


Fig. 12A

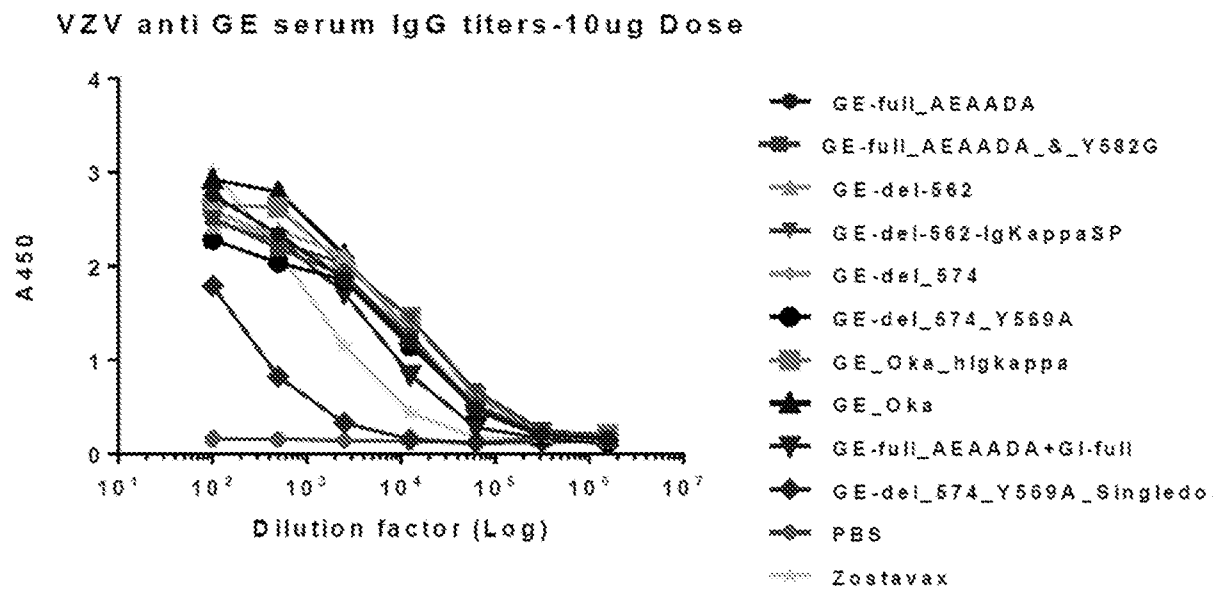


Fig. 12B

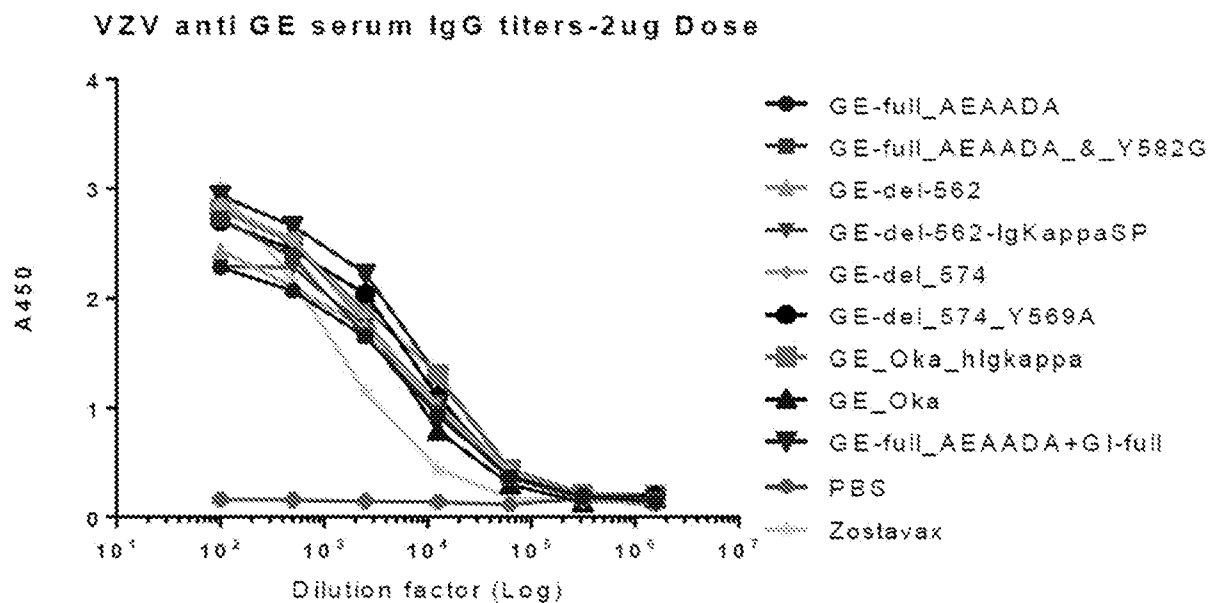


Fig. 13

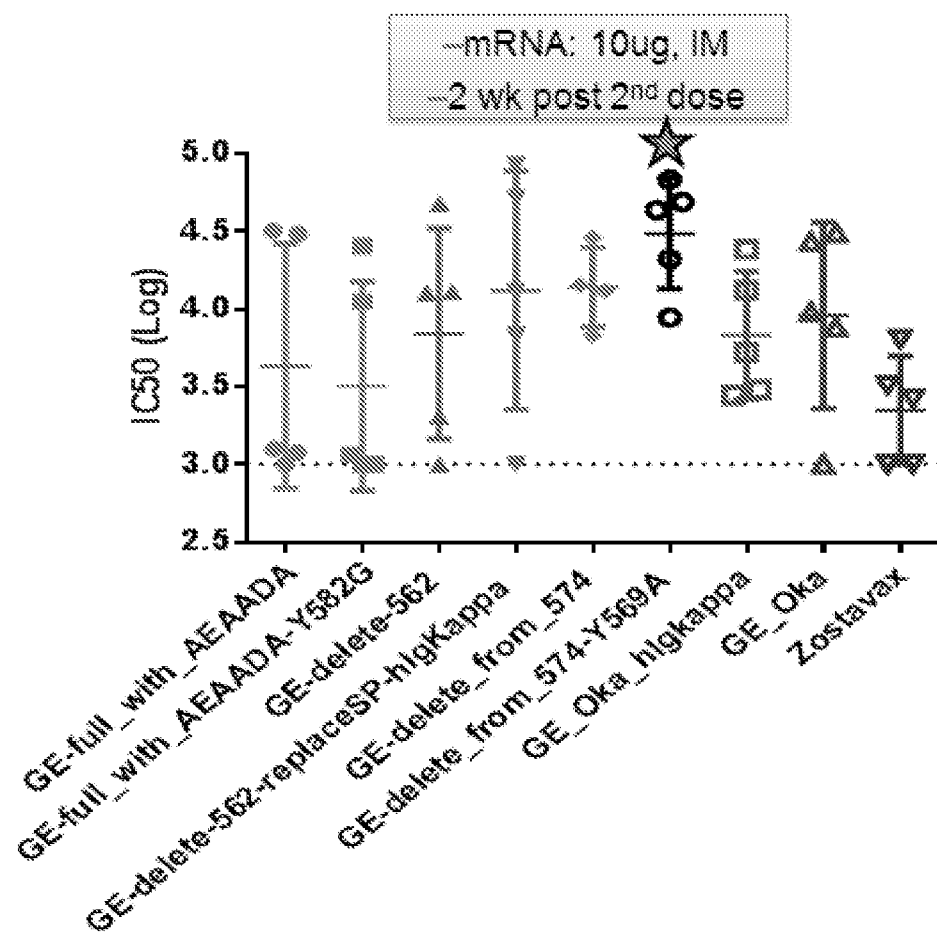


Fig. 14A

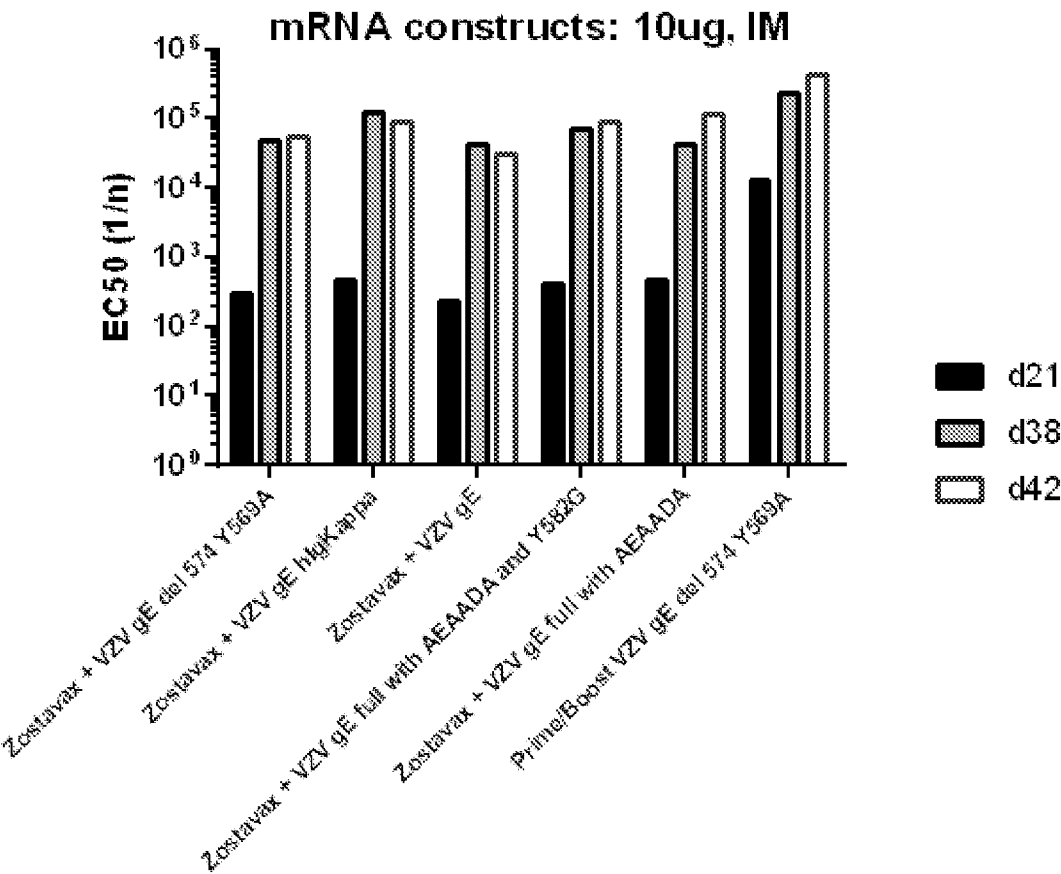


Fig. 14B

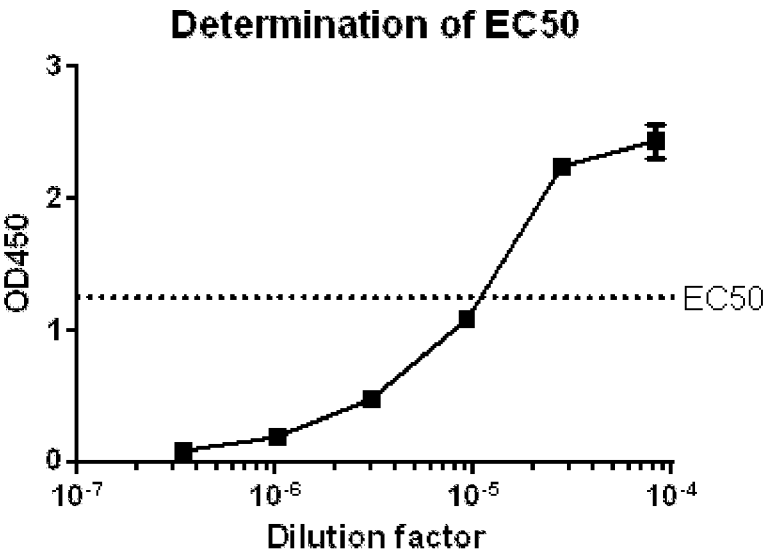


Fig. 15A

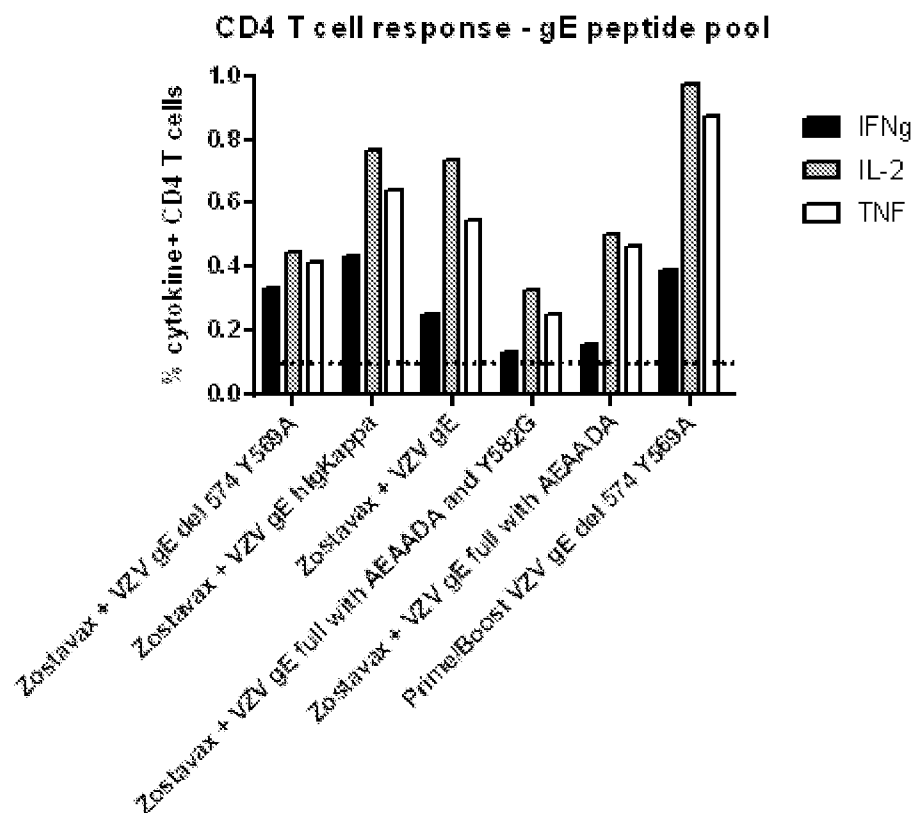


Fig. 15B

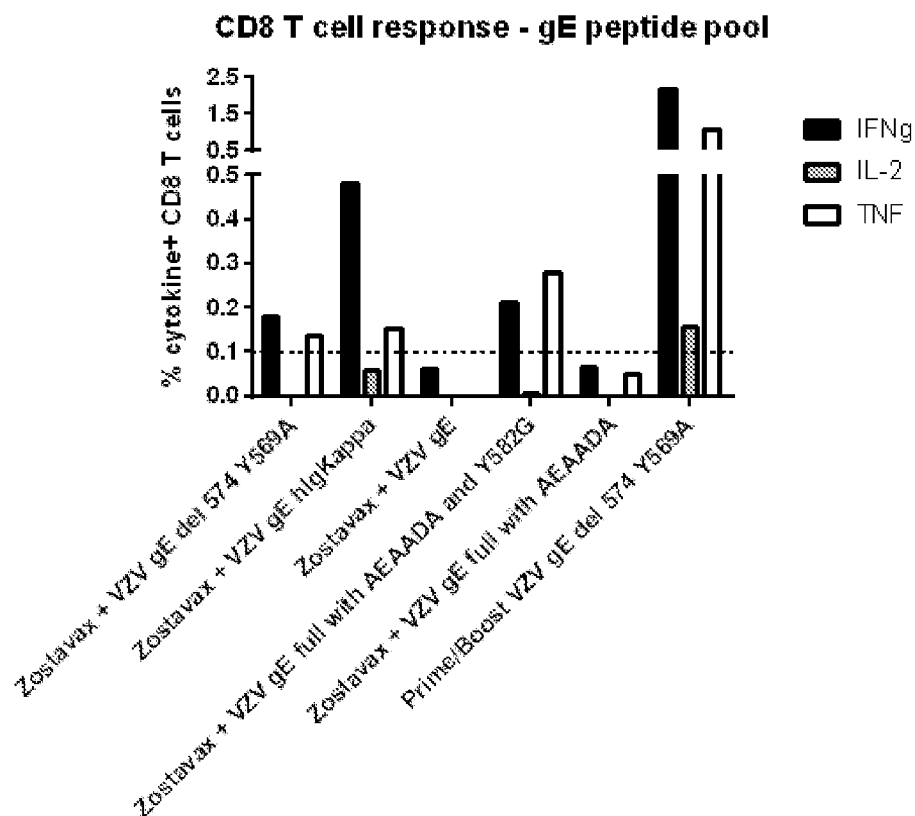


Fig. 16A

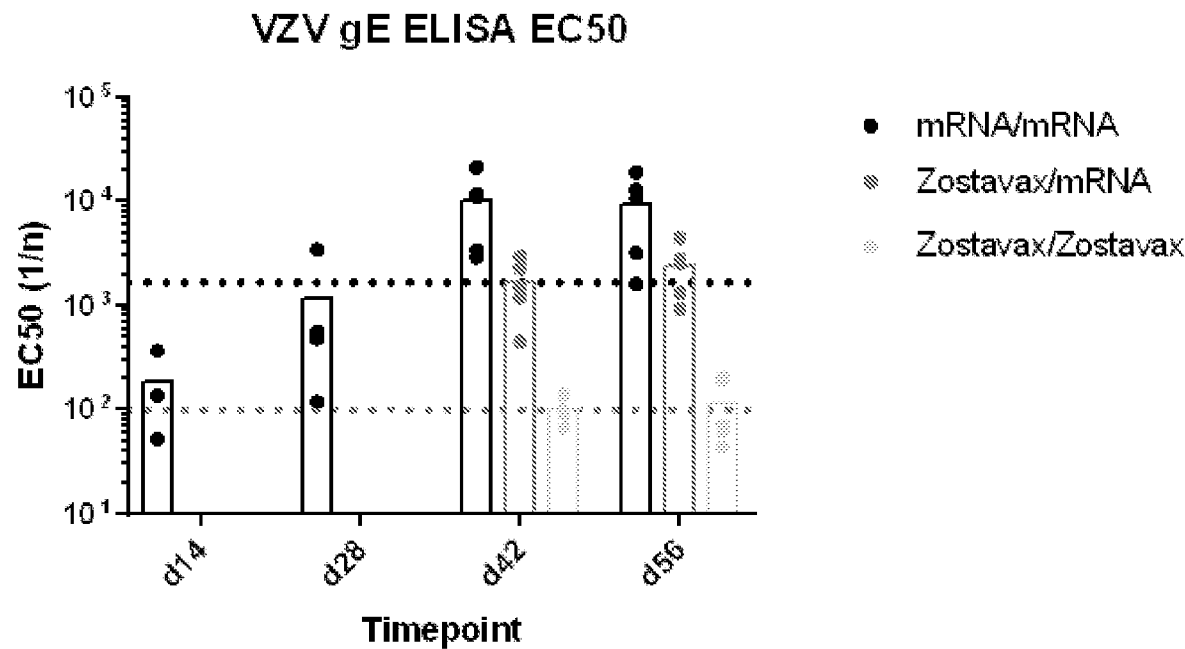


Fig. 16B

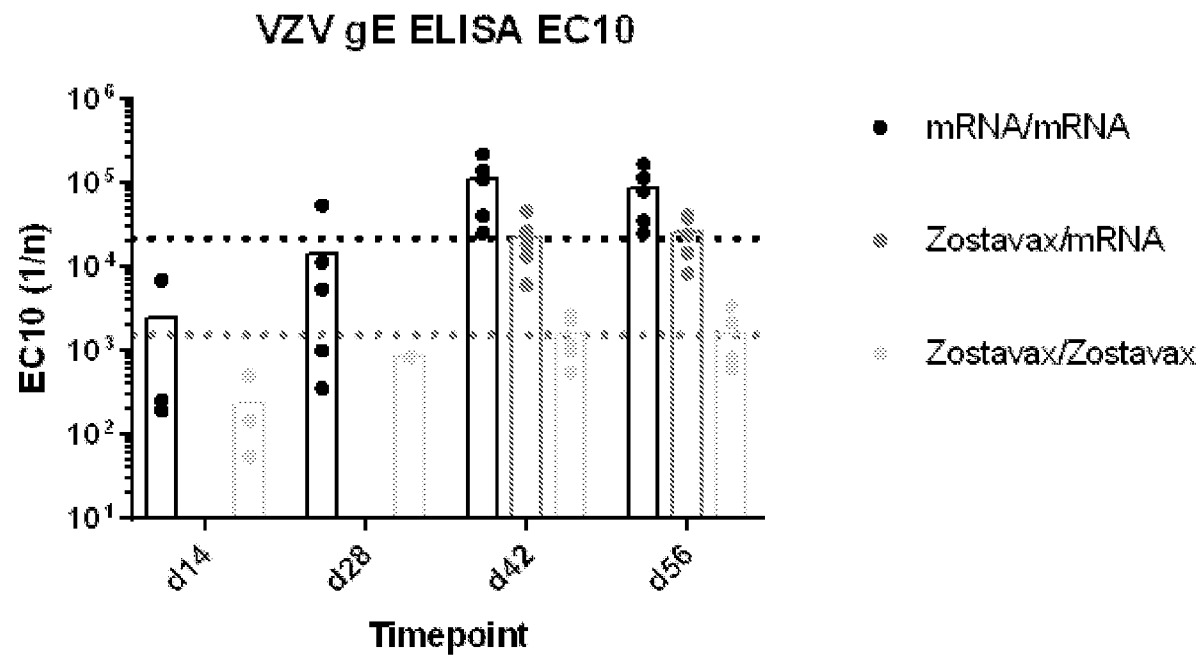


Fig. 16C

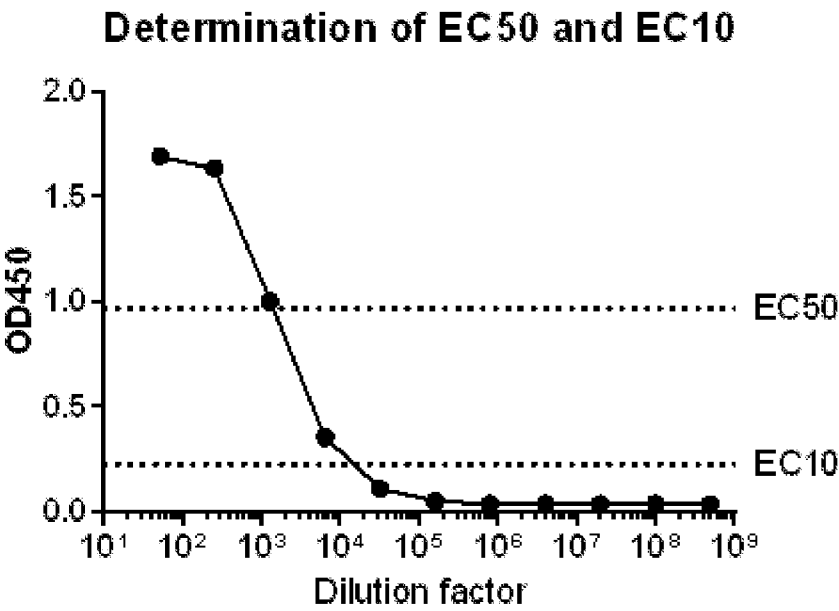


Fig. 16D

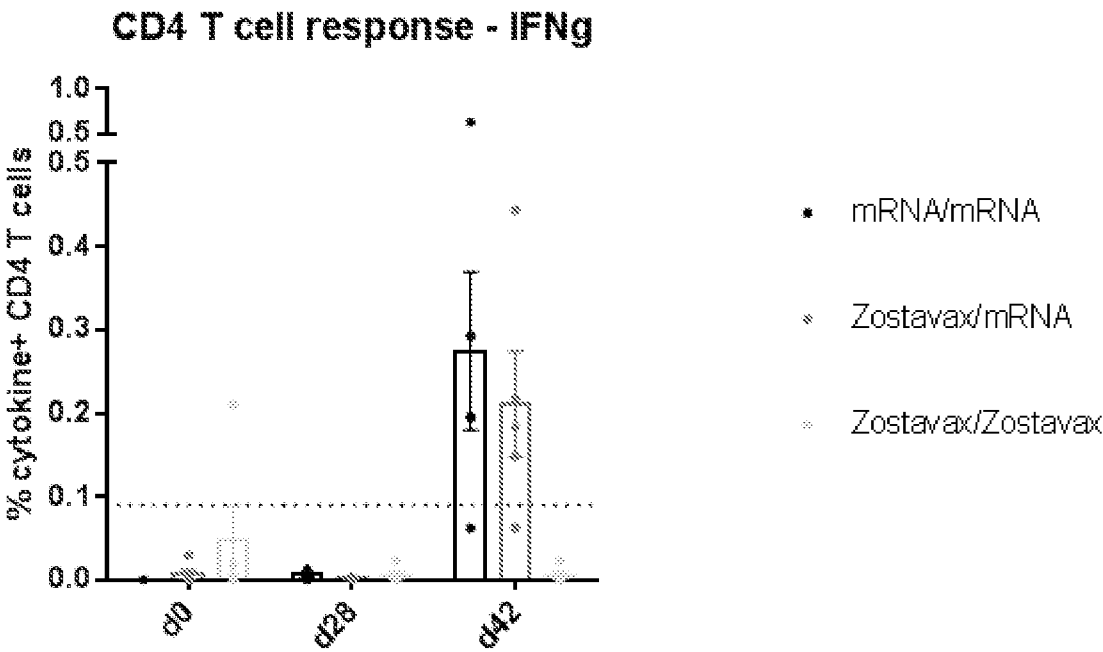


Fig. 16E

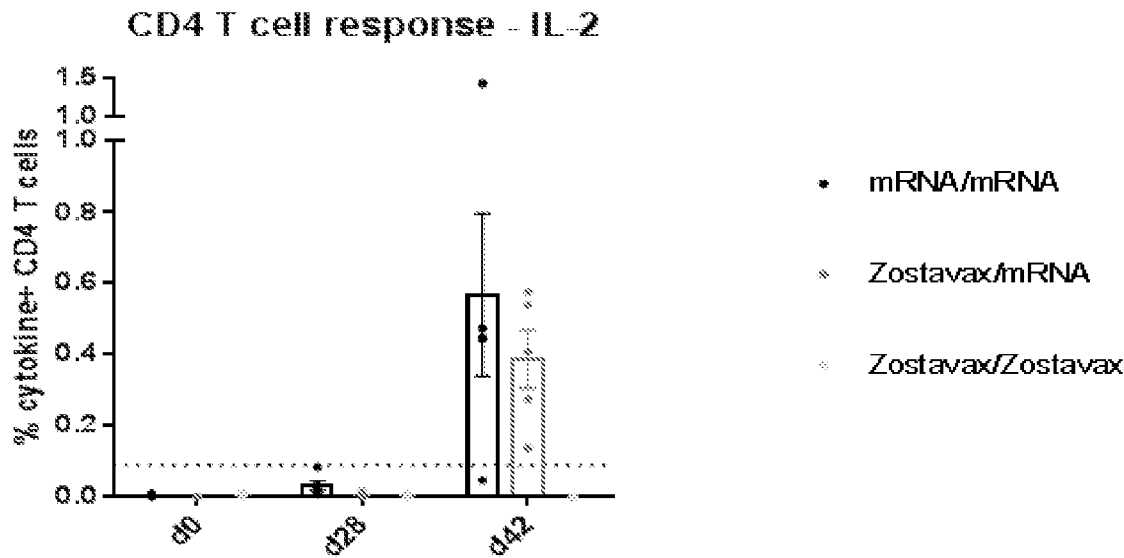
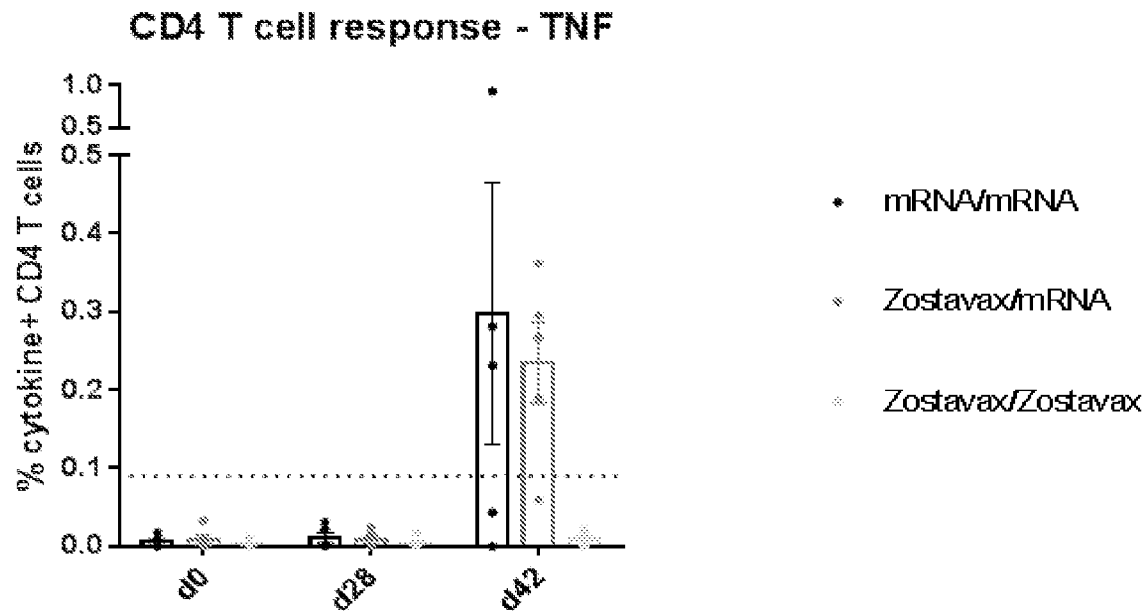


Fig. 16F



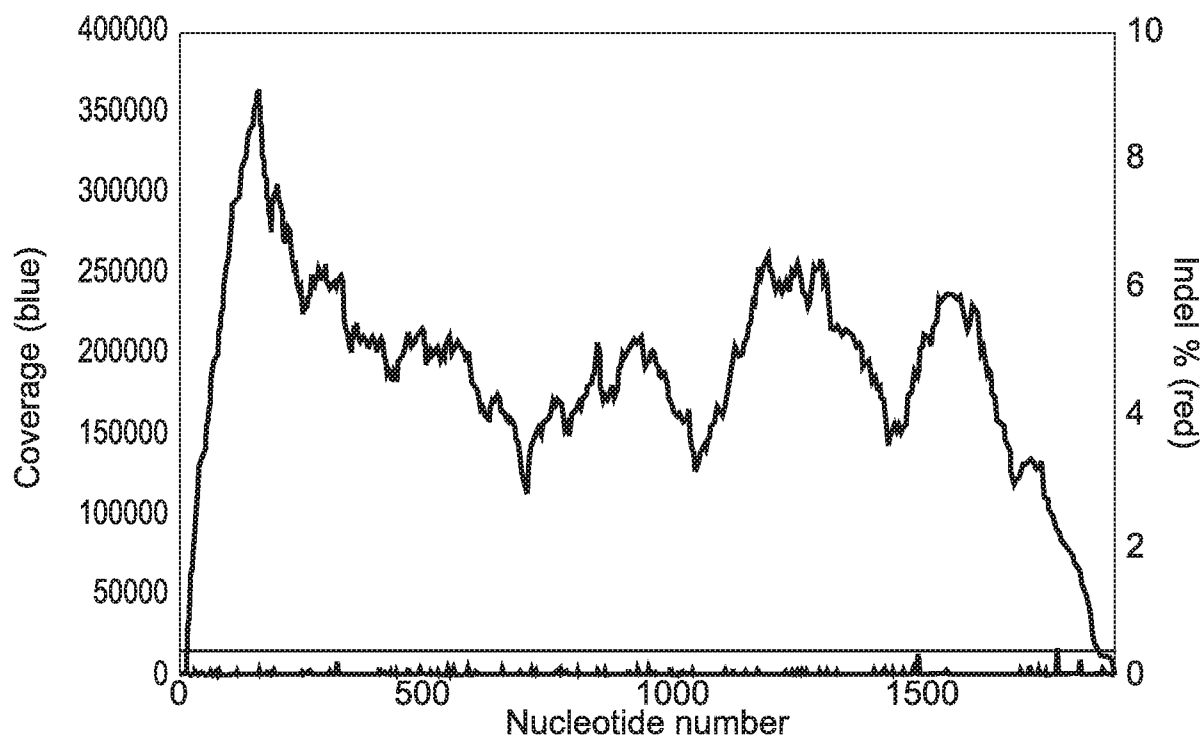


FIG. 17

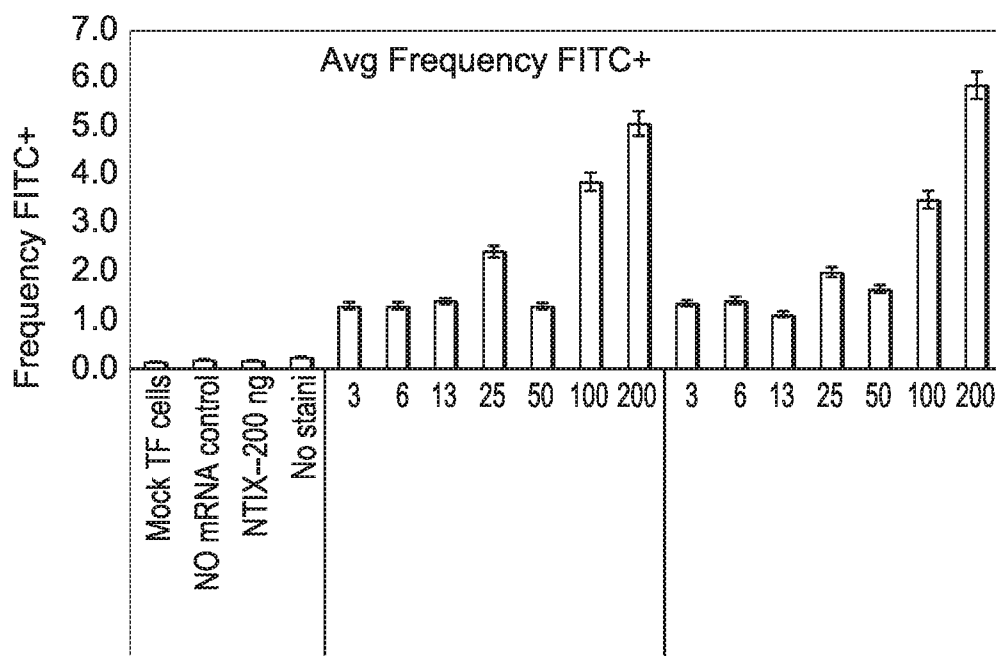


FIG. 18A

Fig. 18B

FITC Geometric Mean

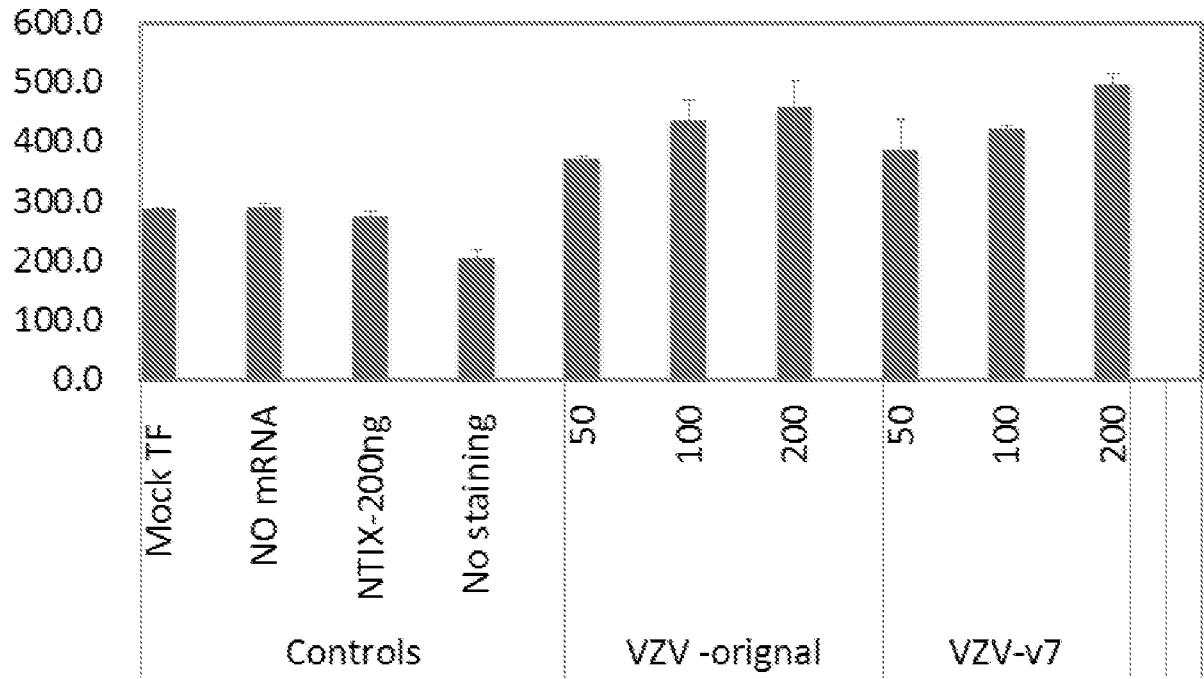


Fig. 19A

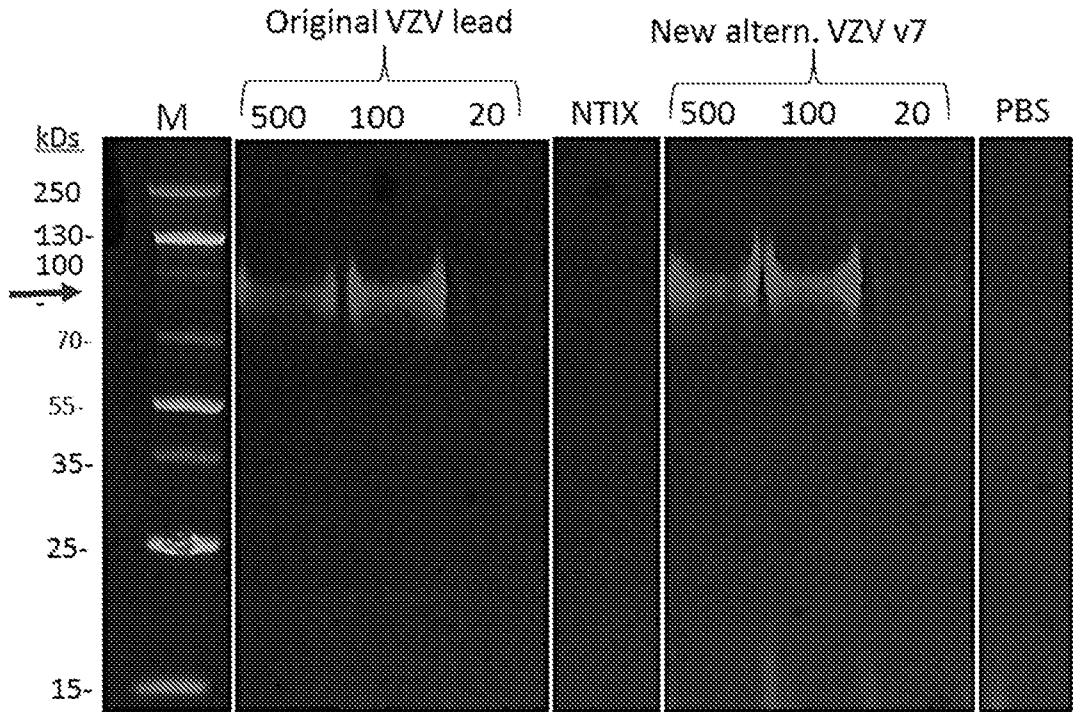


Fig. 19B

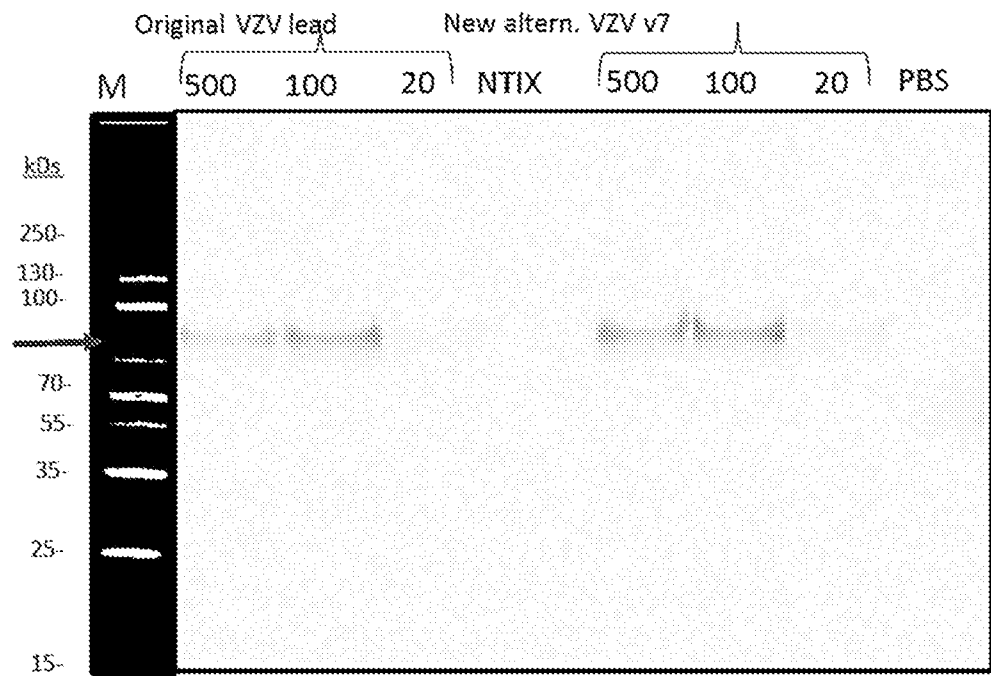


Fig. 20A

Expression

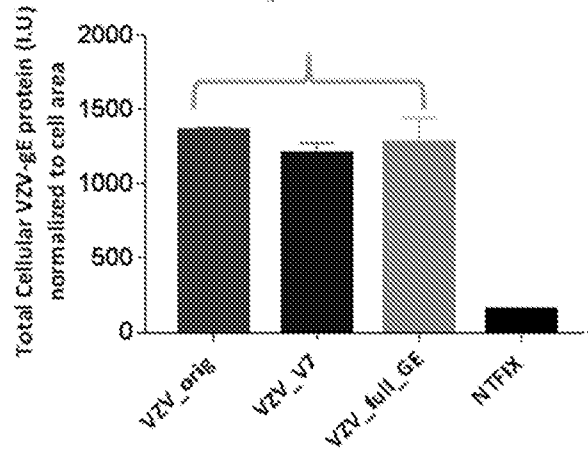


Fig. 20B

Localization

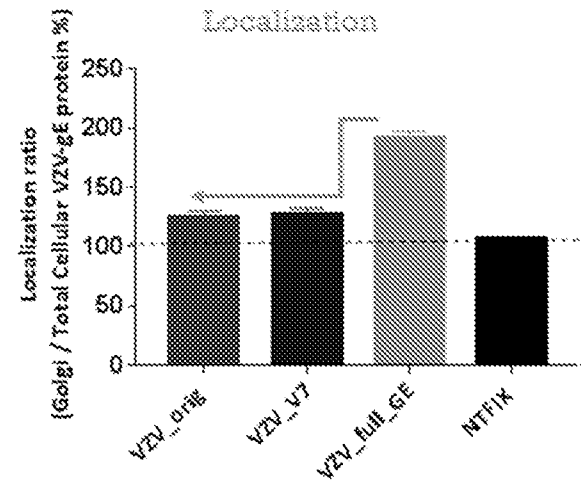


Fig. 21A

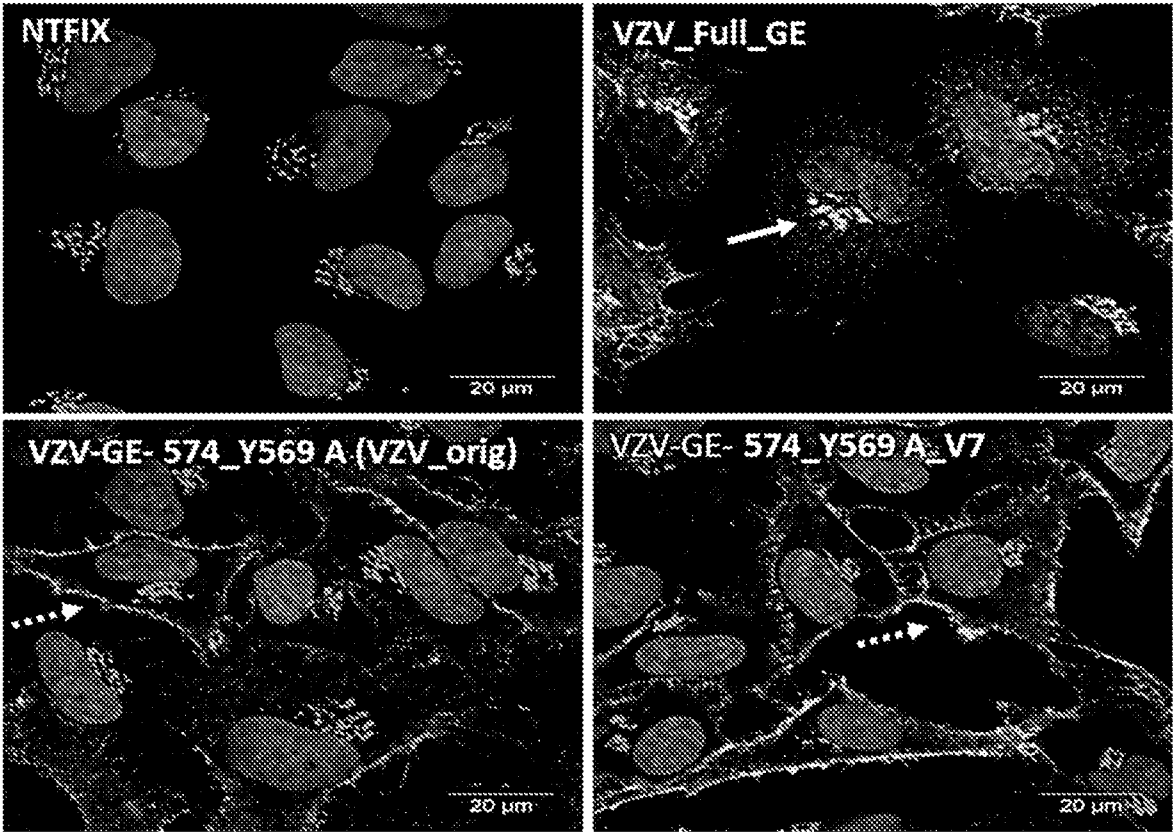


Fig. 21B

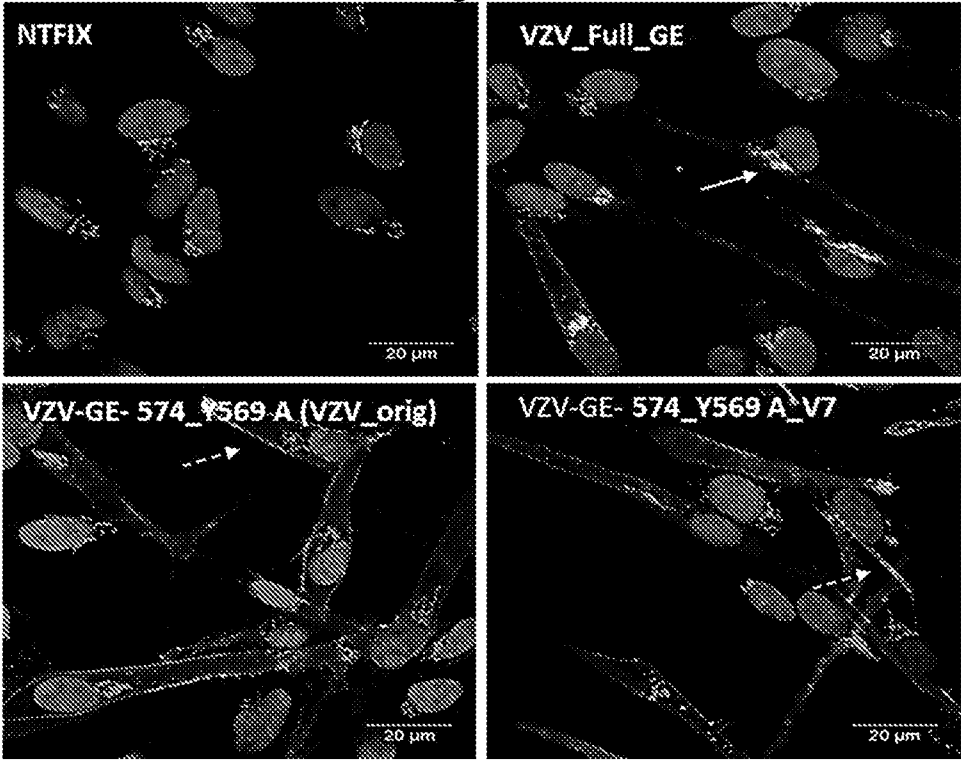
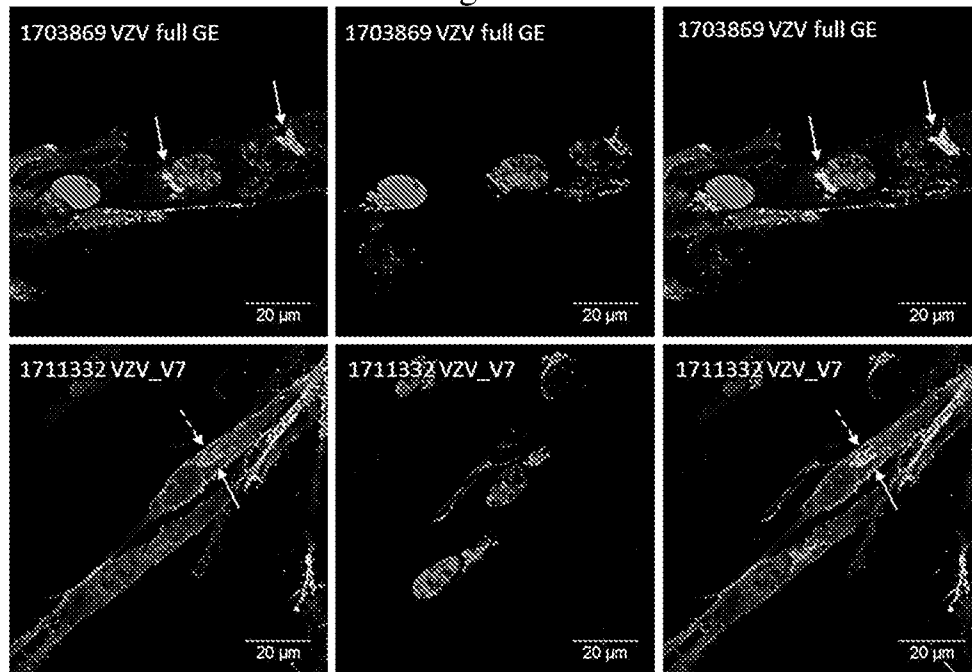


Fig. 21C



A. CLASSIFICATION OF SUBJECT MATTER**A61K 31/7105 (2006.01) A61K 39/25 (2006.01) C12N 15/38 (2006.01) A61P 31/22 (2006.01)**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases: full text English language databases, WIAP, EPODOC, MEDLINE, CAPLUS, EMBASE, BIOSIS, protein and nucleic acid databases of Genomequest, Esp@cenet, Patentscope, Pubmed, and internal databases provided by IP Australia

Keywords: Varicella, chickenpox, zoster, vaccine, immunise, mRNA, glycoprotein, and cationic lipid, NLP & like terms, CPC marks A61K2039/53, A61K39/125, A61K47/6931 and A61K9/51/LOW as well as the recited sequences and the Applicants and Inventors names.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 June 2018	Date of mailing of the international search report 26 June 2018
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Nathalie Tochon-Danguy AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262833101

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/US2018/022643
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/051211 A2 (NOVARTIS AG) 19 April 2012 [12-13], [19], [24], [88-142], [169-173], pages 33-34, 37-43, 147-170, Example 7, Figure 21-22, claims and SEQ ID NO. 68-72	1-116
X	WO 2013/055905 A1 (NOVARTIS AG) 18 April 2013 [12], [61-68], [79-115], [139-140] and [158-162]), claims and SEQ ID NO. 32-36	1-116
X	US 2014/0220083 A1 (BRITO L. et al.) 07 August 2014 Abstract, Figure 4, ([14-34], [70-104], [134-136], [156-158], [189], [240], [337], Table 13, Example 7, claims and SEQ ID Nos: 19 and 20	1-116
A	WANG, Z. et al., "Essential Role Played by the C-Terminal Domain of Glycoprotein I in Envelopment of Varicella-Zoster Virus in the trans-Golgi Network: Interactions of Glycoproteins with Tegument", JOURNAL OF VIROLOGY, 2001, Vol. 75, No. 1, pages 323-340 Whole document	1-116
A	ALCONADA, A. et al., "A tyrosine-based motif and a casein kinase II phosphorylation site regulate the intracellular trafficking of the varicella-zoster virus glycoprotein 1, a protein localized in the trans-Golgi network", The EMBO Journal, 1996, vol. 15, no. 22, pages 6096-6110 Whole document	1-116
A	WO 2015/164674 A1 (MODERNA THERAPEUTICS, INC.) 29 October 2015 Whole document	1-116
A	WO 2014/127917 A1 (CUREVAC GMBH) 28 August 2014 Whole document	1-116
P,X	WO 2017/070601 A1 (MODERNATX, INC.) 27 April 2017 Figures 3, 5-7, 11-16, page 30, Examples, claims, SEQ ID Nos:1-134	1-116

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/US2018/022643	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2012/051211 A2	19 April 2012	WO 2012051211 A2	19 Apr 2012
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